Pedigree Analysis and Risk Assessment

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Abstract

The genetic family history, or pedigree, is a valuable tool for assessment of disease risk. Use of standardized symbols and nomenclature in pedigrees is recommended to ensure accurate communication of information to end users. There are common questions which should always be asked during collection of a family health history; however, questioning is often tailored for the condition under evaluation. Pedigrees may also help assess disease transmission patterns in the family which may be Mendelian (autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant, Y-linked), chromosomal, mitochondrial, or multifactorial. When atypical patterns of inheritance are seen, consideration should be given to other factors which can influence transmission including, imprinting, uniparental disomy, unstable DNA, gene-environment interactions, mosaicism, and synergistic heterozygosity. Recognition of the mode of inheritance within a family can be useful for estimating disease risk for family members or offspring. Risk assessment also may be confounded by logistical factors, such as family dynamics and limited information, or processes such as variable expression of disease, penetrance, heterogeneity, mosaicism, lyonization, or consanguinity. Many different laboratory methods are used for direct detection of genetic mutations associated with disease. When direct mutation analysis is not feasible, gene discovery or assessing risk for disease may be facilitated by linkage analysis or genome/exome sequencing. Bayesian analysis is a statistical construct that allows for the combination of incremental contributors to risk to determine an individual's risk of developing or transmitting a disorder.

Keywords

Family history • Pedigree • Genetics • Relationships • Risk • Inheritance • Traits • Diagnosis • Maternal • Paternal • Mutations • Genotype • Phenotype • Probability • Dominant • Recessive • X-linked • Carrier • Ethnicity • Transmission

The Genetic Family History

The personal and family medical pedigree has evolved from its earliest ancestors in the fifteenth 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-nineteenth century when Pliney Earl published on inheritance

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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 National Society of Genetic Counselors (NSGC) task force surveyed genetic counselors regarding interpretation of pedigree symbols and conformity of usage. Numerous different symbols were being used for very common scenarios, such as pregnancy and miscarriage, and it became evident that standardization was needed. The group established a recommended nomenclature for pedigrees, which has become the widely accepted standard for recording a family health history [2].

The currently recommended methods for documenting pedigree information including symbols, spatial relationships, and nomenclature for clinical/investigative status are detailed in Figs. 4.1, 4.2, 4.3, and 4.4. These standards allow recording of traditional relationships, as well as those nontraditional relationships which are developing as new technologies are applied, in a manner that meets medical-legal requirements and protects patient confidentiality. Because pedigrees contain sensitive information and may be accessed by many individuals, especially if part of the electronic medical record, care should be taken in considering what information to include in a pedigree. To ensure compliance with the Health Insurance Portability and Accountability Act (HIPAA) standards in the USA, the pedigree standardization guidelines recommend including less identifying information on the pedigree [2]. For example, for pedigrees not intended for publication, designating family members using initials or first names (instead of complete names) and listing ages or the year of birth/death (instead of exact dates of birth or death) are preferred. The current standards also serve as a baseline for future additions or modifications as the field continues to evolve.

Pedigrees now form the cornerstone for determination of diagnosis, pattern of inheritance, and recurrence risk. Visually recording elements of family and medical history in the form of a pedigree serves many purposes including: user orientation (to family relationships, source of the information included and reason for pedigree construction), improved readability, risk assessment, validation of information included, compliance with medical documentation standards, communication, and patient education. Well-constructed pedigrees also may result in cost savings by describing evaluations already performed to avoid duplicate testing, and documenting familial mutations necessary for the most cost-effective testing of family members. 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. As the genetic counselor or other healthcare provider explains the purpose of obtaining 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 [3]. This information also is essential for successful counseling of patients in the clinical genetics setting, and while not always recorded in the same fashion, family dynamics are 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. Advance notice to patients and their families about the nature of information to be collected can facilitate the accuracy and completeness of the information provided by the family. In addition, electronic tools such as the US Surgeon General's Family Health Portrait tool (http://www.hhs.gov/familyhistory) are available to engage patients in collecting their family health information.

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 relevant to the reason for referral. For example, genetic evaluations for hereditary cancer syndromes 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. Modifications may be needed depending on the nature of the diagnosis under investigation; recommendations from the NSGC detail additional questions appropriate for individuals being evaluated for hereditary cancer syndromes [4]. Because family medical histories change over time, pedigrees should be updated as new information is learned.

Instructions:
- Key should contain all information relevant to interpretation of pedigree (e.g., define fill/shading)
- For clinical (non-published) pedigrees include:
  a) name of proband/consultand
  b) family names/initials of relatives for identification, as appropriate
  c) name and title of person recording pedigree
  d) historian (person relaying family history information)
  e) date of intake/update
  f) reason for taking pedigree (e.g., abnormal ultrasound, familial cancer, developmental delay, etc.)
  g) ancestry of both sides of family
- Recommended order of information placed below symbol (or to lower right)
  a) age; can note year of birth (e.g.,b.1978) and/or death (e.g.,d.2007)
  b) evaluation (see Figure 4.4)
  c) pedigree number (e.g., I-1, I-2, I-3)
- Limit identifying information to maintain confidentiality and privacy

