Chapter 4

Pedigree Analysis and Risk Assessment

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The Genetic Family History

The personal and family medical pedigree has evolved from its earliest ancestors in the 15th century to its current form and has become an essential tool in many aspects of the clinical genetics evaluation. Originally used primarily to display relationship information, the pedigree was used for the first time to demonstrate inheritance of traits in the mid-19th century when Pliney Earl published on inheritance of color blindness and Francis Galton described inheritance of artistic ability and genius.[1]

Symbols used to document pedigree information have varied, often depending on personal, professional, or national preferences. The key to functionality for pedigrees, however, is the degree to which they are able to communicate information uniformly to all users. In 1993, a task force of the National Society of Genetic Counselors surveyed genetic counselors regarding interpretation of pedigree symbols and conformity of usage.[2] As many as 17 different symbols were used to depict pregnancy, with 16 different symbols being used to denote miscarriage; in both cases, symbols sometimes had several meanings to different users. It became evident that standardization of symbol usage was needed. The group established a recommended nomenclature for pedigrees, which was published in the *American Journal of Human Genetics* in 1995.[3]

The currently recommended methods for documenting pedigree information including symbols, spatial relationships, and clinical/investigative status are detailed in Figures 4-1 through 4-5. These standards allow recording of traditional relationships as well as those developing as new technologies are applied, particularly in reproductive medicine. They also serve as a uniform baseline for future additions or modifications as the field continues to evolve.

These pedigrees now form the cornerstone for determination of diagnosis, pattern of inheritance, and recurrence risk.[4] Use of pedigree information can impact overall risk assessment, medical management decisions, and feasibility of various testing strategies. In addition, collection of family medical information has aided in the understanding of many unique features of hereditary disorders, including natural history, variability, and gene-gene or gene-environment interactions.

Collection of a family pedigree represents an opportunity to build a relationship with the patient and family and to learn about how the family functions.[4] As the genetic counselor or other healthcare provider explains the purpose of the family history, an atmosphere of open communication and respect can be established. This process provides a window to the social relationships and psychosocial and educational needs of patients and families. In the social sciences, genograms are used to graphically depict family dynamics that influence individual behaviors.[5] This information is also essential for successful counseling of patients in the clinical genetics setting, and while not always recorded in the same fashion, it is a vital part of the process of pedigree gathering. Observations about coping mechanisms, assumptions about disease causation, family hierarchy, key life experiences, stress levels, body language, and religious and ethnic influences all are integrated into consideration about the most effective ways to communicate information about a diagnosis, prognosis, or management plan to patients and families.

Ideally, the pedigree is collected in a face-to-face session. This is usually done prior to or at the beginning of the clinical genetics evaluation, but may be done later, particularly when evaluating a pregnancy or a newborn with an unanticipated, newly diagnosed condition. It is helpful to provide patients with advance notice about the nature of information to be collected, as this facilitates accuracy and completeness. At a minimum, a three-generation pedigree should be collected, including all first-degree relatives (parents, children, full siblings), second-degree relatives (grandparents, aunts, uncles, nieces and nephews, half-siblings, grandchildren), and as pertinent, many third-degree relatives (cousins, great-aunts, great-uncles, great-grandparents). This group can be expanded or condensed, depending on the nature of the referral and patient responses to preliminary questioning about features

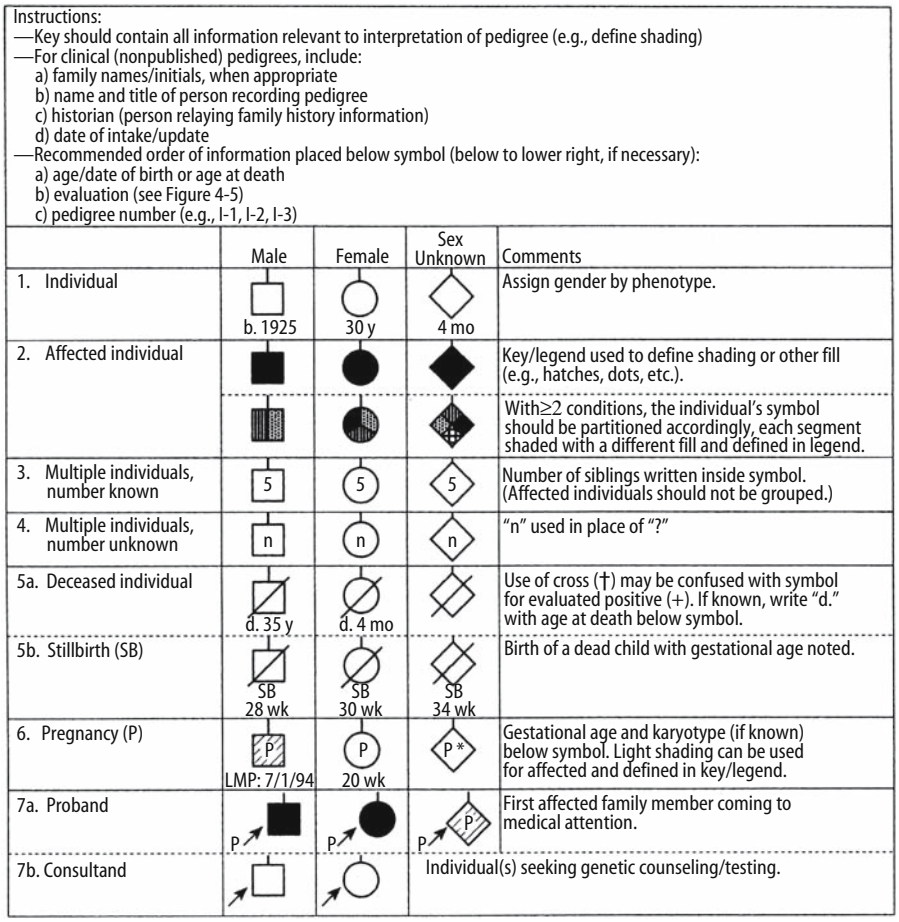


Figure 4-1. Common pedigree symbols, definitions, and abbreviations. (Figures 4-1 to 4-5 reprinted from Bennett RL, Steinhaus KA, Uhrish SB, et al. "Recommendations for standardized human pedigree nomenclature." *American Journal of Human Genetics* 1995;56:745–752, with permission from the University of Chicago Press.)

