Metabolic Disorders

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Abstract

Inborn errors of metabolism represent a highly diverse group of genetic disorders. Although individually the disorders are rare, collectively they are estimated to affect as many as 1 in 600 individuals. This chapter discusses the molecular mechanisms of disease and the available genetic testing for selected metabolic disorders. Mutations in many of the metabolic diseases are genetically heterogeneous and diagnoses are still widely dependent on biochemical testing. DNA testing is critical for confirmatory studies, genetic counseling, carrier and prenatal testing, and 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. With increasing use of next-generation sequencing technologies in the clinical laboratory, DNA testing for confirmatory studies of all newborn screening positive results likely will be available in the near future.

Keywords

Inborn errors of metabolism • Molecular testing • Newborn screening • Phenylketonuria • Medium-chain acyl CoA dehydrogenase deficiency • Maple syrup urine disease • Ornithine transcarbamylase deficiency • Lysosomal storage disorders • Tay-Sachs disease • Gaucher disease • Galactosemia • Hereditary fructose intolerance • Glycogen storage diseases • von Gierke disease • Pompe disease • Cori disease • Andersen disease • Very-long-chain acyl-CoA dehydrogenase deficiency • X-linked adrenoleukodystrophy • Canavan disease

Introduction

Inborn errors of metabolism represent a highly diverse group of genetic disorders. Individually the disorders are rare. The most prevalent disorder, 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, intellectual disability (ID), organomegaly, and high morbidity. The 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.

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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 begin 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 USA and in several countries around the world. In the USA, 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 now screen for PKU, congenital hypothyroidism, galactosemia, maple syrup urine disease, (MSUD), homocystinuria, biotinidase deficiency, congenital adrenal hyperplasia, and tyrosinemia. Since the American College of Medical Genetics and Genomics (ACMG) Newborn Screening Expert Group's recommendation in 2006, tandem mass spectrometry has been added to newborn screening programs in all 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 positive 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. DNA testing often is critical for confirmatory studies, genetic counseling, carrier and prenatal testing, and 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. With increasing use of next-generation sequencing technologies in the clinical laboratory, DNA testing for confirmatory studies of all newborn screening positive results likely will be available in the near future.

Amino Acidopathies

Amino acids derived from protein sources within the diet are metabolized via specific pathways. Enzyme deficiencies within these pathways lead to distinct clinical manifestations of amino acidopathies, such as PKU and MSUD.

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, which encodes the rate-limiting enzyme of the pathway. 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 ID. 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 ID 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 with 13 exons. More than 600 mutations in *PAH* have been reported to date, of which approximately 81 % are point mutations, 14 % are small deletions or insertions and the remaining 5 % are gross deletions or duplications affecting one or multiple exons or even the entire gene (HGMD® Professional 2011.4) [1]. While the majority of the mutations are private mutations, seven of the most prevalent European mutations, p.R408W (31 %), c.1315+1G→A (11 %), c.1066-11G→A (6 %), p.I65T (5 %), p.Y414C (5 %), p.R261Q (4 %), and p.F39L (2 %), account for approximately two-thirds of all mutations [2].

Clinical Utility of Testing

Molecular testing for PKU serves several purposes, including assisting prognosis, confirmation of clinical and newborn screening results, carrier testing, prenatal diagnosis, and information for genetic counseling.

Available Assays

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

- Testing of a panel of common mutations detects up to 50 % of mutations, depending on the number of mutations included as well as the ethnic backgrounds of the patients.

- Denaturing high-performance liquid chromatography (DHPLC) detects approximately 96 % of mutations with scanning of the entire coding region of *PAH* [3]. However, the claimed detection rate is based on limited numbers of variants and does not necessarily reflect the real detection rate for each exonic position in the gene.
- Sanger sequencing, which is considered the gold standard for detecting small nucleotide changes, is utilized much more widely than DHPLC in clinical testing of the *PAH* gene. With high sensitivity and lower cost, Sanger sequencing should be the method of choice for mutation screening of the entire code region of the gene.
- Gross deletions and duplications, which usually cannot be detected by Sanger sequencing, have been reported in the *PAH* gene (HGMD® Professional 2011.4) [1]. The most commonly used methods to detect these types of mutations are array-based comparative genomic hybridization (aCGH), multiplex ligation-dependent probe amplification (MLPA), and quantitative PCR (qPCR). Finally, when molecular analysis fails to detect one or both mutant alleles, linkage studies using polymorphic markers within or very closely linked to the *PAH* gene may be considered.

Interpretation of Test Results

The variant interpretation guidelines from ACMG [4] should be followed, and include assessment of the evidence from in vitro studies, familial segregation data, or population data before a missense variant is classified as deleterious. Genotype may not be a good predictor of phenotype since environmental factors and/or modifier genes also can play a role in the clinical manifestations of the disease. For patients with mild mutations in the BH4 cofactor-binding region (p.V190A, p.R241C, p.A300S, p.A313T, p.E390G, p.A403V, and p.P407S), overloading with BH4 may increase PAH activity and may be used as an alternative to dietary restriction [5].

Maple Syrup Urine Disease

Molecular Basis of the Disease

MSUD is an autosomal recessive disorder characterized by a maple syrup odor in the urine and cerumen, and elevated branched-chain amino acids (leucine, valine, isoleucine, allo-isoleucine) in the blood. MSUD is caused by a deficiency of the branched-chain alpha-ketoacid dehydrogenase (BCKAD) complex. While it is relatively rare in the general population with an incidence of about 1 in 185,000 live births, due to founder effects, select populations such as the Ashkenazi Jewish and the Amish-Mennonites have a much higher incidence (1 in 26,000 and 1 in 380, respectively).

The BCKAD complex is comprised of four subunits; E1a, E1b, E2, and E3. Mutations in E1a (*BCKDHA*), E1b (*BKDHB*), or E2 (*DBT*) that give rise to MSUD are biochemically identical. Because the subunit E3 (*DLD*) also is shared with the pyruvate dehydrogenase and alpha-ketoglutarate complexes, a pathological defect in E3 also will cause lactic and/or metabolic acidosis, abnormal urine organic acids, and Leigh syndrome-like neuropathology, which are features not typically seen in MSUD. E3 deficiency also is called lipoamide dehydrogenase (LAD) deficiency and occurs at a higher frequency in the Ashkenazi Jewish population with two founder mutations.

Based on residual enzyme activity, an affected individual can be phenotypically classified as having classic, intermediate, or intermittent MSUD. Classic MSUD usually presents neonatally with poor feeding, lethargy, dystonia, and seizures. Intermediate types usually present with milder symptoms and later onset with developmental delays or ID. Intermittent types can have normal early development with acute, episodic mental status changes and/or psychosis during times of illness or stress. The treatment goal of protein and branched-chain restrictive diets is to maintain leucine, valine, and isoleucine at a therapeutic level and thus prevent neurotoxic effects from elevated metabolites. Liver transplantation is now a curative treatment for MSUD, with effective normalization of the branched-chain amino acid levels without dietary therapy. MSUD is included in newborn screening programs in all 50 states.