	Male	Female	Gender not specified	Comments
1. Individual	b. 1925	30y	4 mo	Assign gender by phenotype (see text for disorders of sex development, etc.). Do not write age in symbol.
2. Affected individual				Key/legend used to define shading or other fill (e.g., hatches, dots, etc.). Use only when individual is clinically affected.
			With ≥2 conditions, the individual's symbol can be partitioned accordingly, each segment shaded with a different fill and defined in legend.	
3. Multiple individuals, number known	5	5	5	Number of siblings written inside symbol. (Affected individuals should not be grouped).
4. Multiple individuals, number unknown or unstated	n	n	n	"n" used in place of "?"
5. Deceased individual	d. 35	d. 4 mo	d. 60's	Indicate cause of death if known. Do not use a cross (†)to indicate death to avoid confusion with evaluation positive (+).
6. Consultand				Individual(s) seeking genetic counseling/ testing.
7. Proband	P	P		An affected family member coming to medical attention independent of other family members.
8. Stillbirth (SB)	SB 28 wk	SB 30 wk	SB 34 wk	Include gestational age and karyotype, if known.
9. Pregnancy (P)	P LMP 7/1/2007 47,X,Y,+21	P 20 wk 46,XX	P	Gestational age and karyotype below symbol, Light shading can be used for affected; define in key/legend.

Pregnancies not carried to term	Affected	Unaffected	
10. Spontaneous abortion (SAB)	17 wks female cystic hygroma	< 10 wks	If gestational age/gender known, write below symbol, Key/legend used to define shading.
11. Termination of pregnancy (TOP)	18 wks 47,XY,+18		Other abbreviations (e.g., TAB, VTOP) not used for sake of consistency.
12. Ectopic pregnancy (ECT)	ECT		Write ECT below symbol.

Figure 4.1 Common pedigree symbols, definitions, and abbreviations. From Bennett R, French K, Resta R, Doyle D. Standardized human pedigree nomenclature: update and assessment of the recommendations of the national society of genetic counselors. J Genet Couns. 17;2008:424–33 [2]. Reprinted with permission from Springer.

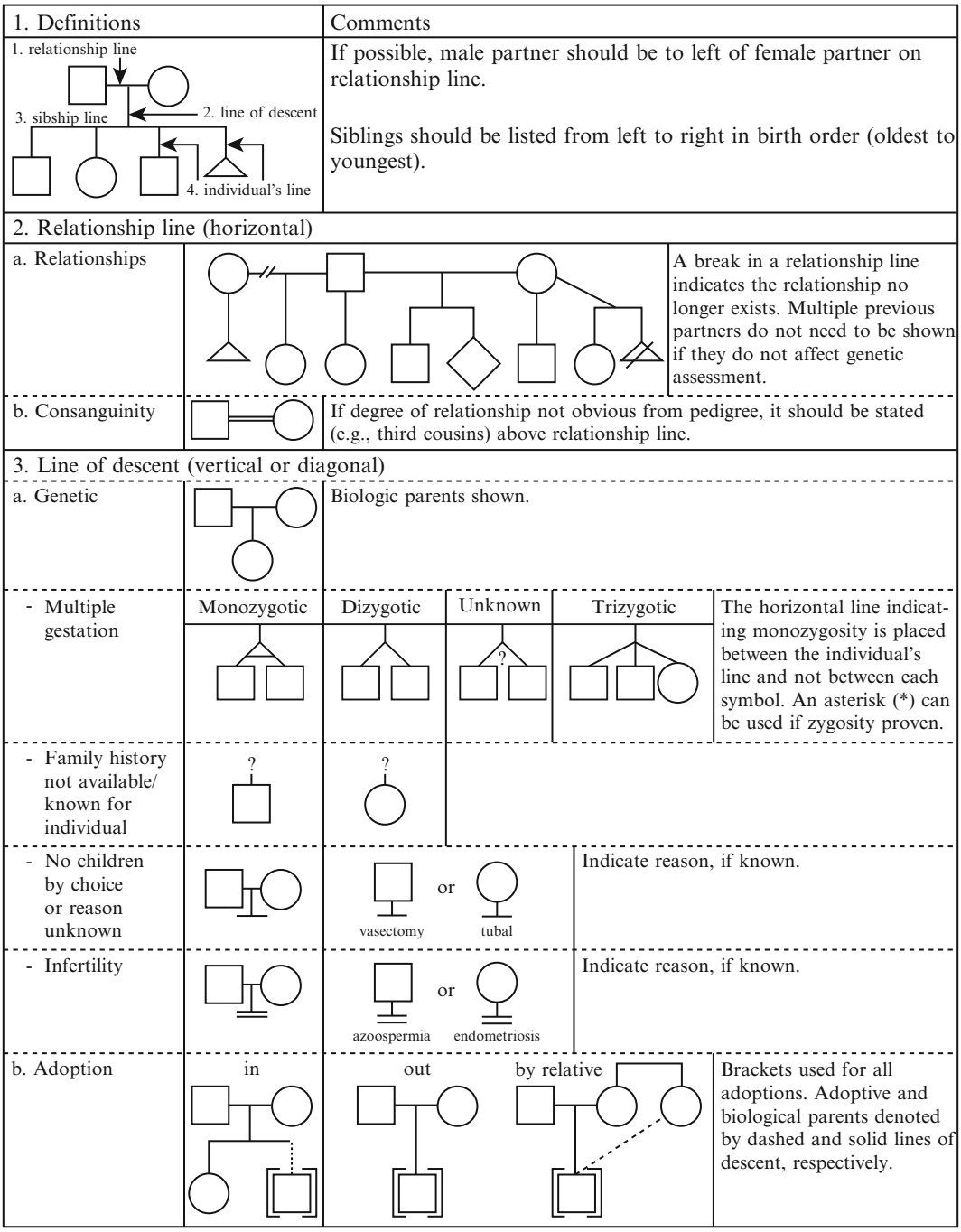


Figure 4.2 Pedigree line definitions. From Bennett R, French K, Resta R, Doyle D. Standardized human pedigree nomenclature: update and assessment of the recommendations of the national society of genetic counselors. J Genet Couns. 17;2008:424–33 [2]. Reprinted with permission from Springer

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 important issue in the use of pedigrees for clinical evaluations and research is the issue of individual confidentiality [5]. Each member of the family has a right to expect that medical information will remain confidential.

