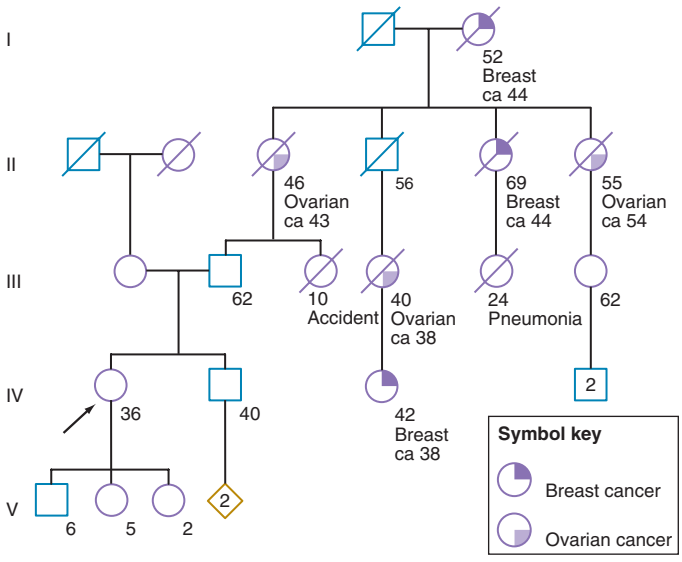

FIGURE 84-1 A 36-year-old woman (*arrow*) seeks consultation because of her family history of cancer. The patient expresses concern that the multiple cancers in her relatives imply an inherited predisposition to develop cancer. The family history is recorded, and records of the patient's relatives confirm the reported diagnoses.

intensive and ideally involves interviews of additional family members or reviewing medical records, autopsy reports, and death certificates.

Although many inherited disorders will be suggested by the clustering of relatives with the same or related conditions, it is important to note that disease penetrance is incomplete for most genetic disorders. As a result, the pedigree obtained in such families may not exhibit a clear Mendelian inheritance pattern, because not all family members carrying the disease-associated alleles will manifest clinical evidence of the condition. Furthermore, genes associated with some of these disorders often exhibit variable disease expression. For example, the breast cancer–associated gene *BRCA2* can predispose to several different malignancies in the same family, including cancers of the breast, ovary, pancreas, skin, and prostate. For common diseases such as breast cancer, some family members without the susceptibility allele (or genotype) may develop breast cancer (or phenotype) sporadically. Such phenocopies represent another confounding variable in the pedigree analysis.

Some of the aforementioned features of the family history are illustrated in Fig. 84-1. In this example, the proband, a 36-year-old woman (IV-1), has a strong history of breast and ovarian cancer on the paternal side of her family. The early age of onset and the co-occurrence of breast and ovarian cancer in this family suggest the possibility of an inherited mutation in *BRCA1* or *BRCA2*. It is unclear however, without genetic testing, whether her father harbors such a mutation and transmitted it to her. After appropriate genetic counseling of the proband and her family, the most informative and cost-effective approach to DNA analysis in this family is to test the cancer-affected 42-year-old living cousin for the presence of a *BRCA1* or *BRCA2* mutation. If a mutation is found, then it is possible to test for this particular alteration in other family members, if they so desire. In the example shown, if the proband's father has a *BRCA1* mutation, there is a 50:50 probability that the mutation was transmitted to her, and genetic testing can be used to establish the absence or presence of this alteration. In this same example, if a mutation is not detected in the cancer-affected cousin, testing would not be indicated for cancer-unaffected relatives.

GENETIC TESTING FOR ADULT-ONSET DISORDERS

A critical first step before initiating genetic testing is to ensure that the correct clinical diagnosis has been made, whether it is based on family history, characteristic physical findings, pathology, or biochemical testing. Such careful clinical assessment can define the phenotype. In the traditional model of genetic testing, testing is directed initially toward the most probable genes (determined by the phenotype), which prevents unnecessary testing. Many disorders exhibit the feature of locus heterogeneity, which refers to the fact that mutations in different genes can cause phenotypically similar disorders. For example, osteogenesis imperfecta (Chap. 427), long QT syndrome (Chap. 277), muscular dystrophy (Chap. 462e), and hereditary predisposition to breast (Chap. 108) or colon (Chap. 110) cancer can each be caused by mutations in a number of distinct genes. The patterns of disease transmission, disease risk, clinical course, and treatment may differ significantly depending on the specific gene affected. Historically, the choice of which gene to test has been determined by unique clinical and family history features and the relative prevalence of candidate genetic disorders. However, rapid changes in genetic testing techniques, as discussed below, may impact this paradigm. It is now technically and financially feasible to sequence many genes (or even the whole exome) at one time. The incorporation of multiplex testing for germline mutations is rapidly evolving.

METHODOLOGIC APPROACHES TO GENETIC TESTING

Genetic testing is regulated and performed in much the same way as other specialized laboratory tests. In the United States, genetic testing laboratories are Clinical Laboratory Improvement Amendments (CLIA) approved to ensure that they meet quality and proficiency standards. A useful information source for various genetic tests is *www.genetests.org*. It should be noted that many tests need to be ordered through specialized laboratories.

Genetic testing is performed largely by DNA sequence analysis for mutations, although genotype can also be deduced through the study of RNA or protein (e.g., apolipoprotein E, hemoglobin S, and immunohistochemistry). For example, universal screening for Lynch syndrome via immunohistochemical analysis of colorectal cancers for absence of expression of mismatch repair proteins is under way at multiple hospitals throughout the United States. The determination of DNA sequence alterations relies heavily on the use of polymerase chain reaction (PCR), which allows rapid amplification and analysis of the gene of interest. In addition, PCR enables genetic testing on minimal amounts of DNA extracted from a wide range of tissue sources including leukocytes, mucosal epithelial cells (obtained via saliva or buccal swabs), and archival tissues. Amplified DNA can be analyzed directly by DNA sequencing, or it can be hybridized to DNA chips or blots to detect the presence of normal and altered DNA sequences. Direct DNA sequencing is frequently used for determination of hereditary disease susceptibility and prenatal diagnosis. Analyses of large alterations of the genome are possible using cytogenetics, fluorescent in situ hybridization (FISH), Southern blotting, or multiplex ligation-dependent probe amplification (MLPA) (Chap. 83e).

Massively parallel sequencing (also called *next-generation sequencing*) is significantly altering the approach to genetic testing for adult-onset hereditary susceptibility disorder. This technology encompasses several high-throughput approaches to DNA sequencing, all of which can reliably sequence many genes at one time. Technically, this involves the use of amplified DNA templates in a flow cell, a very different process than traditional Sanger sequencing which is time-consuming and expensive.

Multiplex panels for inherited susceptibility are commercially available and include testing of a number of genes that have been associated with the condition of interest. For example, panels are available for Brugada syndrome, hypertrophic cardiomyopathy, and Charcot-Marie-Tooth neuropathy. For many syndromes, this type of panel testing may make sense. However, in other situations, the utility of panel testing is less certain. Currently available breast cancer susceptibility panels contain six genes or more. Many of the genes included in the larger panels are associated with only a modest risk of breast cancer, and the clinical application is uncertain. An additional problem of sequencing many genes (rather than the genes for which there is most suspicion) is the identification of one or more variants of uncertain significance (VUS), discussed below.

Whole-exome sequencing (WES) is also now commercially available, although largely used in individuals with syndromes unexplained by