Review

Inborn Errors of Metabolism
Advances in Diagnosis and Therapy

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Inborn errors of metabolism (IEMs) are a large class of genetic disorders characterized by disruption of cellular biochemical functions. Although individual IEMs are rare, collectively they represent a large and diverse class of genetic conditions, with new disorders and disease mechanisms being described regularly. Advances in the understanding of the molecular and biochemical etiologies of many IEMs via modalities such as whole-exome sequencing and metabolomics have led to significant progress in detection and treatment in recent years. In this review, we examine the current state of newborn screening for IEMs, recent advances in therapy for IEMs (including glutaric aciduria type I, urea cycle disorders, mitochondrial disorders, and lysosomal storage disorders), and opportunities for further exploration and discovery.

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Inborn errors of metabolism (IEMs) are a diverse class of genetic disorders involving abnormalities in biochemical cellular processes. Most IEMs are caused by defective enzymes, which result in insufficient or absent conversion of substrates into products. In many cases, problems arise because of accumulation of toxic upstream substances, the effects of reduced downstream essential compounds, or abnormal alternative substrate metabolism.

In recognizing the genetic and biochemical natures of alkaptonuria, cystinuria, pentorusia, and albinism, Garrod[1] developed the idea of "chemical individuality" and became the founder of the field of IEMs. Garrod presented his work entitled "Inborn Errors of Metabolism" in 1908 to the Royal College of Physicians as the Croonian Lectures.[1] This work was first published in 1909, and in the first chapter he eloquently stated,

With regard to the chemical composition of the tissues of living organisms, and the metabolic processes by which those tissues are built up and broken down... the progress of chemical physiology is teaching us that behind a superficial uniformity there exists a diversity which is no less real than that of structure although it is far less obvious.[1(p1)]

The depth to which these words hold true today is exemplified in the vast diversity of biochemical disorders that fall into the category of IEMs. One only has to look as far as the ever-expanding publication *The Online Metabolic and Molecular Bases of Inherited Disease (OMMBID)*[2] for evidence of the continuing growth of this class of disorders, which includes more than 90 chapters discussing IEMs.

Early Detection and Diagnosis via Newborn Screening

Newborn screening provides the opportunity to detect an IEM at an asymptomatic phase and perform medical interventions that positively alter the natural history of the disease. Newborn screening has had a dramatic effect on improving the outcomes in many IEMs, including phenylketonuria (PKU), maple syrup urine disease, and medium-chain acyl–coenzyme A (CoA) dehydrogenase deficiency. Successful newborn screening involves a coordinated system that includes education, evaluation, diagnosis, follow-up, and management.[3]

Massachusetts mandated the first test for newborns in the United States in 1963 for PKU using a metabolite-specific bacterial inhibition test developed by Guthrie and Susi[4] to detect elevated levels of phenylalanine in a dried blood spot. Since then, more than 150 million newborns have been screened for an increasing number of conditions.[5]

In the early 2000s, tandem mass spectrometry was introduced into many newborn screening programs. Mass spectrometry allows for rapid detection and quantification of a wide range of metabolites via identification of ion characteristics (ratios of mass to charge) and comparison with internal standards. Tandem mass spectrometry has replaced most individual metabolite screens and is universally used in state newborn screening programs for detection of disorders of fatty acid oxidation, organic acidemias, and amino acidopathies.[6]

The American College of Medical Genetics developed guidelines for determining if a disorder is suitable to be included in a newborn screening program. These guidelines include the following: the condition can be identified within 24 to 48 hours after birth (when the infant is generally clinically asymptomatic), the disorder has a test that is specific and sensitive, and early detection and effective treatment are proved to positively alter the natural history of the disease.[7]

In 2002, the American College of Medical Genetics recommended 29 conditions to be included in a core screening panel, which was named the Recommended Uniform Screening Panel.[8,9] The panel is continually under revision as new disorders are considered for inclusion. The most recent disorders added to the panel are severe combined immunodeficiency, which was adopted in 2009, and critical congenital cyanotic heart disease, which was adopted in 2010. Pompe disease (glycogen storage disease type II) is under consideration for inclusion. Other conditions have been considered by the advisory committee and were determined to be unsuitable for in-

clusion because they did not meet the above-described American College of Medical Genetics criteria, including Niemann-Pick disease types A and B, Krabbe disease, and others.[10] However, as new advances in detection and therapy are made, these conditions may be reconsidered.