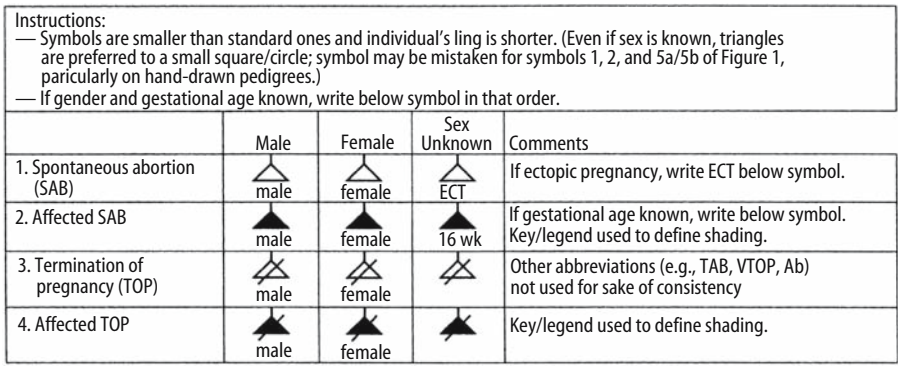


Figure 4-2. Pedigree symbols and abbreviations for pregnancies not carried to term.

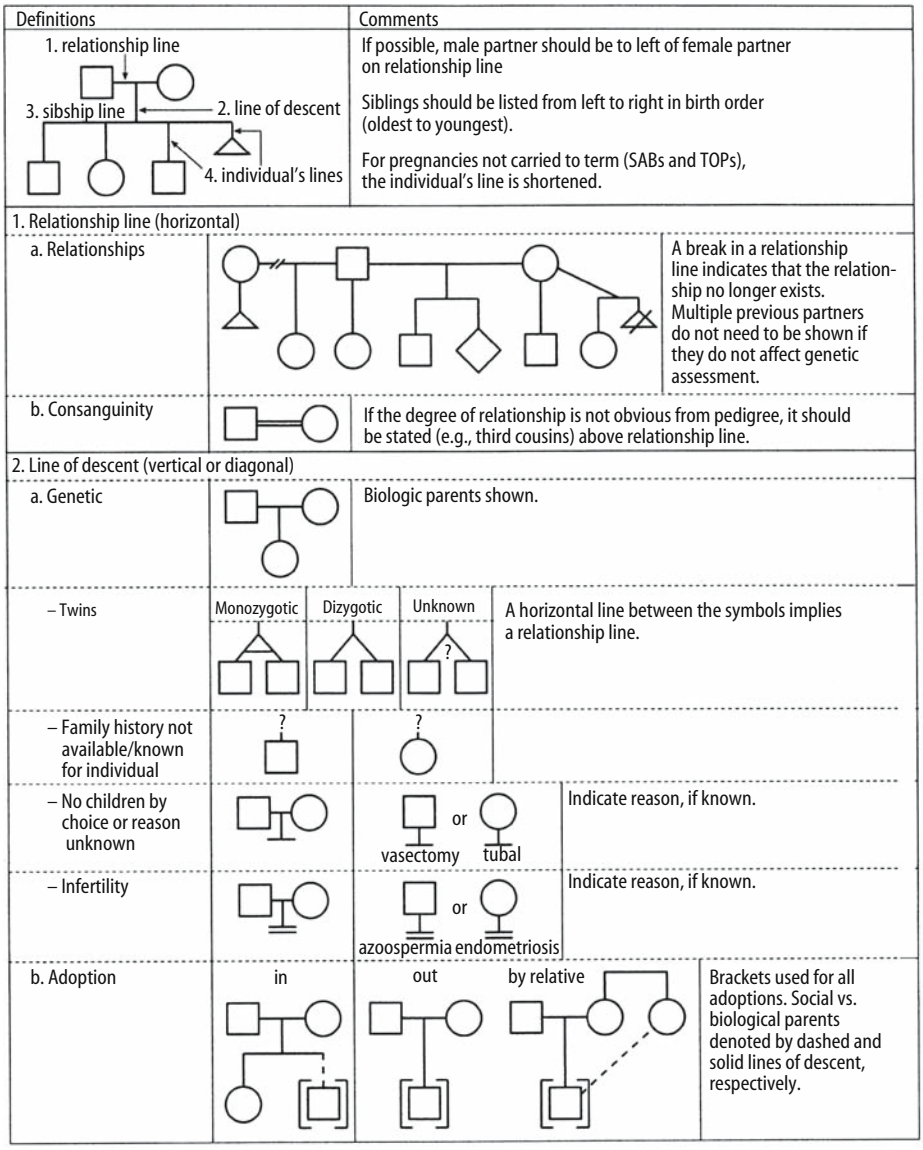


Figure 4-3. Pedigree line definitions.

Definitions:
— Egg or sperm donor (D)
— Surrogate (S)
— If the woman is both the ovum donor and a surrogate, in the interest of genetic assessment, she will be referred to only as a donor (e.g., 4 and 5)
— The pregnancy symbol and its line of descent are positioned below the woman who is carrying the pregnancy.
— Family history can be taken on individuals, including donors, where history is known.