Instructions:
- D represents egg or sperm donor
- S represents surrogate (gestational carrier)
- If the woman is both the ovum donor and a surrogate, in the interest of genetic assessment, she will only be referred to 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
- Available family history should be noted on the gamete donor and/or gestational carrier

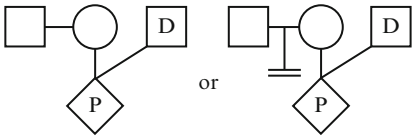
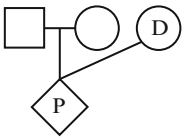
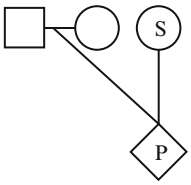
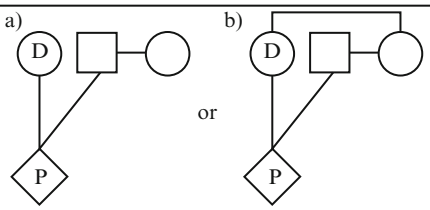
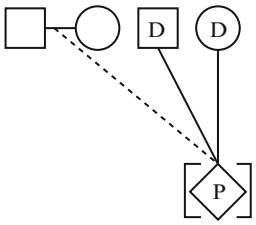
Possible Reproductive Scenarios		Comments
1. Sperm donor		Couple in which woman is carrying pregnancy using donor sperm. No relationship line is shown between the woman carrying the pregnancy and the sperm donor.
2. Ovum donor		Couple in which woman is carrying pregnancy using a donor egg and partner's sperm. The line of descent from the birth mother is solid because there is a biologic relationship that may affect the fetus (e.g., teratogens).
3. Surrogate only		Couple whose gametes are used to impregnate a woman (surrogate) who carries the pregnancy. The line of descent from the surrogate is solid because there is a biological relationship that may affect the fetus (e.g., teratogens).
4. Surrogate ovum donor		Couple in which 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 ovum of the woman carrying the pregnancy and donor sperm.

Figure 4.3 Assisted reproductive technology symbols and definitions. From Bennett R, French K, Resta R, Doyle D. Standardized human pedigree nomenclature: update and assessment of the recommendations of the national society of genetic counselors. J Genet Couns. 17;2008:424–33 [2]. Reprinted with permission from Springer

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 by HIPAA and other medical records privacy legislation may influence how such information is shared. Professional organizations including the American Society of Human Genetics also have developed position statements on this issue [6].

