ZITELLI AND DAVIS'

ATLAS OF
PEDIATRIC
PHYSICAL DIAGNOSIS

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DIAGNOSIS

SEVENTH EDITION

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IN MEMORIUM

Holly W. Davis, MD
August 23, 1945–September 6, 2013

We miss Holly Davis. Holly W. Davis, MD, an original co-editor and contributor to the Atlas, passed away in 2013 after battling cancer. Holly helped to develop the concept of the Atlas and worked tirelessly with contributors over 6 editions to shape the style, provide clinical images and assure the quality of the textbook.

Holly was a consummate clinician–educator. She was widely respected not only for her diagnostic and clinical acumen, but also for her boundless energy, enthusiasm and focus on the child and family.

Holly attended Duke University for undergraduate school and graduated from its medical school in 1971. She completed pediatric residency at Children's Hospital of Pittsburgh and went on to become ambulatory chief resident. In 1978 she became the medical director of the Emergency Department. Her focus on children led to an invitation to "Mr. Rogers' Neighborhood" television program to explain to children in the audience what to expect and how they would be cared for in the Emergency Department. She reassured children and parents that we "had a lot of different ways in which we help children get better." She spearheaded the construction of a new Emergency Department with innovative ideas emulated by many institutions around the country. In 1999 Holly became co-director of the Child Advocacy Center until her retirement in 2005. She became well-known for her skill in evaluation and detection of abuse and neglect in children. She was a fierce advocate for these children.

Holly Davis was an accomplished pediatrician, respected clinician, teacher, mentor, colleague and friend. But even more, Holly loved teaching, loved children and loved life. We were fortunate to know Holly, work with her and honored to call her friend. We miss her.

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ZITELLI AND DAVIS' ATLAS OF PEDIATRIC PHYSICAL ISBN: 978-0-323-39303-4
DIAGNOSIS, SEVENTH EDITION

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To our parents, who were our first teachers:
Hannah L. Zitelli and Patsy A. Zitelli
Thomas P. Nowalk and Lourdes W. Nowalk
George M. McIntire and Charlotte M. McIntire

To my wife, Suzanne, and my children,
Matthew, Daniel, Benjamin, and Anne Zitelli

To my wife, Amy Brinkos, and my children,
James, Max, and Peter Nowalk

To my brother, John, and my children,
Frances and Madeline Marcelle

To those exceptional teachers we have had, whose
dedication, enthusiasm, and creativity helped make the
acquisition, application, and sharing of knowledge more
fun than hard work and who inspired us not only to
perform to the best of our ability but also to
become teachers as well as physicians:

Henry Furrie, Paul C. Gaffney, MD, William H. Zinkham, MD,
J.R. Zuberbuhler, MD, Crystie Halsted, MD,
Ronald Lemire, MD, J. Carlton Gartner Jr., MD,
William I. Cohen, MD

To our residents and students, whose eagerness to learn
and to put their knowledge to use keeps us learning
actively and makes teaching so rewarding

There aren't many texts that stand the test of time, but the book you hold in your hand, or – more likely, given its considerable heft – on your lap or desk, has been proving its worth to students of pediatrics at all stages of their training and careers for 30 years and 7 editions. *Zitelli and Davis* (now ably edited by Drs Zitelli, McIntire, and Nowalk – all widely admired clinicians and diagnosticians) is revered for its breadth and depth, and especially for its extensive use of superb visual material, including striking photographs, radiographs, and data graphs.

It is often said that the art of the physical exam is atrophying from disuse, as are medical libraries of physical books, both victims of the digital age. Yet, the physical exam (along with the medical history) remains essential to the complete physician, and this book is a priceless reminder of the value of an actual book, with pages, stunning pictures, chapters, and indices.

Enjoy this book, and learn from it, and keep going back to refer to it, as generations of clinicians before you have done with previous editions.

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PREFACE

In pediatrics, visual recognition of a variety of disorders is oftentimes the key factor in diagnosis. Experienced clinicians who have seen a wide spectrum of disorders carry a wealth of information for diagnosis and teaching. Despite the increased reliance on technology to make a diagnosis, history and physical examination remain as the foundation for clinical assessment of the patient. The *Atlas of Pediatric Physical Diagnosis* was created to enhance the clinical experience of students, residents, nurses, and practitioners who care for children.

We have been very pleased with the reception of the first six editions of the Atlas, but have heard from many readers for the need of new and updated information. Every chapter in the seventh edition has been reviewed, revised and updated. Additional contributors have provided greater depth and dimension to existing chapters and some chapters have been entirely rewritten. New photographs and diagnostic images have been included in many chapters. New information about vascular anomalies, including recognition and diagnosis, classification, evaluation and prognosis necessitated the addition of a new chapter solely on Vascular Anomalies in children.

The Atlas is by no means encyclopedic, but rather presents an overview of clinical disorders that lend themselves to visual diagnosis. We have attempted to select disorders that are common or important and, when relevant, to describe the spectrum of clinical findings. The accompanying text deliberately emphasizes pertinent historical factors, examination techniques, visual findings, and diagnostic methods rather than therapy. We firmly believe that a careful history and physical examination are the best tools the clinician has for diagnosis and treatment. It is our hope that the seventh edition of the *Atlas of Pediatric Physical Diagnosis* continues to serve as a useful and practical reference for anyone who cares for children.

Basil J. Zitelli, MD
Sara C. McIntire, MD
Andrew J. Nowalk, MD, PhD

VIDEO CONTENTS

The following supplemental videos are available at expertconsult.com

GENETIC DISORDERS AND DYSMORPHIC CONDITIONS

Suneeta Madan-Khetarpal | Georgianne Arnold

The field of pediatric genetics and dysmorphology is complex, interesting, and rapidly evolving. Our knowledge base is gleaned from the careful observations of master clinicians and scientists who recognized clinical characteristics and patterns of malformation in individuals with genetic, teratogenic, developmental, and metabolic problems. They have provided us with a framework for the investigation of patients from clinical and laboratory perspectives. In addition to classic cytogenetics, molecular cytogenetics methods have been increasingly incorporated in clinical settings and have greatly assisted evaluation, enabling far greater understanding of the molecular and physiologic basis of these disorders, and have greatly increased the rate of diagnosis of children with genetic and metabolic disorders. However, even with the availability of an ever-widening array of confirmatory tests, clinical evaluation of patients remains an essential component of the complete assessment of children and adults with genetic diseases and dysmorphic conditions. This stems from the fact that careful evaluation can substantially reduce the number of differential diagnostic possibilities and, thereby, the number of diagnostic tests and the total expense.

Visual identification of dysmorphic features, baseline anthropometrics combined with serial measurements with recognition of patterns of malformation and behavioral phenotypes, remains an integral part of the diagnostic algorithm. As in pediatrics in general, genetic disorders should be investigated on the basis of a careful history, with a family pedigree and a thorough physical examination including evaluation for the presence of major and minor anomalies, and thoughtful laboratory testing. This chapter is designed to present clinicians who care for children with background on the general principles of genetics and dysmorphology, as well as updated information about important advances in our field. Although not exhaustive, it provides a framework for the broad categories of genetic diseases and discusses an approach to the evaluation of the dysmorphic child. Definitions and examples of the types of disorders resulting in genetic and/or congenital anomalies in children are described, including malformations, deformations, disruptions, associations, and sequences. We include examples of disorders inherited through classic mendelian inheritance patterns, including single-gene mutations, such as Marfan syndrome, Rett syndrome, Smith-Lemli-Opitz syndrome, and Conradi-Hünermann syndrome, as well as examples of nonmendelian disorders, such as teratogenic exposures in utero and disruptions or deformations of previously normal fetal structures. New etiologic mechanisms of diseases, such as imprinting abnormalities and expansions of trinucleotide repeats in nuclear deoxyribonucleic acid (DNA), are presented. Last, a newly evolving area of genetics, the investigation of disorders of mitochondrial deoxyribonucleic acid (mtDNA) and/or mitochondrial function, is discussed.

COMMON CHROMOSOMAL DISORDERS
General Principles
The Nature of Chromosomes

Productive insights gleaned from the results of the completed Human Genome Project have dramatically changed some of our understanding of how the human genome functions. However, it is important to introduce to the reader our current understanding of the subject matter. Human hereditary factors are located in *genes* (the *genome*). Approximately 10% are genes that encode proteins that are assembled to form tissue structures or to form enzymes that catalyze chemical reactions within cells. The other 90% have functions that are currently not clear (see also The Nature of Genes and Single-gene Disorders, later). The genes are composed of DNA and are stored in intranuclear cell organelles called *chromosomes.* Each chromosome contains one linear DNA molecule folded over onto itself several times, as well as ribonucleic acid (RNA) and proteins. Because all genes exist in pairs, all chromosomes must likewise exist in pairs. The members of each pair of genes are called *alleles,* and the members of each pair of chromosomes are known as *homologues.* The conventional depiction of the constitution of homologues in the nucleus is called the cell's *karyotype* (Fig. 1.1). If at any gene locus the alleles are identical, that gene locus is *homozygous.* If the alleles are not identical, the gene locus is *heterozygous.*

Except for gametes, normal human cells contain 23 pairs of chromosomes, 46 in all. One of these pairs is concerned in part with inducing the primary sex of the embryonic gonads. These sex chromosomes are called the *X and Y chromosomes,* and they are not genetically homologous except in a few areas. Women have two X chromosomes, whereas men have an X and a Y chromosome. The remaining 22 pairs are called *autosomes,* and they determine non–sex-related (somatic) characteristics.

During most of a cell's life cycle, chromosomes are diffusely spread throughout the nucleus and cannot be identified by morphologic means. Only when the cell divides does chromosome morphology become apparent (Fig. 1.2). The in vitro life cycle and the cellular division, or *mitosis,* of a somatic cell are illustrated in Figs. 1.3 and 1.4, respectively. The life cycle and divisions, or *meiosis,* of a germ cell are much more complex and are not suitable for ordinary clinical evaluation.

Any somatic cell that can divide in tissue culture can be used for chromosomal (cytogenetic) analyses. The most convenient tissue source is peripheral blood, from which lymphocytes can be stimulated to divide during 2 or 3 days of incubation in tissue culture medium. Fibroblasts obtained from skin remain a frequently used alternative when peripheral blood lymphocytes are not clinically
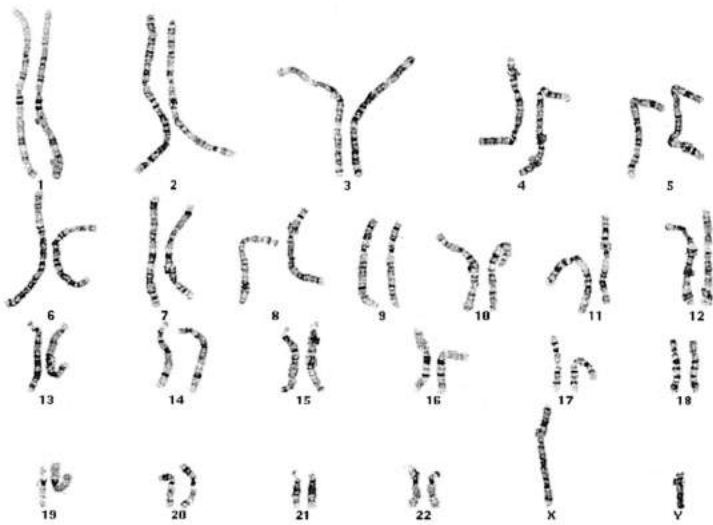
Figure 1.1 Photomicrographs show that this is a G-banded male karyotype. (A female would have two X chromosomes and no Y chromosome.) The horizontal banding produced by the Giemsa staining technique allows for precise identification of homologous chromosomes. *(Courtesy Urvashi Surti, PhD, Pittsburgh Cytogenetics Laboratory.)*

Figure 1.3 The in vitro life cycle of a somatic cell. Interphase lasts 21 hours and can be divided into the following three stages: G_1 (7 hours)—cell performs its tasks; S (7 hours)—deoxyribonucleic acid (DNA) replicates; G_2 (7 hours)—cell prepares to divide. Then mitosis occurs.

suitable, but fibroblasts require an incubation period of 4 to 6 weeks. After death, lung tissue is the best tissue to culture for chromosomal analyses, although the process also requires a 4- to 6-week incubation period. Alternatively, skin fibroblasts are frequently obtained postmortem for various enzymatic and cytogenetic analyses, which may be used to confirm a clinical diagnosis. When a treatment decision requires urgency, preliminary chromosomal evaluation can
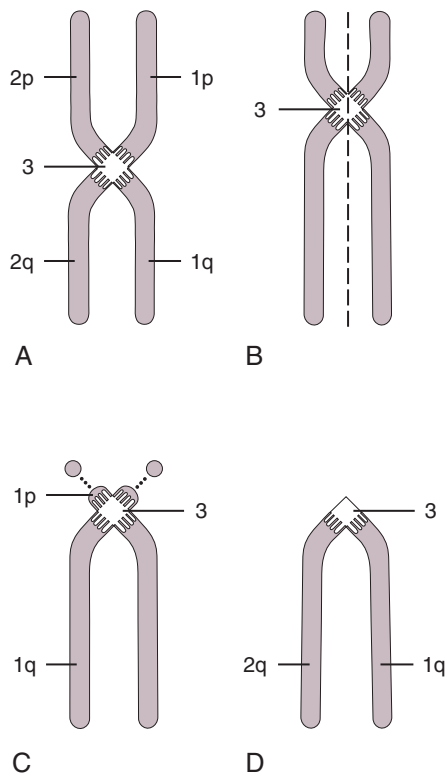
be made within 4 to 24 hours by using uncultured bone marrow aspirate. Oftentimes the karyotype is supplemented within 48 to 72 hours by a molecular cytogenetics technique, either interphase or metaphase fluorescence in situ hybridization (FISH), using rapid culturing and diagnostic techniques. More recently, conventional cytogenetics is being substituted by high-resolution molecular karyotyping using microarray-based comparative genomic hybridization (array-CGH). Array-CGH enables copy number changes at high resolution. This is implemented in the clinical setting and is being recommended as the first step in the investigation of patients with developmental delays, mental retardation, and multiple congenital anomalies. FISH and other molecular techniques are now used primarily to confirm the imbalances detected by array-CGH. This is an ever-evolving area, and pediatric clinicians are advised to discuss clinical and laboratory investigations with clinical geneticists and/or laboratory directors before the initiation of tissue sampling to ensure the most productive use of samples and rapid testing methods.

Aneuploidy

Aneuploidy refers to an abnormality in chromosome number, in humans a chromosome number different from an even multiple of 23 (the haploid number) (Fig. 1.5). In aneuploidy, there are typically

Figure 1.2 Morphology of a chromosome during metaphase. **A,** Metacentric chromosome with centromere *(3)* in the middle. **B,** Submetacentric chromosome with centromere off-center. **C,** Acrocentric chromosome with centromere near one end. **D,** Telocentric chromosome (not found in humans) with centromere at one end. The deoxyribonucleic acid (DNA) of the chromosome has replicated to form two chromatids: *1p* and *1q* represent one complete chromatid, *2p* and *2q* the other complete chromatid (*p* refers to the short arm and *q* refers to the long arm). The chromosome will then divide longitudinally, as shown in **B.**

Figure 1.4 Mitosis lasts about 1 hour, during which time the cell divides. **A,** Interphase cell at the end of G_2. **B,** Prophase: Replicated deoxyribonucleic acid (DNA) condenses and is visible. **C,** Metaphase: 46 duplicated chromosomes align randomly on the spindle and can be photographed for karyotyping. **D,** Anaphase: Chromosomes divide longitudinally, and half of each one moves to the opposite pole of the cell. **E,** Telophase: Cell wall divides. **F** and **G,** Interphase at G_1: Two daughter cells, each with 46 chromosomes.

Figure 1.5 Karyotype of a patient with trisomy 13 demonstrates aneuploidy. Note the extra chromosome 13, causing the cell to have 47 instead of 46 chromosomes. *(Courtesy Urvashi Surti, PhD, Pittsburgh Cytogenetics Laboratory.)*

45 or 47 chromosomes instead of the usual 46. Rarely, multiples of the X or Y chromosome result in individuals with 48 or 49 chromosomes. *Double aneuploidy,* the simultaneous occurrence of two nondisjunctional events, has been described in the literature. In the liveborn, it usually involves one autosome and one sex chromosome. Double autosomal trisomy has been found repeatedly in spontaneous abortion but has not been demonstrated in a liveborn infant.

If aneuploidy occurs in a gamete as a result of an error of chromosomal division (nondisjunction or anaphase lag) during meiosis, all cells are affected in the fertilized embryo. With subsequent pregnancies, the risk for another chromosomal abnormality in the offspring is increased approximately 1% to 2% overall, in addition to the general background risk of abnormalities. The couple would be at risk for aneuploidy states of many types, not just the particular aneuploidy in their affected child. We are not yet aware of the underlying mechanism for the increased risk; however, families may benefit from an understanding of the possibilities for prenatal diagnosis in their individual case and may want to be referred for genetic counseling before the conception of another child (Fig. 1.6).

Mosaic Aneuploidy States Mosaicism, the presence of two or more genetically different cell lines within an individual, can result from an error in division during either meiosis or mitosis. In one possible scenario, aneuploidy originates during meiotic division (i.e., before conception). In such cases, the fetus starts out with an aneuploid chromosomal number and, subsequently, a division error occurs, resulting in the formation of another cell line that is chromosomally normal. In other cases of mosaicism the one-celled embryo (zygote) is chromosomally normal and a division error occurs after fertilization, during mitosis of an embryonic somatic cell, resulting in aneuploidy. Most individuals with mosaicism have only two or three different lines of embryonic cells. It requires considerable laboratory investigation to distinguish the meiotic or mitotic types. Generally speaking, parents are given a 1% to 2% recurrence risk because of the possibility of mosaicism present in a parental gonad, which is not identifiable in usual tissue sample analyses (Fig. 1.7).

Hypomelanosis of Ito is characterized by marbleized or mottled areas of hypopigmented whorls of skin along the Blaschko lines and is of heterogeneous etiology. Individuals with hypomelanosis of Ito can have multiple congenital anomalies, dysmorphic features, variable mental retardation, and other neurologic findings. Karyotyping from skin lesions will reveal mosaic abnormality of chromosomes from normal or hypopigmented and hyperpigmented regions. Balanced and unbalanced chromosome aberrations and uniparental disomy (UPD) may be encountered (Fig. 1.8).

Abnormalities of Chromosome Structure Chromosomes can be normal in number (diploid) but still be abnormal in structure. Inversions (Fig. 1.9), deletions (Fig. 1.10), and translocations (Fig. 1.11) of genetic material are examples of structural chromosomal abnormalities. These can arise as new (sporadic) mutations in the egg or sperm from which the embryo was formed, in which case the parents' recurrence risk for another child with a chromosomal abnormality is again 1% to 2%. However, the abnormality may also be inherited from a phenotypically normal parent who is a "carrier" of a structural chromosomal abnormality (Fig. 1.12). About 1 in 520 normal individuals carries a balanced but structurally abnormal set of chromosomes, called a *chromosome translocation.* The term *balanced,* for the purposes of this chapter, means that on cytogenetic analysis the structural abnormality does not appear to have resulted in any net loss or gain of genetic material. If the apparently balanced
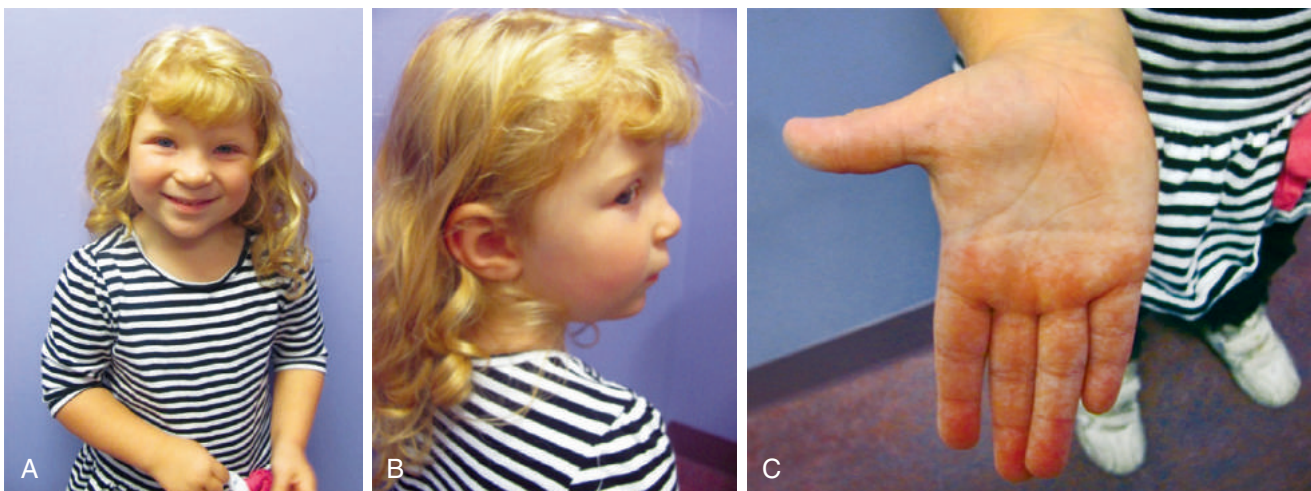
Figure 1.6 A female, 3 years and 8 months old, with double aneuploidy: Aneuploidy depicted by cytogenetic studies. Karyotype and fluorescence in situ hybridization (FISH) studies show predominantly 47XXX; some 47XXX also have an extra 21 (48XXX+21). The patient has some features of Down syndrome. Note the up-slanted palpebral fissures **(A),** low-set ears **(B),** and unilateral simian crease **(C).** An echocardiogram showed a patent foramen ovale. The patient is receiving behavioral and speech therapy; she is not toilet trained and has an individualized education program (IEP) in preschool. Triple X females are tall, and mosaic Down syndrome is similar to full Down syndrome but with a much milder phenotype. Her weight was in the 95th percentile, her height in the 80th, and occipital-frontal circumference (OFC) in the 20th.

Figure 1.7 A 1-year-old with facies suggestive of Down syndrome. Note the facies and short fifth fingers and clinodactyly. The muscle tone and growth parameters were normal. Cytogenetics studies showed 2% of the cells with 47,XY+21; interphase fluorescence in situ hybridization (FISH) studies with an extra cell count showed trisomy 21 in 1.3% of 523 peripheral lymphocytes analyzed.

