Chapter 8
Molecular Genetic Testing for Metabolic Disorders
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Introduction

Inborn errors of metabolism represent a highly diverse group of genetic disorders. Individually the disorders are rare. The most prevalent, phenylketonuria (PKU), affects approximately 1 in 10,000 individuals. However, because numerous metabolic disorders exist, collectively they are estimated to affect as many as 1 in 600 individuals. The clinical consequences of such disorders are broad and can be severe, with progressive neurological impairment, mental retardation (MR), organomegaly, and high morbidity. Their mode of inheritance is usually autosomal recessive but also can be X-linked. Metabolic disorders result from defects in the individual enzymes of pathways that govern many different aspects of metabolism in distinct compartments within the cell.

The onset of disease is most often after birth with the appearance of an apparently normal infant, but in some classes of metabolic disorders multiple congenital anomalies also exist. For most metabolic disorders, disease symptoms present in early infancy or childhood, but in less-severe cases, adolescent or adult onset may occur. Therefore, early recognition with prompt therapeutic intervention when possible is critical for reducing damage due to the metabolic defect. For those diseases that are prevalent and for which early detection and intervention would have a beneficial outcome, neonatal screening is performed in the United States and in several countries around the world. In the United States, each state and the District of Columbia determine the diseases for which newborns are screened and the methods used for screening. With respect to metabolic disorders, all states screen for PKU and congenital hypothyroidism, and all but one screen for galactosemia. A number of states screen for maple syrup urine disease, homocystinuria, biotinidase deficiency, tyrosinemia, and congenital adrenal hyperplasia, or some combination of these. Tandem mass spectrometry has been added to newborn screening programs in many states and can detect more than 20 metabolic disorders, including medium chain acyl CoA dehydrogenase (MCAD) deficiency. DNA testing is currently used as a follow-up to an initial screen for certain disorders, such as MCAD deficiency and PKU.

This chapter discusses the molecular mechanisms of disease and the available genetic testing for selected metabolic disorders. The choice of disorders reflects population prevalence and current availability of molecular testing, as the mutations in many of the metabolic diseases are genetically heterogeneous and diagnoses are still widely dependent on biochemical testing. However, DNA testing is often critical for confirmatory studies, genetic counseling, carrier and prenatal testing, genotype-phenotype correlation, and is widely used for carrier screening for metabolic disorders in certain populations that have a high frequency of specific mutations due to founder effects. As molecular technologies advance, molecular methods will increasingly be used to screen for more metabolic diseases.

AMINO ACIDURIAS (PHENYLKETONURIA)
Molecular Basis of the Disease

PKU is an autosomal recessive disorder caused by the inability of the body to convert phenylalanine to tyrosine. PKU is the most common metabolic disease in caucasians, with an incidence of 1 in 10,000 individuals. About 98% of PKU cases are caused by defects in the phenylalanine hydroxylase (PAH) gene. The other 2% are caused by defects in the biosynthesis or regeneration of the cofactor of PAH, 6(R)-L-erythro-tetrahydrobiopterin (BH4). Accumulation of phenylalanine can damage the development of the central nervous system and result in MR. PKU has a spectrum of phenotypes ranging from classic PKU, which is the most severe type with the least tolerance to dietary phenylalanine, to moderate PKU, mild PKU, and mild hyperphenylalaninemia (MHP). Patients with MHP have no clinical symptoms and do not require dietary treatment.

PKU is included in newborn screening programs in all 50 states and is a classic example of a genetic disease that
meets the criteria for newborn screening: relatively high occurrence, availability of fast and economical screening methods, and therapeutic options. With early diagnosis and intervention, including a low-phenylalanine diet, the major disease phenotypes of mental and growth retardation can be prevented.

The PAH gene is located on 12q23.2 and spans a genomic region of 90 kilobases (kb). The coding region is about 4 kb and is comprised of 13 exons. More than 400 mutations in PAH have been reported to date, most of which are private mutations (http://www.pahdb.mcgill.ca/). The most prevalent European mutations, accounting for approximately two thirds of all mutations, are R408W (31%), IVS12 +1G→A (11%), IVS10–11G→A (6%), I65T (5%), Y414C (5%), R261Q (4%), and F39L (2%).