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

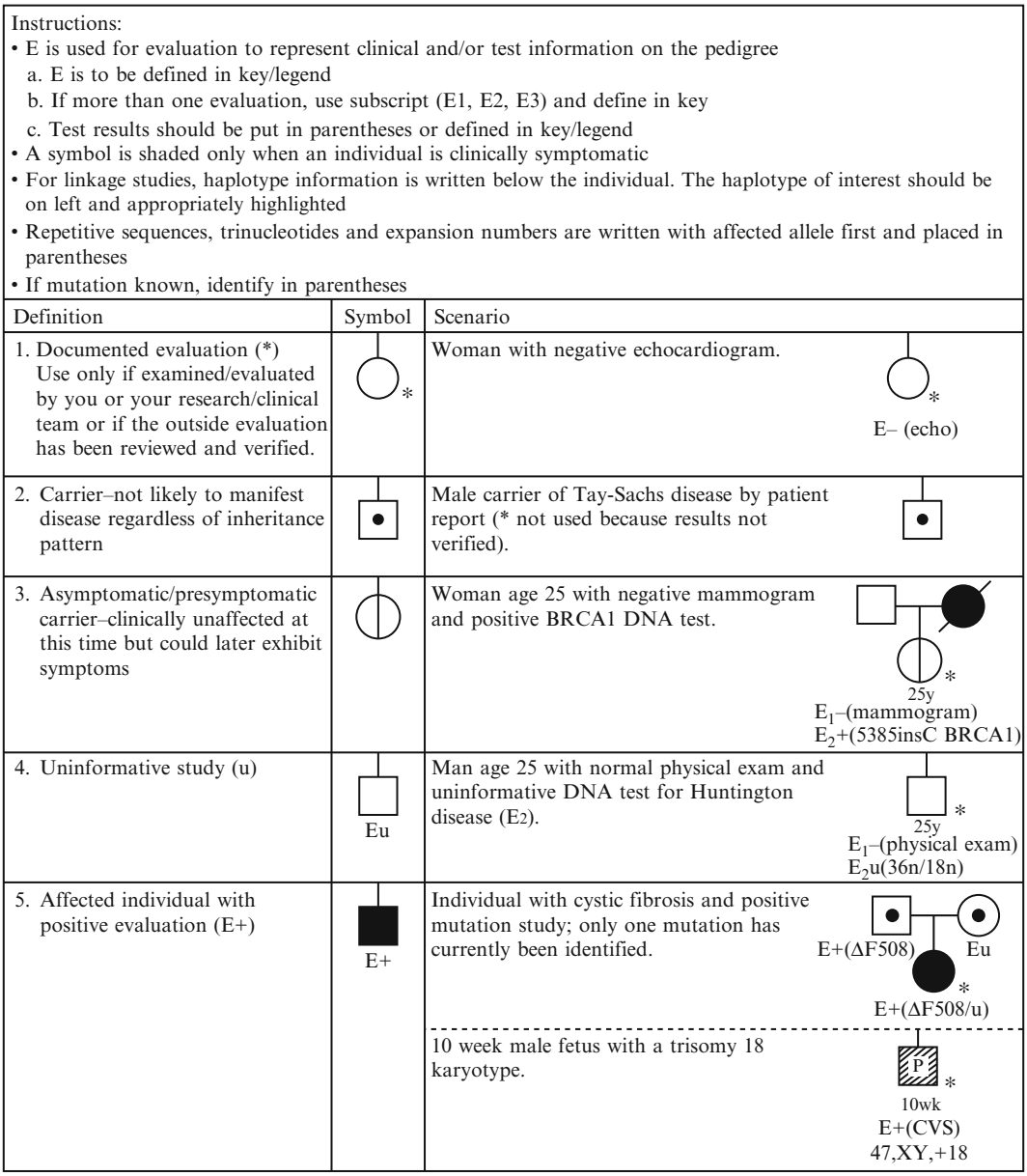


Figure 4.4 Pedigree symbols of genetic evaluation/testing information. From Bennett R, French K, Resta R, Doyle D. Standardized human pedigree nomenclature: update and assessment of the recommendations of the national society of genetic counselors. J Genet Couns. 17;2008:424–33 [2]. Reprinted with permission from Springer

prognosis. The concept of patterns of inheritance extends from the seventeenth-century work of Gregor Mendel, who described transmission of traits associated with single genetic loci. 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 multifactorial inheritance. Other genetic factors which can influence transmission of disease include imprinting, uniparental disomy, unstable DNA, gene-environment interactions, mosaicism, and synergistic heterozygosity. Undoubtedly, additional factors influencing transmission and expression of inherited traits will be elucidated as our understanding of the human genome expands. As of January 2012, the Online Mendelian Inheritance in Man (OMIM), a continuously updated catalog of human genes and genetic phenotypes, listed 13,775 identified genes and 4,520 genetic disorders for which the molecular basis is known [7]. Identified genetic disorders with known patterns of inheritance are commonly inherited as autosomal, X-linked, or mitochondrial.

Table 4.1 Collection of family medical history: what to ask?

For all family members
Current age; year of birth
Exact relationship to the proband
General physical and mental health status
History of major acute or chronic illness, hospitalizations, and surgeries
History of learning problems, diagnosed disabilities, or intellectual disability
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
Ethnicity
Targeted questions relevant to the reason for evaluation, for example, key symptoms or features of the condition in question, pertinent evaluations
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 or disease onset
Method of diagnosis
Evaluations and testing completed
Symptoms
Information about ongoing treatment or management plan
Availability of medical records for review

Mendelian Inheritance Patterns

For a summary of Mendelian inheritance patterns, see Table 4.2.

Autosomal Dominant Inheritance

In classic autosomal dominant inheritance, an affected individual has one non-functional or mutant allele at a particular locus, the presence of which causes disease. Each affected individual in a pedigree has a 50 % chance of passing the disease-associated mutation to each of his or her offspring. Additional genetic and non-genetic factors may influence the occurrence of these conditions in families. A key feature of autosomal dominant inheritance is the observance of male-to-male transmission of the condition or trait. Transmission from fathers to sons is 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 Fig. 4.5.

Table 4.2 Features of Mendelian patterns of inheritance

Autosomal dominant
Male-to-male transmission occurs; both sexes can transmit to offspring
Affected family members in multiple generations; "vertical transmission" typically showing affected descendants of affected individuals and unaffected descendants of unaffected individuals
Males and females affected, typically to comparable extent
Variability of clinical findings
Later/adult onset in some disorders
Homozygotes may be more severely affected than heterozygotes
Homozygosity may be lethal
Occurrence of new mutations
Nonpenetrance; apparent "skipping" of generations
Germline mosaicism reported
Autosomal recessive
Affected family members are usually in one generation; "horizontal" inheritance
Males and females equally likely to be affected; parental consanguinity or a small mating pool may influence disease occurrence
Disease severity is usually consistent 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
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; reflected by a paucity of males or overrepresentation of females in the pedigree
Increased occurrence of miscarriage may be observed
X-linked recessive
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
Y-linked
Male-to-male transmission only
Association with increased rates of male infertility
Discrepancy between chromosomal and phenotypic sex

Autosomal Recessive Inheritance

In autosomal recessive inheritance, an affected individual has two non-functional or mutant alleles at a particular locus. Carriers of autosomal recessive conditions have one nonfunctional allele at the gene locus, but usually have no symptoms as they also have one normal, functional copy of the gene. If both partners of a couple are carriers of the same autosomal recessive condition, with each pregnancy there is a one in four (25 %) chance of having an affected child, a two-in-four