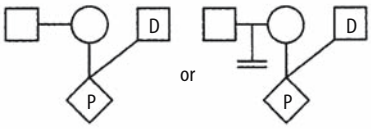
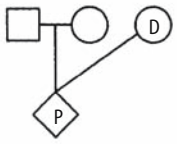
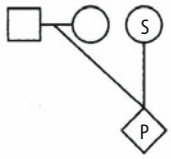
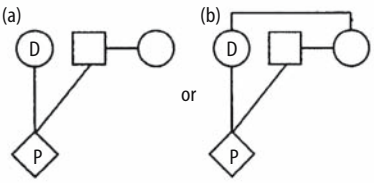
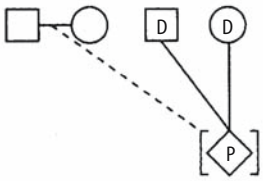
Possible reproductive scenarios		Comments
1. Sperm donor		Couple in which the woman is carrying pregnancy using donor ssperm. No relationship line is shown between the woman carrying the pregnancy and the sperm donor. For a lesbian relationship, the male partner can be substituted with a female partner.
2. Ovum donor		Couple in which the woman is carrying pregnancy using donor egg(s) and partner's sperm.
3. Surrogate only		Couple whose gametes are used to impregnate another woman (surrogate) who carries the pregnancy.
4. Surrogate ovum donor		Couple in which the male partner's sperm is used to inseminate (a) an unrelated woman or (b) a sister who is carrying the pregnancy for the couple.
5. Planned adoption		Couple contracts with a woman to carry a pregnancy using the ovum of the woman carrying the pregnancy and dono sperm.

Figure 4-4. Assisted reproductive technologies symbols and definitions.

Instructions:
— Evaluation (E) is used to represent clinical and/or test information on the pedigree.
  a. E is to be defined in key/legend.
  b. If more than one evaluation, use subscript (E_1, E_2, E_3) and define in key. May be written side by side or below each other depending on available space.
  c. Test results should be put in parentheses or defined in key/legend.
  d. If results of exam/family study/testing not documented or unavailable, may use a question mark (e.g.,"E?").
— Documented evaluation (*)
  a. Asterisk is placed next to lower right edge of symbol
  b. Use only if examined/evaluated by you or your research/clinical team or if the outside evaluation has been personally reviewed and verified
— A symbol is shaded only when an individual is clinically symptomatic.
— For linkage studies, haplotype in formation is written below the individual. The haplotype of interest should be on the left and appropriately highlighted.
— Repetitive sequences, trinucleotides, and expansion numbers are written with affected allele first and placed in parentheses.
— If mutation known, identify and place in parentheses.
— Recommended order of information:
  a. Age/date of birth or age at death
  b. Evaluation information
  c. Pedigree number (e.g., I-1, I-2, I-3)

Definition	Symbol	Scenario	Example
1. Documented evaluation (*)	*	Woman with normal physical exam and negative fragile X chromosome study (normal phenotype and negative test result).	E –*
2. Obligate carrier (will not manifest disease)		Woman with normal physical exam and premutation for fragile X (normal phenotype and positive test result).	E+(100n/35n)*
3. Asymptomatic/ presymptomatic carrier (clinically unaffected at this time but could later exhibit symptoms)		Man age 25 with normal physical exam and positive DNA test for Huntington disease (symbol filled in if/when symptoms develop).	25 y* E+(45n/18n)
4. Uninformative study (u)	Eu	Man age 25 with normal physical exam and uninformative DNA test for Huntington disease (E_1) and negative brain MRI study (E_2).	25 y* E_1u(36n/18n) E_2-
5. Affected individual with positive evaluation (E+)	E+	Individual with cystic fibrosis and positive mutation study, although only one mutation has currently been identified.	E+(ΔF508) Eu E+(ΔF508/u)*
		18- week male fetus with abnormalities on ultrasound and a trisom 18 karyotype.	18 wk* E+(tri 18n)

Figure 4-5. Pedigree symbolization of genetic evaluation/testing information.

relevant to the reason for referral. For example, cancer genetic evaluations may necessitate a more extended family pedigree, while a brief, focused pedigree may suffice when discussing cystic fibrosis carrier testing.

Information that should be collected about each individual in the pedigree is listed in Table 4-1. This, too, may be modified to reflect the nature of the diagnosis under investigation. Ethnicity, consanguinity, and unique biological relationships should be recorded using standard notation. All reported diagnoses or conditions ideally should be confirmed through authorized request and review of medical records. Key records to obtain include pathology reports, test results (particularly for any genetic testing that has been performed), imaging reports, and autopsy reports. In the absence of these documents, family genealogies or death certificates may provide some degree of verification of reported information.

An emerging issue in the use of pedigrees for clinical evaluations and research is the issue of individual confidentiality.[6,7] Each member of the family has a right to expect that medical information will remain confidential. This becomes complicated when one considers the pedigree that may contain both reported ("hearsay") and confirmed information for numerous individuals. Those people may have willingly shared information with the patient but may not want it shared with other family members. If subsequent to an evaluation a patient requests release of his or her pedigree to another family member, a provider should carefully consider the question of ownership of the pedigree information and be attuned to the potential consequences of releasing the (identifiable) information about other family members. Current interpretation of regulations outlined in the Health Insurance Portability and Accountability Act (HIPAA) and other medical records privacy legislation may influence how such information is shared.[8] Professional organizations including the American Society of Human Genetics also have developed position statements on this issue.[9]

Table 4-1. Family History Collection: What to Ask?