Figure 1.8 Hypomelanosis of Ito. Karyotype at birth was normal: 46,XX. At 4 months of age characteristic streaks and whorls of hyperpigmentation and hypopigmentation of the skin were noted. A higher cell-count karyotype showed mosaicism for trisomy 14.
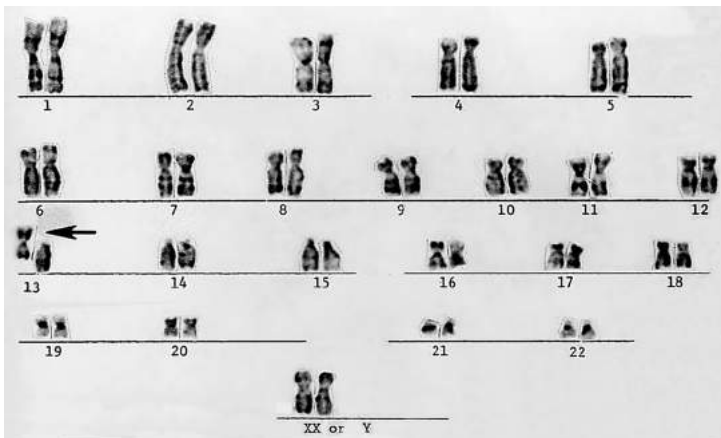
Figure 1.9 Pericentric inversion *(arrow)* of chromosome 13.

Figure 1.10 Deletion *(arrow)* of the p arm of chromosome 5 (cri du chat syndrome). *(Courtesy Urvashi Surti, PhD, Pittsburgh Cytogenetics Laboratory.)*
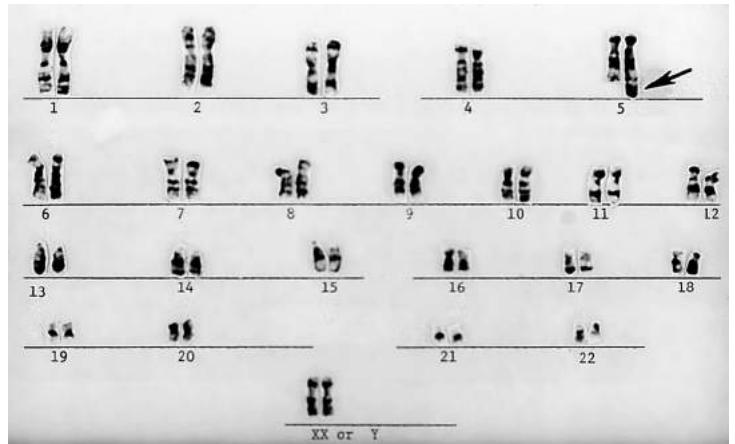
Figure 1.11 Unbalanced translocation. The additional deoxyribonucleic acid (DNA) was translocated onto the q arm of chromosome 5 *(arrow).* The abnormality was inherited from a normal carrier father (see Fig. 1.12) with a balanced reciprocal translocation between the q arms of chromosome 3 and chromosome 5. The patient died of multiple birth defects and in essence had a partial trisomy of the distal portion of the q arm of chromosome 3.
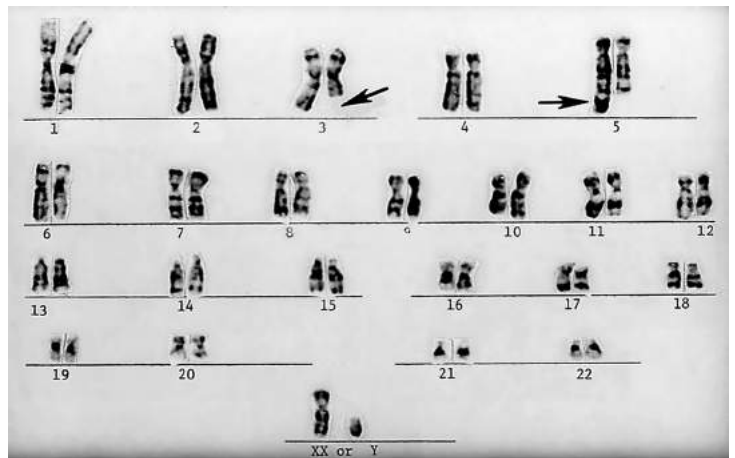
Figure 1.12 A "balanced" reciprocal translocation from chromosomes 3 *(arrow)* to 5 *(arrow)* in a normal man (the father of the chromosomally defective newborn whose karyotype is shown in Fig. 1.11).

chromosomal abnormality has been transmitted by other members of the family who are apparently phenotypically normal, it is considered a familial balanced translocation. Data suggest that a small percentage of individuals with apparently "balanced" translocations are actually mildly affected clinically by variable degrees of cognitive and physical deficits (Warburton, 1991). Thus high-resolution chromosome analyses and molecular cytogenetics techniques, such as array-CGH, are warranted in these instances, including (as needed) in situ hybridization techniques using DNA probes to completely characterize the location of the chromosome breakpoints and to determine on a molecular level whether any genetic material is missing. Molecular studies for imprinting effects may also be warranted.

A frequent way in which families with apparently balanced chromosome translocations present for evaluation occurs when a child is born with structural malformations and on karyotyping is found to have an unbalanced chromosome translocation. This may have occurred de novo in the child's chromosomes only or may be due to a previously undiagnosed familial balanced chromosomal translocation in a parent. Parental karyotypes are used to distinguish the etiology and are crucial in providing accurate genetic counseling regarding future pregnancies for that couple.

Incidence of Chromosomal Abnormalities

Data from Hook (1992) suggest that upward of 50% of human conceptions terminate in a spontaneous abortion. Most of these miscarriages occur so early during gestation that the pregnancy is never recognized. The earlier the abortion occurs, the more likely it is that the miscarried embryo had a chromosomal abnormality. Of recognized first-trimester abortuses, 50% are chromosomally abnormal, compared with 5% of later embryos. Among the chromosomally abnormal abortuses, the most frequent abnormalities are triploidy (69 chromosomes), trisomy 16, and 45,X (Turner syndrome) (Table 1.1). Generally speaking, triploidy and trisomy 16 are not compatible with life and are only occasionally seen among liveborn infants. Despite the fact that Turner syndrome is relatively common among liveborn infants, the majority of conceptuses with 45,X also abort spontaneously. The incidence of chromosomal abnormalities among liveborn infants in general is about 6 in 1000. Among a group including both stillborn infants and infants who die in the immediate perinatal period, the number is increased to approximately 50 in 1000.

Table 1.1	Occurrence of Chromosomal Abnormalities*	
Among Spontaneous Abortuses	**Incidence**	
Overall incidence	32.0%	
First trimester	52.0%	
After first trimester	5.8%	
Type of abnormality seen in		
spontaneous abortions		
Trisomy 16		
Other trisomies		
Triploidy		
45,X		
Miscellaneous		
Among Liveborns	**Number of Cases per 1000**	
Overall incidence	6.20	
Abnormality of autosomes	4.19 (males and females)	
Trisomies		
Balanced rearrangements		
Unbalanced rearrangements		
Abnormality of sex chromosomes	2.03 (males and females)	
In males: XXY, XYY, mosaics		
In females: 45,X (0.08), XXX,		
mosaics (1.43)		

*About one-quarter of all conceptuses are chromosomally abnormal. About 50 in 1000 stillborns have a chromosomal abnormality.

When to Suspect a Chromosomal Abnormality

Chromosomal abnormalities of either number or structure are likely to have a detrimental effect on the phenotype of an affected individual. Aneuploidy of an autosome, or non-sex chromosome, generally significantly impairs physical and cognitive development. However, aneuploidy of a sex chromosome may have little or no apparent effect on the phenotype. One should look for clustering of abnormalities in family members to suggest a problem, although their absence does not rule out a chromosomal abnormality.

Carriers of an inherited or a de novo reciprocal translocation are usually genetically balanced and are subsequently normal. However, their conceptuses are likely to be genetically unbalanced and may abort spontaneously or be born with major congenital anomalies. A history of unexplained infertility, multiple spontaneous abortions (three or more), and particularly of a previous birth to the couple or to a close relative of a child with dysmorphic findings and/or major anomalies may be an indication that one of the parents carries a balanced chromosomal translocation or rearrangement. Thus a chromosome study on the couple is indicated; and if translocation is found, they should seek antenatal genetic counseling. This may also be advisable for extended family members.

A normal person who carries a balanced reciprocal translocation can commonly produce six chromosomal types of gamete. On fertilization, these gamete types can result in several possible fertilized embryos: a normal conceptus, a carrier conceptus like the normal carrier parent, two types of immediately lethal conceptus resulting from gross chromosomal imbalances (i.e., too much or too little DNA), or two types of abnormal conceptus caused by lesser chromosomal imbalances. Whether the latter two types abort spontaneously or come to term as liveborns cannot be predicted in advance solely on theoretical grounds. Therefore genetic counseling in such situations depends somewhat on analysis of what has occurred within the individual family and in other families with similar rearrangements. Rarely, other types of chromosomal imbalances are found in conceptuses of such carrier parents.

Experience suggests the following: If a carrier has already produced a chromosomally unbalanced liveborn child, then it is apparent that it is possible for this to occur again in future pregnancies, and the risk that the translocation carrier might have another chromosomally unbalanced liveborn infant can be as high as 20%. However, if the translocation carrier parent has produced either only healthy liveborn infants or spontaneous miscarriages, then it is less likely that the chromosomally unbalanced gametes are viable. Consequently, that person's risk for producing a chromosomally unbalanced liveborn is only about 4%. Last, if a couple of whom one spouse is a carrier has not yet experienced any pregnancies, their risk for a chromosomally abnormal liveborn is estimated to be about 10%.

New Technologies

Fluorescence in Situ Hybridization

FISH is a laboratory technology that has revolutionized the diagnostic capabilities of clinical cytogenetic laboratories. In this technique, a DNA probe is tagged with a label that fluoresces when viewed under a special microscope. A cocktail of many repetitive DNA probes blanketing a specific chromosome from end to end can be obtained. This is called a *FISH paint*. Using special microscope filters, a clinician can simultaneously FISH paint a slide with probes fluorescing in two or three different colors. FISH paints specific for all chromosomes are available.

Some of the well-recognized syndromes described initially by FISH probes for chromosome microdeletion syndromes include the following:

- Angelman syndrome: del 15q11-13
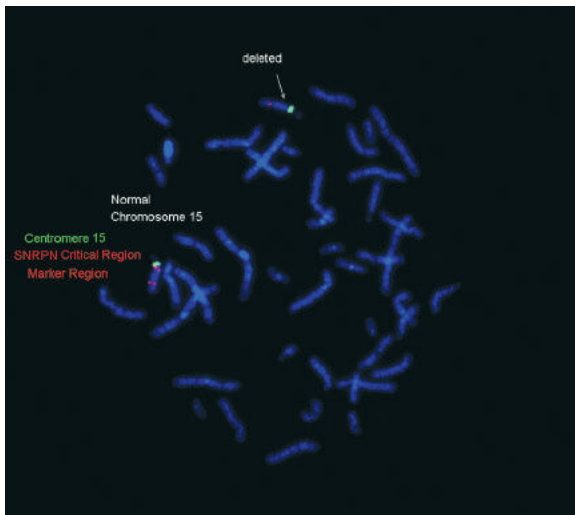- Prader-Willi syndrome: del 15q11-13

Figure 1.13 4′,6′-Diamidino-2-phenylindole (DAPI)–counterstained metaphase and interphase images showing a duplication of the Prader-Willi/Angelman (D15S10 locus) critical region *(red)*. The chromosome 15 centromere is used as a control *(green)*. Adjacent to the centromere in *red* is the normal pattern for D15S10. *SNRPN,* Small nuclear ribonucleoprotein-associated polypeptide N. *(Courtesy Urvashi Surti, PhD, Pittsburgh Cytogenetics Laboratory.)*

- Cri du chat syndrome: del 5p15.2
- DiGeorge sequence/velocardiofacial syndrome: del 22q11.2
- Miller-Dieker/lissencephaly syndromes: del 17p13.3
- Williams syndrome: del 7q11.23
- Smith-Magenis syndrome: del 17p11.2
- Wolf-Hirschhorn syndrome: del 4p16.3
- Severe X-linked ichthyosis: del Xp22.3
- 1p36 deletion syndrome
- 1q21.1 deletion syndrome
- 16p13.11 deletion
- Phelan-McDermid syndrome: del 22q13.3

See Figs. 1.13 and 1.14 for details on Prader-Willi/Angelman syndrome and Wolf-Hirschhorn syndrome.

Array-Based Technology: Microarray for Evaluation of Copy Number Variation

In addition to classic cytogenetics, molecular cytogenetic methods are being incorporated in clinical settings at an increased rate. More
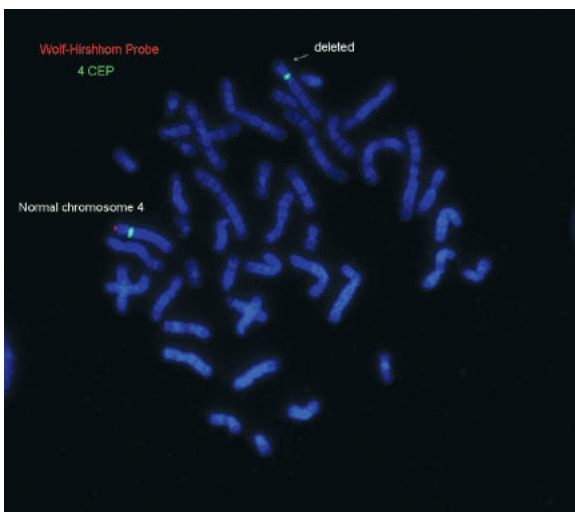

Figure 1.14 Metaphase chromosomes showing a deletion of the Wolf-Hirschhorn syndrome (WHS) critical region *(red)*. A chromosome 4 centromere *(4 CEP)* probe is used as a control, shown here in *green*. Absence of the red probe signal on one chromosome 4 *(arrow)* indicates a deletion of the WHS region at 4p16.3. *(Courtesy Urvashi Surti, PhD, Pittsburgh Cytogenetics Laboratory.)*

recently, conventional cytogenetics is being substituted with high-resolution molecular karyotyping using array-CGH. Array-CGH analyses are proficient in detecting imbalances in the genome and enable detection of copy number changes at high resolution. This technique has been implemented by the American College of Medical Genetics (ACMG) as the first step in the investigation of patients with developmental delays, mental retardation, multiple congenital abnormalities, and autism spectrum disorders and has the highest diagnostic yield—up to approximately 15% to 28%. This is much higher than the diagnostic yield of G-banded karyotypes (on the order of 3%), excluding Down syndrome and other recognizable chromosomal syndrome (Miller et al, 2010). In addition, molecular cytogenetic techniques, such as array-CGH, have demonstrated that approximately 20% of apparently balanced chromosome translocations, de novo or familial, have gain or loss of genetic material at the breakpoints. Therefore, molecular cytogenetic studies are warranted because they completely characterize the location of the chromosome breakpoints and potentially identify additional genetic material that may be duplicated or deleted that would not otherwise be detected by the traditional cytogenetic methods. FISH and other molecular techniques are now used primarily to confirm the imbalances detected by array-CGH. With microarray testing, many new microdeletion and microduplication syndromes have emerged (e.g., deletion 1p36, deletion 1q21.1, and deletion 16p13.11 syndromes) (Fig. 1.15; and e-Figs. 1.1 to 1.3).

Single nucleotide polymorphism (SNP) arrays are being used in clinical settings and allow genome-wide copy number analysis. The copy number changes may provide insight into abnormalities, such as segmental and uniparental disomy, by revealing "copy number–neutral" areas of continuous homozygosity that can give rise to disease, congenital anomalies, and/or cognitive impairment. SNP arrays may be helpful in identifying translocated segments in UPD and in looking for imprinting effects of the chromosomal regions.

Molecular karyotyping and SNP arrays are ultimately more cost-effective tests and have been extremely useful to clinicians in

Figure 1.15 An 8-year-old with del22q.11 and 1p31.1 microdeletion. Patient is short in stature; has a right aortic arch, sacral dimple, left cryptorchidism, and global developmental and significant cognitive and speech delays; and is not toilet trained. He had undergone surgical repair of the palate for velopharyngeal incompetence. Note the low-set, cupped, and posteriorly rotated ears and hypoplastic alae nasi. DiGeorge syndrome was diagnosed in utero by prenatal fluorescence in situ hybridization (FISH) on amniocytes: 46, XY, ish del(22)(q11.2q11.2) (TUPLE1–) was confirmed at 6 years of age by oligonucleotide arrays. In addition, 1p31.1 microdeletion was detected and maternally inherited.
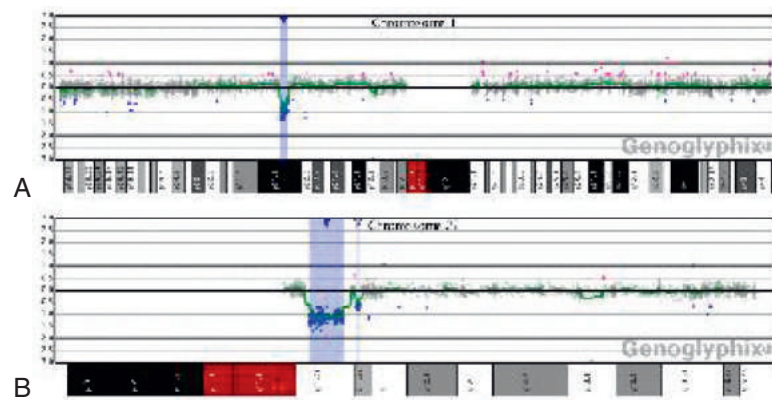
e-Figure 1.1 Microarray characterization of a 1p31 and 22q11.21 deletion in a single proband. **A,** Microarray plot showing single-copy loss of 89 oligonucleotide probes from the short arm of chromosome 1 at 1p31. Probes are ordered on the x axis according to physical mapping positions, with the most distal p-arm probes to the left and the most distal q-arm probes to the right. Values along the y-axis represent $\log_2$ ratios of patient: control signal intensities. **B,** Microarray plot showing single-copy loss of 205 oligonucleotide probes from the long arm of chromosome 21 at 21q11.21. Probes are arranged as in **A** with the most proximal q-arm probes to the left and the most distal q-arm probes to the right. Results are visualized using Genoglyphix (Signature Genomics, Spokane, WA). *(Courtesy Urvashi Surti, PhD, Pittsburgh Cytogenetics Laboratory.)*
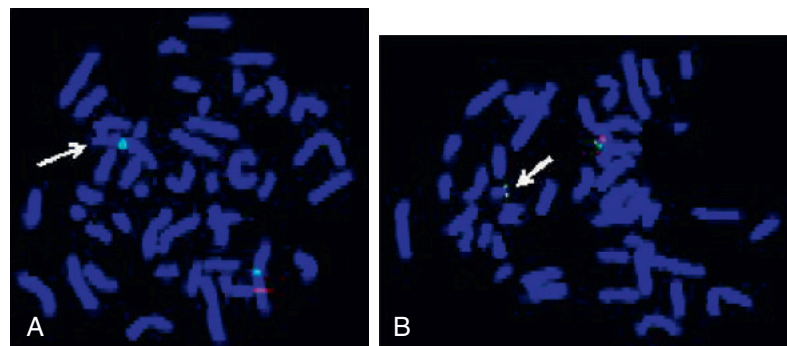
e-Figure 1.2 A, Fluorescence in situ hybridization (FISH) showing a deletion at 1p31.1. Probe 1p31.1 is labeled in *red* and chromosome 1 centromere probe D1Z1 is labeled in *green* as a control. The presence of only one red signal indicates deletion of 1p31.1 on one homologue *(arrow).* **B,** FISH showing a deletion at 22q11.21. Probe 22q11.21 is labeled in *red,* and BAC clone RP11-676E13 from 22q13.33 is labeled in *green* as a control. The presence of only one red signal indicates deletion of 22q11.21 on one homologue *(arrow).* (**A,** *Courtesy Urvashi Sirti, PhD, Pittsburgh Cytogenetics Laboratory.)*

A

B
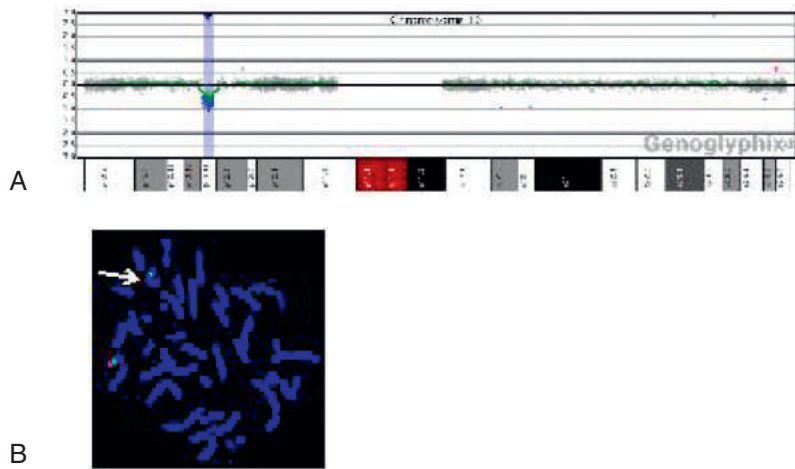
e-Figure 1.3 **A,** Microarray characterization of 16p13.11 deletion. Microarray plot shows a single-copy loss of 170 oligonucleotide probes from the short arm of chromosome 16 at 16p13.11. Probes are ordered on the x axis according to physical mapping positions, with the most distal p-arm probes to the left and the most distal q-arm probes to the right. Values along the y axis represent log$_2$ ratios of patient: control signal intensities. Results are visualized with Genoglyphix software (Signature Genomics, Spokane, WA). **B,** Fluorescence in situ hybridization (FISH) showing a deletion at 16p13.11. Probe 16p13.11 is labeled in *red,* and chromosome 16 centromere probe D16Z2 is labeled in *green* as a control. The presence of only one red signal indicates deletion of 16p13.11 on one homologue *(arrow).* (**A,** *Courtesy Urvashi Surti, PhD, Pittsburgh Cytogenetics Laboratory.)*

identifying necessary medical surveillance and treatment options, and they provide information on recurrence risks and prenatal options for families.

The impact of these newer methodologies continues to emerge, but their usefulness in providing information key to clinical prognosis is clearly becoming evident.

Array-CGH has been increasingly used for genetic testing of individuals with idiopathic mental retardation, developmental delay, autism spectrum disorders, and multiple congenital anomalies. By combining the array-CGH technique with classic cytogenetic and confirmatory FISH and appropriate molecular analyses, we are also able not only to identify cryptic genomic alterations but also to further analyze gross genomic alterations, including marker chromosome or other rearrangements identified by the classic cytogenetic analysis.

In cases of disorders with several etiologic mechanisms (such as Angelman syndrome), the absence of a deletion does not mean the child does not have the condition. An alternative mechanism, such as an imprinting center defect or uniparental disomy, may be the cause and would require methylation studies for detection.

DiGeorge sequence is discussed in Chapter 4, Williams syndrome is discussed in Chapter 5, and Angelman and Prader-Willi syndromes are covered later in this chapter. The remaining syndromes are outlined briefly in Table 1.2 and in Figs. 1.16, 1.17, and 1.18.