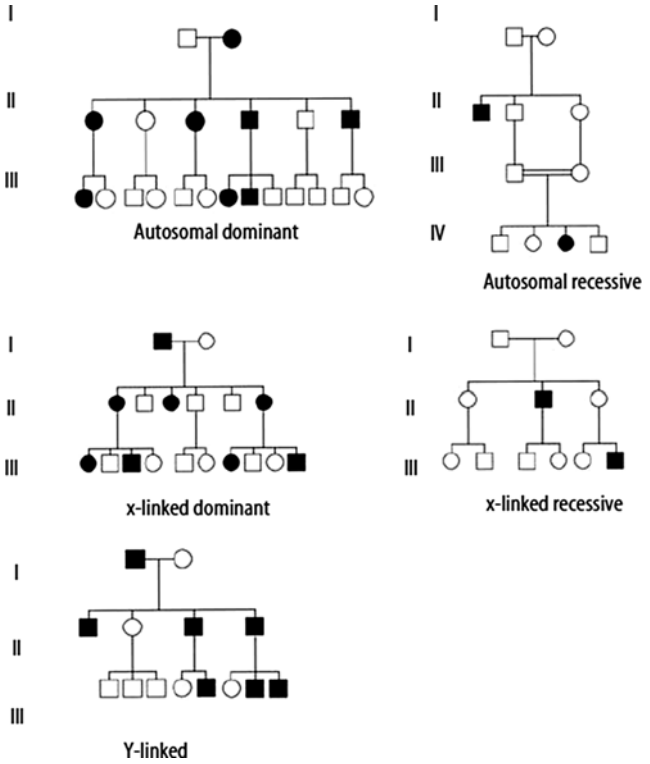


Figure 4.5 Example pedigrees for Mendelian patterns of inheritance

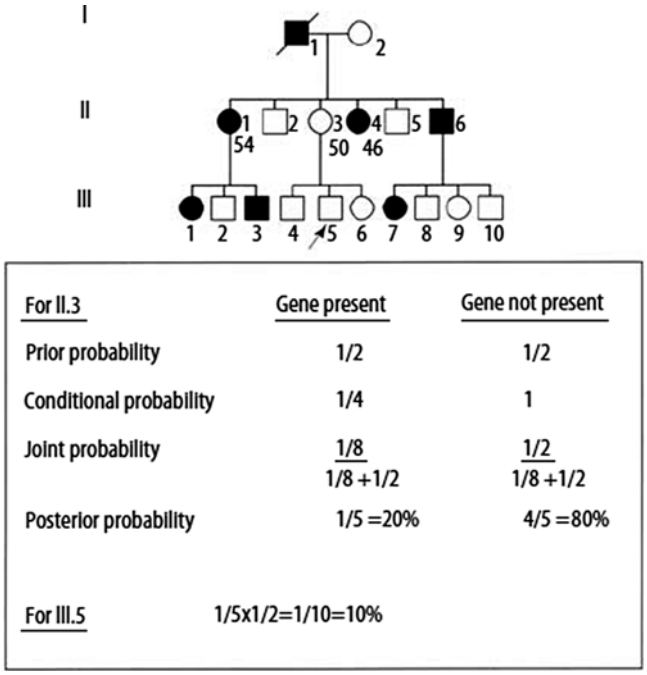


Figure 4.6 Bayesian analysis for risk assessment in an autosomal dominant, adult-onset hereditary cancer disorder. Ages of selected individuals in generation II are shown below the pedigree symbols

(50 %) chance that a child will be a carrier and a one in four (25 %) chance a child will be neither a carrier nor affected. After birth, if a child of a carrier couple is not affected by the condition in question, he or she has a two-in-three 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, which may vary among different ethnic groups or populations. Features of autosomal recessive inheritance are listed in Table 4.2, and an example pedigree is shown in Fig. 4.5.

X-Linked Dominant Inheritance

In X-linked dominant inheritance, an affected individual has one nonfunctional 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 sex of the offspring. Risk to offspring of affected males is sex dependent, with all daughters but no sons inheriting the gene mutation. 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 Fig. 4.6).

X-Linked Recessive Inheritance

Traditional X-linked recessive inheritance is characterized by occurrence of the condition in males having a non-functional or mutant allele for a gene on the X-chromosome. Affected males in the family will be related to each other through females. (See the pedigree in Fig. 4.5, and Table 4.2 for additional features.) Typically, carrier females are unaffected; however, due to lyonization (random inactivation of one X chromosome in each somatic cell in a female), carrier females may have mild symptoms. This occurs when, by chance, the X chromosome with the non-functional allele remains active in a majority of the cells within the critical tissue(s) for the disorder. 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. Fifty percent of the female offspring of carrier females will also be carriers. Offspring of affected males will not be classically affected, but all daughters will be carriers.

Y-Linked Inheritance

In rare cases, a mutation can occur in one of a limited number of genes on the Y chromosome. This can result in disparity between chromosomal and phenotypic sex if the SRY region is involved, or can be associated with hereditary forms of male 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 Fig. 4.5).

Codominant Inheritance

In codominant inheritance, two different alleles of the gene of interest are present and each is expressed. Therefore, the resulting phenotype is influenced by expression of both alleles. Traits inherited in this fashion include the ABO blood group and alpha-1-antitrypsin deficiency.

Non-Mendelian Inheritance Patterns

For a summary of non-Mendelian inheritance patterns, see Table 4.3.

Chromosomal Disorders

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. Chromosomal disorders caused by changes in the number of chromosomes (e.g., Down syndrome) occur most often due to random events during meiosis, and are typically not inherited. Copy number variants (CNVs), small deletions, or duplications of chromosomal material may be benign or disease causing, and may be inherited or de novo. Chromosomal rearrangements, such as translocations (the exchange of parts of nonhomologous chromosomes) and inversions (the breakage and reversal of a single chromosome segment), usually are deleterious when unbalanced. Unbalanced rearrangements are commonly inherited from a parent who carries a balanced version of the rearrangement. Chromosome abnormalities commonly are associated with multiple phenotypic effects as they usually cause deletion and/or duplication of many genes on the chromosomal segment(s) involved.