Diagnosis

Once an infant is identified via newborn screening as potentially having an IEM, the diagnosis must then be confirmed by definitive testing. This testing process involves the same diagnostic procedures that are undertaken in an older child or adult with a suspected IEM and can involve biochemical testing, enzymatic testing, or molecular testing.

Biochemical diagnosis relies on identification of abnormally high levels of a substrate and its substrate by-products upstream of an enzymatic abnormality or abnormally low levels of the product of that enzyme or its downstream metabolites. This is often performed via mass spectrometry. The tests that can identify such biochemical substrates and products include plasma amino acid analysis, urine organic acid analysis, acylcarnitine profile analysis, carnitine analysis, acylglycine analysis, and specific analyte tests.

Direct testing for enzymatic function can be performed for some disorders such as very long-chain acyl-CoA dehydrogenase deficiency (VLCADD). For many of these disorders, there is an overlap between the normal, carrier, and affected enzyme function ranges. However, enzymatic function can be helpful in anticipating the severity of clinical disease in some disorders.

Alternatively, when biochemical testing can neither confirm nor rule out an IEM, molecular sequencing of the suspected abnormal gene can be diagnostic. However, on occasion, neither gene sequencing, enzymatic testing, nor biochemical testing can entirely rule out a disorder, and one must rely on the patient's clinical course.

An example of the complexities of ruling out a disorder suspected via newborn screening is that of VLCADD. Very long-chain acyl-CoA dehydrogenase deficiency is a disorder of mitochondrial fatty acid beta oxidation. This disorder can have a variable clinical picture, including infantile cardiomyopathy and sudden death, childhood hypoketotic hypoglycemia and liver dysfunction, and myopathy and rhabdomyolysis in later childhood to adulthood.[11,12] Timely and aggressive treatment can be critical in averting outcomes with high morbidity and mortality. On the newborn screen, VLCADD can be detected on the acylcarnitine profile with elevations of the C14:1 acylcarnitine.[13]

Follow-up biochemical tests in an affected individual may show elevations of the C14:1, C14, and C14:1 acylcarnitines but may also be without apparent abnormalities.[14] Follow-up molecular testing involves sequencing of the *ACADVL* gene (OMIM 609575), looking for 2 pathogenic gene mutations. However, in some clinically affected individuals, only 1 gene mutation can be found.[14] Even with early screening and diagnosis, long-term risks are not entirely clear.[15] It is often impossible to discern if a positive newborn screen for VLCADD represents a true-positive finding for clinically significant VLCADD, nonsymptomatic VLCADD, a genetic carrier for VLCADD (with no risk for clinical disease), or a false-positive result.[12] Therefore, oftentimes, even with newborn screening and follow-up testing, the clinical outcome is unclear.

At a Glance

- Most inborn errors of metabolism are caused by defective enzymes, which result in insufficient conversion of substrates into products. In many cases, problems arise because of accumulation of toxic upstream substances, the effects of reduced downstream essential compounds, or abnormal alternative substrate metabolism.
- Newborn screening provides the opportunity to detect inborn errors of metabolism at an asymptomatic phase and perform medical interventions that positively alter the natural history of the disease.
- Whole-exome sequencing and untargeted metabolomics are analytic modalities that are increasingly used in diagnosis and new disease discovery.
- Therapies for inborn errors of metabolism use several approaches to overcoming the biochemical aberrations caused by the primary metabolic block. These approaches include reduction of toxic metabolites via dietary modulation of enzymatic precursors, enhanced disposal of toxic metabolites, direct enzyme replacement, and novel compounds with direct pathway effects.

Advances in Diagnosis

The field of IEMs is constantly expanding, with new disorders and disease mechanisms being described regularly. Within the past year, abnormalities in phosphatidylcholine metabolism caused by mutations in *PCYT1A* (OMIM 123695) were shown to cause spondylometaphaseal dysplasia with cone-rod dystrophy.[16] Fatty acyl-CoA reductase 1 deficiency, due to mutations in *FAR1* (OMIM 616107), was shown to cause a peroxisomal disorder with severe intellectual disability, epilepsy, and cataracts, among many other new discoveries.[17]

Whole-exome sequencing (WES) and untargeted metabolomics are analytic modalities that are increasingly used in diagnosis and in new disease discovery. In a clinical setting, WES enables clinicians to evaluate approximately 20 000 genes in a single test when a genetic disorder is suspected but cannot be established by smaller-scale or more specific testing. Whole-exome sequencing uses the technology of next-generation sequencing to examine exons (the approximately 1% of the human genome that encodes for proteins). The patient's sequencing results are compared with various reference sequences, and the genetic variants that are found to differ from the reference sequences are evaluated for likely pathogenicity via tools that take into account previously reported mutations, protein structural disruption, and evolutionary conservation, among other properties.