Table 1.2	Some Syndromes Identifiable With Fluorescence in Situ Hybridization Probes	
Syndrome	Major Findings	Comments
Cri du chat (deletion 5p15.2)	Microcephaly, round face, down-slanting palpebral fissures, epicanthal folds, hypertelorism, catlike cry in infancy	
Isolated lissencephaly	Lissencephaly (incomplete development of brain with smooth surface)	Approximately 30% have deletion 17p13.3
Miller-Dieker phenotype with lissencephaly	Microcephaly, lissencephaly, variable high forehead, vertical furrowing of central forehead, low-set ears, small nose with anteverted nostrils, congenital heart disease, poor feeding	Deletion 17p13.3 in vast majority
Deletion 22q11.2	Phenotypes: • Velocardiofacial syndrome • DiGeorge sequence • Some cases of Opitz syndrome • Conotruncal type of congenital heart disease (in an infant with dysmorphic features)	Appears to be a common deletion and should be considered in the differential diagnosis of children with multiple anomalies even if the features are not classic to any one phenotype
Wolf-Hirschhorn (deletion 4p16.3)	Moderate to severe cognitive impairment, hypertelorism, preauricular pit or tag, broad nasal bridge, micrognathia, cleft palate, short philtrum, growth deficiency	
Smith-Magenis (deletion 17p11.2)	Brachycephaly, flat facies, broad nasal bridge, short stature	Self-hugging behaviors, sleep disturbances

Approach to the Evaluation of a Dysmorphic Child

Approximately 2% to 3% of liveborn infants have an observable structural abnormality. This number rises to about 4% to 5% by the time the child is old enough to attend school. Structural differences can be determined to be either major or minor in character (Table 1.3; Figs. 1.19 and 1.20). Major structural anomalies have functional significance. Examples are polydactyly, colobomas of the iris (see Chapter 20), meningomyelocele, and cleft lip. Minor anomalies are usually of cosmetic importance only. Examples are epicanthal folds of the eyes, single transverse palmar creases, and supernumerary nipples. The incidence of isolated major anomalies in the general newborn population is approximately 1%, and the incidence of minor anomalies is approximately 14%. Both are more common in premature newborns.

The probability of an infant having a major anomaly increases with the number of minor anomalies found. Thus all children with multiple minor anomalies warrant a careful clinical assessment in order to find potentially significant occult major anomalies. Once an anomaly is identified, assessing its significance begins with a determination of whether the anomaly in question is a single localized error in morphogenesis or one component of a multiple malformation syndrome. An understanding of the pathophysiologic mechanisms that produce structural abnormalities or differences provides an opportunity to define the types of structural abnormalities seen. This also assists the process of identifying the etiology and arriving at a specific diagnosis, which then can be useful in determining the prognosis and estimating the risk of recurrence of a similar problem in future pregnancies.

Definitions of the classifications of structural anomalies aid in communication between clinicians and in the process of evaluation and are summarized from Jones (2006):

Malformation: A malformation is an abnormality of embryonic morphogenesis of tissue. It usually results from genetic, chromosomal, or teratogenic influences, but it can be of multifactorial etiology. Malformations are divided into two main categories: (1) those that constitute a single primary defect in development and (2) those that represent a single component of a multiple malformation syndrome. A *multiple malformation syndrome* can be defined as one having several observed structural defects in development involving multiple organ systems that share the same known or presumed etiology. Malformations often require surgical intervention.

Deformation: A deformation represents an alteration (often molding) of an intrinsically normal tissue caused by exposure to unusual extrinsic forces. A classic example is clubfoot, which may be the result of uterine constraint from crowding associated with a multiple gestation. A more severe example is the compressed facial features ("Potter facies") of a child exposed to severe uterine constraint associated with oligohydramnios, due to renal agenesis (see Chapter 14). The vast majority of deformations respond to medical therapy alone and have a relatively good prognosis in contrast to malformations, which frequently require surgical intervention.

Table 1.3	Examples of Congenital Anomalies	
Category	Major	Minor
Craniofacial	Choanal atresia	Plagiocephaly Flat occiput
Eyes	Coloboma of iris	Epicanthal folds
Ears	Microtia	Preauricular pit
Hands	Polydactyly Absent thumbs	Single transverse palmar crease Clinodactyly

A

B

C

D

Figure 1.16 **A** to **D,** Williams syndrome in four different patients. Hallmark features include supravalvular aortic stenosis, hypercalcemia, friendly personality, connective tissue abnormalities, and characteristic facies. Note the periorbital fullness, epicanthal folds, prominent lips, long philtrum, and stellate lacy iris pattern. All cases with clinical features were confirmed on fluorescence in situ hybridization (FISH) alone or microarrays.

Normal chromosome 7

deleted

Williams Region Probe
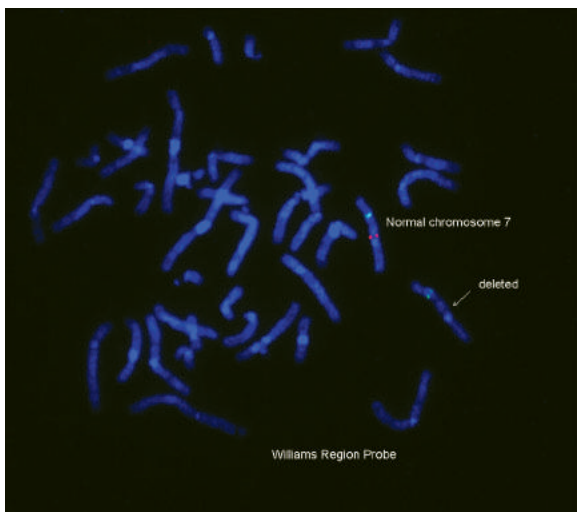
Figure 1.17 Metaphase image showing a deletion of the Williams critical region *(red).* Chromosome 7q31 probe *(green)* is used as a control. Absence of the red probe signal on one chromosome 7 *(arrow)* indicates a deletion of the elastin (ELN) locus at 7q11.23. *(Courtesy Urvashi Surti, PhD, Pittsburgh Cytogenetics Laboratory.)*

Figure 1.18 X-linked steroid sulfatase deficiency. A 14-year-old patient presented with joint laxity, struggles in school, and microcephaly. The karyotype was normal. Note the ichthyosis; the patient's brother was not evaluated but was reported to have ichthyosis, attention-deficit/hyperactivity disorder (ADHD), and seizures. Deletion of the steroid sulfatase (STS) gene from the Xp22.31 region was confirmed by oligonucleotide arrays.
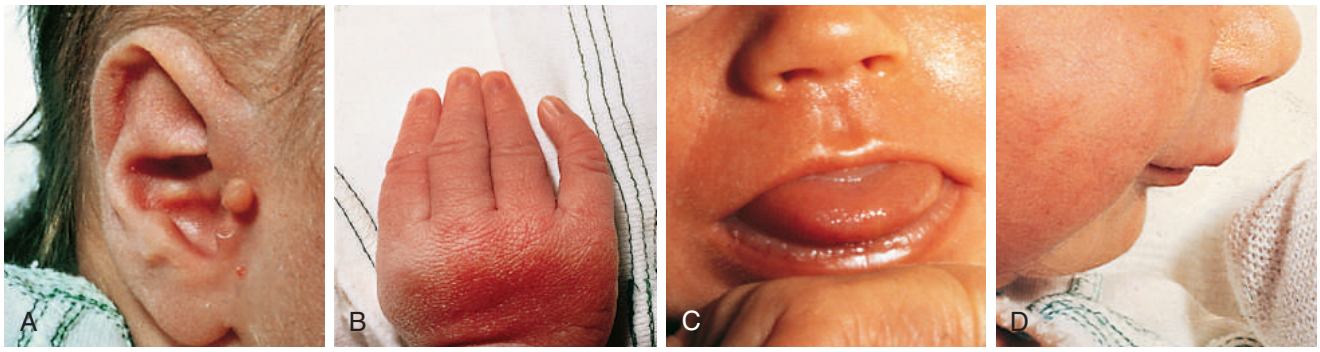
Figure 1.19 Clinical photographs show several minor anomalies seen at birth. **A,** Preauricular skin tag. **B,** Clinodactyly of the fifth finger. **C,** Macroglossia. **D,** Microretrognathia. *(Courtesy Christine L. Williams, MD.)*

Figure 1.20 Clinical photographs show several major anomalies seen at birth. **A,** Encephalocele. **B,** Cleft lip and palate. **C,** Meningomyelocele. **D,** Ectrodactyly (previously termed *lobster-claw deformity*). **E,** Polydactyly (postaxial). **F,** Bilateral clubfoot. **G,** Hypospadias. **H,** Fused labia with enlarged clitoris. **I,** Imperforate anus. *(Courtesy Christine L. Williams, MD.)*

Figure 1.21 Amniotic band syndrome; note the constriction ring at the ankle and amputation of the toes, a sequela to the amniotic bands.

Disruption: A disruption represents a breakdown of normally formed tissue; the breakdown may be the result of vascular accidents or exposure to adverse mechanical forces that are usually more severe than those that produce deformation. A classic example is the combination of clefting, constriction bands, and limb reduction defects associated with the presence of amniotic bands (see Chapter 2). The earlier these vascular accidents or abnormal forces occur during embryogenesis, the more severe the resulting defects (Fig. 1.21).

Dysplasia: Dysplasia is characterized by abnormal organization of cells within tissue, which usually has a genetic basis. An example is achondroplasia, the most frequent form of skeletal dysplasia.

(*Note:* Each of the preceding categories can have a sequence associated with it.)

Sequence: The term *sequence* refers to a recognizable pattern of multiple anomalies that occurs when a single problem in morphogenesis cascades, resulting in secondary and tertiary errors in morphogenesis and a corresponding series of structural alterations. A classic example is the Robin malformation or Pierre Robin sequence, in which the single primary malformation is microretrognathia (see Chapter 24). The resulting glossoptosis, or posterior placement of the tongue in the oropharynx, interferes with normal palatal closure if the lingual displacement occurs before 9 weeks' gestation. The resulting cleft palate is U-shaped, rather than having the V shape that is usually seen in classic cleft palate, a finding that aids in recognition.

Association: An association is a pattern of malformations that occurs together too frequently to be due to random chance alone but for which no specific etiology is yet recognized.

The approach to the evaluation of a child with a dysmorphologic abnormality is similar to a careful diagnostic evaluation of most pediatric problems, starting with a complete history and careful physical examination. In obtaining these, it is helpful to remember that there are six broad etiologic categories to be considered in the differential diagnosis: (1) a known syndrome, (2) an unknown syndrome, (3) a chromosomal abnormality, (4) a teratogen, (5) a congenital infection, and (6) a maternal disease and/or placental abnormalities.

The history should include the following:

- Course of the pregnancy, complications including possible infections or environmental exposures, medications/substance abuse
- Prior pregnancies, spontaneous abortions, stillborns, or infant/child deaths for this couple
- Labor/delivery/perinatal problems
- Past medical history

- Growth and development
- Meticulous family history with family tree going back three generations and including the following:
  - Familial traits and growth characteristics
  - Familial physical or developmental disorders
  - Spontaneous abortions, stillborns, infant/child deaths in extended family

The physical examination entails the following:

- Thorough general examination
- A search for major and/or minor anomalies
- Neurodevelopmental assessment

In addition, focused examination of immediate family members for physical characteristics and growth parameters and review of family photo albums may be helpful.

Determining how the child fits into the norms for growth and development for the general population, for the family's ethnic group(s), and for the extended family is important. One continuing challenge is to determine whether the norms for the family are truly in the normal range for the general population and ethnic background or, in fact, constitute variability of a genetic trait present in its severe expression in the child or family member seen for evaluation.

The identification of a recognizable pattern of both major and minor anomalies provides the clinical dysmorphologist with a diagnosis, or a short list of differential diagnostic possibilities. Thus the detection of major and minor anomalies is critical in the diagnostic process. Identification of specific and unusual malformations that are uncommon and occur in only a few syndromes can be especially helpful. For example, finding that a child has long palpebral fissure length and pronounced fingertip fat pad size in combination with the pattern of anomalies typical of the Kabuki syndrome makes it extremely likely that the diagnosis is the Kabuki syndrome. Training in dysmorphology emphasizes the recognition of key components in patterns of malformation, as well as the specific findings useful in distinguishing syndromes with similarities from one another. Texts that outline currently recognized patterns of malformations can be helpful in assisting the clinician in the identification of specific features that can rule a diagnosis in or out. Commercial computer-based programs exist for syndrome identification; however, these are often more effectively used by experts in the field because of the complexity of terminology and the need for exacting descriptions of the anomalies present in a given child.

A chromosome study should be performed on each child with a syndrome of congenital anomalies. Such a study may establish or confirm the diagnosis of a chromosomal disorder and its hereditary potential and may possibly help map the chromosomal location of genes for those syndromes known to be simple mendelian disorders.

Abnormalities of Autosomes

Down Syndrome

The worldwide incidence of Down syndrome among liveborns is approximately 1 in 660, with 45% of affected individuals born to women older than 35 years old. The incidence of Down syndrome among conceptuses is far greater than among liveborns because the majority of Down syndrome fetuses spontaneously abort.

No single physical stigma of Down syndrome exists; rather, the clinical diagnosis rests on finding a recognizable constellation of clinical characteristics, including a combination of major and minor anomalies (Fig. 1.22).

The most frequent features are up-slanting palpebral fissures and small external ears (by length). Several major anomalies are commonly associated with Down syndrome. Congenital heart disease is found in 45% of cases, particularly atrioventricularis communis and ventricular septal defects. Hence all newborns with Down syndrome

Figure 1.22 Down syndrome. These clinical photographs show several minor anomalies associated with this disorder. **A,** Characteristic facial features with up-slanting palpebral fissures, epicanthal folds, and flat nasal bridge. **B,** Brushfield spots. **C,** Bridged palmar crease, seen in some affected infants. Two transverse palmar creases are connected by a diagonal line. **D,** Wide space between first and second toes. **E,** Short fifth finger. **F,** Small ears and flat occiput.

should undergo cardiac evaluation with echocardiogram. About 5% have a gastrointestinal anomaly—most commonly duodenal atresia or Hirschsprung disease. An increased incidence of thyroid disorders also exists, particularly of the autoimmune type. Thus regular testing of thyroid function is recommended. Acute and neonatal leukemias occur 15 to 20 times more frequently in people with Down syndrome than in the general population. In newborns, much of this is represented by transient leukemoid reactions, with complete remission being the most frequent outcome. Quantitative abnormalities are found in many enzyme systems. People with Down syndrome are shorter than family members and the general population and have premature graying of hair. As adults, most males are infertile, but females may reproduce and can have children who will also have Down syndrome approximately one-third of the time.

Minor anomalies include brachycephaly; inner epicanthal folds; Brushfield spots; flat nasal bridge; a small mouth with protruding tongue that fissures with age; a short neck with redundant skin folds; single transverse palmar (simian) creases; clinodactyly of the fifth fingers, with single digital crease caused by hypoplasia of the middle phalanx; and wide spacing between the first and second toes. The number of such anomalies varies in any particular case.

With rare exceptions, individuals with Down syndrome are cognitively impaired. The degree of impairment varies, with intelligence quotients (IQs) ranging from 20 to 80. Most individuals function in the mild to moderate range of developmental delay. The advent of individualized programs of early intervention therapy, education, and sporting activities has resulted in much improved outcomes and individuals who are much more likely to function at the maximum of their developmental capabilities. Autopsy analyses of brains from individuals with Down syndrome have revealed the neuropathologic changes of Alzheimer disease in 100% of those older than 40 years old. Nevertheless, only about 25% of older individuals with Down syndrome exhibit clinical manifestations of Alzheimer disease. The reason for the clinical/pathologic discordance is not known. However, there does tend to be a progressive loss of cognitive functioning after the fourth decade of life. Longevity, although less than that of the general population, has steadily increased over the years. Individuals with Down syndrome who do not have congenital heart disease may expect to live well into their 60s. The principal causes of death in children with Down syndrome are infection, congenital heart disease, and malignancy.

The etiology of Down syndrome is trisomy 21, the presence of an extra chromosome 21 either as a simple trisomy or as part of a chromosome 21 fused with another chromosome. These fused chromosomes are often robertsonian translocation chromosomes or isochromosomes. Cases of mosaicism, in which trisomy 21 cell lines coexist with cell lines with the standard 46 chromosomes, exist as

Table 1.4	Maternal Age–Specific Risk for Trisomy 21 at Live Birth	
Maternal Age	**Prevalence at Live Birth**	
25 years old	1/1350	
30 years old	1/890	
35 years old	1/355	
40 years old	1/97	
45 years old	1/23	

well and may range in phenotype from normal to that typical of complete trisomy 21. An association between trisomy 21 and advanced maternal age is clear (Table 1.4).

About 5% of Down syndrome cases represent a centric fusion translocation between the long arm of a chromosome 21 and those of a 13, 14, 15, 21, or 22 acrocentric chromosome. Of these, about one-third are inherited from a clinically normal, balanced carrier parent; in the remaining two-thirds, the translocation is new in the affected child. Chromosome studies should therefore be performed on the parents and appropriate family members of an individual with translocation Down syndrome. If a parent carries a 21/21 translocation, all liveborns will have Down syndrome; for the remaining 21/centric fusion translocations, the empiric recurrence risk for a Down syndrome liveborn is less than 2% if the father is the carrier and roughly 15% if the mother is the carrier. The parents of children with trisomy 21 may benefit from genetic counseling to determine their individual risk of having another child with Down syndrome or with other chromosomal abnormalities in future pregnancies.

Trisomy 13

Trisomy 13 is a relatively rare (1 in 5000) genetic condition caused by the presence of additional chromosome material from all or a large part of chromosome 13. The vast majority of embryos with classic trisomy for a complete 13th chromosome abort spontaneously, but approximately 5% survive to be liveborn. They have a severe, recognizable pattern of malformation that allows clinicians to suspect this etiology immediately (Fig. 1.23). The hallmark features are defects of forebrain development related to those seen in holoprosencephaly, aplasia cutis congenita, polydactyly (most frequently of the postaxial type), and narrow hyperconvex nails. A broader listing of features is outlined in Table 1.5, which can be useful in comparing the features frequently seen in infants with trisomy 13 with those seen in trisomy 18. As with many syndromes, trisomy 13 and trisomy 18 share structural abnormalities; however, they usually are distinguishable on the basis of the pattern of anomalies present. Liveborn infants with trisomy 13 represent those who have the least severe structural abnormalities of major organs. Of these, about 5% survive the first 6 months of life. Thus discussions with parents about surgical interventions must take into account the small possibility of long-term survival and require sensitivity to the needs of the child and family.

Milder chromosome abnormalities involving extra material determined to originate from chromosome 13 must be identified and distinguished from classic trisomy 13, because the clinical phenotype and prognosis may be different and, in some cases, less severe. Children with mosaicism, that is, with a normal cell line and a trisomy 13 cell line, as well as those with trisomy of part of chromosome 13, can be identified by chromosome analysis. Careful laboratory investigation must be carried out to identify the exact chromosomal abnormality. The advent of FISH technology has dramatically increased the ability of laboratory specialists to characterize chromosome rearrangements, with the goal being to identify the exact breakpoints of the chromosomes involved in the rearrangements. Molecular studies then may be possible to determine any potential impact of the rearrangement on individual genes and their products. This information is extremely helpful to clinicians in

Figure 1.23 Several physical manifestations of trisomy 13. **A,** Facies showing midline defect. **B,** Clenched hand with overlapping fingers. **C,** Postaxial polydactyly. **D,** Equinovarus deformity. **E,** Typical punched-out scalp lesions of aplasia cutis congenita. (**A,** *Courtesy T. Kelly, MD, University of Virginia Medical Center, Charlottesville.* **B** *to* **E,** *Courtesy Kenneth Garver, MD, Pittsburgh, PA.*)

Table 1.5	Physical Abnormalities and Frequencies of Occurrence in Trisomy 13 and Trisomy 18 Syndromes	
Abnormality	**Trisomy 13**	**Trisomy 18**
Severe developmental retardation	††††	††††
Approximately 90% die within first year	††††	††††
Cryptorchidism in males	††††	††††
Low-set, malformed ears	††††	††††
Multiple major congenital anomalies	††††	††††
Prominent occiput	†	††††
Cleft lip and/or palate	†††	†
Micrognathia	††	†††
Microphthalmos	†††	††
Coloboma of iris	†††	†
Short sternum	†	†††
Rocker-bottom feet	††	†††
Congenital heart disease	††	††††
Scalp defects	†††	†
Flexion deformities of fingers	††	††††
Polydactyly	†††	†
Hypoplasia of nails	††	†††
Hypertonia in infancy	†	†††
Apneic spells in infancy	†††	†
Midline brain defects	†††	†
Horseshoe kidneys	†	†††

Symbols: Relative frequency of occurrence ranges from †††† (usual) to † (rare).

determining prognosis and in providing more realistic information when discussing treatment options. Rarely, children who have the recognizable pattern of clinical features of trisomy 13 have normal chromosomes. If a geneticist/dysmorphologist is not already involved, a consultation is warranted to aid in diagnosis and prognosis counseling and to determine any recurrence risks for the parents in future pregnancies.

Trisomy 18

The chromosomal disorder trisomy 18 occurs in approximately 3 in 10,000 newborns, and females are more likely to be liveborn. Affected infants are small for gestational age and have a frail appearance, and the face tends to appear petite relative to the rest of the craniofacial contour (Fig. 1.24A). They also have a recognizable pattern of malformation, but in these infants hallmark features—clenched hands with overlapping fingers (see Fig. 1.24B), short sternum, and "low arch" fingerprint patterns—are minor anomalies. Major anomalies, especially congenital heart disease, are generally present as well and are the source of significant morbidity and mortality. Other common findings include a prominent occiput, low-set and structurally abnormal ears, micrognathia, and rocker-bottom feet (see Fig. 1.24C). See Table 1.5 for a broader listing of clinical features that can be useful in distinguishing trisomy 18 from trisomy 13, which shares many of the same structural abnormalities.

Trisomy 18 was previously thought to be almost invariably fatal in the neonatal period; however, more recent data suggest that a small percentage of children can live longer and that between 5% and 10% will be alive at their first birthday. Survivors are more frequently female and have less severe structural abnormalities of major organs than most affected infants. Even with optimal neonatal, pediatric, and surgical management and excellent home-based care, children with classic trisomy 18 often "fail to thrive" and have significant developmental and cognitive impairments. Discussions with parents about interventions must take into account the slim possibility of long-term survival and require sensitivity to the needs of the child and family. Great care must be taken in providing a balanced picture to the family when discussing treatment options.

