discussed in previous versions of this chapter, are available for the detection of mutations. In a very broad sense, one can distinguish between techniques that allow for screening of known mutations (screening mode) or techniques that definitively characterize mutations. Analyses of large alterations in the genome are possible using classic methods such as cytogenetics, fluorescent in situ hybridization (FISH), and Southern blotting (Chap. 83e), as well as more sensitive novel techniques that search for multiple single exon deletions or duplications.

More discrete sequence alterations rely heavily on the use of PCR, which allows rapid gene amplification and analysis. Moreover, PCR makes it possible to perform genetic testing and mutational analysis with small amounts of DNA extracted from leukocytes or even from single cells, buccal cells, or hair roots. DNA sequencing can be performed directly on PCR products or on fragments cloned into plasmid vectors amplified in bacterial host cells. Sequencing of all exons of the genome or selected chromosomes, or sequencing of numerous candidate genes in a single run, is now possible with next-generation sequencing platforms.

The majority of traditional diagnostic methods were gel-based. Novel technologies for the analysis of mutations, genotyping, large-scale sequencing, and mRNA expression profiles are undergoing rapid evolution. DNA chip technologies allow hybridization of DNA or RNA to hundreds of thousands of probes simultaneously. Microarrays are being used clinically for mutational analysis of several human disease genes, as well as for the identification of viral or bacterial sequence variations. With advances in high-throughput DNA sequencing technology, complete sequencing of the genome or an exome has entered the clinical realm. Although comprehensive sequencing of large genomic regions or multiple genes is already a reality, the subsequent bioinformatics analysis, assembly of sequence fragments, and comparative alignments remains a significant and commonly underestimated challenge. The discovery of incidental (or secondary) findings that are unrelated to the indication for the sequencing analysis but indicators of other disorders of potential relevance for patient care can pose a difficult ethical dilemma. It can lead to the detection of undiagnosed medically actionable genetic conditions, but can also reveal deleterious mutations that cannot be influenced, as numerous sequence variants are of unknown significance.

A general algorithm for the approach to mutational analysis is outlined in Fig. 82-15. The importance of a detailed clinical phenotype cannot be overemphasized. This is the step where one should also consider the possibility of genetic heterogeneity and phenocopies. If obvious candidate genes are suggested by the phenotype, they can be analyzed directly. After identification of a mutation, it is essential to demonstrate that it segregates with the phenotype. The functional characterization of novel mutations is labor intensive and may require analyses in vitro or in transgenic models in order to document the relevance of the genetic alteration.

Prenatal diagnosis of numerous genetic diseases in instances with a high risk for certain disorders is now possible by direct DNA analysis. *Amniocentesis* involves the removal of a small amount of amniotic fluid, usually at 16 weeks of gestation. Cells can be collected and submitted for karyotype analyses, FISH, and mutational analysis of selected genes. The main indications for amniocentesis include advanced maternal age (>35 years), an abnormal serum triple marker test (α-fetoprotein, β human chorionic gonadotropin, pregnancy-associated plasma protein A, or unconjugated estriol), a family history of chromosomal abnormalities, or a Mendelian disorder amenable to genetic testing. Prenatal diagnosis can also be performed by *chorionic villus sampling* (CVS), in which a small amount of the chorion is removed by a transcervical or transabdominal biopsy. Chromosomes and DNA obtained from these cells can be submitted for cytogenetic and mutational analyses. CVS can be performed earlier in gestation (weeks 9–12) than amniocentesis, an aspect that may be of relevance when termination of pregnancy is a consideration. Later in pregnancy, beginning at about 18 weeks of gestation, percutaneous umbilical blood sampling (PUBS) permits collection of fetal blood for lymphocyte culture and analysis. Recently, the entire fetal genome has been determined prenatally from cells taken from the mother's plasma through deep sequencing and the counting of parental haplotypes, or by inferring it from DNA sequences obtained from blood samples from the mother, father, and umbilical cord. These approaches enable screening for clinically relevant and deleterious alleles inherited from the parents, as well as for de novo germline mutations, and they may have the potential to change the diagnosis of genetic disorders in the prenatal setting.

In combination with in vitro fertilization (IVF) techniques, it is even possible to perform genetic diagnoses in a single cell removed from the four- to eight-cell embryo or to analyze the first polar body from an oocyte. Preconceptual diagnosis thereby avoids therapeutic abortions but is costly and labor intensive. It should be emphasized that excluding a specific disorder by any of these approaches is never equivalent to the assurance of having a normal child.

Mutations in certain cancer susceptibility genes such as *BRCA1* and *BRCA2* may identify individuals with an increased risk for the development of malignancies and result in risk-reducing interventions. The detection of mutations is an important diagnostic and

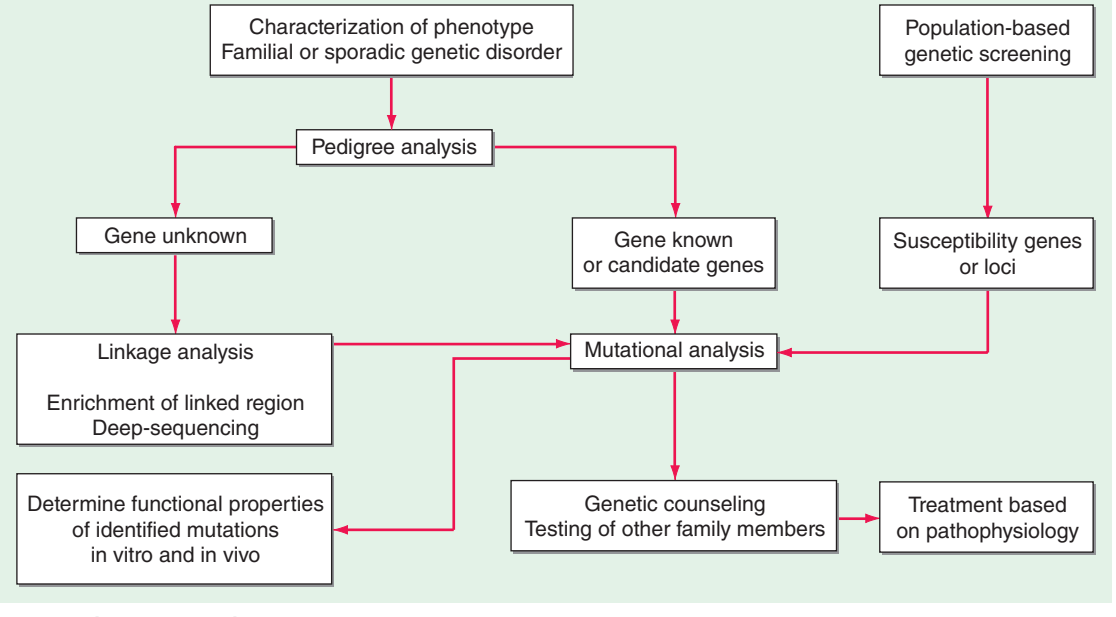

FIGURE 82-15 **Approach to genetic disease.**