For All Family Members
Current age; complete date of birth
Exact relationship to proband
General health status
History of major acute or chronic illness
History of learning problems, diagnosed disabilities, or mental retardation
Highest grade level completed (when relevant)
Employment (when relevant)
Reproductive history, including pregnancies, miscarriages, elective terminations, infertility, and choice not to have children
Gestational age and last menstrual period for ongoing pregnancies
Consanguinity
Targeted questions relevant to the reason for evaluation, for example, key symptoms or features of the condition in question, pertinent evaluations, etc.
Age at death; year of death; cause of death
For Family Member Known to Be Affected by the Condition in Question
Diagnosis
Age at diagnosis
Method of diagnosis
Evaluations and testing completed
Symptoms
Information about ongoing treatment or management plan
Availability of medical records for review

Patterns of Inheritance

One key use of the carefully collected and verified pedigree is determination of the most likely mode of inheritance of a condition in a family. This will have relevance to assessing recurrence risks, approaches to testing, and in some cases, even prognosis. The concept of patterns of inheritance extends from the work of Gregor Mendel, who in the 17th century described transmission of traits associated with single genetic loci.[10] Transmission of human genetic conditions and traits has proven to be more complex, involving not only the single gene patterns first described by Mendel but also chromosomal inheritance, mitochondrial inheritance, and numerous atypical patterns of inheritance, including contiguous gene disorders, imprinting, uniparental disomy, trinucleotide repeat expansion, multifactorial inheritance, mosaicism, epigenetic influences, and synergistic heterozygosity. Undoubtedly, more atypical patterns of transmission will be elucidated as our understanding of the human genome expands. As of June 14, 2006, Victor McKusick's classic reference *Mendelian Inheritance in Man* (12th ed., 1998; http://www.ncbi.nlm.nih.gov/OMIM)[11] lists 16,850 defined gene loci, 2290 of which have been associated with specific clinical entities. There are, however, more than 7500 human traits and/or conditions that have defined classic patterns of inheritance.[10] These primarily fall into three categories, autosomal, X-linked, and Y-linked; however, a number of mitochondrial conditions also have been confirmed.

Autosomal Dominant Inheritance

In classic autosomal dominant inheritance, an affected individual has one non-functional or mutant allele at a particular locus. Each affected individual in a pedigree has a 50% chance of passing the disease-associated mutation to each of his or her offspring. Many factors, however, influence the occurrence of these conditions in families. These will be described as a group following review of the classic modes of inheritance. A key feature of autosomal dominant inheritance is male-to-male transmission of the condition or trait, a pattern not seen in X-linked dominant inheritance, which can be confused with autosomal dominant inheritance on first analysis. Table 4-2 lists additional features of autosomal dominant inheritance, and an example pedigree is shown in Figure 4-6. Codominant inheritance describes equal expression of both alleles of a pair, that is, with equal, coexisting phenotypic effect. An example of this is the ABO blood group.

Autosomal Recessive Inheritance

In autosomal recessive inheritance, an affected individual has two nonfunctional or mutant alleles at a particular locus. One of these is inherited from each of the parents, who are called carriers and who are unaffected by the condition. There is a 1 in 4 (25%) chance of having an affected offspring with each pregnancy of a known carrier couple, and a 2 in 4 (50%) chance that an offspring will be a carrier like the parents. After birth, if a child of a carrier couple is not affected by the condition in question, he or she has a 2 in 3 chance of being a carrier. Risk to future offspring of a known carrier depends on the likelihood that his or her partner is also a carrier. This is influenced by the frequency of the disease gene in the population, which may vary among different populations. Features of autosomal recessive inheritance are listed in Table 4-2, and a pedigree is shown in Figure 4-6.

X-linked Dominant Inheritance

In X-linked dominant inheritance, an affected individual has one non-functional or mutant allele at a locus on an X-chromosome. X-linked dominant conditions can occur in either males or females. Risk for offspring of an affected female is 50%, regardless of the gender of the offspring. Risk to offspring of affected males is gender dependent, with all daughters but no sons inheriting the gene. Many of these conditions, however, are lethal in males, so pedigrees may show overrepresentation of females or increased frequency of miscarriages, presumably of affected male fetuses (see Table 4-2 and Figure 4-6).

Table 4-2. Features of Mendelian Patterns of Inheritance

Autosomal Dominant Inheritance
Male-to-male transmission occurs; both genders can transmit to offspring
Condition occurs in multiple generations
Males and females affected, typically to comparable extent
Variability of clinical findings
Later/adult onset in some disorders
Vertical transmission; affected descendants of affected individuals, unaffected descendants of unaffected individuals (in general)
Homozygotes may be more severely affected than heterozygotes
Homozygosity may be lethal
Occurrence of new mutations
Nonpenetrance; apparent "skipping" of generations
Gender-limited occurrence of conditions (transmission through the unaffected gender)
Germline mosaicism reported
Autosomal Recessive Inheritance
Affected family members are usually in one generation; "horizontal" inheritance
Parental consanguinity or small mating pool may influence disease occurrence
Male and female are affected
Usually consistent in degree of severity among affected family members
Early onset of symptoms more typical
New mutations rare
May see higher frequency of disease in certain ethnic groups
X-linked Dominant Inheritance
No male-to-male transmission
Affected females usually have milder symptoms than affected males
Affected males have no affected sons, but all daughters will be affected
May mimic autosomal dominant inheritance
May be lethal in affected males; paucity of males or overrepresentation of females in the pedigree
Increased occurrence of miscarriage
X-linked Recessive Inheritance
No male-to-male transmission
Males more frequently affected
Carrier females usually unaffected but may have mild symptoms
Affected males in a family are related through females
Occurrence of new mutations, often from maternal grandfather
Y-linked Inheritance
Male-to-male transmission only
Association with increased infertility rates in families
Discrepancy between chromosomal and phenotypic gender

