- In **Prader- Willi syndrome,** deletion of band q12 on long arm of paternal chromosome 15 occurs. Genes in this region of maternal chromosome 15 are imprinted so there is complete loss of their functions. Patients have mental retardation, short stature, hypotonia, hyperphagia, small hands and feet, and hypogonadism.
- In **Angelman syndrome** there is deletion of the same region from the maternal chromosome. Since genes on the corresponding region of paternal chromosome 15 are imprinted, these patients have mental retardation, ataxia, seizures, and inappropriate laughter.

Gonadal Mosaicism

It was mentioned earlier that with every autosomal dominant disorder some patients do not have affected parents. In such patients the disorder results from a new mutation in the egg or the sperm from which they were derived; as such, their siblings are neither affected nor at increased risk for development of the disease. This is not always the case, however. *In some autosomal dominant disorders, exemplified by osteogenesis imperfecta, phenotypically normal parents have more than one affected child.* This clearly violates the laws of Mendelian inheritance. Studies indicate that gonadal mosaicism may be responsible for such unusual pedigrees. Such mosaicism results from a mutation that occurs postzygotically during early (embryonic) development. If the mutation affects only cells destined to form the gonads, the gametes carry the mutation, but the somatic cells of the individual are completely normal. A phenotypically normal parent who has gonadal mosaicism can transmit the disease-causing mutation to the offspring through their mutated gametes. Because the progenitor cells of the gametes carry the mutation, there is a possibility that more than one child of such a parent would be affected. Obviously the likelihood of such an occurrence depends on the proportion of germ cells carrying the mutation.

Molecular Genetic Diagnosis

The nascent field of molecular diagnostics emerged in the latter half of the twentieth century, with the application of low throughput approaches such as conventional karyotyping for recognition of cytogenetic disorders (e.g., Down syndrome) and DNA-based assays such as Southern blotting for the diagnosis of Huntington disease. Over time, a steady stream of technologic breakthroughs has led to ever-increasing capabilities, including notably the development of Sanger DNA sequencing in 1977 and polymerase chain reaction (PCR) in 1983. Used together, these two techniques allowed the routine sequencing of any known segment of DNA, both rapidly accelerating research and providing a straightforward avenue for targeted diagnostics development.

Today, with the completion of the Human Genome Project and with newer and more powerful techniques for genetic and genomic analysis, nucleic acid–based testing is beginning to take a central role in the diagnosis and management of many diseases. Molecular diagnostic techniques have found application in virtually all areas of medicine, and their adoption continues to accelerate.

While an exhaustive discussion of molecular diagnostics is beyond the scope of this book, many of the better known approaches are highlighted in the ensuing sections. It is important to emphasize that, regardless of the technique used, human genetic markers can be either constitutional (i.e., present in each and every cell of the affected person, as with a *CFTR* mutation in a patient with cystic fibrosis) or somatic (i.e., restricted to specific tissue types or lesions, as with mutations in the *KRAS* gene in a variety of human cancers). In suspected infections, the goal is to detect and quantify nucleic acids that are specific to the infectious agent, which may be confined to particular cells or body sites. These considerations determine the nature of the sample used for the assay (e.g., peripheral blood cells, tumor tissue, nasopharyngeal swab).

Diagnostic Methods and Indications for Testing

There are a truly dizzying number of both techniques and indications for performing molecular genetic diagnostic tests on patient specimens, both for inherited and acquired genetic anomalies. The burden of choice can often be problematic, both for molecular pathologists who design tests as well as for clinicians who need to choose the optimal test for their patients.

Laboratory Considerations

On the laboratory side, pathologists focus on the sensitivity, specificity, accuracy, and reproducibility of different methods, as well as practical factors like cost, labor, reliability, and turn-around time. To choose the appropriate diagnostic technique, it is critical to first understand the spectrum of genetic anomalies that are responsible for the disease in the patient population under study. Disease-causing genetic anomalies range in size from single base substitutions up to gains or losses of entire chromosomes, and may vary widely in frequency among ethnic groups. Proper test design requires careful consideration of these factors. For example, standard cystic fibrosis testing for the 23 most common point mutations and small deletions (≤3 base pairs) in the *CFTR* gene has a sensitivity of 94% in Ashkenazi Jews, but identifies less than 50% of affected patients in Asian populations. In cases with negative standard test results and a high clinical suspicion, further tests are needed, such as extensive sequencing that covers all 27 exons of the *CFTR* gene. But even sequencing assays may miss large (kilobase scale) deletions involving one or more exons, which require a different test methodology. Issues like this arise quite frequently in genetic testing, and close communication between primary care clinicians, medical genetics specialists, and diagnosticians is often required in order to select the optimal test strategy in difficult cases.

Indications for Analysis of Inherited Genetic Alterations

Testing for inherited alterations may be required at any age, depending on clinical presentation, although in general most testing is performed during the prenatal or postnatal/childhood periods. Mendelian disorders that have been linked to specific genes number in the thousands, and definitive diagnosis for most of them is possible