Chromosome analysis allows clinicians to evaluate the etiology of the trisomy and can help determine prognosis. Results can demonstrate classic trisomy 18 due to a complete extra chromosome 18, mosaicism for trisomy 18, or a complex chromosome abnormality involving one or more chromosomes. Children with chromosomal rearrangements that result in partial rather than complete trisomy

Figure 1.24 Several physical manifestations of trisomy 18. **A,** Typical profile reveals prominent occiput and low-set, posteriorly rotated malformed auricles. **B,** Clenched hand showing typical pattern of overlapping fingers. **C,** Rocker-bottom feet. *(Courtesy Kenneth Garver, MD, Pittsburgh, PA.)*

18 may have a milder clinical outcome. Trisomy limited to the short arm of chromosome 18 is associated with a significantly milder prognosis, whereas trisomy of the entire long arm of chromosome 18 may be indistinguishable from an individual with classic trisomy 18. An infant with smaller areas of trisomy for the long arm of chromosome 18 may show some, but not all, of the features of classic trisomy 18. Thus chromosomal study of each child is essential.

If a complex chromosome rearrangement is identified in a child, further parental chromosome studies are indicated. Chromosome analysis of the parents will determine whether the rearrangement is new in the child (de novo) or is the result of a familial balanced translocation. Full characterization of the extent of a chromosome rearrangement also allows clinicians to provide more accurate information regarding prognosis, treatment options, and recurrence risk to the family. If a familial balanced translocation is present in one of the parents, other family members may benefit from genetic counseling to discuss recurrence risk and the availability of prenatal diagnosis for future pregnancies.

It has been our experience that parent support organizations can be extremely helpful to family members in the long process of adjustment to having a child with a chromosome problem. If the child dies, these groups can be helpful as a resource to the parents because of the similarity of their collective experience and can assist them in the grieving and healing process. They can also be a source of ongoing support and information to parents of a child with trisomy 13 or trisomy 18 who may live but who will face major medical and developmental challenges due to the chromosomal abnormality.

Abnormalities of Sex Chromosomes

Turner Syndrome

Turner syndrome is one of the three most common chromosomal abnormalities found in early spontaneous abortions. The phenotype is female. About 1 in 2000 liveborn females has Turner syndrome. Primary amenorrhea, sterility, sparse pubic and axillary hair, underdeveloped breasts, and short stature ($4\frac{1}{2}$ to 5 ft) are the usual manifestations. Other external physical features may include webbing of the neck; cubitus valgus; a low-set posterior hairline; a shield chest with widely spaced nipples; and malformed, often protruding, ears (Fig. 1.25). Internally, renal anomalies may be present along with congenital heart disease, particularly bicuspid aortic valve (in 30% of cases) and coarctation of the aorta (in 10% of cases). Affected women have an infantile uterus, and their ovaries consist only of strands of fibrous connective tissue. Newborns often have lymphedema of the feet and/or hands (see Fig. 1.25D and E), which can reappear briefly during adolescence. Mental development is usually normal. Schooling and behavioral problems seem to be the same as

Figure 1.25 Clinical photographs show several physical manifestations associated with Turner syndrome. **A,** In this newborn, a webbed neck with low hairline, shield chest with widespread nipples, abnormal ears, and micrognathia are seen. **B,** The low-set posterior hairline can be better appreciated in this older child who also has protruding ears. **C,** In this frontal view, mild webbing of the neck and small widely spaced nipples are evident, along with a midline scar from prior cardiac surgery. The ears are low set and prominent, protruding forward. **D** and **E,** The newborn shown in **A** also had prominent lymphedema of the hands and feet.

in age-matched control subjects, although difficulty with spatial orientation (such as map reading) may be a problem. The classic physical findings of Turner syndrome may be absent, or the abnormalities may be so minimal in the newborn that the diagnosis is missed. The first indication may be unexplained short stature in later childhood or failure to develop secondary sex characteristics by late adolescence. Thus a chromosome study is indicated as part of the diagnostic workup of adolescent girls with these complaints.

The karyotype in the majority of individuals with Turner syndrome is 45,X. Most often, the missing sex chromosome is paternally derived, so the risk of Turner syndrome does not increase with maternal or paternal age. Another 15% of individuals with Turner syndrome are mosaics (XO/XX, XO/XX/XXX, or XO/XY). The physical stigmata may be less marked in mosaics, some of whom may be fertile. If an XY cell line is present, the intraabdominal gonads should be removed, because they are prone to malignant change. The remaining cases of Turner syndrome have 46 chromosomes, including one normal plus one structurally abnormal X. The latter may have a short (p) arm deletion or may be an isochromosome duplication of the long (q) arm of the X chromosome; usually it is paternally derived. Cases of Turner syndrome with one normal and one abnormal X chromosome are more likely to have other, more serious major anomalies, including cognitive deficits. A structurally abnormal X chromosome may lead to abnormal X inactivation, resulting in a deleterious dosage effect for X-linked genes. Karyotypes such as 46,XYp- or 46,Xi(Yq) result in a female with Turner syndrome.

Moreover, loss of the short arm of an X chromosome results in full-blown Turner syndrome; deletion of the long arm usually produces only streak (fibrous) gonads with consequent sterility, amenorrhea, and infantile secondary sex characteristics without the other somatic stigmata of Turner syndrome. If the diagnosis is clinically suspected, a chromosome study should be ordered. Should the affected child be 45,X or a mosaic, the parental risk for recurrence of a chromosomally abnormal liveborn is 1% to 2% but may be higher if a parent carries a structurally abnormal X chromosome.

Antenatal diagnosis of chromosomally abnormal fetuses should be discussed with the parents, and the relatively good prognosis for Turner syndrome liveborns should not be overlooked. Girls with Turner syndrome should receive appropriate hormone therapy during adolescence to enable development of secondary sex characteristics and stimulate menses. Rarely, 45,X women with Turner syndrome have been fertile for a limited number of years.

Klinefelter Syndrome

One in 500 newborn boys has Klinefelter syndrome. The physical stigmata are subtle and usually not obvious until puberty, at which time the normal onset of spermatogenesis is blocked by the presence of two X chromosomes. Consequently the germ cells die, the seminiferous tubules become hyalinized and scarred, and the testes become small. Testosterone levels are below normal adult male levels, although the level varies from case to case (the average being about half as much as normal). Hence there is a wide range in degree of virilization. At one extreme is the man with a small penis and gynecomastia (Fig. 1.26); at the opposite extreme is the virile mesomorph with a normal penis. Scoliosis may develop during adolescence. The average full-scale IQ of men with Klinefelter syndrome is 98, which is about the same as the general population. Behavioral problems may be more common than in the population at large, however.

The karyotype in Klinefelter syndrome is XXY in 80% of cases and mosaic (XY/XXY) in the other 20%. Rarely the latter type may be fertile. About 60% of cases reflect a chromosome error in oogenesis, and an error in spermatogenesis occurs in 40%. The risk of
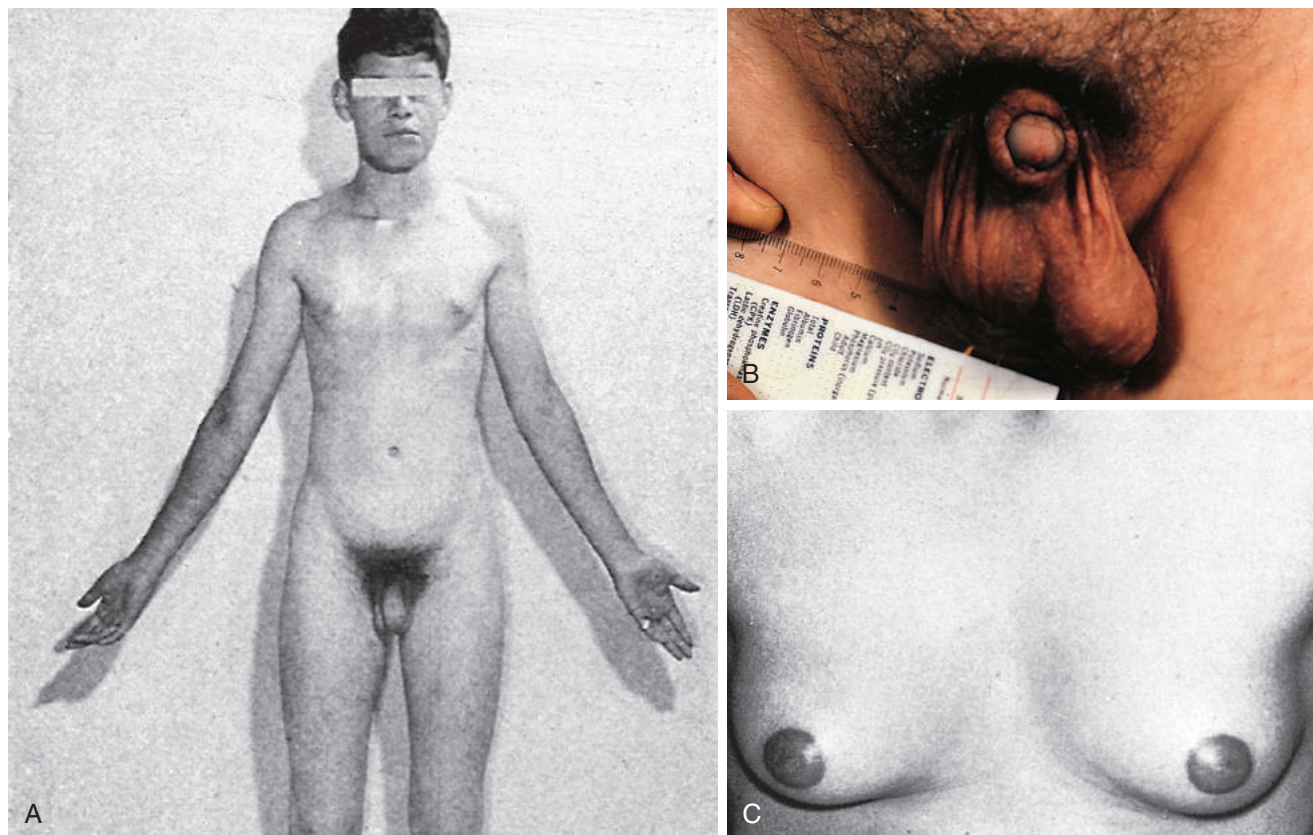
Figure 1.26 Clinical photographs show several physical manifestations of Klinefelter syndrome. **A,** Relatively narrow shoulders, increased carrying angle of arms, female distribution of pubic hair, and normal penis but with small scrotum due to small testicular size. **B,** Small testes and penis. **C,** Gynecomastia. *(**B,** Courtesy Peter Lee, MD, Hershey Medical Center, Hershey, PA. **C,** From Gardner LI, editor: Endocrine and genetic diseases of childhood, ed 2, Philadelphia, 1975, WB Saunders.)*

having an affected child increases with maternal age. Males with more than two X chromosomes (XXXY, XXXXY) are usually cognitively impaired and are more likely to have skeletal and other major congenital anomalies, such as cleft palate, congenital heart disease (particularly a patent ductus arteriosus), and microcephaly. The parents' recurrence risk for another chromosomally abnormal liveborn is 1% to 2%; antenatal diagnosis with subsequent pregnancies is possible.

XXX and XYY

Triple X females have a karyotype result of 47,XXX. The incidence is approximately 1 in 1000 liveborn females. Affected individuals have no characteristic abnormal physical features. Although usually within the normal range of intelligence, their IQ scores may be lower than those of their normal siblings, delays in development of motor skills and coordination are common, and approximately 60% require some special education classes. Behavioral problems occur in approximately 30% and are usually mild. XXX women are fertile, and their children are usually chromosomally normal.

XYY males have a karyotype result of 47,XYY. The incidence is 1 in 840 liveborn males. They tend to be tall in comparison with their own family members, but generally their phenotypic appearance is normal. As for 47,XXX females, their IQ is usually within the normal range but may be lower than that of siblings. Affected boys often come to medical attention because of problems with fine motor coordination, speech disorders, and learning disabilities. Early reports raised concerns about significant behavioral problems; however, long-term prospective studies now suggest that these boys do not have any greater incidence of problem behaviors than the general population.

The risk of recurrence for a couple with a child with an XXX or XYY karyotype depends on many factors including the parents' own karyotype results and advancing maternal age. Therefore it is recommended that they be referred for individualized genetic counseling when considering future pregnancies.

Molecular Cytogenetic Syndromes

Advances in molecular genetics have provided new insights into the genetic pathogenesis of several syndromes often associated with specific cytogenetic abnormalities. The recent advances in molecular techniques enabling higher resolution analysis of genomic DNA from uncultured cells by microarray platforms containing both array-CGH and SNP probes, known as *combo-chips,* are increasingly and routinely utilized as first tier tests in clinical setting for making a diagnosis. This molecular cytogenetics test therefore simultaneously interrogates and not only identifies copy number variations (CNVs) but is also capable to detect *r*egion(s) with long or short stretches *o*f *h*omozygosity (ROH) known as *DNA copy number neutral alterations.* The clinical implications of CNVs are well recognized in the delineation of diagnosis, as well as the management of the patient. Identification of the ROH is equally important, because these ROH contribute to the phenotype of an individual due to autosomal recessive inherited conditions or imprinting disorders and impact patients' clinical management. The application of whole-genome array-CGH in establishing of the specific genetic defect has important consequences for genetic counselling of the families and follow-up of the patients. Detailed molecular analysis of the rearranged regions may help to identify the gene(s) associated a specific phenotypic presentation. Chromosomal microarray has been widely adopted as the first-tier clinical test for individuals with multiple congenital anomalies, developmental delay, intellectual disability, and autism spectrum disorders. Chromosomal microarray has been extensively shown to provide a 10- to 15-fold higher diagnostic yield than conventional cytogenetic methods, and the health insurers have started to initiate the coverage recognizing the impacts(s) on the patients' immediate and long-term clinical management for isolated neurodevelopmental disorders, growth disorders, and

multiple congenital anomalies. Recommendations are included but not limited to specific surveillance, pharmacological treatment, cancer-related screening or exclusion of screening, contraindications, and referrals for further evaluation.

Fragile X Syndrome

It has long been recognized that there is a significant excess of males in moderately to severely mentally retarded populations. Much of this inordinate male representation is the result of altered X-linked recessive genes. These may represent new mutations or inheritance of the abnormal gene from normal heterozygous (carrier) mothers. About 1 in 150 individuals, usually male, has some form of X-linked mental retardation. Of these, it is estimated that between 30% and 50% have fragile X syndrome.

In 1969, Herbert Lubs noted in short-term lymphocyte cultures the in vitro cytogenetic marker now called *fragile X.* However, its clinical significance was not realized until a 1977 report by G. R. Sutherland in Australia. Under tissue culture conditions that starve the cell of its ability to synthesize thymidylic acid, a chromosome break at Xq27, the distal part of the long arm of the X chromosome (Fig. 1.27), is visible in cells of individuals clinically affected with fragile X syndrome. By pedigree analysis, about 1 in 4000 males has the fragile X gene.

Fragile X syndrome is the first recognized example of a trinucleotide repeat disorder. The gene involved, located at Xq27.3, is called *FMR1* and is active in brain cells and sperm. At the start of the gene is the DNA trinucleotide cytosine-guanine-guanine (CGG), which in the general population is normally repeated about 5 to 50 times (the average being 30). The presence of from 55 to 200 CGG repeats is considered a fragile X *premutation.* Individuals with a premutation appear clinically normal. The finding of more than 200 linear CGG repeats is considered a *full mutation* and results in fragile X syndrome in males. In females with more than 200 linear CGG repeats, there are clinical effects in 50% and apparently little or no effect in 50%. The explanation for this disparity in females most likely is the phenomenon known as *X chromosome inactivation* (Fig. 1.28). Premutations and, in females, random X inactivation explain the lack of penetrance of the fragile X gene.

No cases of new mutations for these *FMR1* gene CGG trinucleotide expansions or repeats have been found. That is, all such expansions are inherited from a parent. A man with a premutation passes it on to all of his daughters as a premutation. Men with a full mutation generally do not reproduce. Men with premutations, however, do not have affected sons, because they give their Y chromosome to all of their male offspring.

Figure 1.27 Fragile X chromosome marker in lymphocyte culture. Partial metaphase plate shows the chromosome break at Xq27 *(arrow)* characteristic of fragile X syndrome (solid Giemsa stain).
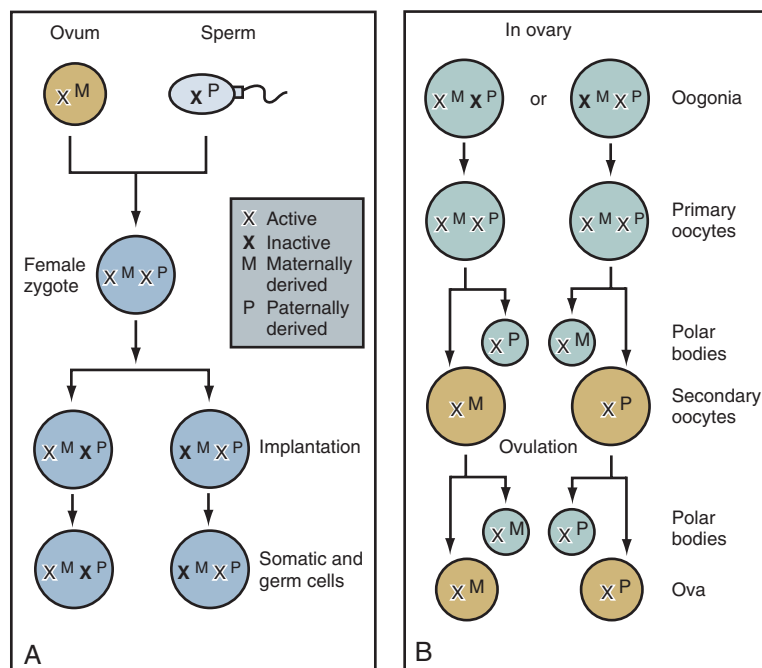
X-CHROMOSOME INACTIVATION

Figure 1.28 Functional behavior of the X chromosome in XX females. **A,** Somatic and premeiotic germ cells. Implantation occurs 5 days after conception, at which time in each female cell either XM or XP is randomly genetically inactivated and remains so in each of the cell's descendants. Because the process is random, by determining the proportion of cells with an inactive XM or inactive XP in each of a large population of women, a gaussian population distribution of women is generated. That is, most women in the population will have an approximate 50:50 mix of cells, in which each cell expresses either XM or XP. However, some women will by chance have more cells with an inactive XM and vice versa. **B,** Meiotic germ cells. When a female germ cell enters first prophase of meiosis, X inactivation is abolished; both X chromosomes become genetically active through fertilization and continue so until embryonic uterine implantation. Then, as in **A,** random X inactivation in XX females occurs all over again.

Women heterozygous for either a premutation or full mutation have a 50% chance of passing it on to each child as follows: If she has a full mutation, she passes it on as a full mutation in most instances; if she has a premutation, she passes it on to her child as either a premutation or expanded into a full mutation, depending on the size of her own premutation. As the number of repeats in the premutation increases, the greater the likelihood that her premutation will expand to a full mutation in her offspring. The relative risk is shown in Table 1.6.

Males affected with fragile X syndrome have cognitive impairment, ranging from severe to borderline in degree. The majority have an IQ between 20 and 49, and the remainder fall in the 50 to borderline IQ range. Furthermore, IQ may decline with age. The majority have speech delay, short attention span, hyperactivity, persistence of mouthing objects, and poor motor coordination. Many exhibit a variety of disordered behaviors, including disciplinary problems, temper tantrums, poor eye contact, perseverative speech, hand flapping, avoidance of socialization, and rocking. Physical stigmata may include long, wide, or protruding ears; long face; a prominent jaw; flattened nasal bridge; "velvety" skin; hyperextensible joints; and mitral valve prolapse. Relative macrocephaly is more likely than microcephaly. Macroorchidism is found in most mature males.

Approximately 50% of females affected with full-mutation fragile X are clinically normal. The 50% who are affected usually have lesser degrees of cognitive impairment than males; about 35% fall in the 20 to 49 IQ range, and the remainder falls in the 50 to borderline range. However, learning disabilities, mood disorders, schizoid personality, and significant disturbances in affect, socialization, and communication are common. The physical features often seen in males with fragile X syndrome are less common in females.

Laboratory testing for fragile X mutations is done by molecular genetic techniques. The standard molecular genetic test is Southern blot analysis of DNA extracted from cells, usually in blood. Another molecular genetic technique, polymerase chain reaction (PCR) analysis of DNA, can be done with less blood. These techniques can also be applied to fetal cells for the purpose of antenatal diagnosis. However, if the fetus is a female with a full mutation, it is impossible to predict with certainty whether the child will be clinically affected with fragile X syndrome after birth because of the influence of X inactivation.

Rarely, an individual may seem to have a mild form of fragile X syndrome but tests are negative using these molecular genetic laboratory techniques. Another fragile X gene site *(FRAXE)* distal to the fragile X gene on Xq is associated with mild mental retardation and a positive fragile X cytogenetic laboratory test.

The number of known trinucleotide expansion disorders is increasing. Three other examples are Huntington disease, caused by a CAG trinucleotide expansion in its gene at the end of

Table 1.6	Relative Risk of Maternal Transmission of a Fragile X Premutation to Her Offspring as a Full Mutation
Number of CGG Repeats in Mother's Premutation	**Risk of Expansion to Full Mutation in Offspring (%)**
55-59	3.7
60-69	5.3
70-79	31.1
80-89	57.8
90-99	80.1
100-109	100
110-119	98.1
120-129	97.2

From Saul RA, Tarleton JC: *FMR1*-related disorders. GeneReviews. Available at: www.genetests.org. Accessed August 26, 2016.

chromosome 4p; myotonic dystrophy, resulting from a CTG expansion in its gene on chromosome 19q; and spinobulbar muscular atrophy caused by a CAG expansion in its gene on the proximal part of chromosome Xq.

Disorders of Imprinting (Epigenetic Phenomena): Prader-Willi and Angelman Syndromes

Etiologic Mechanisms

Prader-Willi and Angelman syndromes are disorders that derive from abnormalities of imprinted genes. The concept of *imprinting* refers to the fact that the function of certain genes is dependent on their parental origin: maternal versus paternal. This appears particularly true of the 15q11-q13 region of chromosome 15, a region that contains several imprinted genes that, when abnormal, result in recognizable constellations of physical and behavioral problems.