Table 11.1 shows the four genes implicated in MSUD, the protein involved, and notable pathological alleles. The p.T438N mutation on E1a has a carrier frequency of about 1 in 10 in the Amish-Mennonite population. The three common mutations on E1b found in the Ashkenazi Jewish population (p.R183P, p.G278S, and p.Q372X) have a combined carrier frequency of approximately 1 in 80 [6]. A pathological allele for LAD deficiency (p.G229C) has a carrier frequency of 1 in 94 in Ashkenazi Jews [7]. Excluding those with LAD deficiency, 45 % of patients have mutations in the *BCKDHA* gene, 35 % in the *BCKDHB* gene, and 20 % in the *DBT* gene [8].

Clinical Utility of Testing

Molecular testing for MSUD confirms clinical and newborn screening results. Carrier screening for the Mennonite and Ashkenazi Jewish populations is available and allows for preconceptual and prenatal genetic counseling and for immediate treatment of an affected infant after birth.

Available Assays

Targeted mutation analysis of the common alleles via multiplex PCR is a cost-effective and quick method for the Mennonite and Ashkenazi Jewish populations, with a mutation detection rate of over 98 % for *BCKDHA* and

Table 11.1 Genes, protein subunit, and founder alleles associated with MSUD

Gene	MSUD type	Locus	Size	Protein subunit	Exons	Founder mutations
BCKDHA	Ia	19q13	28 kb	E1a	9	p.T438N
BCKDHB	Ib	6q14	240 kb	E1b	11	p.R183P, p.G278S, p.Q372X
DBT	II	1p31	56 kb	E2	11	
DLD	III (aka. LAD deficiency)	7q31	30 kb	E3	14	p.G229C

BCKDHB, respectively [8]. For the general population, exon sequencing and deletion analysis of *BCKDHA*, *BCKDHB*, and *DBT*, and *DLD* are available.

Interpretation of Test Results

Mutations in *BCKDHA*, *BCKDHB*, and *DBT* do not have clear phenotype correlations, and treatment modality is essentially the same, regardless of the degree of biochemical phenotype. In a small study of 13 Ashkenazi Jewish families affected with LAD deficiency, homozygosity for the p.G229C allele appeared to be associated with a milder and later-onset disease, with no neurological manifestations [7].

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, and apnea due to hyperammonemia, leading to coma or death. Recurrent episodes of metabolic crisis can result in ID. 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 15–20 % of carrier females, symptoms are evident. Symptomatic carrier females typically have a later onset but the disease can be fatal, presumably due to an unfavorable pattern of X-inactivation in the liver [9].

OTC is a homotrimeric mitochondrial matrix enzyme that catalyzes the synthesis of citrulline from ornithine and carbamyl phosphate, and is found almost exclusively in the liver and intestinal mucosa. Loss of OTC activity results in high plasma glutamine and ammonium, low plasma citrulline, and an excess of orotic acid in the urine, a combined metabolic profile that is diagnostic for OTC deficiency. However, a direct assay of OTC activity performed on tissue isolated from a liver biopsy specimen may be necessary to obtain unequivocal biochemical results.

The *OTC* gene is located on Xp21 and spans a region of 73 kb that contains ten exons and encodes a protein of 354 amino acids. The overall prevalence of the disease is estimated at 1 in 50,000 in the USA, with similar statistics reported in Japan. Mutations have been identified in all ten exons; however, disease-causing mutations are less frequent in exons 1 and 7, the least conserved exons, most likely reflecting their lesser relevance to the function of the enzyme [10].

Clinical Utility of Testing

Diagnosis of OTC deficiency by molecular testing is preferable to the more invasive liver biopsy that is necessary for the enzymatic test. Molecular screening for *OTC* mutations identifies approximately 80 % of mutations, while the remaining undetected mutations are expected to affect promoter function or splicing [11]. The *OTC* gene has an approximately 50:1 sperm-to-egg mutation rate ratio [9]. From 66–93 % of male probands inherit the mutation from their mothers, while only 20 % of manifesting females inherit the mutation, taking into account differences in new mutation rates between sperm and eggs, and lyonization effects for a female to manifest symptoms. Thus, a woman who has a son with OTC deficiency has a much higher chance of harboring the mutation than a woman who has an affected daughter. Due to the inheritability and the severity of the disease with the limited treatment available, molecular screening of at-risk couples for the purpose of prenatal testing may be beneficial. Additionally, mutation identification may be of prognostic value in OTC deficiency (see "Interpretation of Test Results" below).

Available Assays

Molecular testing using bidirectional sequencing of the entire coding region and intron-exon boundaries of the *OTC* gene is available in a number of clinical molecular laboratories. In addition, MLPA is used to detect large *OTC* coding region deletions/duplications. High-resolution aCGH can be used to detect exonic deletions/duplications or larger rearrangements that include the *OTC* gene as part of a contiguous gene deletion syndrome [12]. Approximately 300

mutations have been reported (http://www.cnmcresearch.org/OTC/). Most mutations (86 %) in the *OTC* gene are point mutations, with G to A transitions accounting for 34 % and C to T transitions accounting for 21 % of the total. Approximately one-third of all point mutations are at CpG dinucleotides, and 15 % are at splice junctions. Although the CpG sites are recurrent mutation sites, none accounts for more than 4 % of the total single-base substitutions.

Interpretation of Test Results

In general, the genotypic spectrum correlates with the severity of the phenotype, and mutations that result in complete loss of function or amino acid changes near the active site of the protein result in neonatal onset of disease. In contrast, amino acid changes that are not close to the active site and result in protein with residual enzymatic activity are associated with later onset and a milder disease course [12].

Lysosomal Storage Disorders

Lysosomal storage disorders are a group of diverse inherited metabolic diseases that result from the disruption of the lysosomal system and catabolism of macromolecules (for review, see Ref. 13). Mutations in genes encoding hydrolyzing enzymes, activator proteins, lysosomal membrane proteins, or proteins involved in the posttranslational modification or transport of lysosomal proteins can cause such storage disorders. More than 40 lysosomal storage disorders are known, and they have a collective incidence of approximately 1 in 5,000–8,000 live births in the USA. Most of the genes responsible for lysosomal storage disorders have been cloned, permitting molecular testing once a diagnosis is established by biochemical analyses. This information is valuable for genotype-phenotype correlation, selection of therapy, and genetic counseling. In this section, two lysosomal storage disorders are discussed: Tay-Sachs disease (TSD), which serves as a model for population screening, and Gaucher disease (GD), for which much effort has been concentrated on genotype-phenotype correlations.

Tay-Sachs Disease

Molecular Basis of the Disease

TSD is a neurodegenerative disorder resulting from deficiency of the lysosomal enzyme hexosaminidase A (HEX A), resulting in accumulation of the cell membrane glycolipid G_{M2} ganglioside within lysosomes (for review, see Refs. 14, 15). The clinical course of TSD is characterized by normal development for the first few months of life followed by progressive loss of motor skills, macrocephaly, seizures, blindness, and death usually before 4 years of age. Infantile TSD always is fatal and there is no effective treatment. Later-onset forms of TSD have slower disease progression. TSD is an autosomal recessive disease and has a carrier frequency of approximately 1 in 30 among Ashkenazi Jewish individuals and 1 in 250–300 in most other populations. Genetically isolated populations such as the French Canadians of Quebec, Cajuns from Louisiana, and the Amish in Pennsylvania also have carrier frequencies similar to the Ashkenazi Jews. The first carrier screening programs began in 1970 and used the measurement of HEX A activity in serum, leukocytes, or tears. When the *HEXA* gene encoding HEX A was cloned in 1987, disease-associated mutations were identified. Current testing for TSD utilizes both biochemical and molecular testing by various methods.