X-linked Recessive Inheritance

Traditional X-linked recessive inheritance is characterized by occurrence of the condition in males with a non-functional or mutant allele on the X-chromosome who are related through females. (See the pedigree in Figure 4-6, and Table 4-2 for additional features.) Typically, carrier females are unaffected; however, due to lyonization (random inactivation of one X chromosome in each cell in a female), carrier females may have mild symptoms. This occurs when, by chance, more of the X chromosomes with the nonfunctional allele remain active in the cells. The likelihood of symptoms in carrier females varies considerably among disorders. Risk to offspring of carrier females is 25% overall, or 50% for affected status if the fetus/offspring is male. Offspring of affected males will not be classically affected, but all daughters will be carriers.

Y-linked Inheritance

In rare cases, one of a limited number of genes on the Y chromosome can be mutated. This can result in disparity between chromosomal and phenotypic gender if the SRY region is involved, or can be associated with genetic/hereditary forms of infertility. This may be identified more frequently as reproductive technologies such as intracytoplasmic sperm injection (ICSI) are used to aid in achieving pregnancies for previously infertile males, due to Y-chromosome deletions, for example (see Table 4-2 and Figure 4-6).

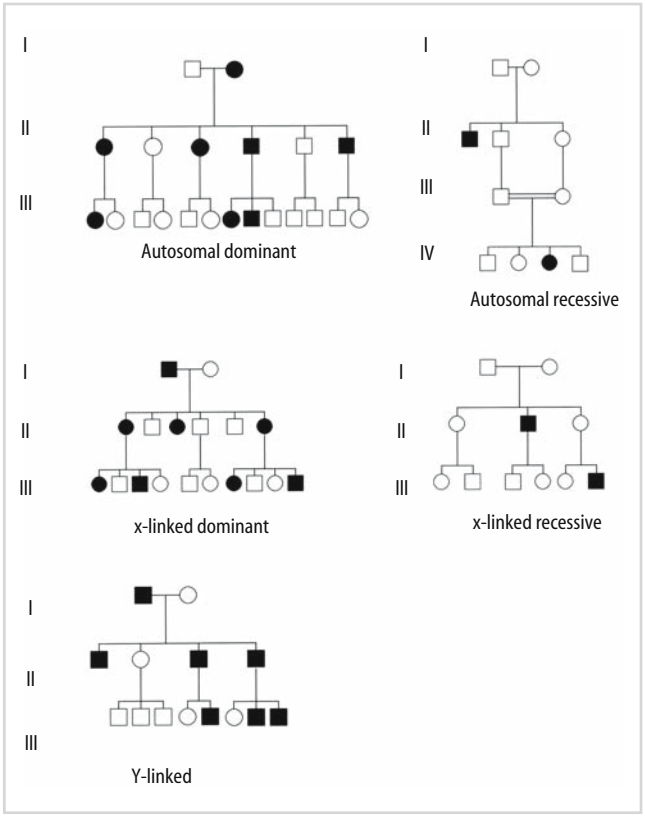


Figure 4-6. Example pedigrees for Mendelian patterns of inheritance.

Non-Mendelian Inheritance Patterns

For a summary of non-Mendelian inheritance patterns, see Table 4-3.

Chromosomal

Chromosome abnormalities can occur sporadically or can be caused by familial transmission of duplications, deletions, or rearrangements that can result in imbalance of genetic material in the offspring.[12] Due to the presence of many genes along the segment of chromosome involved, multiple phenotypic effects usually are seen. Risks to offspring of familial cases depend on parent of origin and size and location of the involved chromosomal segment, and vary depending on loss or gain of material in a particular region. In apparently sporadic cases, parental status with respect to the chromosomal abnormality should be assessed for all cases of offspring with chromosomal rearrangements. Absence of a parental chromosomal abnormality significantly reduces the risk to future offspring.

Contiguous Gene Disorders/ Microdeletion Syndromes

Contiguous gene disorders are the result of loss of several adjacent genes along a segment of chromosome and may consist of symptoms of one known hereditary disorder, more than one closely linked group of hereditary disorders, or either of these in conjunction with mental retardation, dysmorphic features, or both.[13] The condition results from loss of one copy of a group of closely linked genes (haploinsufficiency) that may be detectable by high-resolution chromosome analysis or fluorescence in situ hybridization (FISH) using region-specific probes. Approximately 5% to 10% of monogenic diseases are associated with gene deletions that cannot be detected through routine cytogenetic analysis.[10] The microdeletions occur in regions of repeated genomic sequences that lead to rearrangements (recombination), resulting in loss or gain of genetic material during transmission, both of which have been documented.