Mechanisms that can produce the Prader-Willi phenotype include the following:

- A chromosome deletion of 15q11-q13, including the Prader-Willi critical region of the paternally derived chromosome 15 (majority of cases)
- A structural chromosome abnormality involving the Prader-Willi critical region of 15q11-q13 (translocation, and so on)
- Maternal UPD in which the child has two maternally derived chromosome 15s and no paternally contributed chromosome 15 (25% of cases) (*Note:* The association of UPD with older maternal age suggests that in these cases the fetus may originally have had trisomy 15 but that owing to a phenomenon known as *trisomic rescue,* one of the three chromosome 15s was lost, returning the fetus to the normal chromosome number. If the "lost" chromosome was paternally derived, then UPD-derived Prader-Willi results.)
- Mutations of imprinting control center genes (1% of cases)

The critical region of chromosome 15 for Angelman syndrome is located adjacent to the Prader-Willi critical region. However, when deletion of the Angelman critical region is causative, it is the maternally derived chromosome that is deleted. The six currently identified etiologic mechanisms of Angelman syndrome include the following:

1. A large chromosome deletion of 15q11-q13 including the Angelman critical region of the maternally derived chromosome 15 (68% of cases)
2. A structural chromosome abnormality involving the Angelman critical region of 15q11-q13 (translocation, and so on)
3. Paternal UPD of chromosome 15 (7% of cases)
4. Mutations of imprinting control center genes (3% of cases)
5. Mutations of the ubiquitin-protein ligase gene (*UBE3A)* (11% of cases)
6. Classic phenotype, with no identifiable etiologic mechanisms but a positive family history of other affected individuals (11% of cases)

Note: Mechanisms 4, 5, and 6 account for approximately 25% of cases of Angelman syndrome.

Because of etiologic variability and complexity of the diagnostic process, families of children suspected of having either of these disorders should be referred for genetic evaluation and diagnostic testing to ensure the most accurate determination of etiologic mechanism, and therefore, of recurrence risk.

Current diagnostic testing for these disorders includes the following:

- Karyotype with high-resolution cytogenetic technology
- Methylation studies, which determine whether genes within the 15q11-q13 critical region are functional

- Appropriate oligonucleotide arrays that cover and encompass small nuclear ribonucleoprotein-associated polypeptide N (SNRPN) for Prader-Willi syndrome and D15S10 for Angelman syndrome
- In some cases of Angelman syndrome, direct analysis of the *UBE3A* gene

Clinical Findings in Prader-Willi Syndrome

Newborns affected with Prader-Willi syndrome usually are markedly hypotonic and often have a history of decreased fetal movement in utero and breech fetal position. Although birth is usually at term, birth weights tend to be below 3000 g. In neonates, in addition to hypotonia, poor sucking and swallowing are common and predispose to choking episodes that can cause respiratory problems. Although the baby's cry may be weak and Moro and deep tendon reflexes are often decreased, the neurologic evaluation is otherwise unremarkable. Subsequently motor development is delayed, speech even more so, and most patients have cognitive impairment in the mild to moderate range. Hypotonia abates over the first 2 to 3 years, and patients develop an insatiable appetite that rapidly results in morbid obesity. The distribution of excess fat is particularly prominent over the lower trunk, buttocks, and proximal limb. Although the facies are not particularly dysmorphic, they are similar in most Prader-Willi patients. The bifrontal diameter is narrow, the eyes are often described as "almond shaped," and strabismus is not unusual. Hypopigmentation is common, the patient usually having blond to light brown hair, blue eyes, and sun-sensitive fair skin. Picking of skin sores can become a problem. Hands and feet are noticeably small from birth, and the stature of the older child and adult is short. The penis and testes are hypoplastic in males with Prader-Willi syndrome, although the penile size can be enlarged by testosterone therapy. If the testes are cryptorchid, surgical correction should be attempted. Menarche in females is delayed or absent, and menses, when present, are sparse and irregular. Gonadotropic hormone levels are reduced in both sexes. Infertility is the rule, but there are two known exceptions.

Of particular concern in older children with Prader-Willi syndrome are problems of emotional lability and extreme temper tantrums. These conditions and the overeating often can be partly ameliorated by intensive inpatient behavioral modification programs followed by longitudinal parental support and follow-up in the home. Interestingly, despite a normal basal metabolic rate, weight reduction requires significantly more severe caloric restriction in these patients than in normal persons. Diabetes mellitus can develop in the older child, and its incidence is correlated with the severity of obesity. Although it tends to be insulin resistant, the condition responds well to treatment with oral hypoglycemic agents. Life expectancy can be shortened by cardiorespiratory complications related to the extreme obesity (pickwickian syndrome).

Clinical Findings in Angelman Syndrome

Angelman syndrome, first recognized in 1956, has an incidence of 1 in 15,000 to 1 in 20,000 live births. Except for the tendency to have hypopigmentation, the clinical phenotypes of Prader-Willi and Angelman syndromes are quite different. The latter have severe cognitive deficits, speech is impaired or absent, and inappropriate paroxysms of laughter are common. Physical features include microbrachycephaly, maxillary hypoplasia, large mouth, prognathism, and short stature (in adults). The gait is ataxic, with toe-walking and jerky arm movements. Akinetic or major motor seizures are common. Although survival to adulthood is possible, to date only one patient with Angelman syndrome has been known to reproduce.

Note: Some other examples of imprinting disorders are Beckwith-Wiedemann syndrome (Fig. 1.29) and Russell-Silver syndrome.

Figure 1.29 A, Beckwith-Wiedemann syndrome. Note the macrosomia, macroglossia, and asymmetry with hemihypertrophy and omphalocele and/or umbilical hernia. Craniofacial features also include unusual ear creases. **B,** At 3 months old, note the macroglossia, right facial prominence, and ear creases. **C,** At 27 months old; note the resolving umbilical hernia. The elevated alpha-fetoprotein (AFP) levels from infancy have normalized. The patient has nephromegaly, and is under surveillance for the detection of embryonal tumors by serum AFP and abdominal and renal sonograms every 4 months. Chromosomes were normal male complement, 46,XY. The abnormal methylation pattern of the *LIT1* gene at the 11p15 region confirmed the clinical diagnosis of Beckwith-Wiedemann syndrome.

The Nature of Genes and Single-Gene Disorders

A gene consists of a sequence of DNA that contains the code for production of a "functional product" along with sequences that ensure "proper expression" of the gene. Its product may be an RNA molecule or a polypeptide chain or protein that ultimately becomes a structural component of a cell or tissue, or of an enzyme. The latter may catalyze a step in formation or modification of another product, a step in cell metabolism, or one of a number of steps involved in the breakdown or degradation of molecules that are no longer necessary. "Proper expression" includes production of the product at the right time, in the needed amount, in the correct cell type, and ensures its transport to its proper site of biologic action.

Approximately 30,000 genes are arranged in linear fashion on the chromosomes, all having their own specific locus. Genes range in length from about a thousand to hundreds of thousands of bases in length (any of which can be subject to mutation). Coding sequences for a gene's product, termed *exons,* vary in length and are not continuous but occur in sections with noncoding sequences, termed *introns,* interspersed between them. Exons are further subdivided into triplets of bases, termed *codons,* each of which encodes a specific amino acid within the polypeptide product. Because there are 64 possible triplet combinations of the four nucleotide bases (adenine, guanidine, thymine, and cytosine) and 20 amino acids, most amino acids have more than one codon that can specify them, the exceptions being methionine and tryptophan, which have only one specific codon each. In addition, three triplets encode stop codons in the messenger ribonucleic acid (mRNA) that signal the termination of mRNA translation.

The process of going from DNA code to polypeptide product has many steps and begins with *transcription,* during which the DNA of the gene serves as a template for the formation of an mRNA molecule. RNA synthetase, proteins called *transcription factors,* and regulatory elements all participate in this process, which is initiated and concluded by DNA sequences that signal where to start and stop transcription. After this, both ends of the mRNA molecule undergo modification. Thereafter, the introns are excised and the exons spliced together. Then the mRNA is transported to the rough endoplasmic reticulum within the cytoplasm, where it attaches to ribosomes, and the process of *translation* from mRNA template to polypeptide chain begins. During translation, transfer ribonucleic acid (tRNA) molecules, each of which is specifically designed to attach to a particular amino acid, find their target moieties and bring them into position at the correct time over a codon on the mRNA that specifies for their particular amino acid.

After assembly, the polypeptide chain is released from its template and then may be subject to *posttranslational modification.* Steps may include folding, bonding into a three-dimensional conformation, being combined with another or other polypeptide chains as part of a protein complex, being split into smaller segments, and addition of phosphate or carbohydrate moieties. Thereafter, it is transported to its site of action via directional terminal sequences, which are then cleaved from the finished product. Mutation of a gene encoding the polypeptide product or for any molecule used at any step along the entire process can adversely influence the end product.

A single-gene mutation produces a permanent change in a gene's DNA sequence and may involve anywhere from one to several thousands of nucleotides. Most appear to affect only one to a few to several base pairs via substitution of one base for another or by deletion or insertion of one or more bases.

Some mutations have no effect on phenotype or cell function. One example is a base substitution within a codon for an amino acid that changes it to another codon specifying the same amino acid. Still other mutations have no adverse effect but rather encode normal variations in human characteristics (e.g., eye or hair color). Other mutations do have adverse effects and are causative in disease. Examples of these include *missense* mutations, in which a base substitution changes a codon specific for one amino acid into one specifying another; *frameshift* mutations, in which a deletion or insertion is not an exact multiple of three bases, and thereby shifts the reading frame for transcription (and later translation) from that point on; and *nonsense* mutations, in which a base substitution changes a codon for an amino acid into one specifying one of the three possible stop codons in mRNA, thereby stopping translation prematurely.

A single-gene disorder is the result of a mutation altering the DNA sequence within a single gene on one (dominant) or both (recessive) of a pair of chromosomes. Correspondingly, this change may result in alteration of the amount of the gene's product, failure to produce the product at all, and/or compromise of its functional integrity. The greater the degree of functional loss, the more severe the clinical manifestations of the disorder and often the earlier their onset. are representative of single-gene disorders.

Figure 1.30 Classic presentation for features of X-linked recessive hypohidrotic ectodermal dysplasia at 1 to 20 months of age. At 1 month the infant was admitted to "rule out sepsis" with high fever, but all the workup was negative. **A,** Note the thin, sparse, fine hair. **B,** Severe hypodontia and anterior conical teeth. **C,** Low nasal bridge, periorbital wrinkling, full forehead, prominent lips, and prominent supraorbital ridges. Deoxyribonucleic acid (DNA) analysis of the *EDA1* gene at the Xq12 region showed the missense mutation and confirmed the clinical diagnosis. The mother and her maternal female relatives have variable and milder clinical features.

Mutation(s) of gene(s) within the nuclear genome are also recognized as mendelian disorders. The occurrence and/or recurrence are in fixed proportions (Mendel's laws). These disorders are compiled in a catalog, the Online Mendelian Inheritance in Man (OMIM; http://www.ncbi.nlm.nih.gov/omim), which is a great resource. Phenotype/genotype correlations are unfolded by detailed clinical evaluation, recognition at a clinical level, and confirmation by molecular diagnostics confirming the genotype.

Pedigree analyses are usually helpful. Autosomal, X-linked, recessive, and dominant patterns are recognized. Gene penetrance, disease expressivity, genetic (locus) heterogeneity, and allelic heterogeneity are some of the well-recognized complexities characterizing mendelian disorders.

The family of disorders known as *osteogenesis imperfecta* (OI; see also Chapter 22) provides a good example of the effects of mutations that alter the precursors of a structural protein, type I collagen. Collagen is a triple helix made up of two pro-α1 chains and one pro-α2 chain. The latter are composed of hundreds of amino acid triplet repeats, with glycine (the smallest amino acid) being the first member of each triplet and forming the apex of each bend in the helical structure. A base substitution in a codon specifying glycine at any one of the hundreds of such points along either the *COL1A1* gene (on band 17q21) or the *COL1A2* gene (on band 7q22.1) may result in the production of an unstable mRNA molecule that is degraded in the nucleus, or in the production of structurally abnormal pro-α1 or pro-α2 chains. The assembly of these may be slowed; they may be subject to excessive posttranslational modification, may be unstable and subject to degradation, or may have difficulty conforming and associating with other pro-chains to form the triple helix. The earlier the altered mRNA codon appears in the translation process, the more abnormal is the resulting prochain structure, and the greater is the degree of compromise of collagen strength and function within connective tissues. Also, because there are two pro-α1 chains for each pro-α2 chain, mutations in the *COL1A1* gene are more likely to be deleterious. These types of mutations, which result in the synthesis of structurally abnormal products, are the basis for clinical abnormalities found in types II to IV OI.

In type I OI, the causative mutations in the *COL1A1* gene (often nonsense or splicing mutations) usually result in the production of mRNA that is so abnormal it is degraded before it can leave the nucleus and be translated, or in the synthesis of a prochain that is unstable and degraded. Hence the mutant gene is unexpressed, that is, a *null mutation*. The end result of this is that the patient can make only 50% of the expected amount of type I collagen, although the entire product is structurally normal. Being the mildest form of OI, it demonstrates the fact that in many cases of mutations involving genes that encode structural polypeptides or proteins, it can be better to have no gene product than to have an abnormal one.

The phenomenon of excessive posttranslational modification of a structurally abnormal gene product is also seen in some types of Ehlers-Danlos syndrome (EDS; see the Ehlers-Danlos Syndrome section, later).

When the gene product is an enzyme or a component of an enzyme, this results in interruption of its step in a chain of reactions that may be involved in the formation or modification of a product, a step in cell metabolism, or in the degradation of molecules no longer needed by the cell. The missed step results in a build-up of substrate from the step preceding the one in which the affected enzyme acts. In some instances, this accumulated substrate can be toxic, as in phenylketonuria (PKU). In others, ever-expanding storage of substrate can adversely affect cell function, as in the lysosomal storage diseases.

Figure 1.31 Incontinentia pigmenti syndrome. **A** to **C,** At 7 weeks old this patient manifested erythema, and blisters on the trunk and extremities. Hyperkeratotic lesions have already started. This X-linked dominant condition is lethal in males. One-third of the patients have psychomotor delays, microcephaly, and seizures, which were not observed in this patient. Mutation(s) in the *NEMO* gene at Xq28 are encountered in the majority of patients. By 5 months old, the rash was already resolving. **D,** Rash replaced with hyperpigmentation on the trunk and pale hairless patches or streaks subsequently on the lower limbs. Patient was confirmed to have intragenic microdeletion of the *NEMO* gene, involving exons 4 through 10.

Figure 1.32 A girl, 3 years and 3 months old, with macrocephaly, macrosomia, and pervasive developmental disorder, not otherwise specified (PDD-NOS). A missense mutation was found in the *PTEN* gene.

Figure 1.33 A 4-month-old male with classic features of Treacher Collins syndrome. Note the down-slanted palpebral fissures, malar hypoplasia, malformed auricle, and mandibular hypoplasia. The mutation was identified as a single sequence variation (Nt2897insC) in the *TCOF1* gene in the 5q31 region that introduces a premature stop codon.

Table 1.7	Differentials for Connective Tissue Disorders		
Diagnosis	**Inheritance**	**Molecular Basis**	
Stickler syndrome	AD	COL2A1 gene	
		COL11A1 gene	
		COL11A2 gene	
		COL9A1 gene	
EDS type IV	AD	COL3A1 gene	
EDS type VI	AR	PLOD1 gene	
Beals contractural arachnodactyly	AD	FBN2 gene	
Homocysteinemia	AR	Defect in cobalamin synthesis	
Arterial tortuosity syndrome	AR	SLC2A10 gene	
MASS phenotype	AD	FBN1 gene	
Loeys-Dietz syndrome	AD	TGFBR1 gene	
		TGFBR2 gene	
Familial aortic aneurysm	AD	ACTA2 gene	
		MYH11 gene	
Klinefelter syndrome (47,XXY) or triple X syndrome (47, XXX)	Chromosomal	Chromosomal	
Fragile X syndrome	X-linked	FMR1 gene	
Shprintzen-Goldberg syndrome		Unknown	

AD, Autosomal dominant; *AR,* autosomal recessive; *EDS,* Ehlers-Danlos syndrome; *MASS, m*itral valve prolapse, borderline nonprogressive *a*ortic enlargement, *s*triae and marfanoid *s*keletal features, which overlap with those seen in Marfan syndrome.

Connective Tissue Disorders of Genetic Origin

See Table 1.7 for examples of some connective tissue disorders of genetic origin.

Marfan Syndrome

Marfan syndrome is a genetic disorder of connective tissue that is inherited as an autosomal dominant trait, although approximately 25% to 30% of cases represent new mutations. The site of the genetic abnormality or mutation is the fibrillin gene *(FBN1)* located at band 15q21.1 on chromosome 15. As a result, the molecular structure of the protein fibrillin, an intrinsic component of connective tissue, is abnormal. Clinical consequences are most notable in the musculoskeletal, cardiovascular, and ocular systems and in the dura. Approximately 70% of cases are familial. Classic phenotypic findings include arachnodactyly (Fig. 1.34A and B); joint hyperextensibility due to ligamentous laxity (see Chapter 5); tall stature with long, thin extremities; a decreased upper-to-lower segment ratio; an arm span that exceeds height; and moderate to severe pectus excavatum or carinatum (see Fig. 1.34C). Pes planus and thoracolumbar kyphoscoliosis are other common skeletal features (see Fig. 1.34D). A defect in the suspensory ligaments of the eye is responsible for subluxation of the lens (seen in 50% to 60% by 10 years old), which is usually displaced in an upward direction. Myopia and astigmatism are common, and affected individuals are also at risk for developing glaucoma, cataracts, and retinal detachment in adulthood. Mitral valve prolapse may progress to mitral insufficiency (at times associated with arrhythmias). Of great concern is progressive aneurysmal dilatation of the ascending aorta and, less commonly, the thoracic or abdominal aorta. The latter is the major source of morbidity and mortality because it can result in acute dissection and death. Dural ectasia in the lumbosacral region, assessed by computed tomography (CT) or magnetic resonance imaging (MRI) of the spine, is observed in 65% of cases. The presence of a high arched palate is common. The incidence of hernias, both inguinal and femoral, is increased, and patients often have striae of the skin in unusual places such as the shoulder. Although most Marfan individuals are of normal intelligence, an occasional patient may have learning disabilities.

The disorder is currently diagnosed primarily on clinical grounds. In addition, family history and multiorgan manifestations are variable and may have age-dependent expressivity. All the manifestations of this condition are classified as either major or minor diagnostic criteria. The diagnostic criteria for Marfan syndrome (first established in Berlin; Beighton et al, 1988) were revised as the Ghent criteria (de Paepe et al, 1996). These have continued to be revised, and the most recent revised Ghent diagnostic criteria were established in 2010 (Loeys et al, 2010). The diagnostic criteria are based on cardiovascular, ocular, and skeletal features; the presence of a dural ectasia; and family history. These revisions have placed an increasing emphasis on the cardinal features of Marfan syndrome. Because it takes time for a number of the major abnormalities to develop or to become clinically evident, a firm diagnosis is generally impossible in early childhood, especially in the absence of a positive family history. The molecular testing of an individual who clinically meets the diagnostic criteria for Marfan syndrome is not usually necessary. Molecular testing is being used more frequently in children with an emerging clinical phenotype, especially in the absence of family history. The recurrence risk for affected individuals to their offspring is 50%.

When the diagnosis of Marfan syndrome is strongly suspected or confirmed, patients should be monitored closely during growth spurts for signs of onset and progression of kyphoscoliosis; in addition, they should undergo regular ophthalmologic evaluations, and have regular echocardiograms and electrocardiograms. When aortic dilatation is detected, administration of β-blockers can slow progression by decreasing blood pressure and the force of myocardial contractions. Subacute bacterial endocarditis (SBE) prophylaxis may or may not be indicated for patients with evidence of cardiovascular involvement. Patients also should be cautioned to avoid weight lifting and contact sports.

The differential diagnosis of Marfan syndrome includes Loeys-Dietz syndrome; Beals congenital contractural arachnodactyly (Figs. 1.35 through 1.39); homocystinuria; the MASS phenotype (MASS being an acronym for *m*itral valve prolapse, borderline nonprogressive *a*ortic dilatation, *s*triae and marfanoid *s*keletal features, without ocular findings); familial ectopia lentis; Klinefelter syndrome (47,XXY), triple X syndrome (47,XXX), and many syndromes characterized by joint hypermobility, such as Stickler syndrome and EDS types IV and VI; familial thoracic aortic aneurysm and aortic dissection (TAAD); neuromuscular disorders; fragile X syndrome; and some of the rare dysmorphologic entities, such as Shprintzen-Goldberg syndrome.

Loeys-Dietz syndrome is a more aggressive connective tissue disorder than Marfan syndrome; it is characterized by craniofacial, cutaneous, and skeletal manifestations along with vascular manifestations; and aneurysms and dissection of the aorta (in the root, thoracic, and/or abdominal regions) and cerebral vessels. Tortuosity of blood vessels and heart defects, involving the bicuspid aortic valves and atrial septal defects, may be observed. Translucent skin and organ rupture, specifically in postpartum females, have been reported. Mutations in transforming growth factor-β (TGF-β) receptor type 1, *TGFBR1,* and *TGFBR2* result in Loeys-Dietz syndrome. Research in the area of therapy has found TGF-β to be involved in the formation of aortic aneurysms. Losartan, an angiotensin II type 1 receptor blocker, inhibited TGF-β in a mouse model of Marfan syndrome (as shown by H.C. Dietz; Habashi et al, 2011), leading to inhibition of aortic growth. These results are promising; treatment of patients with Marfan syndrome may reduce aortic enlargement. At present, double-blind studies are being conducted in adults and pediatric patients to look into treatment with losartan (Fig. 1.40).