The classic microdeletion syndromes (e.g., DiGeorge syndrome, Williams syndrome) are clinically recognizable disorders resulting from the loss of many adjacent genes along a defined segment of a chromosome and usually result from a de novo event. The mechanism responsible for common microdeletion/microduplication syndromes is homologous recombination between stretches of nearly identical sequence that either remove or duplicate the unique intervening sequence [8]. Microdeletion/duplication syndromes are most reliably detected using array comparative genomic hybridization (aCGH) or fluorescence in situ hybridization (FISH) because the abnormalities are usually not detectable using standard cytogenetics.

Risks to offspring of individuals with chromosomal rearrangements or CNVs depend on the specific chromosome region(s) involved, size of the abnormality, and sometimes the sex of the transmitting parent. In apparently sporadic cases of unbalanced chromosomal rearrangements or disease-associated CNVs, parental testing with respect to the chromosomal abnormality should be performed to assess recurrence risk. Absence of a parental chromosome abnormality in such cases reduces the risk to future offspring. It is important to note that novel CNVs identified in an affected individual and in an unaffected parent should not be assumed to be benign; such CNVs may be disease causing but exhibit variable expressivity or reduced penetrance [9].

Table 4.3 Features of non-Mendelian patterns of inheritance

Chromosomal
Occurrence of congenital anomalies involving two or more organ systems
Occurrence of intellectual disability with dysmorphism or congenital anomalies
Multiple pregnancy losses or infertility in carriers of balanced translocations
Many occur as sporadic conditions with negative family history
Mitochondrial
Extreme variability of clinical symptoms; multiple organ systems involved
Degenerative/neuromuscular disorders predominate
Maternal transmission (fathers do not transmit disease)
Multiple generations affected (matrilineal)
Males and females equally likely to be affected
Environmental factors may influence symptoms
Multifactorial inheritance
Implicated in common adult-onset disorders
Males and females affected
The number and sex of affected relatives influence recurrence risk
Degree of relationship to affected relatives influences recurrence risk

Mitochondrial Disorders

The mitochondrial (mt) genome is a 16.5 kb circular strand of DNA located within the mitochondria. Unlike nuclear genes, the mt genome has a very high mutation rate due to lack of DNA repair mechanisms. In general, large deletions in the mtDNA arise as new mutations and confer low risk to relatives, while point mutations and duplications are commonly maternally transmitted [10]. Individuals inherit essentially all their mitochondrial DNA from their mothers; thus, transmission of mtDNA mutations is maternal. Affected males do not transmit mtDNA mutations to offspring. In each cell, including egg cell progenitors, there may be up to 1,000 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 daughter cells may inherit only mutant mtDNA or no mutant mtDNA (homoplasmy), or a mix of mutant and nonmutant mtDNA (heteroplasmy). When the proportion of mutant mtDNA exceeds a critical threshold in the cell, mitochondrial dysfunction results. As the degree of heteroplasmy may differ among individuals in a family, predicting risk to offspring of affected females is difficult. The level of heteroplasmy may differ in cell populations of different organs or tissues of an affected individual; therefore, conditions caused by mtDNA mutations often result in phenotypes affecting multiple organ systems and exhibit highly variable expression. Mutations in nuclear genes which affect mitochondrial function also can result in mitochondrial diseases. Such disorders are inherited in either an autosomal or X-linked fashion and tend to have expression that more closely resembles other Mendelian disorders.

Multifactorial Disorders

Multifactorial disorders are the result of the interaction or additive effect of multiple genetic and environmental factors. The likelihood of expression of a trait or disease is based on the relative contributions of each of the factors involved. With a relatively low concentration of contributing factors, no effect will be seen. 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. For multifactorial disorders that are more common in one sex, such as pyloric stenosis or neural tube defects, the risk for recurrence is higher for relatives when the affected individual is of the less commonly affected sex. Empiric risk figures for multifactorial disorders may be used for genetic counseling, but should be modified based on individual factors including number of affected relatives, relationship of affected family members to the counselee, severity of disease, and sex.

Genetic Mechanisms Influencing Transmission

For a summary of several known genetic factors which influence transmission, see Table 4.4.

Table 4.4 Genetic factors influencing transmission

Genomic imprinting
Gender of transmitting parent modifies gene/disease expression (parent-of-origin effects)
May appear to skip generations
Uniparental disomy
Single/isolated case in a family
Documentation of only one carrier parent in individuals with an autosomal recessive disease
X-linked recessive disorders occurring in 46,XX females
Unstable DNA
Anticipation (increasing severity with subsequent generations)
Gender of the transmitting parent may influence disease likelihood and severity
Synergistic heterozygosity
Unaffected, unrelated parents have multiple affected offspring
Described for genes with autosomal recessive inheritance
Implicated genes act in the same biological pathway or their proteins form complexes

Genomic 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 are then reimprinted as female before being transmitted to the next generation, and so on. Genomic imprinting is thought to occur early in development, most likely in the germ cells [11]. Imprinting errors have been described in a number of disorders including about 3 % of individuals with Angelman syndrome and an estimated 1 % of patients with Prader-Willi syndrome. If an affected individual has a mutation in an imprinting control center, which controls gene expression by regulating methylation, recurrence risks may be as high as 50 %.