Rates of diagnosis from WES range from 22% to 41% in cohort studies.[18-20] There are shortcomings in the technology used in WES that limit detection of some causative genetic mutations. Included among these issues is incomplete sequencing coverage of some exons because of the properties of their DNA (ie, exons with a high percentage of cytosine and guanine can be difficult to sequence), difficulty in interpreting sequence variants of unclear clinical significance, and poor detection of certain types of mutations that involve changes in DNA copy number.[19,21] In addition, WES will not detect variants in noncoding regions of the genome (regions that are not in exons), including introns, where some disease-causing mutations are known to exist.

Clinical WES studies differ from research WES studies in that, in general, clinical WES results are reported only for genes of known function and disease association. In contrast, research WES analysis is a discovery tool that can be used for novel gene identification, disease association, and further experimental validation.

With additional advances in understanding the complex nature of the nonexonic genome, whole-genome sequencing will increasingly be established as another tool for disease diagnosis for those genetic abnormalities not confined to the exons,[21] however, WES and whole-genome sequencing are inherently limited in their ability to identify causative gene variants in many genes involved in multifactorial disorders in which there are genetic and environmental contributors.

Metabolomics analysis involves the study and characterization of small-molecule metabolites in a biological sample and can provide an overview of the biochemical status of the individual from whom the sample was obtained. When metabolomics analysis uncovers abnormalities in the same pathway as genetic sequence analysis in a patient, this provides powerful evidence for the pathogenicity of the genetic sequence variant.

Targeted metabolomics analysis involves looking at a specific metabolite or group of metabolites thought to be related to a specific disease state, such as urine methylmalonic acid measurements and urine organic acid analysis, in an individual suspected of having methylmalonicacidemia. Untargeted metabolomics is a global survey of detectable metabolites without a presupposition of the associated disease state. Although clinical applications of untargeted metabolomics are not as well developed as WES or whole-genome sequencing, untargeted metabolomics analysis has shown progress in biomarker discovery and understanding biochemical disturbances in disorders such as chronic neuropathic pain.[22]

Successful Therapeutic Advances

Once an IEM is diagnosed through newborn screening or other means, the next challenge is determining the most effective therapeutic options. Therapies for IEMs use several approaches, all of which share a similar principle of overcoming the biochemical aberrations caused by the primary metabolic block. Such approaches include reduction of toxic metabolites via dietary modulation of enzymatic precursors, enhanced disposal of toxic metabolites, direct enzyme replacement, and novel compounds with direct pathway effects (**Figure**).

Dietary Modulation

Dietary therapy has been instrumental in successful treatment of glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I). Included in the Recommended Uniform Screening Panel, glutaric aciduria type I is caused by a defect in the breakdown pathway for tryptophan, lysine, and hydroxylysine. This defect leads to an abnormal accumulation of glutaryl CoA, which is trapped in the cerebral space because of limited efflux capacity across the blood-brain barrier and causes neurotoxicity.[23] Untreated children have striatal lesions and a subsequent movement disorder in approximately 90% of cases.[24] For many years, the mainstays of therapy were early metabolic interventions, including carnitine to promote glutaric acid clearance, a carefully controlled protein-limited diet when healthy, and a high-calorie and low-protein diet and aggressive hydration during times of illness. These interventions, which must be implemented before a neurologic crisis occurs, were shown to reduce basal ganglia injury from approximately 90% to 35%.[24]

In 2002, a mouse model for glutaric aciduria type I was developed,[25] and subsequent experiments with this model verified that glutaryl CoA and its derivatives become trapped in the cerebral space.[26] When the mice were administered a diet low in L-lysine (which is a precursor to glutaric acid) and high in L-arginine (which competes with L-lysine for transport at the blood-brain barrier), a lower concentration of glutaric acid was observed in the brain.[25]