Ehlers-Danlos Syndrome

EDS is composed of a group of inherited connective tissue disorders, the major features of which consist of hyperextensibility and fragility of the skin and ligamentous laxity with secondary joint

Figure 1.34 Marfan syndrome. **A** and **B,** This young man has prominent arachnodactyly of both fingers and toes. Note the clubbing due to associated cardiopulmonary problems and the flattening of the arch of his foot. He also has severe pectus carinatum **(C)** and significant kyphosis and joint contractures **(D).** Also note his long arms.
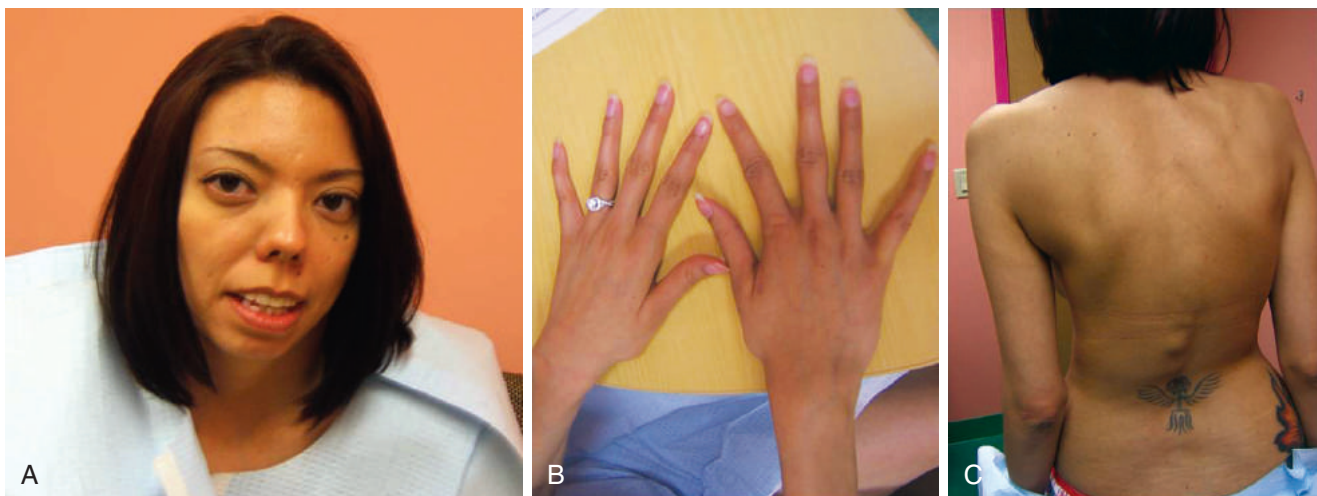
Figure 1.35 A to **C,** An adult female presented during the first trimester of pregnancy with a long-standing diagnosis of Marfan syndrome. On clinical evaluation, she had hypertelorism, low-set ears, and bifid uvula. Arachnodactyly, kyphoscoliosis, and marfanoid habitus were encountered. Cardiology evaluation was consistent, with an echocardiogram showing dilatation at the aortic root (with aortic sinus measurement of approximately 4.5 cm) and mitral valve prolapse. Cardiac surgery immediately after delivery was recommended. Deoxyribonucleic acid (DNA) tests confirmed Loeys-Dietz syndrome due to mutation in the *TGFBR1* gene. *FBN1* and *TGFBR2* analyses were normal.

Figure 1.36 Infant with Loeys-Dietz syndrome at 3 months old. Note the retromicrognathia, hypertelorism, low-set ears, and failure to thrive.

Figure 1.37 The mother has clinical features of Loeys-Dietz syndrome. She has a pathogenic mutation in the *TGFBR1* gene. Her infant son, seen here at 5 months old, is clinically affected and already has a dilated aorta. His mother had undergone valve-sparing aortic root repair on an emergent basis, due to possible dissection complicated by right coronary artery injury and bypass procedure.

Figure 1.38 A to **C,** Beals contractural arachnodactyly. Note the crumpled ears, arachnodactyly, and joint contractures. The patient was confirmed to have a missense mutation in the fibrillin II gene *(FBN2).*

Figure 1.39 Beals syndrome variant. This child was found to have an abnormality of fibrillin-2 secretion in fibroblasts. **A,** She was tall and had arachnodactyly with contractures. **B,** Her broad forehead and hypertelorism are physical features that help distinguish her case from classic Beals syndrome and Marfan syndrome.
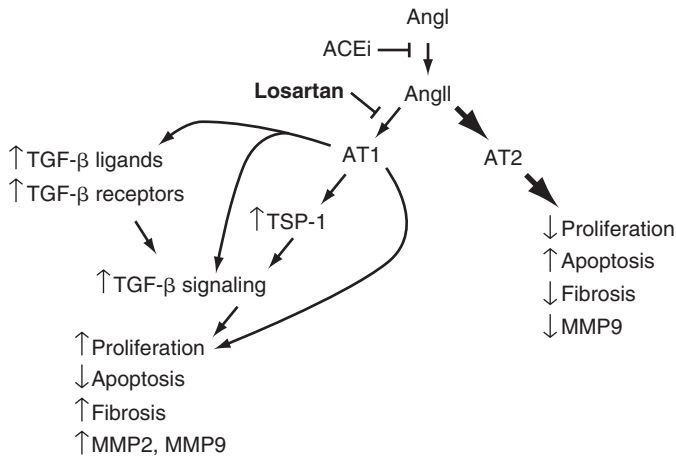
Effects of Losartan on AT1 Signaling

Figure 1.40 Diagram of events mediated by angiotensin II subtype 1 (AT1) signaling. *ACEi,* Angiotensin-converting enzyme inhibitor; *AngI* and *AngII,* angiotensins I and II; *AT1* and *AT2,* angiotensin II subtypes 1 and 2 receptors; *MMP2* and *MMP9,* matrix metalloproteinases 2 and 9; *TGF-β,* transforming growth factor-β; *TSP-1,* thrombospondin-1.

hypermobility. Each type stems from a defect in synthesis of type I, III, or V collagen, resulting in decreased tensile strength of connective tissues. Previously divided into types I to XI, it has been reclassified into six major subgroups on the basis of their predominant clinical features; mode of inheritance; and, when known, underlying defect. Table 1.8 presents these along with their estimated incidence. Given limitations of space, we focus on the clinical features of the four most common types.

Classic Type In the classic form of EDS (previously known as subtypes I and II), cutaneous manifestations are especially prominent, although they may have a wide spectrum of severity. Skin hyperextensibility is prominent (Fig. 1.41A), the texture is smooth and "velvety," and the skin is abnormally fragile with easy bruising and tearing. Wound healing is impaired and slower than average, often resulting in the formation of unusually wide atrophic scars that have a thin papery quality, sometimes likened to cigarette paper (see Fig. 1.41B).

When these children incur lacerations necessitating wound closure, use of glue or tape is preferable to sutures because the latter tend to tear away from the fragile skin. Staples are better tolerated for closure of operative incisions, and postoperatively development of incisional hernias is not uncommon.

Two features unique to this type are the tendency to form pseudotumors under scars located over bony prominences and to develop subcutaneous fatty tumors over the forearms and shins.

Although usually not as severe as in the hypermobility type, ligamentous laxity and joint hypermobility also are features (see Fig. 1.41C and D) and predispose to sprains, subluxations, and dislocations, and to early onset of chronic musculoskeletal pain.

Hypotonia and gross motor delays are seen in some infants and young children with this type of EDS.

Hypermobility Type In hypermobility-type EDS, the most common type, ligamentous laxity and attendant joint hypermobility are the major source of symptomatology. All joints, large and small, are affected, and patients are prone to frequent and recurrent subluxations and dislocations, especially of the patella, shoulder, and temporomandibular joints. Chronic limb and joint pain, which is due to the excessive pull placed on periarticular structures and to dislocations, develops early on and can become increasingly debilitating over time.

Cutaneous manifestations vary widely in severity and include a smooth "velvety" texture, hyperextensibility, and easy bruisability.

Vascular Type Vascular-type EDS is the most serious form of EDS because fragility of vascular and visceral tissues accompanies cutaneous and joint abnormalities. Many affected children are born with clubfeet, and they tend to have rather characteristic facial features that include prominent eyes and sunken cheeks (due to decreased subcutaneous facial fat), a thin nose, small chin, and lobeless external ears. Scalp hair is sparse in some.

The skin is thin and appears translucent, giving prominence to the underlying venous pattern, especially over the chest and abdomen. Easy bruisability and skin fragility are significant features, and postoperative wound dehiscence is not unusual. Premature aging of the skin over the distal extremities and early development of varicose veins are also seen. Joint hypermobility is present but is limited to the small joints of the fingers and toes.

As noted earlier, the feature that makes this type of EDS so serious clinically is the fragility of the walls of medium-size arteries, the intestines, and the uterus. This predisposes to wall rupture with potentially catastrophic results. Arterial and intestinal ruptures are heralded by sudden onset of severe abdominal and/or flank pain, which is promptly followed by signs of shock. Risk of uterine rupture is greatest intrapartum and is associated with significant hemorrhage. Other reported problems include pneumothoraces and development of arteriovenous fistulas.

Because the major complications of this form of EDS tend to occur in the third or fourth decade, exact diagnosis in early childhood can be difficult in patients without a positive family history or in those whose other clinical findings are subtle.

Kyphoscoliosis Type Newborns with the kyphoscoliosis form of EDS tend to have severe hypotonia with delayed gross motor development and congenital scoliosis, which is progressive. Some patients

Table 1.8	Classification of Types of Ehlers-Danlos Syndrome			
Type	**Former Type**	**Mode of Inheritance**	**Approximate Incidence**	**Underlying Abnormality**
Classic	I and II	AD	1 per 20,000-40,000	Abnormal electrophoretic mobility of pro-α1 and pro-α2 chains of type V collagen
Hypermobility	III	AD	1 per 10,000-15,000	No specific biochemical defect identified
Vascular	IV	AD	1 per 100,000-200,000	Mutation in *COL3A1* gene resulting in structurally abnormal pro-α1 chain of type III collagen, posttranslational overmodification, thermal instability, or increased sensitivity to proteases
Kyphoscoliotic	VI	AD	Rare	Deficiency of the collagen-modifying enzyme lysyl hydroxylase
Arthrochalasis	VIIA and VIIB	AD	Very rare	Mutations resulting in deficient processing of amino-terminal ends of pro-α1 or pro-α2 chains of type I collagen
Dermatosparaxis	VIC	AR	Very rare	Deficiency of procollagen 1 amino-terminal peptidase

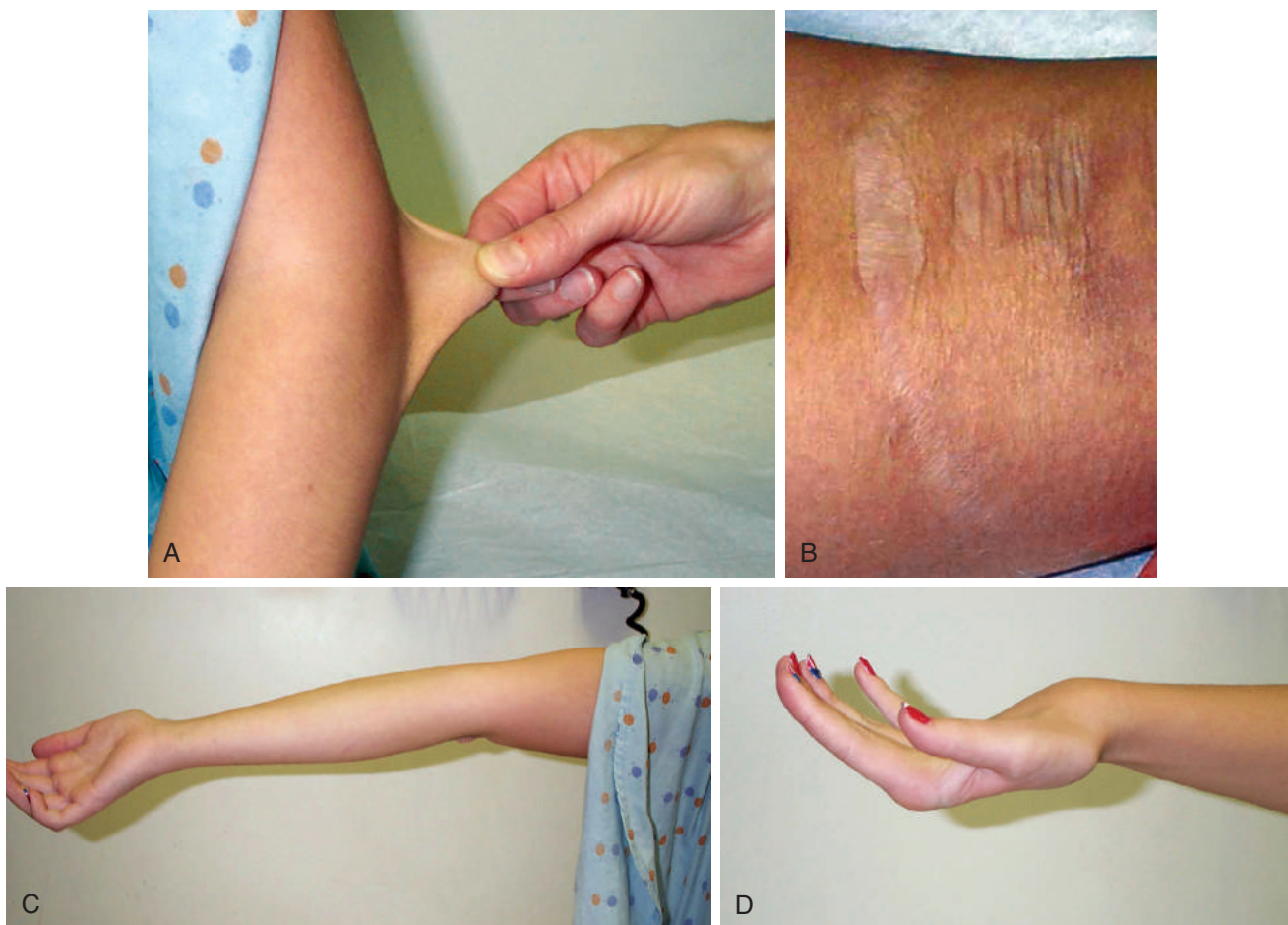AD, Autosomal dominant; AR, autosomal recessive.

Figure 1.41 Ehlers-Danlos syndrome (EDS)—classic type. **A,** Note the marked hyperextensibility of the skin over this child's arm. **B,** These widened atrophic scars have the thin papery texture that is characteristic of EDS. **C** and **D,** Hyperextensibility of the joints of the elbow and fingers is seen as well.

develop a marfanoid body habitus with growth. Generalized ligamentous laxity and joint hypermobility may be so severe that the ability to ambulate is lost in the teens or twenties. Osteopenia is seen radiographically, perhaps partly from disuse.

Other features include easy bruisability, skin fragility, and formation of atrophic scars. In contrast to other forms of EDS, children with this type have scleral fragility, which places them at risk for globe rupture following even minor trauma. High myopia and microcornea are seen in some.

Diagnosis The diagnosis of EDS should be suspected in children who present with unusually distensible skin, especially when atrophic scars are seen, and in those with unusual degrees of joint hypermobility who suffer recurrent joint dislocations. The presence of skin hyperextensibility is best tested over the volar forearm by grasping the skin and pulling until resistance is felt. Evidence of significant joint hypermobility includes the following:

- Ability to touch palms to the floor on forward bending
- Hyperextensibility of knees and elbows greater than 10 degrees
- Ability to appose thumb to the volar forearm
- Passive dorsiflexion of the fifth fingers past 90 degrees

Finding these and other clinical features described earlier in a child with a positive family history is especially helpful. With the exception of the kyphoscoliotic type, for which a urine test is available, confirmatory diagnostic tests usually require skin biopsy.

Depending on type, differential diagnostic considerations may include Marfan syndrome and cutis laxa. Easy bruisability can be mistaken for child abuse.

Osteogenesis Imperfecta

OI is a family of genetic connective tissue disorders characterized predominantly by brittle bones. The vast majority of patients have OI type I, II, III, or IV, all of which involve mutations in the *COL1A1* gene and/or *COL1A2* gene that either reduce the amount or alter the structure of type I collagen. A description of some of the many causative mutations and their structural consequences is presented in an earlier section, The Nature of Genes and Single-gene Disorders. Clinical features are presented in Chapter 22. Other, rarer forms of OI include types V through VIII. Of these, OI type V is an autosomal dominant disorder, and OI type VII is autosomal recessive; the mode of inheritance of OI type VI is unclear, but patients with OI type VI have rhizomelic shortening of the limbs.

Figs. 1.42 through 1.44 show examples of defects in collagen synthesis.

Associations

As noted earlier, an *association* is a pattern of malformations that occurs together too often to be the result of chance alone, but for which no specific cause has yet been identified. This results in a spectrum of anomalies with a wide and variable clinical spectrum.

Facio-Auriculo-Vertebral Anomalies Spectra

Facio-auriculo-vertebral anomalies (FAVA) encompasses a spectrum of hemifacial microsomia and Goldenhar syndromes resulting from developmental defects of the first and second branchial arches. Varied facial defects observed include hypoplasia of the maxilla and/or mandible, a lateral cleft at the angle of the mouth resulting in

Figure 1.42 Clinical features suggestive of type III osteogenesis imperfecta (OI); collagen screen results on skin fibroblasts are consistent with type III or type IV OI. The patient would not consider deoxyribonucleic acid (DNA) diagnosis. He is wheelchair bound but lives independently.

Figure 1.44 A grandfather and granddaughter with Stickler syndrome type II. Grandfather has high myopia and status post–unilateral retinal detachment. Grandfather was confirmed to have a frameshift mutation in the collagen II gene, confirming Stickler syndrome type II. The mutation was confirmed in his 5-year-old granddaughter; note the early-onset high myopia and midface hypoplasia. She also had generalized joint laxity. She had surgery for cleft palate and is under surveillance by audiology for conductive hearing loss.

macrostomia, microtia and preauricular tags and/or pits, deafness, and tongue and palatal involvement with abnormal functioning of the palate.

Vertebral defects are predominantly in the cervical region, observed as a short neck and/or torticollis. Radiologic imaging is able to decipher underlying hemivertebrae and hypoplasia of the vertebrae in the cervical region but may involve the thoracic and lumbar regions. Microphthalmia and/or epibulbar eyelid coloboma are observed as infrequent features. Congenital heart defects include ventricular septal defect, tetralogy of Fallot, and coarctation of the aorta. Renal defects are occasional features and may present as ectopic, fused kidney or renal agenesis, as well as multicystic dysplastic kidney. Ureteral aberrations may be associated as well. Central nervous system (CNS) involvement is an occasional feature. Intelligence is usually preserved but may be compromised in

association with microphthalmia and CNS anomalies. Genetic delineation of etiology is at a research level, and clinical cases are not confirmed by mutation analyses. FAVA may overlap with clinical findings of VATER association (see later) and branchio-oto-renal syndrome, also known as *Melnick-Fraser syndrome.*

CHARGE Association

CHARGE is an acronym for a nonrandom association of features including *c*oloboma of the retina, less commonly the iris; *h*eart abnormalities; *a*tresia of the choanae; *r*etarded growth and mental development; *g*enital hypoplasia in males; and *e*ar anomalies that can include deafness. The minimal diagnostic criteria should include abnormalities in four of the six categories, at least one of which must be coloboma or choanal atresia. Cleft lip and/or palate and renal abnormalities are sometimes found. The association includes congenital heart disease, particularly abnormalities of the aortic arch, right subclavian artery, or ventricular septal defect; agenesis or hypoplasia of the thymus with decreased T-cell production and impaired cell-mediated immunity; partial or less often complete absence of the parathyroid glands, manifest by hypocalcemia and neonatal tetany; and often a facies characterized by wide-spaced, slightly down-slanting palpebral fissures, anteverted nares, a short philtrum, and small, dysmorphic ears (Fig. 1.45). Infants with CHARGE association often die early as a result of their congenital anomalies, but many of them survive to adulthood. Although developmental delay exists, the IQ range is broad (<30 to 80).

CHARGE has been found to be related to mutations in the *CHD7* gene located on chromosome 8q12. In the neonate, CHARGE association must be differentiated from other chromosomal disorders, such as deletion 22q11.2 or trisomy 13 or 18, and from the more benign nonchromosomal VATER association. When the DiGeorge sequence is present in patients with CHARGE association, a small interstitial deletion of chromosome 22 at q11 is occasionally found with high-resolution cytogenetic techniques and FISH DNA probe.

The etiology of CHARGE association is most likely heterogeneous. Although most cases are sporadic, instances of affected siblings and an affected parent and offspring have been reported. The risk of recurrence must be determined after genetic evaluation and ranges from 4% to 6% to as high as 50%.

VATER Association

VATER is another acronym for a nonrandom association of *v*ertebral and *a*nal anomalies, *t*racheoesophageal fistula with *e*sophageal atresia, and *r*adial and/or *r*enal abnormalities. Most affected

Figure 1.43 Stickler syndrome: Short stature, early-onset myopia, midface hypoplasia, submucous cleft palate, sensorineural hearing loss, mild skeletal dysplasia, and joint pain. A missense mutation in the *COL2A1* gene confirmed the clinical diagnosis and is consistent with type I Stickler syndrome.

Figure 1.45 CHARGE association. **A,** Note the short palpebral fissures and ptosis; low-set, dysplastic ears; and small chin. Choanal atresia necessitated tracheotomy. **B,** Another example of an infant with CHARGE association has clinical features that include a prominent forehead, hypertelorism, narrow palpebral fissures, hypoplasia of the right naris, low-set ears, and a cupid's-bow mouth. (**A,** *Courtesy W. Tunnessen, MD.* **B,** *Courtesy Timothy McBride, MD, Fairfax, VA.*)

newborns have anomalies in all five categories. The acronym can be expanded to VACTERL to include *c*ongenital heart disease (particularly ventricular septal defect) and, less often, other *l*imb defects. Vertebral anomalies include hemivertebrae and sacral abnormalities. Limb deformities consist of ray abnormalities, such as radial aplasia or hypoplasia, abnormal thumbs, preaxial poly-dactyly, and syndactyly (Fig. 1.46). Renal abnormalities include unilateral agenesis and less commonly ectopic or horseshoe kidney. The etiology of VATER association is unknown. Virtually all cases are sporadic. Detection of an abnormal karyotype rules out this disorder. The prognosis for growth and development in newborns who survive infancy is good. Most have normal intelligence and eventually achieve normal stature. Consequently, to make optimal

management decisions, it is important to distinguish VATER syndrome from more dire chromosomal abnormalities (such as trisomy 18) or nonchromosomal disorders (such as CHARGE association).

For the purpose of genetic counseling, VATER association must also be differentiated from Townes-Brocks syndrome, an autosomal dominant, simple mendelian genetic disorder with features repre-senting a combination of the findings observed for FAVA and VATER associations. However, in Townes-Brocks syndrome there is often a positive family history of autosomal dominant inheritance of ear, thumb, and anal abnormalities, whereas vertebral anomalies and tracheoesophageal fistula are unusual. The prognosis for growth and development in patients with Townes-Brocks syndrome is good.
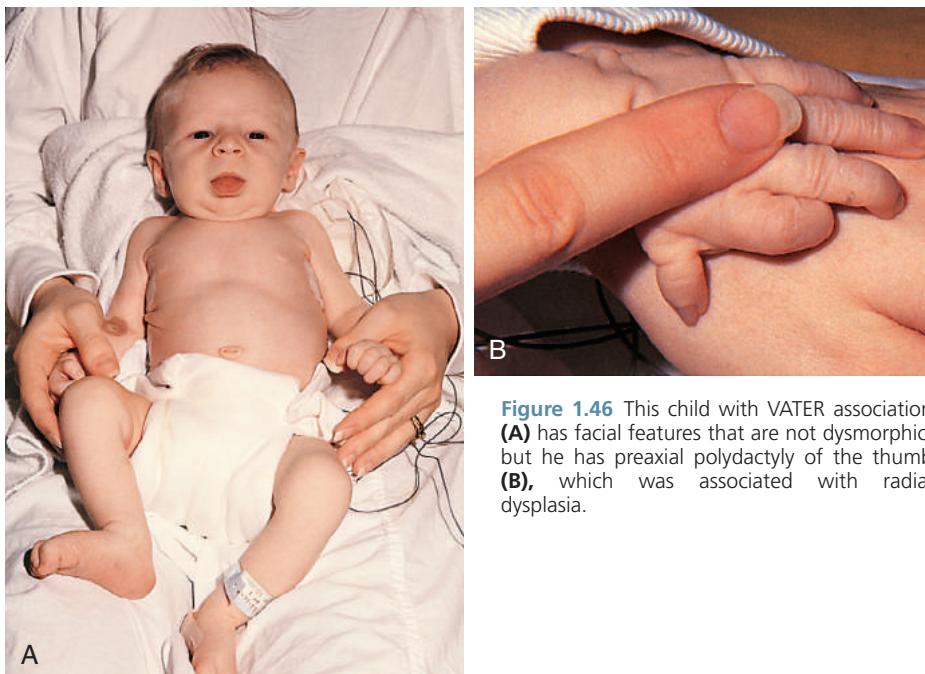

Figure 1.46 This child with VATER association **(A)** has facial features that are not dysmorphic, but he has preaxial polydactyly of the thumb **(B),** which was associated with radial dysplasia.