Clinical Utility of Testing

Carrier screening for TSD, which began in the 1970s and was later endorsed by the American College of Obstetricians and Gynecologists (ACOG) and the ACMG, has been a model for population screening programs. As a result, there has been a 90 % reduction in the incidence of TSD in the North American Ashkenazi Jewish population, such that the incidence of TSD is now three- to fourfold higher in non-Jews by comparison.

Available Assays

Clinical laboratories use several strategies to incorporate mutation testing into their screening programs. Some laboratories initially screen by enzyme analysis followed by molecular testing for individuals with a result in the carrier or inconclusive ranges. Other laboratories use molecular testing alone for selected populations. Molecular tests are performed using a variety of methods, including PCR amplification followed by allele-specific primer extension analysis, allele-specific oligonucleotide (ASO) hybridization or restriction enzyme digestion, allele-specific amplification, TaqMan probe technologies, or ligation chain reaction amplification. In Ashkenazi Jewish individuals, two common mutations in *HEXA* are associated with infantile TSD and one associated with an adult-onset form of the disease. A four-base pair insertion (c.1274_1277dupTATC) in exon 11 accounts for approximately 80 % of mutant alleles in the Ashkenazi Jewish population, and a splice defect in intron 12 (c.1421+1G>A; IVS12) accounts for another 15 %. A missense mutation, p.G269S, leads to an adult-onset form of TSD and accounts for approximately 2 % of carriers.

Interpretation of Test Results

A pseudodeficiency allele, p.R247W, is present in approximately 2 % of Ashkenazi Jewish individuals who are carriers by the enzymatic assay. The p.R247W variant

decreases the activity of HEX A for the artificial substrate used in the laboratory but does not cause TSD since it does not affect HEX A activity for its natural substrate, GM2 ganglioside. About 36 % of non-Jewish individuals who are carriers by enzyme analysis have a pseudodeficiency allele (32 % p.R247W and 4 % p.R249W). In addition, screening for the three common Ashkenazi Jewish mutations and an additional mutation (c.1073+1G>A; IVS9) will identify approximately 95 % of Ashkenazi Jewish carriers, but only 40–50 % of disease-causing alleles in the non-Ashkenazi Jewish populations. Other populations who are at high risk, such as the French Canadians, Cajuns, and Pennsylvania Dutch, have their own common alleles. Therefore, the mutations included for population screening must target the specific mutations of the ethnic background of the individuals being tested.

Gaucher Disease

Molecular Basis of the Disease

GD is another prevalent autosomal recessive lysosomal storage disorder that is found with higher incidence in the Ashkenazi Jewish population. The carrier frequency is 1 in 15 in the Ashkenazi Jewish population and 1 in 100 in other populations [16] (for comprehensive review on GD, see Ref. 17). A defect in the enzyme glucocerebrosidase leads to the accumulation of glucocerebrosides in lysosomal compartments in macrophage/monocyte-derived cells, particularly in the liver, bone marrow, spleen, and lung. Several forms of GD exist. Type 1 GD has a wide range of clinical presentations, from asymptomatic to symptoms including bone disease, hepatosplenomegaly, anemia, and thrombocytopenia, but without primary central nervous system involvement. Types 2 and 3 have primary central nervous system involvement that varies by age of onset and rate of disease progression. Type 2 GD patients usually have an earlier age of onset than type 3 patients, with acute disease progression and death by approximately 2 years of age. Type 3 patients have onset in early childhood to adolescence and survive into their first to fourth decade of life. A perinatal-lethal form of GD also can occur, as well as a cardiovascular form characterized by aortic and mitral valve calcification, ophthalmologic abnormalities, and hydrocephalus.

The glucocerebrosidase gene (*GBA*) and its transcribed pseudogene (ψ*GBA*) are located on chromosome 1q21. Approximately 200 mutations causing GD have been identified. Many of the mutations are most likely due to gene conversion events with the pseudogene.

Clinical Utility of Testing

The demonstration of deficient glucocerebrosidase activity in leukocytes establishes a diagnosis of GD but is unreliable for carrier detection. Therefore, molecular testing is useful for carrier identification, prenatal testing, and genetic counseling.

Available Assays

Four mutations (p.N409S, c.115+1G>A, c.84dupG, p.L483P) are responsible for approximately 95 % of disease-causing alleles in Ashkenazi Jewish individuals and 50 % of disease-causing alleles in non-Ashkenazi Jewish individuals [16]. Most clinical molecular laboratories performing GD molecular testing assess at least these four mutations. Several factors must be considered when designing molecular testing for GD. Primers must be selected that avoid amplification of the pseudogene which is located 16 kb downstream and is approximately 96 % identical to the functional gene. Recombinant alleles, which are thought to result from unequal crossovers between exons 9 and 10 of the functional gene and pseudogene, contain two or more point mutations, including p.L483P. If p.L483P alone is tested, misdesignation of the genotype may occur. This may be important, as the recombinant allele is typically associated with a more severe genotype. Mistyping also is possible when a 55 base pair (bp) deletion in exon 9 (c.1263_1317del), is present in combination with the common p.N409S allele. Homozygosity of p.N409S would be observed even though the true genotype is p.N409S/55 bp deletion. Therefore, the 55 bp deletion should be analyzed in patients who are found to be homozygous for p.N409S.

Interpretation of Test Results

Genotype-phenotype correlations have been widely investigated in GD [18]. While overlaps occur, some generalizations can be made. The presence of a p.N409S allele is predictive of type 1 disease. Individuals with p.L483P in the presence of a null allele will usually have type 2 GD, while homozygosity for p.L483P typically results in type 3 GD. Homozygosity for the p.D448H allele has been associated with the rarer cardiovascular form of GD.

Disorders of Carbohydrate Metabolism

Galactosemia, fructosemia, and the glycogen storage diseases are discussed in this section, which addresses disorders of carbohydrate metabolism.

Galactosemia

Molecular Basis of the Disease

Galactosemia is an autosomal recessive disorder caused by deficient or absent activity of one of three enzymes involved in the metabolic pathway to convert galactose to glucose: galactokinase (GALK), galactose-1-phosphate uridyl transferase (GALT), and UDP-galactose 4′-epimerase (GALE). Classic

galactosemia is due to a severe reduction or absence of the GALT enzyme, and has an incidence of 1 in 40,000–60,000 in European newborns (for review, see Ref. 19).

The symptoms of classic galactosemia in neonates include poor feeding, vomiting, failure to thrive, lethargy, jaundice, occasionally diarrhea, and *E. coli* sepsis. The symptoms in an affected newborn can be obviated if a lactose-free diet is initiated within the first 2 weeks of life. Newborn screening for galactosemia is included in all 50 states. Newborns with a positive screen are followed up with immediate dietary treatment and confirmatory biochemical analysis.