Uniparental Disomy

Uniparental disomy (UPD) is defined as both copies of a chromosome or chromosome segment being derived from the same parent. The frequency of this phenomenon is unknown. UPD can occur as heterodisomy (the presence of both copies of a chromosome from one parent) or isodisomy (two copies of the same parental chromosome). 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 for a recessive disorder in that region [11]. Some disorders which can be caused by UPD include Beckwith-Wiedemann syndrome, Angelman syndrome, and Prader-Willi syndrome. UPD has been reported as a rare cause of autosomal recessive disorders, including cystic fibrosis and sickle cell anemia, and should be considered as a mechanism for autosomal recessive disease when only one parent can be confirmed as a carrier. In addition, X-linked recessive disorders occurring in 46,XX females may be caused by UPD.

Unstable DNA

Most classic hereditary disorders are caused by static or stable mutations in one or a few genes. For trinucleotide repeat disorders (e.g., fragile X syndrome, Huntington disease, myotonic dystrophy) the causative gene alterations are unstable and consist of a variable number of copies of a tandemly repeated three-nucleotide sequence. Trinucleotide repeats are stable and usually inherited without alteration when the repeat size falls with a specific range, which is gene specific. DNA replication of the repetitive sequence may result in errors leading to expansion (additional copies of the trinucleotide sequence) or contraction (loss of copies of the trinucleotide sequence) of the number of repeat copies. With expansion, the gene segment becomes less stable and thus more likely to expand further. Intermediate lengths of expanded trinucleotide repeats are called pre-mutations, which are extremely unstable and highly likely to undergo further expansion. Individuals who carry pre-mutations typically do not have classic symptoms of the associated disorder but may show mild signs or develop associated symptoms at later ages.

If the trinucleotide repeat expands into the disease-associated size range, gene function is disrupted and symptoms occur in the individual. 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 and more severe disease in subsequent generations) is explained mechanistically by the progressive expansion of the trinucleotide repeat region from one generation to the next. Sex of the transmitting parent also can influence the likelihood and degree of expansion, and is gene specific (the significant parent of origin varies by disease). Many common trinucleotide repeat disorders are associated with neurological phenotypes.

Synergistic Heterozygosity

This phenomenon can be described as the interaction of genes at multiple loci needed to express a phenotype. Heterozygous mutations in two or more distinct genes may lead to an overall decrease in function if the gene products form a complex or participate in the same developmental or metabolic pathway [12]. Examples of disorders resulting from synergistic processes include non-syndromic hearing loss resulting from heterozygous mutations in both the *GJB2* gene (encoding Connexin26) and the *GJB6* gene (encoding Connexin30). The connexins co-localize in the inner ear tissues to form gap junctions which are important for cellular communications. The presence of a heterozygous large deletion within the *GJB6* gene results in the loss of expression of *GJB2* on the same chromosome by removing a cis-acting regulatory element [13]. Thus, individuals carrying a *GJB2* mutation on one chromosome and a *GJB6* mutation on their other chromosome present with non-syndromic hearing loss. Digenic inheritance, or heterozygosity for a recessive mutation at two distinct loci, has been reported for inherited disorders including severe insulin resistance, primary congenital glaucoma, and retinitis pigmentosa. Recurrence risks for conditions resulting from synergistic heterozygosity depend on the number of loci involved, the specific genes implicated, genetic linkage of the loci, and the degree of decreased function conferred by each mutation. For classic digenic inheritance of recessive gene mutations which are not linked, risk for recurrence is expected to be 25 %.

Other Factors Affecting Risk and Risk Assessment

Understanding modes of inheritance provides a framework for risk assessment for 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 distant relatives, or relatives may not wish to share those details by providing medical records. 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 4.5). 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, gaps in an otherwise

Table 4.5 Factors affecting risk and risk assessment

Variable expressivity/pleiotropy
Typical age of disease onset
Penetrance
Heterogeneity
Phenocopies
Sex-influenced expression (sex-limited vs sex-influenced)
Family size/paucity of at-risk sex
Nonpaternity
Consanguinity/inbreeding
Lyonization
New mutation
Mosaicism (somatic or germline)
Modifying genes
Environmental and lifestyle effects

classic pedigree can occur due to variability in age of onset, particularly with adult-onset disease due to penetrance, or likelihood that an individual who carries the gene mutation(s) for a condition will show signs or symptoms. Some conditions show genetic heterogeneity, that is, can be caused by mutations in a number of different genes or by multiple distinct mutations at the same loci. Phenocopies, similar conditions with different genetic or nongenetic etiologies or both, also may 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 sex in sex-influenced disorders (sex-limited vs sex-influenced expression) may result in failure to recognize a hereditary disorder and underestimation of risk.

Accurate reporting of relationships within a pedigree is critical. Nonpaternity, estimated at 3 % in Western industrialized nations [14], may explain transmission patterns that seem to deviate from the expected. Consanguinity, or sharing of common ancestors, is particularly important when considering transmission of autosomal recessive traits. In a consanguineous union, there is an increased chance that a gene mutation present in a common ancestor and associated with an autosomal recessive condition may be transmitted through both sides of the family and occur in the homozygous state in offspring. In general, the risk for congenital malformations or adverse medical outcomes in the offspring of consanguineous unions is increased over the general population and varies depending on the degree of relatedness of the couple [15].