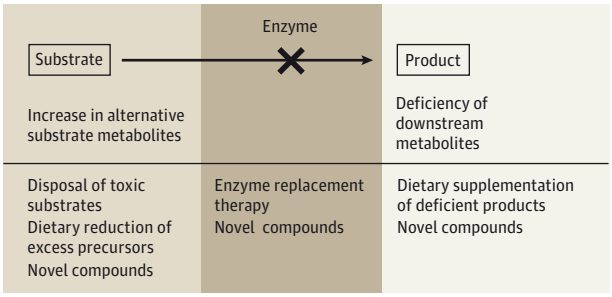
Based on this and other similar observations, a controlled, high-arginine, low-lysine diet was developed for patients with glutaric aciduria type I and, used in conjunction with carnitine and emergency sick-day interventions, resulted in a 36% reduction of neurologic risk.[23] In fact, the first 12 patients treated with this combination of interventions were healthy after 28 aggregate patient-years of follow-up, with appropriate growth and developmental milestones and no reported neurologic events.[23]

Toxic Metabolite Disposal

Urea cycle disorders are IEMs that result from abnormalities in the enzymes involved in the production of urea from waste nitrogen, resulting in hyperammonemia. Depending on the severity of the defect, these disorders can manifest in the neonatal period with severe hyperammonemia and acute cerebral edema or later in life.[27,28] In late-onset disease, the hyperammonemia may be less severe and the symptoms more subtle than in the patients with early-onset disease.[27,28] Avoidance of hyperammonemia through dietary protein control, prevention of catabolism, and alternative nitrogen disposal mechanisms is the mainstay of treatment.[28]

In 1979, Brusilow and colleagues[29] introduced the idea of treating urea cycle disorders by using alternative biochemical pathways to eliminate excess nitrogen. This led to the use of oral sodium phenylbutyrate as a maintenance medication for disposal of excess nitrogen, intravenous sodium phenylacetate (the active, metabolic product of sodium phenylbutyrate), and sodium benzoate, used in conjunction with arginine hydrochloride for emergency management of hyperammonemia.[28,30] In situations where ammonia levels cannot be controlled by the above interventions, hemodialysis is used.

Figure. Therapeutic Approaches in Inborn Errors of Metabolism



Therapies for inborn errors of metabolism are used to correct biochemical aberrations caused by an enzymatic block. Such approaches include reduction of toxic metabolites via dietary modulation of enzyme precursors, enhanced disposal of toxic metabolites, direct enzyme replacement, and novel compounds with direct pathway effects. These novel compounds can affect any part of the enzyme pathway or have other indirect cellular targets. The "X" indicates the enzyme at that step is not functional.

Another prodrug of phenylacetate, glycerol phenylbutyrate, was recently developed. Glycerol phenylbutyrate is composed of 3 molecules of phenylbutyrate linked to a glycerol backbone. This compound is hydrolyzed by pancreatic lipases in the small intestine to release phenylbutyrate.[31] It has a slower release than sodium phenylbutyrate, resulting in favorable pharmacokinetics and superior overnight ammonia control.[31,32] In addition, this preparation is sodium free, tasteless, and available as an oral liquid, while sodium phenylbutyrate is in a powder or tablet form and has an unpleasant taste. The clinical outcomes include a 40% reduction in hyperammonemic crises and improved executive functioning in the pediatric population.[32]

Enzyme Replacement Therapy

Enzyme replacement therapy (ERT) is an approach to treating specific IEMs, including lysosomal storage disorders (LSDs), that replaces a deficient or absent enzyme. This treatment is not curative of the underlying genetic defect but is instead an ongoing, supportive therapy typically given via regular intravenous infusions. Enzyme replacement therapy is available for Gaucher disease, Fabry disease, Pompe disease, and mucopolysaccharidosis types I, II, IV, and VI and is under development in other disorders.[33]

Enzyme replacement therapy for Pompe disease exemplifies how this therapeutic approach can have a dramatic effect on the natural history of an LSD. Pompe disease is an autosomal recessive disorder that is caused by a deficiency of acid α-glucosidase and leads to abnormal accumulation of glycogen in the lysosome. The disease represents a spectrum of disease depending on the amount of residual enzyme activity, and 2 classic phenotypes of infantile and late-onset forms have been described, although there are also intermediate phenotypes. Individuals with the infantile form have severe cardiorespiratory disease and hypotonia and typically die of their disease by age 1 year.[34] The late-onset form can manifest with variable rates of progression and clinical presentations, including an isolated elevation in creatine kinase, muscle weakness, and acute respiratory abnormalities.[34,35] Before 2006, therapy for Pompe disease was largely palliative.[34]