The *GALT* gene is located at 9q13, is about 4 kb in length, and consists of 11 exons. More than 150 mutations in the *GALT* gene have been reported, most of which are private mutations [20]. The p.Q188R mutation is the most frequent mutation associated with classic galactosemia in many populations, and accounts for 64 % of disease alleles in Europeans, 60–70 % in Americans, and 50–58 % in Mexican Hispanics [20, 21]. Ethnic-specific mutations include p.K285N, p.S135L, c.253-2A>G, and a 5 kb deletion in Caucasian, African-American, Hispanic, and Jewish patients, respectively.

Clinical Utility of Testing

Molecular testing is used for confirmation of diagnosis, carrier detection, prenatal diagnosis, prognosis, and genetic counseling. Because the detection rate of molecular testing is less than 100 % and biochemical testing is highly accurate, mutation detection is carried out in parallel with biochemical analysis.

Prenatal diagnosis for galactosemia can provide the opportunity for immediate dietary restriction of the newborn. Although galactosemia is considered "treatable," symptoms such as ID, growth delays, speech dyspraxia, abnormal motor function, and premature ovarian failure in women may still occur even with early intervention and lifetime dietary restrictions. These long-term phenotypes often are associated with specific mutations; for example, p.Q188R can cause premature ovarian failure and speech dyspraxia. Genetic counseling is very important for parents of affected fetuses before a choice regarding pregnancy outcome is made.

Available Assays

Initial molecular testing for the diagnosis of galactosemia focuses on the most prevalent mutations or the p.N314D mutation associated with Duarte galactosemia (Duarte-2) by quick and cost-effective methods, such as multiplex PCR followed by restriction enzyme digestion [22]. If only one mutation or no mutations are found, screening of all 11 exons and exon-intron boundaries by DNA sequencing can be performed [22]. A detection rate of 96 % can be achieved by a combination of testing for prevalent mutations and DNA sequencing [23]. Gross deletions and duplications are detected by aCGH or MLPA.

Interpretation of Test Results

Genotype-phenotype associations have been established for some mutations [20]. For example, p.Q188R, p.K285N, and p.L196P alleles have undetectable GALT activity and are associated with severe phenotypes, whereas p.S135L and p.T138M are less severe and are usually associated with a good prognosis. In addition to the classic form of galactosemia, which has less than 5 % of the normal GALT activity, the Duarte-2 variant associated with the p.N314D variant has 50 % of the normal GALT activity. The p.N314D allele is in linkage disequilibrium with four polymorphisms, c.329-27G>C, c.378-24G>A, c.507+62G>A, and c.-119_-116delGTCA. The symptoms of Duarte-2 patients are mild compared to the classic type, but Duarte-2 still results in long-term phenotypes such as ID and growth delays, speech dyspraxia, abnormal motor function, and premature ovarian failure in women.

Hereditary Fructose Intolerance

Molecular Basis of the Disease

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder caused by deficient or absent activity of aldolase B. The aldolases are a group of tetrameric enzymes that are highly conserved and involved in the cleavage of fructose-1,6-biphosphate. Aldolase B also cleaves fructose-1-phosphate, and thus is a key player in fructose metabolism, as well as in gluconeogenesis and glycogenolysis. Aldolase A is found predominantly in muscles, whereas aldolase B is found predominantly in the liver, kidney, and intestine, and aldolase C is predominantly in the brain. Severe reduction or absence of only the aldolase B enzyme causes HFI, and has an incidence of 1 in 20,000–30,000 in European newborns (for review, see Ref. 24).

Typically in HFI, symptoms do not arise until an infant is exposed to fructose, sucrose, or sorbitol-containing foods, usually at the weaning of breast milk and introduction of table foods. Acute ingestion of the offending sugar can result in a variable presentation of abdominal pain, vomiting, progressive liver dysfunction, hypoglycemia, uric and lactic acidosis, and renal tubulopathy, depending on the dose of the sugar ingested, and timing and length of exposure. Chronic, low-dose ingestion of fructose-containing foods can cause a subacute picture of hepatomegaly and failure to thrive. Affected individuals typically have an aversion to sweets. The acute and chronic symptoms are, for the most part, reversible, if a fructose-free diet is initiated. Once on treatment, the prognosis is good with a normal life expectancy.

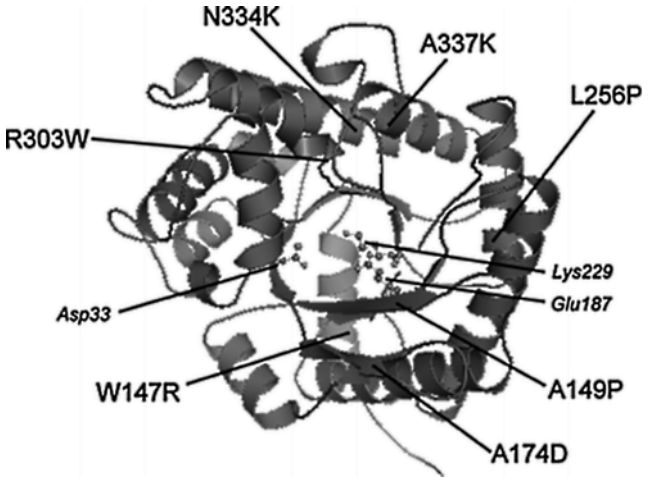

Figure 11.1 Location of some naturally occurring aldolase B mutations, shown on the crystal structure of human aldolase B (based on Protein Data Bank [PDB] structure file 1QO5, chain A) [26]. Only one monomer is shown for clarity. Active site residues are shown in a *ball and stick* representation and labeled in *italics*. Reprinted from Bouteldja N, Timson DJ. The biochemical basis of hereditary fructose intolerance. J Inherit Metab Dis 2010;33(2):105–12 [24], by permission of Springer

The *ALDOB* gene is located at 9q22.3, is about 14 kb in length, and consists of nine exons, eight of which are translated. More than 40 mutations have been identified (http://www.bu.edu/aldolase/HFI/hfidb/hfidb.html). The most common allele, p.A149P, accounts for 50–65 % of all mutations in the European population, and together with two other common mutations, p.A174D and p.N334K, account for more than 80 % of mutant alleles in the European population [25]. Most of the missense mutations are located in exons 5 and 9. Studies show that the p.A149P decreases the thermal stability of the enzyme. Figure 11.1 illustrates the locations of the most common variants in the aldolase B protein structure.

Clinical Utility of Testing

Molecular testing is used for confirmation of diagnosis, carrier detection, prenatal diagnosis, and genetic counseling. Enzymatic testing of aldolase B activity can only be performed from liver, kidney, or intestinal tissue. Thus, molecular testing is now the preferred method. Prenatal diagnosis for HFI can provide the opportunity for early dietary restriction and prevention of acute symptoms and liver disease.

