8.2.1 Introduction

“The natural world is not famous for making life easy for human geneticists”
D. B. Goldstein [5]

Over recent years, molecular genetic investigations have attained an important place in the diagnostic work-up of patients with known or suspected inborn errors of metabolism. Identification of a known disease-causing mutation may provide the ultimate proof of diagnosis, particularly in those disorders in which a biochemical or enzymatic diagnosis is not possible, not reliable or requires invasive procedures. This may be the case when an enzyme is expressed in specific organs only (e.g. liver or brain) or when the disease is caused by a deficiency of structural, receptor or membrane proteins. Mutation analysis may be the first follow-up method in disorders that are caused by one or a few common mutation(s) such as long-chain hydroxyacyl-coenzyme A dehydrogenase (LCHAD) deficiency. Knowledge of the mutation may provide information about the course of disease and prognosis in disorders with established genotype-phenotype correlations. Finally, knowing the causative mutations in a family may be valuable for genetic counselling and prenatal diagnosis.

DNA studies may be quite expensive, and there are several aspects that should be considered before mutation studies are requested or when the results are available:

1. Are mutation studies necessary? Enzyme studies or other phenotypic or functional investigations may be more sensitive and more (cost-)effective for reaching a diagnosis.

2. How unlikely is the diagnosis when no mutation is found? There is virtually no inborn error of metabolism for which all mutations are detected, even with the most sophisticated methods. Negative results do not usually rule out a diagnosis, and for their interpretation it is essential to know the sensitivity of the method used in the analysis. This information must be provided in the DNA analysis report.

3. How likely is the diagnosis when mutations are found? Novel DNA variants may be erroneously regarded as disease-causing when they are in fact silent. Nowadays it is easier to sequence a gene than to interpret the results correctly. Make sure that the laboratory staff is familiar with the full spectrum of mutations in the genes studied.

4. Cis or trans? When two mutations are found in a recessive disorder, inheritance in trans (on different chromosomes) should be confirmed. Two mutations may
occasionally be in cis (on the same chromosome), with another or no mutation on the second chromosome. It may be useful to confirm mutations in parental samples but beware of non-paternity.

5. How good are genotype-phenotype correlations? Is the clinical picture fully explained by the genetic findings? Is the disorder fully penetrant? Are there additional, non-genetic factors of pathogenesis?

6. Mutation data should be communicated to the family through genetic counselling. Many patients do not fully understand the genetic aspects of a metabolic disorder in their child, and genetic counselling should be offered to all. Genetic counselling is absolutely essential when other members of the family could be at risk of being affected or when prenatal diagnosis is considered an option.

7. Who benefits from the analysis? Mutation analyses in children should only be performed if there is an important medical consequence in childhood. In particular, carrier analyses in healthy siblings of children with metabolic disorders are not indicated and should not be carried out even when requested by the parents.

A range of molecular methods is available for the identification of genetic alterations. Most depend on amplification of specific genomic areas with the polymerase chain reaction (PCR). Which method is employed by a particular laboratory depends on a range of factors including experience, costs and billing. For practical purposes it is important to differentiate between mutation scanning methods, mutation screening methods, direct sequencing and genomic quantification.

1. The aim of mutation scanning methods is to detect known or novel mutations in a gene by scanning base-by-base and exon-by-exon. Abnormalities found are confirmed by direct sequencing. In this chapter we present in more detail denaturing gradient gel electrophoresis (DGGE) as a sensitive and cost-efficient mutation scanning method.

2. Mutation screening methods involve testing for specific (previously selected) mutations in a gene. This approach is relatively inexpensive and may be useful for disorders that are caused by one or few common mutations. It is important to take the origin of the patient into consideration, since the frequency of mutations differs markedly between populations. As an example for such a method we discuss restriction enzyme analysis in more detail in this chapter.

3. Direct sequencing is the gold standard of mutation detection; is also covered in this chapter. Sequencing does not usually detect large deletions or genomic rearrangements. Keep in mind that quality control schemes for DNA sequencing consistently show an error rate of at least 1% even in expert laboratories. If the results do not fit the clinical picture it may be justified to check the results in another laboratory.

4. Genomic quantification is necessary to identify large deletions or duplications that occasionally cause single gene disorders. A novel molecular method (multiplex ligation-dependent probe amplification, MLPA) has been recently developed for this purpose and is presented in this chapter.

8.2.2 Properties of the Analyte

Modern genetics started with the elucidation of the double-helical structure of the DNA molecule by James Watson and Francis Crick in 1953 [20]. It was already known