Mitochondrial Inheritance

Individuals inherit essentially all their mitochondrial DNA from their mothers; thus, any disease associated with a mitochondrial DNA mutation is transmitted from the mother to the offspring. In each cell, including egg cell progenitors, there may be up to 1000 mitochondria. If a mutation occurs in one of these mitochondria, as the mitochondrion divides over time, the mutation becomes present in a percentage of the overall mitochondrial population in the cell. When the cell divides, the mitochondria are distributed stochastically to the daughter cells. The

Table 4-3. Features of Non-Mendelian Patterns of Inheritance

Chromosomal Disorders

Increased frequency in individuals with 2 or more major birth defects, 3 or more minor birth defects, or 1 major and 2 minor birth defects
Occurrence of multiple pregnancy losses or infertility
Occurrence of mental retardation with dysmorphism
Occurrence of mental retardation with multiple congenital anomalies
Many occur as sporadic conditions with negative family history

Contiguous Gene Disorders/Microdeletion Syndromes

Involvement of multiple organ systems
Negative family history; frequent/isolated or sporadic cases
May appear as recognized single-gene disorder
May involve occurrence of mental retardation with an otherwise recognized hereditary or medical disorder typically lacking mental retardation
May involve occurrence of dysmorphism with an otherwise recognized hereditary or medical disorder typically lacking dysmorphism

Mitochondrial Inheritance

Maternal transmission (fathers do not transmit disease)
Males and females affected
Extreme variability of clinical symptoms; multiple organ systems involved
Multiple generations affected (matrilineal)
Degenerative/neuromuscular disorders predominate
Gender can influence variability of symptoms
Environmental factors may influence symptoms (pseudomultifactorial)

Imprinting

Gender of transmitting parent modifies gene/disease expression (parent-of-origin effects)
May appear to skip generations

Uniparental Disomy

Documentation of only one carrier parent
Single/isolated case in a family

Trinucleotide Repeat Disorders

Anticipation
Increasing severity with subsequent generations
Gender of transmitting parent may influence disease severity
Disorders may have variable age at onset, degree of severity
May see skipping of generations (transmission of premutation)

Synergistic Heterozygosity

Described in inborn errors of metabolism
Variability in severity of symptoms among affected family members
Complex phenotypes, multisystem involvement
Multiple partial enzyme deficiencies in affected individuals
Environmental factors may influence severity of disease

Multifactorial Inheritance

Males and females affected
Gender of affected individual influences recurrence risk
Classically, few affected family members, but now also implicated in common adult-onset disorders
Degree of relationship to affected individual influences recurrence risk
Recurrence risk correlates with number of affected family members

daughter cells may inherit only mutant mitochondrial DNA (homoplasmy), a percentage of mutant mitochondrial DNA (heteroplasmy), or no mutant mitochondrial DNA. The degree of heteroplasmy affects the overall function of the cell or population of cells and thus correlates with disease severity. It is not possible to predict for any given cell what the degree of heteroplasmy will be; thus, it is extremely difficult to predict recurrence risk or severity of disease. Furthermore, different cell populations in different organs can have different degrees of heteroplasmy, yielding a variable multisystem disease (pleiotropy).

Imprinting

Imprinting refers to differential expression of genes depending on the parent of origin. The process is reversible, as it affects the action of the gene but not the gene structure; genes that are passed from a male (imprinted as male) to a female and then passed by the female are reimprinted as female, and so on. This is thought to occur early in development, most likely in the germ cells.[14] A number of disorders have been described that are caused by imprinting. Depending on the underlying mechanism and assuming transmission from the critical parent of origin, recurrence risks could be as high as 50%, particularly if a mutation exists in an imprinting control center that regulates methylation status and, thus, gene expression.

Uniparental Disomy

Uniparental disomy is defined as both copies of all or part of a chromosome in a cell or individual being derived from only one parent. This can appear as heterodisomy (the presence of copies of both of one parent's chromosomes) or homodisomy (a single chromosome or chromosome segment present in two identical copies). This becomes clinically relevant when males and females differentially imprint the chromosomal segment in question, or when the parent who transmits the disomic region carries a mutation in that region.[14] This process has been seen in cystic fibrosis, Prader-Willi and Angelman syndromes and other disorders, and may need to be considered for any autosomal recessive disorder when only one parent is a confirmed carrier, and for X-linked recessive disorders occurring in 46, XX females. The frequency of this phenomenon is unknown.

Trinucleotide Repeat Disorders

Most classic hereditary disorders are caused by static or stable mutations in one or a few genes. For trinucleotide repeat disorders, alterations in the causative gene are unstable, called dynamic mutations, and characterized by a variable number of copies of a tandemly repeated three-nucleotide sequence within the gene.[14] These trinucleotide repeats are normal, do not generally cause disease, and can be inherited stably within certain, usually small, tandem repeat size ranges that are gene specific.

Due to the structure of the repeated gene sequence, however, miscopying during DNA replication can occur, leading to expansion (creation of additional tandem copies of the trinucleotide sequence) or, rarely, contraction (loss of one to five copies of the trinucleotide sequence) of the gene segment. With expansion, the gene segment becomes less stable and thus more likely to expand further. Intermediate lengths of expanded gene segment are called premutations, which are extremely unstable and highly likely to undergo further expansion. Individuals who carry premutations typically do not have symptoms of the associated disorder but may show mild signs or develop associated problems at later ages.

Once the gene segment has expanded into the disease-associated repeat size range, disease symptoms occur in the individual. Degree of disease severity typically correlates with the size of the repeated segment, with earlier age of onset and more severe symptoms with increasing repeat size. The clinical phenomenon of anticipation (earlier onset of disease in subsequent generations) is explained mechanistically by the progressive expansion of the trinucleotide repeat region from one generation to the next, with earlier and more severe disease for each generation. Gender of transmitting parent also influences likelihood and degree of expansion, and is gene specific (the significant parent of origin varies by disease).

Synergistic Heterozygosity

A phenomenon described primarily to date in inborn errors of metabolism, synergistic heterozygosity results from relative decreases in function in several components of a complex biological pathway.[15] Effects of mutations in a single copy of each of multiple genes encoding components of a pathway accumulate and lead to an overall decrease in function of the pathway. This is much more akin to multifactorial or at least polygenic inheritance than classical Mendelian inheritance typical of the majority of inborn errors of metabolism. Recurrence risks depend on the degree of decreased function of each of the components, which components are involved, genetic linkage of the components, or the potential for environmental influences on the pathway, or some combination of these factors.