Available Assays

Targeted mutation analysis of the common alleles using multiplex PCR is a cost effective and rapid method for the European population [27]. If only one mutation or no mutations are found, DNA sequencing of the entire coding region and exon-intron boundaries should be performed. For those not of European descent, DNA sequencing should be performed as a first-line method.

Interpretation of Test Results

Genotype-phenotype correlations are not clear. HPI patients with the same genotype have varied presentations and reactions to ingestion of fructose.

Glycogen Storage Diseases

Molecular Basis of the Disease

Glycogen storage diseases (GSD) are a group of heterogeneous genetic disorders characterized by the accumulation of glycogen in tissues and have an overall incidence of 1 in 20,000–25,000 live births [28]. Fourteen types of GSD that vary significantly in clinical phenotypes, age of onset, and affected organs have been identified (www.omim.org). They are caused by defects in one of 18 genes in glycogen metabolism. A summary of the 14 GSD types is presented in Table 11.2. The most common and severe types, GSD I, II, III, and IV, are discussed below.

GSD I (von Gierke disease) is characterized by hepatomegaly, kidney enlargement, growth retardation, hypoglycemia, hyperuricemia, and hyperlipidemia. GSD I has two major subgroups, GSD1a and GSD1b. The subgroup GSD1a has a deficiency of glucose-6-phosphatase (G6Pase), which converts glucose-6-phosphate to glucose and phosphate, the last step in glycogenolysis. The *G6PC* gene encoding G6Pase is located on 17q21. The subgroup GSD1b has a deficiency in glucose-6-phosphate translocase, encoded by *SLC37A4* gene located on 11q23. Common mutations vary in different ethnic groups [20]. The prevalent mutations for GSD1a in different ethnic groups are: p.R83C and p.Q347X in Caucasians; p.R83C in Ashkenazi Jews; c.459insTA and p.R83C in Hispanics; p.V166G in Muslim Arabs; p.R83H and p.G727T in Chinese; and p.G727T in Japanese. For GSD1b, two common mutations, p.G339C and c.1211delCT, are present in whites, while p.W118R is prevalent in Japanese.

GSD II, also known as Pompe disease, is a lysosomal storage disease caused by the inability to degrade glycogen due to defects in acid α-1,4-glucosidase. The phenotypes range from the most severe infantile disorder to juvenile- and late-onset adult myopathy. Patients with the infantile form usually die from cardiomyopathy before 2 years of age. Acid α-1,4-glucosidase is encoded by the gene *GAA* located at 17q25, and different forms of the protein are obtained by different proteolytic processing. Common mutations have been identified in different ethnic groups.

Patients affected with GSD III, also known as Cori disease, have symptoms similar to but milder than those associated with GSD I. GSD III is caused by mutations in the

Table 11.2 Glycogen storage disease types and subgroups

Disorder(s)	Defective enzymes	Gene symbol	Gene location	Inheritance pattern
GSD I (von Gierke disease)	GSD1a: Glucose-6-phosphatase, catalytic	*G6PC*	17q21.31	AR
	GSD1b: Solute carrier family 37 (glycerol-6-phosphate transporter), member 4	*SLC37A4*	11q23.3	AR
GSD II (Pompe disease)	Glucosidase, alpha acid	*GAA*	17q25.3	AR
GSD III (Cori disease)	Amylo-1,6-glucosidase, 4-alpha-glucanotransferase	*AGL*	1p21.2	AR
GSD IV (Andersen disease)	Glucan (1,4-alpha-) branching enzyme	*GBE1*	3p12.2	AR
GSD V (McArdle disease)	Phosphorylase, glycogen (muscle)	*PYGM*	11q13.1	AR
GSD VI (Hers disease)	Phosphorylase, glycogen (liver)	*PYGL*	14q22.1	AR
GSD VII	Phosphofructokinase, muscle	*PFKM*	12q13.11	AR
GSD IX (GSD VIII)	Phosphorylase kinase alpha 1 (muscle)	*PHKA1*	Xq13	XR
	Phosphorylase kinase alpha 2 (liver)	*PHKA2*	Xp22.13	XR
	Phosphorylase kinase, beta subunit	*PHKB*	16q12.1	AR
	Phosphorylase kinase, testis/liver, gamma 2	*PHKG2*	16p11.2	AR
GSD X	Phosphoglycerate mutase 2, muscle	*PGAM2*	7p13	AR
GSD XI	Lactate dehydrogenase A	*LDHA*	11p15.1	AR
GSD XII	Aldolase A	*ALDOA*	16P11.2	AR
GSD XIII	Enolase 3 (beta, muscle)	*ENO3*	17p13.2	AR
GSD XIV	Phosphoglucomutase 1	*PGM1*	1p31.3	AR

amylo-1-6-glucosidase (*AGL*) gene. GSD IV, also known as Andersen disease, is caused by deficiencies of the glycogen-branching enzyme encoded by the *GBE1* gene, resulting in abnormal and insoluble glycogen. Intracellular glycogen accumulations occur in the liver, brain, heart, skeletal muscles, and skin fibroblasts. Neonates with GSD IV appear normal at birth but develop hepatomegaly and failure to thrive in the first year of life. Patients develop progressive cirrhosis and usually die of liver failure by 2–5 years of age.

Clinical Utility of Testing

Due to the complex nature of molecular testing for GSD (large genes with numerous mutations), enzyme assays are usually used for the diagnosis of GSD. However, for prenatal diagnosis of GSD I, gene-based mutation testing or linkage analysis is the preferred method, since the enzymes are not present in amniocytes or chorionic villi, requiring a liver biopsy to obtain tissue containing the relevant enzyme. For prenatal diagnosis of other types of GSD, DNA testing can be used to complement and confirm biochemical results. Therapy for GSD I, II, III, and IV includes dietary management, and for GSD II, enzyme replacement is available (for complete review on treatment for GSD, see Refs. 28, 29).

Available Assays

Sanger sequencing tests are available clinically for all the GSD genes.

Fatty Acid Oxidation Disorders

During periods of fasting or prolonged aerobic exercise when glycogen stores are depleted, fatty acids become a main energy source by β-oxidation in the liver, and skeletal and cardiac muscles, via synthesis of acetyl-CoA and ketone bodies. The pathway for fatty acid oxidation occurs in the mitochondria and is complex, involving many steps. A number of disorders involving different enzymes in the pathway have been identified. Although the symptoms of the disorders have phenotypic overlap, several biochemical measurements can aid in the diagnosis of these disorders, including plasma carnitine levels which are usually low, plasma acylcarnitines, and urine acylglycines (for review, see Ref. 30). The most common of these disorders is medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. Since the addition of the acylcarnitine profile to newborn screening, the prevalence for very-long-chain acyl-CoA

dehydrogenase (VLCAD) deficiency is thought to be more common, and a new role for clinical molecular testing for VLCAD is emerging. At present, all 50 states within the USA employ tandem mass spectrometry in their newborn screening program, which allows detection of the abnormal plasma acylcarnitine profile characteristic of MCAD and VLCAD deficiencies.