However, the risk for recurrence of Townes-Brocks syndrome with a positive family history may be up to 50%, whereas for VATER association the risk may be less than 2%. Antenatal diagnosis for both conditions depends on detecting structural anomalies in the fetus by high-resolution ultrasound.

Other disorders in the list of differential diagnostic possibilities include Fanconi anemia and Holt-Oram syndrome (see Chapter 5). Once chromosome studies are completed and found to be normal, when other disorders are deemed less likely, and a child has the pattern of malformation characteristic of the VATER association, the diagnosis (which remains one of exclusion) can be made.

Recognizable Multiple Malformation Syndromes

de Lange, Cornelia de Lange, or Brachmann–de Lange Syndrome

de Lange syndrome is characterized by intrauterine growth retardation, persistent postnatal failure to thrive, moderate to severe cognitive impairment, and microcephaly with a flat occiput and low hairline. Facial features are quite distinctive and include long eyelashes; a fine, almost "brushed-on" appearance of the arch to the eyebrows; occasional synophrys due to hirsutism; small nose with anteverted nostrils; long philtrum; downturned upper lip with cupid's-bow shape; and micrognathia (Fig. 1.47A and B).

Extremities are notable for small hands and feet, and varying abnormalities can include proximally placed thumbs (see Fig. 1.47C), flexion contractures of the elbows, hypoplastic limbs, and even overt phocomelia. Hirsutism is generalized and distinctive, and cutis marmorata is a frequent feature. In males, hypospadias with cryptorchidism is common, and females may have a bicornuate uterus. Most affected adults are quite short in stature.

In general, most cases are believed to be the result of new autosomal dominant mutations, and mutations in the *NIPBL* gene on chromosome 5p13 have been identified as responsible for approximately 50% of cases of classic de Lange syndrome. In evaluating cases, careful physical examination of family members must be performed to determine recurrence risks for individual families. Clearly, this disorder can be so mild in expression that many cases may go unrecognized. Families have been identified with severely affected children whose parents have been determined to be subtly affected. In those families, autosomal dominant inheritance would apply, with a 50% recurrence risk for any affected individual to have a child with the same disorder. If parents are not thought to be affected, the recurrence risk has been shown to range from 1% to 5%. A few individuals have somewhat similar features, most notably synophrys, and have been found to have an abnormality of the short arm of chromosome 3; thus careful high-resolution chromosome studies are indicated, with particular attention to chromosome 3.

Figure 1.47 Cornelia de Lange syndrome. **A** and **B,** Facial features seen in an infant and an older child include finely arched heavy eyebrows, long eyelashes, small upturned nose, long smooth philtrum, and cupid's-bow mouth. **C,** Small hands, hypoplastic proximally placed thumb, and short fifth finger with mild clinodactyly are examples of commonly associated extremity anomalies. (**A** *and* **C,** *Courtesy A. H. Urbach, MD, Children's Hospital of Pittsburgh, Pittsburgh, PA.*)

Noonan Syndrome

Noonan syndrome is an autosomal dominant, simple mendelian genetic disorder that shares a number of clinical features with 45,X (Turner syndrome). The disorder has been found to be associated with mutations in the *PTPN11* gene on chromosome 12q24.1 (in 50% of classic cases) and in the *KRAS* gene on chromosome 12p12.1 in 5% to 10% of the cases that are negative for the *PTPN11* mutation. Thus a chromosome study should be performed on any individual in whom this diagnosis is suspected. It is relatively common and thought to be present in 1 in 1000 to 1 in 2500 individuals. Like many other autosomal dominant disorders, it is seen in both males and females, and there is significant variability in clinical expression. Hence careful examination of close relatives of an index case may identify other affected individuals within the extended family, which is helpful when attempting to determine recurrence risks, because that risk would be 50% for offspring of an affected individual. In cases in which the child is considered to be the first in the family with Noonan syndrome, the empiric recurrence risk to apparently unaffected parents is 5%.

The pattern of malformations in Noonan syndrome is characterized by webbing of the neck, sternal abnormalities, pulmonic stenosis, and cryptorchidism in males. Facial characteristics include widely spaced eyes with down-slanting palpebral fissures, ptosis, and retrognathia (see Chapter 5). Ears are often low-set and can be posteriorly rotated. Hair can be coarse and curly, and the posterior hairline is often low. Sternal abnormalities include both pectus excavatum and carinatum, and often there are differences in the number of sternal ossification centers. Many have congenital heart disease with pulmonary valvular stenosis being the most common, followed by septal hypertrophy or defects. Hypertrophic cardiomyopathy is found in approximately 20% and can be sufficiently severe as to necessitate cardiac transplantation. Coagulation abnormalities are found in approximately one-third of cases.

Puberty can be delayed in individuals with Noonan syndrome. Cryptorchidism, when present in males, can result in sterility. Females are fertile. Stature is often less than the third percentile, but head circumference and intelligence are usually normal.

Several recognizable patterns of malformation share features in common with Noonan syndrome. Cardio-facio-cutaneous syndrome has additional features suggesting abnormal development of tissues derived from ectoderm, and affected individuals usually have significant CNS abnormalities. The phenotypic features of Noonan syndrome and neurofibromatosis type I may overlap (Watson syndrome). In the Costello syndrome, macrocephaly; coarse facial features; papillomas in the oral, nasal, and anal areas; cutis laxa; and cognitive impairments are seen in addition to findings shared with Noonan syndrome. Genetic heterogeneity is encountered; at least 10 gene loci are involved for the Noonan syndrome and allied disorders, that is, Noonan, cardio-facio-cutaneous, and Costello syndromes. Molecular testing at the clinical level is currently available for *PTPN11* at 12q24.1, *RAF1* at 3p25, *SOS1* at 2p22-p21, *KRAS* at 12p12.1, *HRAS* at 11p15.5, *BRAF* at 7q34, *MEK1* at 15q21, *MEK2* at 7q32, *NRAS* at 1p13.2, and *SHOC2* at 10q25 (Fig. 1.48). Refinement of molecular techniques has enabled the simultaneous analyses of all the genes in a single diagnostic test known as the "Noonan Chip," currently offered by some laboratories. Watson syndrome is characterized by mutation of the neurofibromatosis gene but also shows phenotypic overlap with Noonan syndrome. In addition to short stature, pulmonic stenosis, café au lait spots, and cognitive disabilities, freckling and neurofibromas are also observed.

Kabuki syndrome, characterized by physical growth and psychomotor delays in association with skeletal, cardiac, and craniofacial features, may have an overlapping phenotype with Noonan spectrum disorders. Exome sequencing has identified mutation(s) in the *MLL2* gene at 12q12-14 among patients with Kabuki syndrome (Ng et al, 2010).
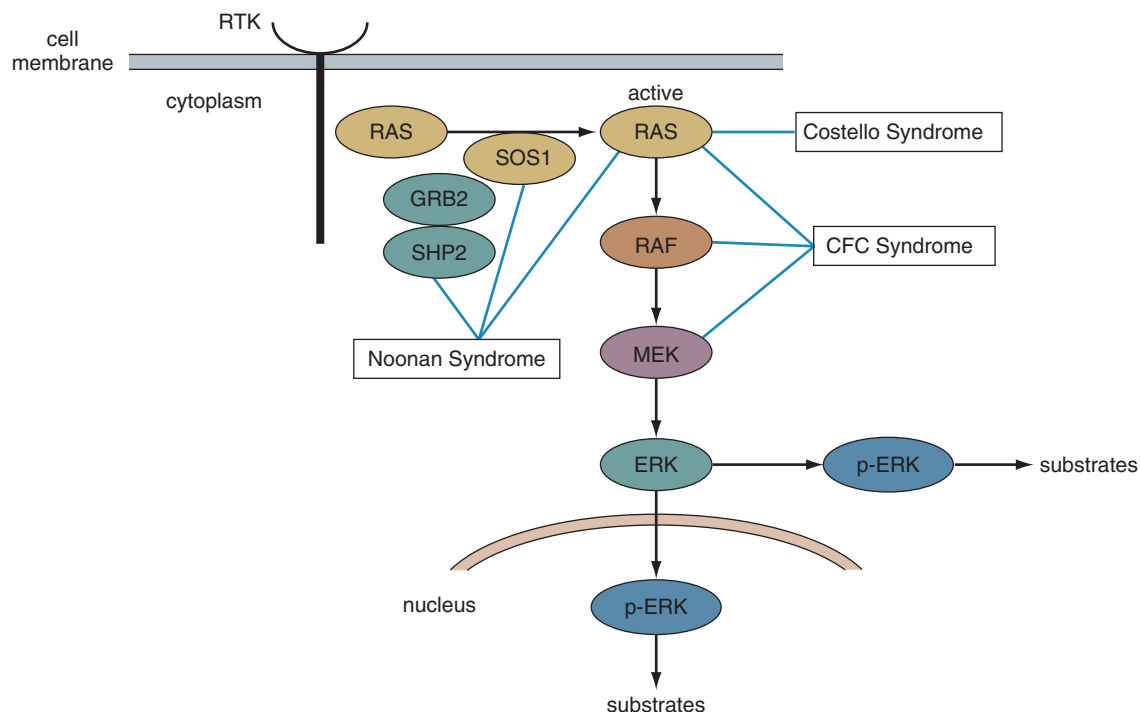
Figure 1.48 The mitogen-activated protein kinase (MAPK) signaling cascade, also known as the *RAF/MEK/ERK signaling cascade*. Germline mutations that affect components of the RAS-MAPK pathway are involved in the pathogenesis of Noonan syndrome and allied disorders (Noonan phenotype with overlapping features: CFC, LEOPARD, Costello and neurofibromatosis-1). *CFC,* Cardio-facio-cutaneous; *ERK,* extracellular signal–regulated kinase; *GRB2,* growth factor receptor-bound protein 2; *MEK,* MAPK/ERK kinase; *p-ERK,* phosphorylated ERK; *RAF,* murine sarcoma viral oncogene; *RAS,* rat sarcoma viral oncogene; *RTK,* receptor tyrosine kinase; *SHP2,* src homology region 2-domain phosphatase 2; *SOS1,* son of sevenless 1. *(Courtesy of Partners Health Care, Laboratory for Molecular Medicine, Harvard Medical School.)*

Figure 1.49 Noonan syndrome. Note the down-slanting palpebral fissures, ptosis, and low-set posteriorly rotated ears. Patient has bilateral simian creases and underwent cardiac surgeries for severe pulmonic stenosis and atrial septal defect. She has short stature, developmental delays, alternating esotropia, optic nerve cupping, and unilateral pelvicaliectasis. A missense mutation in exon 3 of the *PTPN11* gene resulted in the classic features of Noonan syndrome.

Figure 1.51 Cardio-facio-cutaneous syndrome. The patient underwent surgical repair of ventricular septal defect and pulmonic stenosis during infancy. He presented at 8 years old with short stature while receiving growth hormone injections; he also had seizures and intellectual disabilities. Patient has optic nerve hypoplasia, relative macrocephaly, curly hair, deep palmar creases, and prominent finger pads. Note the prominent forehead, hypertelorism, ptosis, and low-set posterior rotation of ears. The patient carried a clinical diagnosis of Noonan syndrome and tested negative for *PTPN11, SOS1, RAF1,* and *KRAS* genes. Noonan Chip analysis confirmed the missense pathogenic mutation in the *BRAF* gene, which is consistent with cardio-facio-cutaneous syndrome.

The differential diagnosis also includes several well-known teratogenic exposures, including fetal hydantoin syndrome and fetal alcohol syndrome (see Fig. 1.54). Hence careful dysmorphologic assessment of all individuals suspected of having Noonan syndrome is indicated before making a final diagnosis.

Patients with Noonan, LEOPARD (LEOPARD is an acronym for the cardinal features: *l*entigines, *e*lectrocardiographic conduction abnormalities, *o*cular hypertelorism, *p*ulmonic stenosis, *a*bnormal genitalia, *r*etardation of growth, *s*ensorineural *d*eafness), cardio-facio-cutaneous, or Costello syndrome (Figs. 1.49 through 1.52, respectively) are demonstrated by pathogenetic pathways. Interaction of the many respective gene(s) at the genetic level results in clinical overlap among these entities of "Noonan syndrome and allied disorders." These are therefore an excellent example of locus or genetic heterogeneity. Allelic heterogeneity is observed among classic Noonan phenotype and LEOPARD syndrome clinical picture (see Fig. 1.49).

Next generation sequencing (NGS) methodologies are rapidly becoming a mainstay of clinical diagnosis for heritable disorders. The first clinical application of NGS was for disease gene discovery

in cancer involving analyses of patients' tissue samples with suspected inherited monogenic aberrations in the nuclear and mitochondrial genome. Newer applications of NGS with immense promise for clinical diagnostic utility include whole genome sequencing (WGS) and diagnostic whole exome sequencing (WES). WES allows the unbiased simultaneous interrogation of the coding regions, or exons, and the exon boundaries of approximately 20,000 genes by massive parallel sequencing, and is thus a powerful tool for making a diagnosis in complex clinical cases with a suspected heritable etiology that is becoming increasingly applicable to the clinical

Figure 1.50 A 10-year-old girl with LEOPARD syndrome. LEOPARD is an acronym for the cardinal features: *l*entigines, electrocardiographic conduction abnormalities, *o*cular hypertelorism, *p*ulmonic stenosis, *a*bnormal genitalia, *r*etardation of growth, sensorineural *d*eafness. Not all features need to be present to be diagnosed with LEOPARD syndrome.

Figure 1.52 Costello syndrome. Note the macrocephaly, curly sparse hair, short neck, and hypotonia. The patient had a tracheostomy and is gastrostomy tube dependent, and has had global delays with some communication by sign language at 4 years old. Skin was very lax and soft and a somewhat darker color for the family. A mutation in exon 2 of the *HRAS* gene was classic for Costello syndrome.

setting. Most disease-causing changes in the DNA sequence are found in the exons (exome) consisting of about 3% of the genome. WES can be further streamlined in a manner known as *targeted exome sequencing (TES),* in which analysis of the WES is first targeted to regions most relevant to a patient's clinical findings, thus reducing analytical time. WES must be interpreted in context of familial segregation of any changes detected in the patient's sequence, the patient's medical and family history, and detailed knowledge of clinical features including other lab testing. WES has limitations in that it does not detect certain types of deleterious genetic changes, such as deletions, duplications, tri-nucleotide repeats, or methylation defects. Although mapping of the genes is complete, our understanding of the function of most genes is in its infancy. Ultimately, WES provides profound diagnostic and economic advantages in the diagnosis of pediatric and adult heritable disease presenting in the medical genetics clinic. The impact of achieving a definitive diagnosis and identification of the heritable etiology helps to streamline the multidisciplinary medical needs and implication(s) on the patient and his or her family. Diagnosis in patients with a comorbid diagnosis and blended phenotypes may only be possible through utilization of WES. Similarly, WGS and WES are critical in the identification of new mutations and genetic causes of novel phenotypic presentations (Fig. 1.53).

Patterns of Malformation Associated With in Utero Teratogen Exposure

Fetal Alcohol Syndrome

The effect of exposure to significant levels of serum alcohol during gestation results in a pattern of microcephaly, prenatal and postnatal growth deficiency, short palpebral fissures, long smooth philtrum, and a thin upper lip (Fig. 1.54). Other features include a short nose and hypoplasia of the nails and distal phalanges (particularly the fifth toes). On occasion, affected infants have eyelid ptosis, epicanthal folds, strabismus, small raised hemangiomas, cervical vertebral abnormalities, and congenital heart disease.

Newborns with fetal alcohol syndrome are small for gestational age and have poor catch-up growth postnatally. They may have increased or decreased muscle tone and can be irritable and tremulous. Most older children tend to be thin and hyperactive, and more than 80% have some delay in development, especially fine motor function.

The diagnosis of fetal alcohol syndrome should be reserved for those infants who have a history of in utero exposure to large amounts of alcohol and who have the characteristic physical features of the disorder. The past practice of labeling children with developmental disorders who do not have the clinical stigmata as having fetal alcohol effects should be abandoned.

Although there may be no absolutely safe level of maternal alcohol consumption throughout pregnancy (particularly in the first trimester), the risk of teratogenesis increases dramatically with increasing degrees of maternal ethanol consumption. Major evidence of fetal alcohol syndrome is observed in 30% to 50% of offspring of mothers who are chronic severe alcoholics, whereas more subtle effects result from ingestion of lesser quantities of alcohol. The risk to the fetus of occasional maternal alcoholic binges is not clear, but such drinking is best avoided. Why some babies are affected and others are not, despite equivalent degrees of maternal alcoholism, is also unclear.

Other examples of teratogen-induced disorders include fetal hydantoin syndrome and fetal retinoic acid (isotretinoin [Accutane]) embryopathy.

Genetic Disorders of Metabolism

Inborn errors of metabolism encompass a wide range of inherited disorders. These include disorders of intermediary metabolism, disorders of organelle function (lysosomal, peroxisomal, or

Figure 1.53 Use of whole exome sequencing (WES) and whole genome sequencing (WGS) in diagnosis. **A,** Three-years and 5-months-old girl with intellectual disabilities, neurobehavioral presentation suggesting Rett syndrome. WES disclosed a de novo frame shift mutation in SMC1A(c.2394dupA; p.R799fs), the gene, a rare form of X-linked Cornelia de Lange syndrome. **B,** Ten-month-old girl with failure to thrive, microcephaly, congenital hearing loss, complex heart defect with mesocardia and altered surface morphology. Patient underwent a series of diagnostic testing prior to WGS. WGS delineated a de novo missense mutation (c.418G>A p.G140R) in the HDAC8 gene, yet another infrequent cause for an X-linked type of Cornelia de Lange syndrome.

mitochondrial), disorders of cholesterol biosynthesis, disorders of protein glycosylation, disorders of metals, disorders of transport, and others. Although most are enzyme deficiencies that are inherited in an autosomal recessive pattern, various forms in inheritance (dominant, recessive, X-linked, and maternal) are found with metabolic disorders. This brief review is limited to some of the more common metabolic disorders and should be supplemented with a more detailed text on metabolic disorders as needed.

Disorders of Intermediary Metabolism

Intermediary metabolism involves conversion of the nutrients—protein, carbohydrate, and fat—into energy (Fig. 1.55). The common endpoint in the metabolism of all three nutrients passes through the Krebs cycle, and then through the electron transport chain where oxidative phosphorylation occurs, consuming oxygen and yielding adenosine triphosphate (ATP). A substantial number of errors of intermediary metabolism are detected by expanded newborn screening, but some are not detectable or can be missed in some cases.

Figure 1.54 Fetal alcohol syndrome. Note the short palpebral fissure length, mild ptosis, and long simple philtrum.

The *feed/fast cycle* refers to changing patterns of metabolism depending on available energy sources. After eating, the body uses circulating fuels, which are fairly quickly depleted postprandially. The body then largely relies on stored glycogen until these stores are exhausted (approximately 4 to 8 hours, depending on age and clinical circumstances). After glycogen stores are exhausted protein catabolism and fatty acid oxidation become the most important

energy sources, and gluconeogenesis is required to support glucose stores. Thus, disorders of glycogen metabolism tend to present after a short fasting interval. However, most disorders of protein or fatty acid oxidation metabolism tend to present after a longer fasting period, after normal glycogen stores are depleted.

Disorders of Protein Metabolism

Proteins are broken down into their basic components, amino acids. Essential amino acids cannot be synthesized by the body and must be consumed in the diet. Amino acids can be used to support tissue growth or maintenance, can be converted into other amino acids (transamination), can be excreted in the urine, or can be catabolized for energy (see Fig. 1.55). Some characteristic physical findings of protein metabolism disorders are noted in Table 1.9.