Multifactorial Inheritance

Multifactorial disorders are the result of interactions among multiple genetic and environmental factors. A threshold effect defines the likelihood of disease based on the relative contributions of each of the factors involved. With a relatively low concentration of contributing factors, no effect will be seen. However, above a critical cutoff of accumulated factors, the condition occurs. Risk to relatives of affected individuals increases as more family members are affected, presumably reflecting the presence of a higher "dose" of critical factors in the family or shared environmental factors. The threshold for affected status

may, however, be different in males and females. In classic conditions, such as pyloric stenosis or neural tube defects, a higher dose of risk factors is needed to push the less-frequently affected gender above the critical threshold; close relatives are therefore more likely to have a similar clustering of risk factors and be above the threshold, particularly if they are of the more commonly affected gender and thus are presumed to have a lower threshold.

Other Factors Affecting Risk and Risk Assessment

Classic and atypical modes of inheritance provide a framework for assessment of risk to close relatives of individuals affected by hereditary disorders. However, many factors influence the ability to clearly define patterns of inheritance in families. From a logistical perspective, family members may not know details about medical conditions in more distant relatives, or relatives may not wish to share those details by medical record request. For some, there may be stigma or guilt attached to discussion of hereditary conditions in themselves or their children. Mechanistically, there are a number of processes that may confound pedigree interpretation (Table 4-4). Variable expressivity and pleiotropy relate, respectively, to the presence of different degrees of severity of symptoms and the presence of varying phenotypic features in affected individuals. These could lead to misclassification of affected status, or failure to recognize the presence of a single clinical entity in affected family members. Further, variability in age of onset, particularly with adult-onset disease, may leave gaps in an otherwise classic pedigree, as can penetrance, or the likelihood that an individual who carries the gene(s) for a condition will show signs or symptoms of that condition. Some conditions show genetic heterogeneity, that is, can be caused by mutations in a number of different genes. While mutations in these genes may be rare, theoretically more than one type of gene mutation could lead to symptoms within a family. Phenocopies, similar conditions with different genetic or nongenetic etiologies or both, may also occur within a family and lead to misinterpretations of patterns of inheritance and, thus, of risk to family members. Small family size or relatively low frequency of the at-risk gender in gender-influenced disorders (sex-limited vs. sex-influenced expression) may result in failure to recognize a hereditary disorder and underestimation of risk.

Table 4-4. Factors Affecting Risk and Risk Assessment

Variable expressivity/pleiotropy
Age of onset
Penetrance
Heterogeneity
Phenocopies
Gender-influenced expression (sex-limited vs. sex-influenced)
Family size/paucity of at-risk gender
Nonpaternity
Consanguinity/inbreeding
Lyonization
New mutation
Mosaicism (somatic vs. germline)
Modifying genes
Environmental effects

Accurate reporting of relationships within a pedigree is critical. Nonpaternity, estimated at 10% in the United States,[4] and consanguinity, or inbreeding (shared common ancestors), are particularly important when considering possible autosomal recessive traits. A recent review confirmed only modest contribution of consanguinity to overall risk,[16] but it can be of critical importance when an autosomal recessive disorder is under consideration in a symptomatic individual due to potential presence of shared nonfunctional genes in related parents. Expression and risk assessment of X-linked disorders are influenced by lyonization, or the random inactivation of one X chromosome in each cell in a female. The percentages of the active and inactive nonfunctional X-chromosome gene could lead to full expression, intermediate symptomatology, or lack of symptoms altogether for an X-linked recessive condition in a female. The occurrence of spontaneous new mutations could lead to failure to recognize risk due to autosomal dominant or X-linked conditions, in particular. Similarly, mosaicism, or the presence of a mixture of at least two populations of cells with some containing a functional and others a nonfunctional gene, could lead to partial expression of a condition in an individual (somatic mosaicism). Mosaicism also could lead to unrecognized or indefinable risk to future offspring if only the germ cells (egg or sperm) are affected or only a percentage of germ cells are affected (germline mosaicism).

Finally, factors outside of the critical gene can influence the expression of that gene and thus the assessment of risk. Expression of some genes is influenced by variant forms of other, so-called modifying genes. Polymorphisms or mutations in these modifying genes can change gene-gene or protein-protein interactions to affect expression of the condition in question. Similarly, environmental factors such as shared environment, dietary practices, and specific exposures (medications, smoke, etc.) may positively or negatively affect gene function or expression of clinical symptoms.

Each of these factors must be carefully considered in the overall diagnostic and risk assessment, initially based on collection of a family pedigree and continued through clinical evaluation, including physical examination and indicated diagnostic testing.

Direct Mutation Analysis and Linkage Analysis

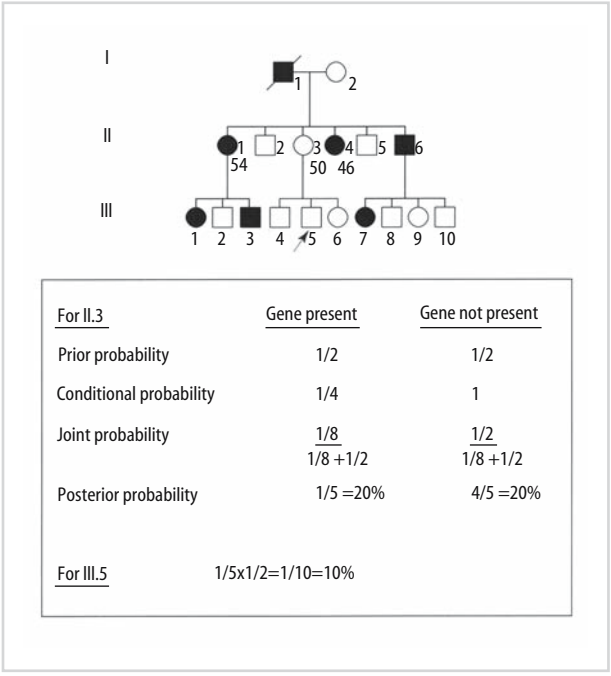
The ability to define mutations or gene regions associated with disease removes much of the art of risk assessment from evaluation of the pedigree and provides a more