Medium-Chain Acyl-CoA Dehydrogenase Deficiency

Molecular Basis of the Disease

MCAD is an intramitochondrial enzyme that is encoded by a nuclear gene. The normal function of MCAD is the initial dehydrogenation of acyl-CoAs with chain lengths of 4–12 carbons. Defective function leads to the accumulation of metabolites of the medium-chain fatty acids, mainly the dicarboxylic acids, acylglycine in urine, and acylcarnitine in plasma. These metabolites are at their highest concentration in the blood in the first few days of life, making the newborn period the ideal time for detection. Accordingly, the specificity of the newborn screening test is 100 %, as no false negatives have been reported. MCAD enzymatic activity also can be assayed in several different tissue types.

Because fatty acid oxidation fuels hepatic ketogenesis, the symptoms of MCAD deficiency appear after periods of prolonged fasting or intercurrent infections and include hypoketotic hypoglycemia, lethargy, seizures, coma, and, without treatment, death. Complications of the disease can include hepatomegaly, acute liver disease, and brain damage. The disease typically presents before 2 years of age but after the newborn period. However, individuals have been described who present with symptoms within the first few days of life as well as those who present as adults.

MCAD deficiency is an autosomal recessive disorder that is prevalent in individuals of northwestern European ancestry, with the highest overall frequency of 1 in 4,900 in northern Germany. The incidence in the USA is somewhat lower and is estimated to be 1 in 15,700. The MCAD gene, *ACADM,* spans a 44 kb region on chromosome 1p31 and contains 12 exons encoding a protein of 421 amino acids. A single founder mutation in exon 11, 985A>G, which results in the substitution of the acidic amino acid, glutamate, for the basic amino acid, lysine (p.K304E), represents 90 % of all alleles in the northern European population. However, recent studies of the US population, attributable to the expansion of newborn screening for MCAD deficiency, indicate that this mutation accounts for 79 % of the total mutant alleles in the US population (Table 11.3) [31]. The discrepancy between the two results is presumably due to the greater ethnic diversity of the US population.

Table 11.3 Genotypes of 57 MCAD-deficient newborns detected

Using MS/MS to screen more than 1.1 million newborns (Neo Gen Screening, Pittsburgh, PA, USA)	
Mutation position and type	**Number of patients identified**
c. 985 A→G/985 A→G	35
c. 985 A→G/199T→C (exon 3)	8
c. 985 A→G/deletion 343–348	2
c. 985 A→G/other[a]	5
c. 985 A→G/unidentified	5
c. 799 G→A/254 G→A	1
Unidentified/unidentified	1
Other mutations: seen with c. 985A→G	
c. 244 insertion T (exon 4)	
c. 362 C→T (exon 5)	
c. 489T→G (exon 7)	
IVS 5+1G→A	
IVS 8+6G→T	

Clinical Utility of Testing

Molecular testing for *ACADM* mutations usually is offered as confirmatory testing after the initial diagnosis of MCAD deficiency by biochemical testing. In addition, carrier testing for MCAD deficiency cannot be performed using biochemical metabolite profiles and must be done by molecular testing or by direct assay of MCAD activity in cultured fibroblasts.

MCAD deficiency is a disease that can be treated if promptly diagnosed in the early postnatal period. Precautions, such as avoidance of fasting and saturated fats and ingestion of carbohydrates prior to bedtime, can eliminate the symptoms and related complications of the disease. Although prenatal diagnosis on chorionic villus sampling or cultured amniocytes using biochemical or molecular testing, or both, is possible, with the inherent risks of the procedures, prenatal testing may offer no advantage to postnatal testing of acylcarnitines and other metabolites characteristic of the disease. Therefore, genetic counseling and discussion of the issues related to this disease are warranted when prenatal testing is being considered.

Available Assays

Initial molecular testing usually focuses on the high-prevalence p.K304E allele and is performed by PCR amplification followed by restriction enzyme digestion or other methods that can discriminate between single nucleotide changes, such as ASO hybridization or ligation chain reaction amplification. Clinical testing for this mutation is widely available. When an affected individual is found to be heterozygous for the p.K304E mutation or in the rare instance when an affected individual is negative for the mutation, all 12 exons of the *ACADM* gene are sequenced; however, relatively few laboratories offer *ACADM* gene sequencing. Additional mutations

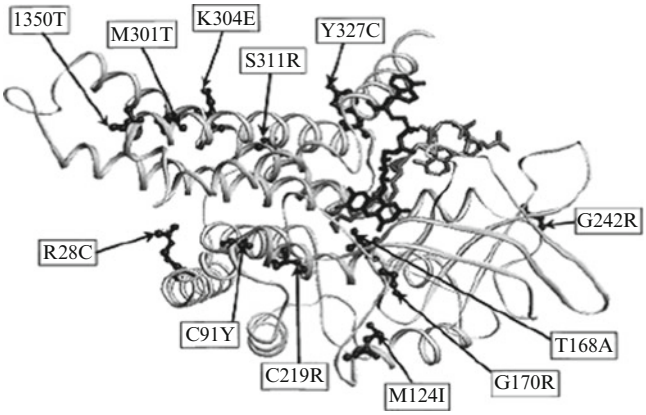

Figure 11.2 Schematic representation of a human MCAD monomer based on the crystal structure with cofactor flavin adenine dinucleotide (FAD) (*black*) and bound C8-CoA substrate (*darker gray*). The side chains for residues in which missense mutations have been published are shown in ball-and-stick representation. Only one of these mutations (T168A) is located in close proximity to the active site, forming a hydrogen bond to the flavin N(5) of FAD. Reprinted from Gregersen N, Andresen BS, Corydon MJ, et al. Mutation analysis in mitochondrial fatty acid oxidation defects: exemplified by acyl-CoA dehydrogenase deficiencies, with special focus on genotype-phenotype relationship. Hum Mutat 18(3):169–89 [32], by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc., ©2001

have been identified throughout the gene with no obvious mutation hotspot.

Interpretation of Test Results

The majority of mutations identified in *ACADM* are missense mutations located away from the active center of the enzyme, and are thought to affect the overall stability of the protein by affecting proper protein folding (Fig. 11.2) [32]. Most patients exhibit the classic MCAD phenotype; however, a small subset of patients has been identified that is compound heterozygous for the p.A304E mutation or for two other mutations, where at least one mutation is present that does not eliminate MCAD activity. These patients are much less likely to experience metabolic decompensation; however, even mildly symptomatic patients should avoid circumstances that could precipitate a metabolic crisis, since there is variability in age of onset even with classic MCAD deficiency.

Very-Long-Chain Acyl-CoA Dehydrogenase Deficiency

Molecular Basis of the Disease

VLCAD is an inner mitochondrial membrane enzyme encoded by the nuclear gene, *ACADVL*. VLCAD initiates the first reaction in β-oxidation of long, straight-chained acyl-CoAs of 14–20 carbons in length. Since the majority of dietary and endogenous fats are long chains, VLCAD function is an early crucial step for energy and ketone production from fat metabolism. Like MCAD deficiency, defective VLCAD activity leads to the accumulation of abnormal metabolites, mainly long-chain fatty acids and their conjugated moieties in the urine and plasma. Again, these metabolites are at their highest concentration in the first few days of life, due to the stressful and catabolic early neonatal period.