Risk assessment for disorders caused by mutations in X-linked genes or for chromosomal rearrangements involving the X chromosome may be influenced by lyonization. Female carriers of X-linked recessive disorders may be symptomatic if the affected X chromosome is preferentially active while female carriers of X-linked dominant disorders may be asymptomatic if the affected X chromosome is preferentially inactivated. Spontaneous new mutations, which are not inherited from either parent but may be transmitted to offspring, are a common cause for some autosomal dominant or X-linked conditions. Parental testing may be necessary to determine if a mutation occurred sporadically and can be helpful in clarifying recurrence risk and establishing risk to family members. Somatic mosaicism, or the presence of at least two populations of cells with different genetic makeup in the same individual, may result in an atypical or mild disease phenotype depending on the type and percentage of cells affected. Mosaicism which is restricted to the egg or sperm cells (germline mosaicism) can lead to unrecognized or indefinable risk to future offspring because such individuals are asymptomatic and it is difficult to estimate the percentage of germ cells affected.

Finally, factors outside of the critical gene can influence expression of traits 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 the expression of the condition. Similarly, environmental or lifestyle factors such as dietary habits, medical screening practices and specific exposures (medications, radiation, smoke, etc.) may positively or negatively affect gene function and 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 and Indirect Mutation Analysis

The ability to directly interrogate mutations or gene regions associated with disease often provides a more definitive answer about individual risk than pedigree evaluation. Currently, there are clinical or research tests offered for over 2,500 different genetic diseases (http://www.ncbi.nlm.nih.gov/, accessed on 17 January 2012), and this list will continue to increase as additional genes are implicated in disease. Molecular methods of gene analysis vary and technologies selected by clinical molecular laboratories may be influenced by numerous factors including the number of gene(s) and sample(s) to be analyzed, size of the gene(s), gene structure, and the number and type of gene mutations to be interrogated. Sanger sequencing is considered the gold standard for DNA sequencing and is frequently used in clinical testing. Mutation scanning techniques such as conformation-sensitive gel electrophoresis (CSGE), denaturing gradient gel electrophoresis (DGGE), denaturing high-performance liquid chromatography (DHPLC), single-strand conformation polymorphism (SSCP), and high-resolution melting (Wittwer) are commonly utilized for genes which may contain a variety of disease-causing sequence alterations [16, 17]. If a sequence alteration is suspected by a scanning method, sequencing to confirm the presence of the variant can be targeted only to the suspicious gene region which makes scanning technologies economical. For analysis of a small number of defined mutations within a gene, including small insertions, deletion rearrangements, or changes in the number of repeats, polymerase chain reaction (PCR) and fragment analysis may be used [16]. For detection of defined, disease-causing single-nucleotide polymorphisms (SNPs), allele-specific PCR and fluorescent monitoring, single-nucleotide extension (SNE), or oligonucleotide ligation assays (OLA) may be used. Southern blot analysis, multiplex ligation-dependent probe amplification (MLPA), or array hybridization techniques may be utilized to identify loss or

gain of entire genes or gene segments. Southern blot analysis also may be used to identify large trinucleotide repeats.

New high-throughput sequencing technologies, collectively referred to as next-generation sequencing (NGS), use DNA synthesis or ligation processes for massively parallel sequencing of numerous DNA templates. NGS technologies have improved sequencing speed and accuracy and lowered costs of testing multiple genes for a single disease dramatically [18]. By targeting regions of the genome or genes of interest, NGS technologies are being applied to clinical molecular testing. Panels of genes associated with a particular disease phenotype (e.g., cardiomyopathy or X-linked intellectual disability) can be assembled for analysis by NGS, which is especially cost effective for diseases demonstrating genetic heterogeneity. Exome sequencing targets only the protein-encoding regions of the genome, which harbor the majority of identified disease-causing mutations, yet represent only 1 % of the entire genome. Exome sequencing is being used to identify causative mutations for Mendelian disorders difficult to identify by targeted sequencing of specific genes [19]. Genome sequencing also is being applied in clinical settings to identify rare Mendelian disorders [20]. A detailed discussion of molecular methods is provided in Chap. 2 and NGS technologies in Chap. 59 and 60.

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. The classic method for determining the causative gene for a Mendelian disorder is linkage analysis. Linkage analysis uses polymorphic genetic markers near the genomic locus of interest to assess association with the disease phenotype in the family. As linkage analysis requires samples from multiple family members, both affected and unaffected, this type of indirect mutation analysis may not be feasible for genetic assessment of very rare Mendelian disorders, sporadic cases, or unrelated cases. In addition, linkage analysis may not be an ideal method of gene discovery for conditions demonstrating diverse clinical phenotypes, those resulting from mutations in more than one gene or influenced by gene-environment interactions. Integration of exome or genome sequencing with linkage and homozygosity data can help elucidate previously unidentified causative mutations or candidate genes [19].

Bayesian Analysis Used in Risk Modification

Numerous factors, some listed above, influence the likelihood that a given individual in the family may be affected by, or a carrier of, the presenting condition. When it is not possible to do direct diagnostic testing for the condition (e.g., 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 mutation or genetic condition [12]. Some factors that may be considered in genetic risk assessment using Bayesian analysis include ethnicity, degree of relationship to affected family members, inheritance pattern, laboratory results, incidence of the disease, and natural history of the condition. The probability assigned based on past events is called the prior probability. The probability 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. The example pedigree in Fig. 4.6 demonstrates an autosomal dominant cancer predisposition syndrome affecting males and females equally. Based on Mendelian inheritance alone, the risk that individual III.5 carries the disease-causing mutation is 25 %. However, knowing that 75 % of gene carriers have been diagnosed with cancer by age 50, risk can be recalculated as demonstrated. (See Chap. 5 for a complete discussion of Bayesian analysis.)

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