Transamination Disorders

Phenylketonuria A common disorder of transamination is PKU, in which the transamination of phenylalanine to tyrosine is impaired. PKU is a relatively common autosomal recessive disorder of protein metabolism (affecting 1 in 10,000 to 1 in 15,000), and it is most commonly diagnosed by newborn screening. Affected patients follow a diet restricted in phenylalanine (containing just enough natural protein from fruits, vegetables, and limited starches to meet essential phenylalanine needs for growth and maintenance), supplemented with a medical food "formula" containing tyrosine and other essential amino acids and nutrients, and specially formulated low-phenylalanine foods. If untreated, the disorder results in mental retardation, eczematous skin changes, a "mousy" odor, and other changes. Well-treated patients may have mild tremor, but physical examination is otherwise normal. Although cognitive outcomes are normal in most diet-adherent patients, some patients experience mild to moderate difficulties with executive functioning (attention,
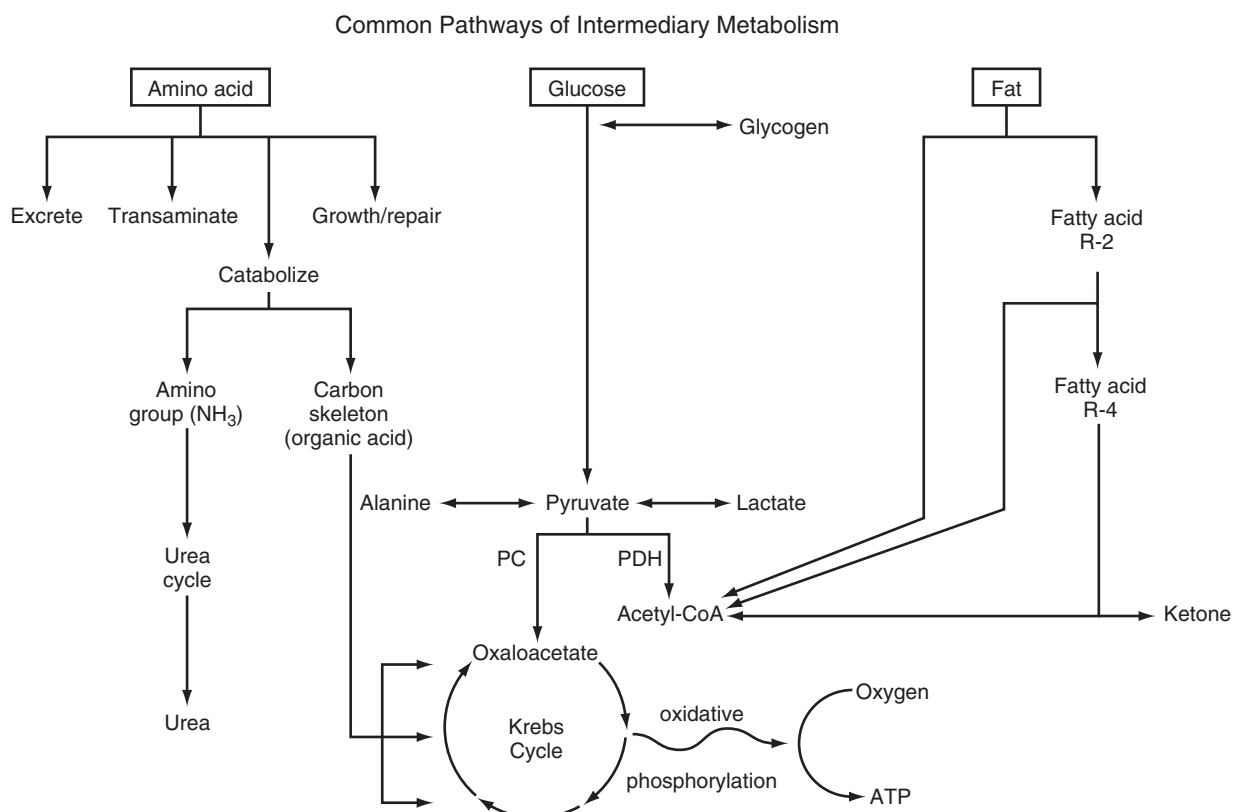

Figure 1.55 The conversion of protein, carbohydrate, and fat into energy. *ATP,* Adenosine triphosphate; *NH₃,* ammonia; *PC,* pyruvate carboxylase; *PDH,* pyruvate dehydrogenase; *R-2* and *R-4,* fatty acids shortened by successive two-carbon units, the result of β-oxidation.

Table 1.9	Common Findings in Disorders of Protein Metabolism
Disorder	**Common Findings**
PKU	Untreated: MR, eczema, mousy odor, fair coloring
	Treated: Minimal to mild tremor, executive function deficits
Homocystinuria	Untreated: Variable MR, ectopia lentis, marfanoid habitus, vascular thromboses
	Treated: Variable persistent vascular issues
MSUD	Untreated: Ketosis, acidosis, coma, death, MR, maple syrup odor, some late onset with intermittent symptoms
	Treated: Intermittent ketosis/acidosis during intercurrent illnesses, variable developmental delay to normal cognition
Organic acidemia (MMA, PA, IVA)	Untreated: Ketosis, acidosis, death, neutropenia, hyperammonemia, hypotonia, some late onset with failure to thrive, hypotonia
	Treated: Intermittent ketoacidosis during intercurrent illnesses, metabolic strokes, low to normal tone, delay to normal cognition
Urea cycle defect	Untreated: Hyperammonemia, coma, death, hyperreflexia, MR, variable acidosis, some late onset with intermittent symptoms
	Treated: Intermittent hyperammonemia with intercurrent illnesses, variable developmental delay to normal cognition

IVA, Isovaleric acidemia; *MMA,* methylmalonic acidemia; *MR,* mental retardation; *MSUD,* maple syrup urine disease; *PA,* propionic acidemia; *PKU,* phenylketonuria.

organization, and working memory). When a woman with PKU is pregnant, she must follow her diet especially carefully to prevent mental retardation and birth defects (particularly defects of the heart and esophagus) in her fetus.

Most PKU is due to a defect in the enzyme phenylalanine hydroxylase. A small proportion of patients with PKU have normal phenylalanine hydroxylase enzyme but have a defect in synthesis or recycling of the enzyme's biopterin cofactor. Because this biopterin cofactor is shared with two other enzymes (tyrosine hydroxylase [a precursor of dopamine, epinephrine, and norepinephrine] and tryptophan hydroxylase [a precursor of serotonin]), patients with biopterin defects have neurotransmitter deficits in addition to PKU. These patients require supplementation with biopterin and/or neurotransmitter replacements in addition to a phenylalanine-restricted diet, and the neurologic outcome is not always normal.

Homocystinuria Homocysteine is an intermediate in the multistep metabolism of methionine to cysteine. In homocystinuria, the metabolism of homocysteine is blocked at the level of the enzyme cystathionine β-synthase. When untreated, in addition to developmental delays, physical findings include various degrees of ectopia lentis; a marfanoid habitus; and, by young adulthood, vascular thromboses (see Table 1.9). Homocystinuria is most commonly diagnosed by newborn screening (by detection of elevated methionine), but the sensitivity of the screen is not complete. Homocystinuria may be present in older children and adults who were born before this disorder was added to the current newborn screening panel. Rarer forms of homocystinuria exist caused by failure to return homocysteine back into methionine, typically due to defects in vitamin B$_{12}$ metabolism.

Maple Syrup Urine Disease The branched-chain amino acids (valine, leucine, and isoleucine) share a common initial step in catabolism, catalyzed by branched-chain keto acid decarboxylase. A genetic defect in this enzyme leads to maple syrup urine disease (MSUD), named because of the characteristic odor of the urine. Affected individuals develop elevations of these three branched-chain amino acids resulting in ketosis and, if untreated, acidosis,

coma, and death. Physical examination may identify the odor of maple syrup in cerumen and concentrated urine. The patient may exhibit varying degrees of spasticity or developmental delay, especially if late or poorly treated (see Table 1.9). Elevated leucine levels are intoxicating, and they can chronically impair learning and social interactions when poorly controlled. Patients must follow a diet restricted in these three essential amino acids, supplemented with other essential amino acids and nutrients. The most important component of dietary treatment is the prevention of stressful fasting, because this would lead to the increased catabolism of amino acids for energy and thus increased formation of toxic metabolites. Milder variants are known to exist with symptoms, including altered mental status or ketosis during intercurrent illnesses. MSUD is most commonly diagnosed by newborn screening, but the sensitivity of screening for milder or intermittent variants is not known, and an infant may already be symptomatic before the screening results are available.

Urea Cycle Disorders Amino acids are further catabolized by deamination. The amino group is removed and forms ammonia, and the residual carbon skeleton is an organic acid, typically metabolized in the Krebs cycle (see Fig. 1.54). Ammonia is toxic when accumulated and is normally detoxified in the urea cycle. Metabolic errors are well described in each enzymatic step of the urea cycle. Affected infants classically present with hyperammonemic coma in the neonatal period, typically progressing through poor feeding, respiratory alkalosis, decreasing mental status, vomiting, and neurologic irritability to coma (see Table 1.9). The presence of unexplained respiratory alkalosis or neurologic irritability, including hyperreflexia, increased startle, or clonus in an infant with depressed mental status, should always prompt a search for an inborn error of metabolism (although these findings will eventually be blunted as coma deepens). Acidosis (rarely even ketoacidosis) may develop as the infant's clinical status deteriorates. Any patient of any age undergoing a lumbar puncture for otherwise unexplained mental status changes should have their plasma ammonia level checked. Analysis of plasma amino acids and orotic acid is key in helping to define the specific urea cycle defect, and additional information on this is available in textbooks dedicated to metabolic disorders.

A significant percentage of patients with urea cycle defects can present later in life with failure to thrive, developmental delays, friable hair (trichorrhexis nodosa, in argininosuccinate lyase deficiency), or in some cases acutely with hyperammonemia during intercurrent illnesses or weaning from breast milk to formula (which is higher in protein). Some patients have presented in adulthood during profound metabolic stress (such as after bariatric surgery or childbirth); thus age is no barrier to diagnosis. Treatment involves careful titration of protein intake to meet requirements for growth and maintenance without providing excess, with supplementation of essential urea cycle intermediates (citrulline or arginine, as determined by the position of the enzymatic block), and with drugs that complex with glutamine or glycine to form urine-soluble nitrogen complexes and provide an alternative excretion pathway for nitrogen. It is particularly important to provide alternative energy intake during fasting to prevent the catabolism of amino acids for energy and the release of ammonia.

Organic Acidemias Once the ammonia is removed from an amino acid, the remaining carbon skeleton is an *organic acid*. Most organic acids are catabolized to specific Krebs cycle intermediates (see Fig. 1.55). Various errors in catabolism lead to specific organic acid disorders.

Propionic Acidemia and Methylmalonic Acidemia The most common series of errors is in the catabolism of the carbon skeletons of *v*aline, *o*dd-chain fatty acids, *m*ethionine, *i*soleucine, and *t*hreonine (VOMIT), which pass through propionyl-CoA, methylmalonyl-CoA, and then succinyl-CoA (a Krebs cycle intermediate). Inborn errors of propionyl-CoA carboxylase lead to propionic acidemia,

and errors of methylmalonyl-CoA mutase lead to methylmalonic acidemia. These can present in the neonatal period with acute ketoacidosis, hyperammonemia, and bone marrow suppression (from secondary inhibition of the urea cycle and bone marrow); or at a later age with acute symptoms during an intercurrent illness; or at any time with chronic failure to thrive, hypotonia, or developmental delay. Physical examination is most remarkable for central hypotonia, often with hyperreflexia (see Table 1.9). Patient management includes careful titration of dietary protein to meet the needs for the essential amino acids valine, methionine, isoleucine, and threonine, supplemented with other essential amino acids and nutrients. Alternative forms of calorie supplementation are important during fasting/intercurrent illnesses to prevent protein catabolism.

Disorders of Leucine Catabolism A number of other organic acidemias are relatively common, including isovaleric acidemia, an inborn error in the catabolism of leucine (a complete review of which is beyond the scope of this chapter). The incidence of this disorder on newborn screening is somewhat higher than expected. Expanded newborn screening also has identified an unexpectedly high incidence of 3-methylcrotonyl-CoA carboxylase deficiency, another inborn error of leucine catabolism. Previously believed to be associated with developmental delays, failure to thrive, and other problems, the clinical significance of this disorder is now unclear. Most organic acidemias are detectable by newborn screening, although the sensitivity for late-onset or milder forms is not yet known.

Disorders of Carbohydrate Metabolism

The basic unit of carbohydrate metabolism is glucose. Glucose is metabolized for energy through glycolysis. Complex carbohydrates and alternative carbohydrates (such as galactose or fructose) are converted to glucose or glycolytic intermediates for catabolism. Some of the common disorders of carbohydrate metabolism are listed in Table 1.10.

Glycogen Metabolism When carbohydrate intake exceeds immediate need, glucose is stored as glycogen, predominantly in the liver. During the interprandial fast, glycogen is used preferentially to meet energy needs by converting it back to glucose. After glycogen stores are exhausted, protein and fat catabolism are enhanced.

Glycogen Storage Disease Type 0 In the case of glycogen storage disease (GSD) type 0, glycogen cannot be made. Patients typically have postprandial hyperglycemia and interprandial hypoglycemia, but no other specific physical findings. The liver is not enlarged because glycogen is not stored. In some cases, the interprandial hyperglycemia can be mild or overlooked, and the disorder manifests as hypoglycemia during fasting or intercurrent illness.

Other Forms of Glycogen Storage Disease Other forms of GSD affect glycogen metabolism in the liver or muscle. The most common forms of liver GSD are types I, III, VI, and IX, in which glycogen can be stored in the liver but not efficiently returned to glucose. The patient most commonly develops hepatomegaly (from stored glycogen), and interprandial hypoglycemia begins within a few hours of eating, when circulating fuels are exhausted. The patient may also manifest "cherubic" cheeks from glycogen storage. Other findings can include elevations in triglycerides, uric acid, and lactate. There are a number of enzymes involved in the formation, branching, debranching, and catabolism of glycogen, and various subtypes of GSD are associated with defects in the various enzymatic steps. Some glycogen disorders present predominantly in muscle with weakness, rhabdomyolysis, and other predominantly muscle findings but not significant hypoglycemia. Infantile-onset Pompe disease (GSD type II) presents with progressive weakness in the skeletal and respiratory muscles, as well as cardiomyopathy. The electrocardiogram demonstrates a characteristic high-voltage pattern. Some patients with residual enzyme protein respond to enzyme replacement therapy.

Defects in Glycolysis, Gluconeogenesis, and Metabolism of Other Carbohydrates Glycolysis, the process of metabolizing glucose to pyruvate, does not have commonly associated inborn errors of metabolism. However, in some cases alternative carbohydrates (such as galactose or fructose) have impaired conversion into a glycolytic substrate.

Galactosemia Patients who are unable to metabolize galactose have galactosemia. After ingestion of galactose (one of the sugars in lactose), patients can present in infancy with hepatomegaly, liver disease, gram-negative sepsis, cataracts, or later with failure to thrive and excretion of galactose (a reducing substance) in the urine. Treatment includes exclusion of galactose from the diet.

Hereditary Fructose Intolerance Patients with hereditary fructose intolerance are unable to metabolize fructose (a common fruit sugar and one of the components of sucrose). They can present with acute decompensation with hypoglycemia and hypophosphatemia from fructose ingestion, but can also present chronically with failure to thrive and liver disease.

Gluconeogenesis The reverse of glycolysis (gluconeogenesis, the production of glucose from distal metabolic substrates) can be impaired by metabolic errors. Fructose-1,6-bisphosphatase deficiency is a gluconeogenic enzyme deficiency that results in fasting hypoglycemia. Patients typically tolerate an interprandial fast because glycogen metabolism is intact, but they are at risk for hypoglycemia after glycogen stores are exhausted. Most patients present in infancy during an intercurrent illness with hypoglycemia and varying degrees of elevated ketones, lactate, and glycerol.

Other defects in gluconeogenesis, as well as ketone synthesis and use disorders, are described in more detailed texts.

Defects of Fatty Acid Oxidation

Fatty acid oxidation is a significant source of energy after liver glycogen stores are exhausted. Fatty acids are essentially chains of carbon atoms. β-Oxidation of fatty acids up to 18 carbons in length takes place in the mitochondria. One cycle of β-oxidation removes two carbons from the fat, releasing the two-carbon piece as acetyl-CoA (a primary substrate for the Krebs cycle), which also can be

Table 1.10	Common Findings in Disorders of Carbohydrate Metabolism
Disorder	**Findings**
GSD 0	Postprandial hyperglycemia, interprandial hypoglycemia
GSD I	Interprandial hypoglycemia, hepatomegaly, "cherub" cheeks, neutropenia (type 1b), elevated uric acid, lactate
GSD II	Pompe's disease, cardiomyopathy (infantile form), progressive skeletal and respiratory weakness, elevated CK
GSD III	Hypoglycemia, hepatomegaly, variable myopathy, disorder of glycogen debranching
GSD IV	Hepatomegaly, cirrhosis, disorder of glycogen branching
GSD VI and IX	Hepatomegaly with variable mild hypoglycemia, variable myopathy
Fructose-1,6-bisphosphatase deficiency	Hypoglycemia with prolonged fast/intercurrent illness, may be fructose sensitive, urine organic acids may reveal keto-lactic acidosis with elevated glycerol
Galactosemia	Untreated: Hepatomegaly, cataracts, failure to thrive, liver dysfunction, MR Treated: Variable cognitive deficits
Hereditary fructose intolerance	With fructose ingestion: Hypoglycemia, hypophosphatemia, shock Untreated: Chronic liver disease and failure to thrive

CK, Creatine kinase; *GSD,* glycogen storage disease; *MR,* mental retardation.

Table 1.11	Common Findings in Fatty Acid Oxidation Disorders	
Disorder	**Findings**	
Carnitine uptake disorder	Cardiomyopathy, variable weakness, sudden death	
CPT I	Infantile onset: Hypoglycemia, acidosis	
	Later onset: Fasting intolerance, exercise intolerance	
CPT II	Infantile onset: Hypoglycemia, acidosis	
	Later onset: Fasting or exercise intolerance, rhabdomyolysis	
SCAD deficiency	Possible hypotonia, developmental delay, neurologic abnormalities. Phenotype now in question	
MCAD deficiency	Potentially fatal hypoglycemia with prolonged fasting	
VLCAD deficiency	Infantile onset: Cardiomyopathy, juvenile onset: fasting intolerance	
	Later onset: Exercise intolerance	
LCHAD deficiency	Hypoglycemia, rhabdomyolysis, pigmentary retinopathy, fasting intolerance	

CPT I, Carnitine palmitoyltransferase I; *CPT II*, carnitine palmitoyltransferase II; *LCHAD*, long-chain 3-hydroxyacyl-CoA dehydrogenase; *MCAD*, medium-chain acyl-CoA dehydrogenase; *SCAD*, short-chain acyl-CoA dehydrogenase; *VLCAD*, very long–chain acyl-CoA dehydrogenase.

converted to a ketone body that can be exported to more distal tissues for energy (see Fig. 1.55). The shortened fatty acid undergoes successive cycles of oxidation removing two-carbon units at a time until it is fully metabolized. The enzymes used in fatty acid oxidation change as the fatty acid becomes successively shorter. Absence of ketosis in a patient older than 3 months of age who has hypoglycemia or is undergoing a stressful fast should raise concern for a possible fatty acid oxidation defect. Some of the most common defects of fatty acid oxidation are listed in Table 1.11.

Carnitine Disorders

Carnitine Uptake Disorder and Carnitine PalmitoyltransferaseI, Carnitine-Acylcarnitine Translocase, and Carnitine Palmitoyltransferase II Deficiency Long-chain fatty acids (that is, those having approximately 12 to 18 carbons) must first esterify carnitine in order to pass through the mitochondrial membrane for oxidation. Patients with carnitine uptake disorder have impaired transport of carnitine and develop profound carnitine deficiency. Symptoms can include cardiomyopathy, weakness, or simply sudden death. Plasma carnitine levels are very low while urine carnitine is generally elevated. Patients with a deficiency of carnitine palmitoyltransferase I (CPT I) are unable to esterify fatty acids to carnitine. The fatty acids are thus unable to penetrate the mitochondria for oxidation. The disorder has variable presentation, including life-threatening neonatal hypoglycemia and acidosis, or later onset fasting or exercise intolerance/rhabdomyolysis. Biochemically, the patient has low levels of acylcarnitines in the plasma. Defects in the carnitine-acylcarnitine translocase (CACT) enzyme (which carries fatty acylcarnitines across the mitochondrial membrane) are rare but reported and can be life-threatening; they often resemble CPT II deficiency. Patients with defects in carnitine palmitoyltransferase II (CPT II) are unable to release the carnitine from the fatty acid after it has passed inside the mitochondria. Like CPT I deficiency, it has variable severity and can present at any age. Biochemically, patients have elevated acylcarnitines in the plasma. Most carnitine disorders are now diagnosed by newborn screening (by measurement of acylcarnitines), and more mildly affected patients often do not appear ill in the newborn period but still require follow-up and treatment. Carnitine is not required for medium- and short-chain fatty acids to penetrate the mitochondria, and thus patients with severe carnitine disorders may benefit from dietary restriction of long-chain fat and supplementation with medium-chain fat.

Acyl-CoA Dehydrogenase Deficiencies

Very Long–Chain, Medium-Chain, and Short-Chain Acyl-CoA Dehydrogenase Deficiencies, and Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency Inside the mitochondria, the β-oxidation process progressively removes two carbon atoms, forming ketones or acetyl-CoA (see Fig. 1.55). Many of the enzymes in this process have chain length specificity, preferring fats that are long chain (12 to 18 carbons), medium chain (6 to 10 carbons), or short chain. The first step of β-oxidation is performed by an acyl-CoA dehydrogenase with chain length specificity. Deficits in the long-chain enzyme (very long–chain acyl-CoA dehydrogenase, also known as VLCAD or ACADVL, deficiency) present with variable severity, from neonatal cardiomyopathy, to later onset fasting or exercise intolerance. Genotype/phenotype correlations between residual enzyme activity and severity are emerging. Treatment includes supplementation with medium-chain fat and prevention of fasting. Defects in the medium-chain enzyme (medium-chain acyl-CoA dehydrogenase, also known as MCAD or ACADM, deficiency) are among the most common metabolic disorders (approximately 1 in 15,000 births). Affected patients most commonly present in the toddler period with potentially fatal hypoketotic hypoglycemia or Reye's syndrome–like symptoms during fasting associated with an intercurrent illness. Treatment is primarily by prevention of fasting, although some patients require carnitine supplementation. Defects in the short-chain enzyme (short-chain acyl-CoA dehydrogenase, also known as SCAD or ACADS, deficiency) were originally believed to cause hypotonia, hypoglycemia, and developmental abnormalities. However, the clinical significance of this disorder is now in question. Defects are also described in the third step of β-oxidation, another acyl-CoA dehydrogenase enzyme with chain length specificity. The most common enzyme deficiency at this step is long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency. This disorder can also have variable presentation from early hypoketotic hypoglycemia to later fasting or exercise intolerance. More severely affected patients demonstrate a pigmentary retinopathy and episodes of rhabdomyolysis. An increased incidence of HELLP (an acronym that stands for the three features of the disease—*h*emolysis, *e*levated *l*iver enzyme levels, and *l*ow *p*latelet levels) syndrome is reported in pregnant mothers carrying an affected fetus. Some patients have a deficiency of a trifunctional protein that results in defects in multiple steps of the long-chain fatty acid oxidation pathway.

Most disorders of fatty acid oxidation are now detected on newborn screening. However, some infants experience significant morbidity or mortality quickly (early presentation), before the newborn screening results are available. Thus, this group of disorders should always be considered in ill neonates.

Organelle Dysfunction

Important metabolic processes take place in various organelles. These include the peroxisomes, the lysosomes, and the mitochondria (Tables 1.12 through 1.14). Defects in each of these organelles are considered.

Peroxisomal Disorders

A number of metabolic processes take place in the peroxisome, including the oxidation of very long–chain fats (greater than 20 carbons), metabolism of phytanic acid (present in food), initiation of plasmalogen formation (the most abundant phospholipid in myelin), peroxidation/detoxification, and other processes. Peroxisomal diseases fall into two major categories: (1) those that interfere with assembly of the peroxisome itself (thus affecting all enzyme functions), and (2) those that affect a single enzyme.

Disorders of Peroxisome Biogenesis A number of mutations in 12 different peroxisome assembly *(PEX)* genes have been identified, leading to a continuum of phenotypes. The most severe of these disorders is Zellweger syndrome (Fig. 1.56). Infants with Zellweger syndrome (cerebrohepatorenal syndrome) typically have