Enzyme replacement therapy with alglucosidase alfa was approved for use in Pompe disease in 2006. Clinical trials in individuals with the infantile form have shown improved overall survival, ventilator-free survival, heart disease, and motor development.[34,35] Patients with late-onset disease have been shown to have disease stabilization with improvement in motor and pulmonary function.[35] Furthermore, earlier initiation of therapy in the infantile form of this disease is associated with better health outcomes.[34] Therefore, Pompe disease is a strong candidate for inclusion in the Recommended Uniform Screening Panel and, as mentioned above, is under consideration.[36]

One of the major drawbacks of ERT is that the replacement enzymes do not cross the blood-brain barrier and do not alter neuronopathic features. In addition, other somatic clinical effects of certain mucopolysaccharidoses, including cardiac valve disease and skeletal disease, do not usually improve if pathologic changes have already occurred before the initiation of ERT.[37] Nonetheless, ERT offers a successful therapeutic approach to the somatic effects of several LSDs, and vigorous research in this field is ongoing.

An alternative approach to treatment in LSDs is substrate reduction therapy, which uses small molecules to inhibit an enzymatic step upstream of the formation of abnormally accumulated molecules. One of these small molecules, miglustat, inhibits glucosylceramide synthase, which catalyzes the synthesis of glycosylceramide, the precursor to the accumulating substances in some LSDs.[38,39] Miglustat, an oral drug, is able to cross the blood-brain barrier and can potentially mitigate the neuronopathic features of some LSDs nontreatable via ERT. Miglustat has been shown to have positive effects on some neuronopathic features in Niemann-Pick disease type C but no measureable effect on the neuronopathic features of other LSDs such as Gaucher disease type 3.[40]

Novel Compounds

Mitochondrial disorders represent a broad array of IEMs. Mitochondria are present in every cell of the body, with the exception of mature red blood cells, and are responsible for oxidative phosphorylation, programmed cell death, and fatty acid oxidation, among many other critical cellular functions. Mitochondrial disorders can result from abnormalities of the 37 genes encoded in the mitochondrial DNA (mtDNA) itself or from abnormalities of nuclear encoded genes that have a role in mitochondrial function, including mtDNA replication and maintenance, mitochondrial protein translation, and mitochondrial membrane maintenance and transport.[41]

Although mitochondrial disorders are common among the classes of IEMs, specific targeted therapies have proved elusive. This is in part because of the vast diversity of this group of diseases (in clinical presentation and genetic origin) and a limited understanding of the precise nature of the organelle dysfunction. However, oxidative stress has long been recognized as a consequence of many mitochondrial disorders, and various antioxidants (eg, coenzyme Q_{10}, vitamin E, and others) have been used in an attempt to modulate this stress.[42]

EPI-743 is a parabenzoquinone analogue that targets reduced intracellular glutathione and has protective activity against cellular oxidative stress.[43,44] It has been shown to affect the natural history of several mitochondrial disorders. In a trial of EPI-743 among 10 children with Leigh syndrome, the patients experienced a significant improvement in all measures of outcomes, including the Newcastle Pediatric Mitochondrial Disease Scale, Gross Motor Function Measure, and PedsQL Neuromuscular Module.[45] In a trial of EPI-743 among 5 patients with Leber hereditary optic neuropathy, 4 patients had arrested disease progression and vision improvement; in fact, in this disease previously thought to be irreversible, 2 of the 5 patients showed a total recovery of visual acuity.[46] Other, larger clinical trials with EPI-743 are in progress.

EPI-743 and other compounds are being evaluated in trials that target redox abnormalities associated with mitochondrial dysfunction. They hold the potential for therapeutic advances in the field of mitochondrial medicine that have not been seen previously.

Conclusions

Inborn errors of metabolism are a diverse class of genetic abnormalities affecting the biochemical processes of cells. Advances in IEMs range from early detection via newborn screening, to new therapies that include progress in dietary modulation, to novel compound development and enzyme replacement therapeutics, to diagnosis via WES and untargeted metabolomics analysis. The study and treatment of IEMs represent a rapidly advancing field filled with opportunities for discovery.

ARTICLE INFORMATION

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