definitive answer in many cases. Currently, there are clinical or research tests being done for 1,269 different diseases (http://www.genetests.org, accessed on June 14, 2006), which continues to increase as definitive mutations are identified in newly described disease-associated genes. Methods of gene analysis vary among different laboratories (see chapter 2 and Reference 17). For large deletions and gene rearrangements, Southern blot analysis is used. Dosage analysis (determination of gene copy number utilizing densitometry, multiplex ligation-dependent probe amplification [MLPA] or similar techniques) may be used in cases where an affected individual is not available for study and deletion is a common form of mutation, as is the case in Duchenne muscular dystrophy. Southern blot analysis also may be needed for sizing of large trinucleotide repeats, while smaller repeats can be identified by targeted polymerase chain reaction (PCR) analysis. PCR is also used in conjunction with allele-specific oligonucleotides (ASOs) for analysis of conditions with a single or few common mutations. In disorders where many unique, private mutations have been found, mutation-screening techniques may be utilized, including conformation sensitive gel electrophoresis (CSGE), denaturing gradient gel electrophoresis (DGGE), denaturing high-performance liquid chromatography (DHPLC), two-dimensional gel scanning (TDGS), and single-strand conformation polymorphism (SSCP). Once gene segments with probable variants have been identified by these techniques, DNA sequencing is utilized to verify the presence of a mutation, polymorphism, or variant of unknown significance.

These direct methods of identifying mutations are invaluable when the disease-associated gene is known; however, historically and even today, for many conditions the causative gene has not been identified or is not characterized adequately to allow for mutation-specific testing. In these situations, it is possible to offer an indirect testing method, called linkage analysis, to clarify the risk status of family members if the responsible gene has been localized to a specific genomic map location. For some families, the most significant issue in linkage analysis is the need for specimens from a number of family members, both affected and unaffected, to ensure useful interpretation of results. Linkage analysis requires that the clinical status of the relatives and their relationships be accurately reported for accurate interpretation. Linkage analysis involves determination of "markers" for the disease gene, often variant forms of highly polymorphic short tandem repeats, within or near the genomic map location. It requires that a marker or markers near the genetic locus be informative; that is, key individuals in the family must be heterozygous, or have two different forms of the marker (alleles) at the locus in question. These markers are then tracked as they are passed from one individual to the next. It is thus essential to know which allele(s) is associated with the disease gene and which alleles track with the normal gene copy (setting phase). This typically involves analysis of DNA from a number of affected family members or a carefully selected group of affected and unaffected relatives. In addition, if the marker(s) is closely associated (linked) with the disease gene or is within the gene, presence of the disease-associated marker allele will correlate with presence of the disease gene. If the marker is genetically distant from the disease gene, it may become separated from the disease gene through recombination, and predictions about gene transmission may be inaccurate. Caution is required when doing linkage analysis of very large genes because a marker at one end of the gene may, through recombination, become unlinked from the (unknown) mutation if it resides at the other end of the gene. Accuracy of linkage analysis can be further increased by assessing more than one linked marker, preferably within or flanking opposite ends of the gene.

Bayesian Analysis Used in Risk Modification

When collected pedigrees are used to provide risk assessment, a variety of data may be relevant to the overall assessment. Numerous factors, some listed above, influence the likelihood that a given individual in the family may be affected by the condition in question or may be a carrier of the gene in question. When it is not possible to do direct diagnostic testing for the condition (for example, if the causative gene is unknown), when the affected relative is not available for testing, or for complex traits, it is possible to combine incremental contributors to risk by utilizing Bayesian analysis. Bayesian analysis is a statistical construct that uses information about the likelihood of occurrence of past events or conditions, and the current status of those events or conditions for the individual, to predict the likelihood of a future event or condition, in this case, the presence or absence of a particular gene or genetic condition.[18] Some factors that may be considered in genetic risk assessment using Bayesian analysis include number and pattern of affected and unaffected family members, laboratory data, and natural history of the condition. The probability assigned based on past events is called the prior probability; that based on current information or observations is called the conditional probability. The calculated probability for each possible outcome of an event or condition is the joint probability, and the final probability of one outcome as a percentage of all possible outcomes is the posterior probability. Calculations often utilize data from multiple generations and are usually done in tabular form. In the example pedigree in Figure 4-7 for an autosomal dominant cancer predisposition syndrome affecting males and females equally, based on Mendelian inheritance alone, the risk that individual III.5 is a gene carrier is 25%. However, knowing that 75% of gene carriers have been diagnosed with cancer by age 50, risk can be recalculated as demonstrated. (See chapter 5 for a complete discussion of Bayesian analysis.)



For II.3	Gene present	Gene not present
Prior probability	1/2	1/2
Conditional probability	1/4	1
Joint probability	1/8 / 1/8 +1/2	1/2 / 1/8 +1/2
Posterior probability	1/5 =20%	4/5 =20%

For III.5 1/5x1/2=1/10=10%

Figure 4-7. Bayesian analysis for risk assessment in an autosomal dominant, adult-onset hereditary cancer disorder. Ages of selected individuals in generation II are shown below the pedigree symbols.

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