by direct sequencing. Some disorders, most with recessive inheritance, are associated with a limited number of recurrent mutations. Many others, especially those with dominant inheritance, are caused by mutations scattered throughout the responsible gene and represent a considerable diagnostic challenge.

Prenatal testing should be offered for all fetuses at risk for a cytogenetic abnormality. Possible indications include:

- Advanced maternal age
- A parent known to carry a balanced chromosomal rearrangement (because these greatly increase the frequency of abnormal chromosome segregation during meiosis and the risk of aneuploidy in the fertilized ovum)
- Fetal anomalies observed on ultrasound
- Routine maternal blood screening, indicating an increased risk of Down syndrome or another trisomy

Prenatal testing may also be considered for children at known risk for many other genetic disorders (e.g., cystic fibrosis, spinal muscular atrophy) using targeted analysis based on familial mutations or family history. At present it is usually performed on cells obtained by amniocentesis, chorionic villus biopsy, or umbilical cord blood. However, as much as 10% of the free DNA in a pregnant mother's blood is of fetal origin, and new technologies are opening the door to an era of noninvasive prenatal diagnostics utilizing this source of DNA.

Beyond prenatal testing, parents known to be at risk for having a child with a genetic disorder can choose to have genetic testing performed on embryos created in vitro prior to uterine implantation, eliminating the chance of generational transmission of a familial disease.

Following birth, testing is ideally done as soon as the possibility of constitutional genetic disease arises. It is most commonly performed on peripheral blood DNA and is targeted based on clinical suspicion. In newborns or children, indications may be as follows:

- Multiple congenital anomalies
- Suspicion of a metabolic syndrome
- Unexplained mental retardation and/or developmental delay
- Suspected aneuploidy (e.g., features of Down syndrome) or other syndromic chromosomal abnormality (e.g., deletions, inversions)
- Suspected monogenic disease, whether previously described or unknown

In older patients, testing logically becomes more focused toward genetic diseases that manifest at later stages of life. Again, the possibilities are vast, but the more common indications include:

- Inherited cancer syndromes (triggered by either family history or an unusual cancer presentation)
- Atypically mild monogenic disease (e.g., attenuated cystic fibrosis)
- Neurodegenerative disorders (e.g., familial Alzheimer disease, Huntington disease)

Indications for Analysis of Acquired Genetic Alterations

In this era of molecularly targeted therapies it is becoming increasingly important to identify nucleic acid sequences or aberrations that are specific for acquired diseases (e.g., cancer and infectious disease). The technical approaches are the same as those used for germ line Mendelian disorders, and the common indications include:

- Diagnosis and management of cancer (see also Chapter 7)
  - Detection of tumor-specific acquired mutations and cytogenetic alterations that are the hallmarks of specific tumors (e.g., *BCR-ABL* fusion genes in chronic myelogenous leukemia, or CML)
  - Determination of clonality as an indicator of a neoplastic condition
  - The identification of specific genetic alterations that can direct therapeutic choices (e.g., HER2 [official name *ERBB2*] amplification in breast cancer or EGFR [official name *ERBB1*] mutations in lung cancer)
  - Determination of treatment efficacy (e.g., minimal residual disease detection of *BCR-ABL* by PCR in CML)
  - Detection of drug-resistant secondary mutations in malignancies treated with genetically tailored therapeutics
- Diagnosis and management of infectious disease (see also Chapter 8)
  - Detection of microorganism-specific genetic material for definitive diagnosis (e.g., HIV, mycobacteria, human papillomavirus, herpesvirus in central nervous system)
  - The identification of specific genetic alterations in the genomes of microbes that are associated with drug resistance
  - Determination of treatment efficacy (e.g., assessment of viral loads in HIV, Epstein-Barr virus, and hepatitis C virus infection)

PCR and Detection of DNA Sequence Alterations

PCR analysis, which involves the synthesis of relatively short DNA fragments from a DNA template, has been a mainstay of molecular diagnostics for the last few decades. By using appropriate heat-stable DNA polymerases and thermal cycling, the target DNA (usually less than 1000 base pairs) lying between designed primer sites is exponentially amplified from as little as one original copy, greatly simplifying secondary sequence analysis. Many options exist for subsequent analysis, each with different strengths and weaknesses:

- *Sanger sequencing.* Here, the amplified DNA is mixed with a DNA polymerase, a DNA primer, nucleotides, and four dead-end (di-deoxy terminator) nucleotides (A, T, G, and C) labeled with different fluorescent tags. The ensuing reaction produces a series of DNA molecules of all possible lengths up to a kilobase or so, each labeled with a tag that corresponds to the base at which the reaction stopped due to incorporation of one of the terminator nucleotides. After size separation by capillary electrophoresis, the exact sequence can be "read" and compared with the normal sequence to detect the presence of mutations. Many applications of Sanger