Because long-chained fats are the primary source of energy for the heart and during sustained skeletal muscle activity, the primary features of VLCAD deficiency, besides fasting hypoketotic hypoglycemia, are cardiomyopathy and skeletal myopathy with three main phenotypes. The severe, infantile-onset form presents with cardiomyopathy and arrhythmias which can lead to sudden death, hypotonia, and hepatomegaly. The childhood-onset form presents primarily with fasting hypoglycemia and liver disease, and rarely with cardiomyopathy. The late-onset form is mostly myopathic in presentation, with exercise intolerance, muscle cramping, and intermittent rhabdomyolysis during physical stress or times of illness. Patients are treated with a low-fat diet with supplemental medium-chain triglycerides, avoidance of fasting, intravenous glucose during hypoglycemic episodes, and intravenous fluids for rhabdomyolysis.

VLCAD deficiency is an autosomal recessive disorder with a prevalence of approximately 1 in 30,000. The VLCAD gene, *ACADVL*, is located on chromosome 17p13, is 5.4 kb in length, and contains 20 exons, encoding a protein of 701 amino acids. Unlike MCAD deficiency, there does not appear to be a prevalent mutant allele among a specific population. Mutations in all 20 exons have been reported.

Clinical Utility of Testing

An elevated C14:1-carnitine on the newborn screen taken at 48–72 h of life is highly indicative of VLCAD deficiency. However, borderline high levels can be equivocal in determining affected individuals from healthy carriers and normal neonates [33]. In addition, C14:1-carnitine levels can normalize with time, and attempts to confirm an abnormal acylcarnitine profile after 5 days can lead to a false-negative diagnosis [34]. Thus, molecular testing for *ACADVL* mutations is now offered as confirmatory testing of positive newborn screening results. VLCAD enzyme activity in lymphocytes or fibroblasts also is available. If two pathological *ACADVL* mutations are found, the diagnosis of VLCAD deficiency is confirmed. If only one mutation is found, an enzyme test using lymphocytes or fibroblasts is recommended.

Once diagnosis is confirmed, VLCAD deficiency is a disease that can be treated promptly in the early postnatal period

to avoid cardiomyopathy, liver disease, and sudden hypoglycemic coma. With an identified *ACADVL* mutation, genetic counseling and carrier and prenatal testing can be made available to the patient and family, and genotype-phenotype correlations can be deduced (see below).

Available Assays

Because mutations causing VLCAD deficiency are heterogeneous, molecular testing is done through sequencing and deletion/duplication analysis. Sequencing of all 20 exons has a mutation detection rate of 85–93 % for individuals with clinical disease [35].

Interpretation of Test Results

Deleterious mutations identified in *ACADVL* include missense mutations, deletions, insertions, and splice-site changes. Mutations that lead to a null mutation, as in a truncation of protein or a premature stop codon, result in elimination of enzyme activity, while missense or single-amino acid deletions can still confer residual enzyme activity. The presence of two null mutations correlates with the severe infant-childhood phenotype. Conversely, patients with a milder disease phenotype more frequently have missense or single-amino acid deletions [36].

Peroxisomal Disorders (X-Linked Adrenoleukodystrophy)

Genetic disorders of peroxisomal biogenesis and function have severe phenotypic consequences that often result in death in early childhood. A number of important metabolic processes, including β-oxidation of long- and very-long-chain fatty acids and the degradation of peroxide (H_2O_2), take place in the unique microenvironment of the single-membrane-bound matrix of the peroxisome. Whereas the symptoms of most metabolic diseases manifest after birth, disorders of peroxisome biogenesis, such as Zellweger syndrome, are associated with multiple congenital anomalies (for review, see Ref. 37). In this section, X-linked adrenoleukodystrophy (X-ALD) is highlighted, as it is the most common disorder.

Molecular Basis of the Disease

X-ALD is a severe, often fatal, disease that manifests in a progressive demyelination of the central nervous system, dysfunction of the adrenal cortex, and testicular dysfunction in hemizygous males. The most common form has an early onset that typically appears at 4–8 years of age and results in a progressive irreversible dementia and often death. Less severe presentations of the disorder include adrenomyeloneuropathy (AMN), which has a later age of onset, neurological complications that are limited to the spinal cord and peripheral nerves, and frequently includes adrenal insufficiency [38]. Although the disease is inherited in an X-linked recessive manner, up to 20 % of carrier females manifest late onset neurological symptoms similar to AMN. More than 93 % of X-ALD patients inherit mutations from their mothers, while the remaining 5–7 % carry de novo mutations; mosaicism has been reported in less than 1 % of patients [39]. The primary biochemical defect is impaired peroxisomal β-oxidation of fatty acids with the subsequent accumulation of very-long-chain fatty acids (VLCFAs), most notably C26, in the plasma and tissues. Therefore, X-ALD is not a disorder of peroxisomal biogenesis, but rather a specific defect of peroxisomal function. The accumulation of VLCFAs and the accompanying inflammatory response are thought to mediate the severity of the disease phenotype. In the great majority of hemizygous males (99 %) and approximately 85 % of carrier females, the plasma concentration of VLCFAs is elevated, a measurement that can be used as a diagnostic marker for the disease [40].

Defects in the peroxisomal membrane protein, ALDP, a member of the ATP-binding cassette family of molecular transporters, cause the severe juvenile form of X-ALD and its milder associated forms. The X-ALD gene, *ABCD1*, is located on Xq28, spans 19 kb, contains ten exons, and encodes a protein of 745 amino acids. The overall incidence of X-ALD and all variant forms is 1 in 15,000, making X-ALD the most common genetic determinant of peroxisomal disease. More than 1,000 different mutations have been found in the *ABCD1* gene, with the vast majority being point mutations, although deletions and duplications also have been identified (http://www.x-ald.nl/). In addition, mutations in all ten exons have been reported. No genotype-phenotype correlations are apparent, and wide phenotypic variation has been reported within families.

Clinical Utility of Testing

Molecular testing is most useful for determining the carrier status of at-risk women and for prenatal diagnosis, since 15 % of carrier females will not have elevated VLCFA levels and therefore will have a false-negative result by biochemical methods.

Available Assays

Molecular testing of the *ABCD1* gene is clinically available with many of the mutations identified as private mutations specific to a particular family. PCR amplification and SSCP or direct sequencing of all ten exons successfully identifies mutations in the majority of cases [41], whereas Southern blotting and MLPA can be used to assess deletion and duplication status. Complications can arise during PCR amplification due to the presence of paralogous gene segments of *ABCD1* spanning exons 7–10 on chromosomes 2p11, 10p11,