### Clinical Utility of Testing

Molecular diagnosis of PKU serves several purposes, including prognosis, confirmation of clinical and newborn screening results, carrier testing, prenatal diagnosis, and information for genetic counseling. The genotype-phenotype correlations can be used to direct the degree of restriction of phenylalanine in the diet (Table 8-1). Moreover, for patients with mild mutations in the BH4 cofactor-binding region (V190A, R241C, A300S, A313T, E390G, A403V, and P407S), overloading with BH4 can increase PAH activity and may be used as an alternative to dietary restriction. Prenatal diagnosis allows for the termination of an affected fetus or can ensure immediate therapeutic intervention after birth. Proper genetic counseling assists parents in making informed decisions.

### Available Assays

Several methods are currently used for the molecular detection of mutations in PAH associated with PKU. These methods include:

- Testing for a panel of common mutations with a detection rate of approximately 50%, depending on the number of mutations included.
- Mutation scanning of all 13 exons and the intron-exon junction regions. DNA sequencing detects approximately 94% of mutations; however, this method can be expensive. A recently developed system for mutation scanning, denaturing high-performance liquid chromatography (DHPLC), which also has a high detection rate (~96%) and is more cost-effective than DNA sequencing, may be the method of choice for PKU molecular testing.
- Finally, when molecular analysis fails to detect one or both mutant alleles, linkage studies can be performed and are highly accurate if polymorphic markers within or very closely linked to the PAH gene are used.

### Table 8-1. Genotype-Phenotype Correlations for the Most Common PAH Mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Prevalence</th>
<th>PAH Activity in COS Cells</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>R408W</td>
<td>31%</td>
<td>&lt;1%</td>
<td>Classic PKU</td>
</tr>
<tr>
<td>IVS12+nt1G→A</td>
<td>11%</td>
<td>&lt;1%</td>
<td>Classic PKU</td>
</tr>
<tr>
<td>IVS10–11G→A</td>
<td>6%</td>
<td>Not available</td>
<td>Classic PKU</td>
</tr>
<tr>
<td>I65T</td>
<td>5%</td>
<td>26%</td>
<td>Variant PKU</td>
</tr>
<tr>
<td>Y414C</td>
<td>5%</td>
<td>50%</td>
<td>Variant PKU</td>
</tr>
<tr>
<td>R261Q</td>
<td>4%</td>
<td>&lt;30%</td>
<td>Non-PKU PAH</td>
</tr>
</tbody>
</table>

Source: “PAH Activity in COS Cells” and “Phenotype” from the PAHdb Phenylalanine Hydroxylase Locus Knowledgebase [database online]. Available at: http://www.pahdb.mcgill.ca/.

### Interpretation of Test Results

The heterogeneity of the clinical phenotypes results mainly from the great variety of mutations in the PAH gene. Null alleles eliminate almost all the enzyme’s activity and cause classic PKU, while mutations with residual PAH activity result in milder forms. Like many single-gene disorders, genotype-phenotype correlations exist in most but not all cases. Environmental factors and/or modifier genes can also play a role in the clinical manifestations of the disease. The correlations of the most common mutations and their biochemical and clinical phenotypes are summarized in Table 8-1.

### UREA CYCLE DISORDERS (ORNITHINE TRANSCARBAMYLASE DEFICIENCY)

#### Molecular Basis of the Disease

Defects in the urea cycle constitute a rare group of disorders resulting in the accumulation of urea precursors, mainly ammonium and glutamine. Ornithine transcarbamylase (OTC) deficiency, the most common inborn error of ureagenesis, is an X-linked disorder. Affected hemizygous males typically present in the neonatal period or later in childhood, with symptoms that include vomiting, lethargy, hypothermia, apnea due to hyperammonemia, and leading to coma or death. Recurrent episodes of metabolic crisis can result in MR. The only available treatment after an acute metabolic episode is liver transplantation, which should be performed as early as possible to prevent brain damage. In 15% to 20% of carrier females, symptoms of disease are evident. Symptomatic carrier females typically have later onset but disease also may be fatal, presumably due to an unfavorable pattern of X-inactivation in the liver.