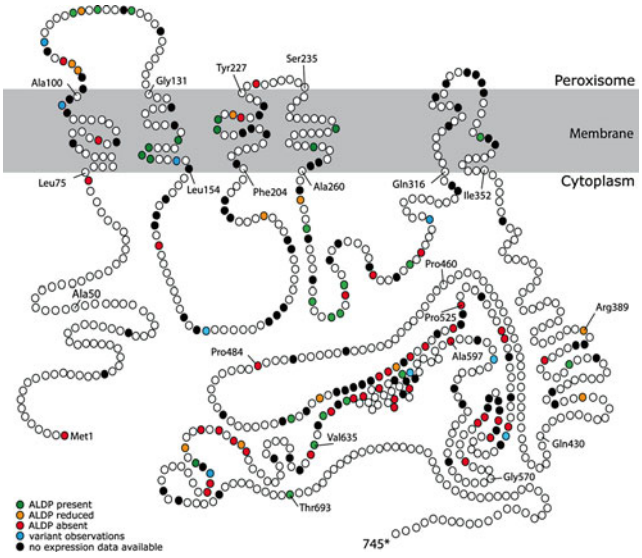

Figure 11.3 A hypothetical model of ALDP is shown. Individual amino acids are represented by *circles*. Missense mutations may affect the stability of ALDP. The effect of a missense mutation on ALDP stability by means of immunofluorescence (IF) in primary fibroblasts was investigated for >200 independent (non-recurrent) missense mutations. The results are presented in this figure. *Green circles* indicate missense mutations that do not affect protein stability; *red circles* those that result in no detectable ALDP (see remark below); *orange circles* represent mutations that result in reduced ALDP expression; *blue circles* indicate amino acid residues at which multiple missense mutations have been reported that result in different outcomes. The *black circles* indicate amino acids at which a missense mutation was identified, but for which no expression data are available. Overall, 69 % of all missense mutations result in reduced levels or absence of detectable ALDP [42]. All other mutations, including in-frame amino acid deletions and truncations near the carboxy terminus, result in the absence of detectable levels of ALDP. Reproduced from the Web site http://www.x-ald.nl/, with permission ©J. Berger and S. Kemp

16p11, and 22q11, but can be overcome by primer design that avoids amplification from the other chromosomes [41]. Although mutations have been identified throughout the entire *ABCD1* gene, a 2 bp AG deletion in exon 5 was found in 10.3 % of families with X-ALD and is therefore the most common mutation identified in the *ABCD1* gene [42]. Interestingly, this mutation, which is associated with all X-ALD phenotypes, does not represent a founder allele and is therefore a mutation hotspot within the *ABCD1* gene.

Interpretation of Test Results

The majority of mutations in *ABCD1* are missense mutations (61 %), with frameshifts and nonsense mutations accounting for 20 % and 10 % of mutation alleles, respectively, and amino acid insertions and deletions and whole exon deletions accounting for 4.6 % and 4 %, respectively. A recent study of the effects of missense mutations on ALDP stability demonstrated that approximately 70 % resulted in absent or reduced ALDP, indicating that most mutations in *ABCD1* result in complete loss of protein function (Fig. 11.3) [42]. These findings are consistent with the observed lack of genotype-phenotype correlation and lend support to the existence of additional genetic and environmental factors that modify the X-ALD phenotype.

Leukodystrophies (Canavan Disease)

The leukodystrophies are a group of degenerative metabolic diseases that involve the white matter of the brain, resulting predominately from disruption in the synthesis, transport, or catabolism of myelin. Examples include Krabbe disease and metachromatic leukodystrophy which affect lysosomal function and degradation of myelin, and Pelizaeus-Merzbacher disease, which is due to the abnormal synthesis of proteolipid protein. Another example, discussed in detail below, is Canavan disease, characterized by loss of axonal myelin sheaths and spongiform degeneration of the brain. Neurological deterioration in most leukodystrophies occurs after a period of normal development, and therapy is usually limited to the alleviation of symptoms.

Molecular Basis of the Disease

Canavan disease (CD) is an autosomal recessive disorder found mainly in Ashkenazi Jewish families and is caused by deficiency in the activity of the enzyme aspartoacylase (for review, see Ref. 43). The pathophysiologic relationship between the loss of this enzymatic activity and the development of CD remains to be elucidated. Diagnosis usually is established by the demonstration of increased levels of the substrate N-acetylaspartic acid in urine because enzymatic studies have been shown to be quite variable. Clinical symptoms associated with CD include macrocephaly, hypotonia, severe developmental delay, optic atrophy, poor head control, and death in childhood.

The gene encoding aspartoacylase (*ASPA*), located on the short arm of chromosome 17, is relatively small, with six exons spanning 30 kb of genomic sequence. Two point mutations, p.E285A and p.Y231X, are responsible for more than 97 % of mutant alleles in Ashkenazi Jews. Mutations in non-Jewish individuals are more heterogeneous; however, a panethnic mutation, p.A305E, accounts for 40–48 % of non-Jewish European alleles [44].

Clinical Utility of Testing

The genes responsible for many of the leukodystrophies have been cloned and characterized. Because the mutations in most of these genes are diverse, biochemical testing is still widely used for diagnosis, although molecular testing may be performed for carrier and subsequent prenatal testing. CD, however, occurs at increased frequency in the Ashkenazi Jewish population and screening for a limited number of specific mutations is feasible.

CD population screening of Ashkenazi Jewish individuals has demonstrated a carrier frequency of 1 in 40–59 (Refs. 45, 46 and our unpublished data). The carrier frequency for non-Jewish individuals has not been determined adequately, but it is far lower than that seen in the Ashkenazi Jewish population. As the carrier frequency is so high in the Ashkenazi Jewish population and the sensitivity of the assay is well above 90 %, ACOG and the ACMG recommended in 1998 that carrier screening for CD be performed preconceptually on couples with Ashkenazi Jewish ancestry.

Available Assays

A number of laboratories test for the two common Ashkenazi Jewish mutations, while some also test for p.A305E. A few laboratories also test for the less frequent non-Jewish mutation, c.433-2A>G. Testing methods commonly used for the detection of these mutations include PCR followed by ASO hybridization or restriction enzyme digestion, or allele-specific amplification. Miami Children's Hospital Research Institute holds a patent on the *ASPA* gene and CD testing, and requires laboratories to obtain a license, with a royalty fee for each test performed.

Interpretation of Test Results

If carrier screening indicates that both partners are carriers, prenatal testing should be offered. The preferred method of testing is DNA analysis of known mutations. For couples in which one partner is shown to be a carrier and the other partner is negative by targeted molecular testing, particularly if they are not Jewish, biochemical testing by measurement of the substrate in amniotic fluid is possible when the fetus has been shown to carry the one identified parental mutation.

Laboratory Issues

As discussed in this chapter, molecular genetic testing for metabolic disorders is used primarily as a follow-up to biochemical analyses for confirmation of findings, prognosis, carrier screening, or prenatal testing. Readers interested in learning which clinical laboratories perform individual tests should refer to the Genetic Test Registry (http://www.ncbi.nlm.nih.gov/gtr/). As testing for many of these disorders is performed in only a few laboratories, commercial test kits and proficiency testing is limited. Proficiency testing for TSD is available through the California Tay-Sachs Disease Prevention Program. For other disorders, interlaboratory exchange of samples is common practice. Several companies have developed assays for a panel of Ashkenazi Jewish disorders that include TSD, CD, and GD, among others. Although a large number of clinical laboratories offer Sanger sequencing and additional dosage sensitive methods for metabolic disorders, next-generation sequencing will allow assessment of large panels of genes related to metabolic disorders, though improved genotype-phenotype correlation will be needed before sequencing will replace biochemical testing as a first-line test.

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