VOLUME - XVI
ISSUE - XCVI
NOV/DEC 2019

The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

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Editorial

Newborn screening is a form of preventive health care in which babies are tested within the first days of their life to discover evidence of diseases for which the principal symptoms may not yet be apparent.

In order for screening to be successful a simple and reliable test must exist. Also, there must be a treatment that makes a difference when the disease is detected early.

PRIMARY NEWBORN SCREENING DISORDERS

- Biotinidase Deficiency
- Congenital Hypothyroidism
- Galactosemia
- Congenital Adrenal Hyperplasia
- Sickle Cell and other Hemoglobinopathies
- Severe combined immunodeficiency (SCID)
- Cystic Fibrosis
- Phenylketonuria (PKU)
- Glucose-6-phosphate dehydrogenase deficiency (G6PD)
- Duchenne muscular dystrophy (DMD)

FATTY ACID OXIDATION DISORDERS

- Carnitine Uptake Defect (CUD)
- Long Chain L-3 hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)
- Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)

ORGANIC ACID DISORDERS

- 3-Hydroxy-3-Methylglutaric Aciduria (HMG)
- Glutaric Acidemia Type I (GA I)
- Isovaleric Acidemia (IVA)
- 3-Methylcrotonyl-CoA Carboxylase Deficiency (3-MCC)

AMINO ACID DISORDERS

- Argininosuccinic Aciduria / Citrullinemia
- Homocystinuria (HCY)
- Maple Syrup Urine Disease (MSUD)
- Tyrosinemia Type-1 (TYR I)

LYSOSOMAL STORAGE DISORDERS

- Lysosomal storage disorders (LSDs)

This is a special issue as Tulip Diagnostics is launching complete system for seven New Born Inborn errors of metabolism. As this protocol has been a standard protocol the world over but somehow wasn't mandated of all live births in India and other third world nations.

We present to you a cogent write up on INHERITED METABOLIC DISORDERS and the status of socio-clinical-diagnostic aspects of NEW BORN SCREENING. Yes, BOUQUET hasn't been forgotten. TULIP NEWS proudly announces our new arrival in the FAMILY - BORNSAFE. Hurrah !

INHERITED METABOLIC DISORDERS

Overview

The field of inherited disorders of the nervous system has undergone major revolutions in the past 150 years. The 19th century saw the first systemic approach to disease through the use of rational, consistent outlines for taking histories and doing physical examinations. Scientific methods were applied to pathology and clinical medicine because of discoveries in physics and chemistry (both organic and inorganic). The discoveries led to knowledge of the organic chemistry of dyes, tissue staining, and improved microscopy. The blood pressure apparatus, thermometer, stethoscope, tuning fork, and later, the reflex hammer were added to the clinician's armamentarium. With these tools, physicians and pathologists (often the same person) were able to apply Sir Francis Bacon's dictum to medicine: "inquire carefully into the origin of things."

Garrod summarized the initial discoveries of the 19th century and the turn of the 20th in his book, Inborn Errors of Metabolism, some 80 years ago. By the mid 1960s, defects that led to the accumulation of metabolic products in the urine, blood, or neural tissues were identified. These defects were largely problems in the catabolism of lipids and amino acids or in the rapid breakdown of glycogen. The identification of the metabolites that accumulated in a disease made possible the identification of the enzyme whose activity was deficient.

Although direct identification of abnormal protein structure still was not possible, such defects could be inferred, indirectly, by demonstrating alterations in the kinetic properties of patients' enzymes (ie, rates of reaction as concentrations of cofactor or substrate were varied) or in the rate at which heating the enzyme altered its catalytic properties. Such changes allowed a strong inference of a change in the structure of the enzyme-protein. A change in structure was in those days thought to be due to a change in an amino acid somewhere in the peptide chain of the protein. In the case of some hemoglobin variants, demonstrating substitutions of one or another amino acid was actually possible.

Over the next 2 or 3 decades, errors in glycolysis, the Krebs cycle, and adjacent pathways were elucidated by such methods, although some of these errors are debated still. By the mid 1980s, techniques largely had switched from those of the biochemistry of intermediates and enzymes to the identification of mutations in genes. This was done by a large number of techniques that make use of DNA fragments (restriction fragment-length polymorphisms) so as to permit linkage mapping and gene sequencing. As a result, we now know many genetic defects responsible for neurological disease, but frequently we do not know much about the resulting protein product and therefore the pathophysiologic basis for the disease.

Pathophysiology

The human genome at one time was estimated to have 70,000-100,000 genes. New data from the Human Genome Project suggest this number may be closer to 30,000. Many of these appear to code for proteins produced in the brain. Data from the Human Genome Project surely will be useful in identifying mutations in the thousands of genes that must underlie inherited diseases of the central and peripheral nervous system. Genetic data also will be useful in identifying mutations and polymorphisms that predispose to some of the acquired diseases of the nervous system, some of which are discussed in this article. One of the major principles of pathophysiology that has appeared in recent decades is that many acquired diseases have one or several genetic bases (predispositions). Another principle is that diseases often appear only after 2 or 3 things go wrong. Some examples of each are discussed in this article.

Until the genes and their mutations that underlie neurological disease are characterized, inherited disorders have to be defined the way clinicians have been classifying disease over the last 2 centuries. These classifications make use of clinical descriptions in the living patient correlated with pathologic changes found at autopsy; with chemical changes in excreta, blood, cerebrospinal fluid (CSF), and tissues, or sometimes with abnormalities found on images of the brain and other organs. The methods of genetic analysis first described by Mendel and the correlations with human disease first collected by Garrod were fundamental to the knowledge of inherited diseases that was acquired in the latter two thirds of the 20th century.

Until the end of the 1970s, only a few methods were available for investigating inherited disease. They included the following:

- Identifying the patterns of inheritance of a syndrome in families as well as unique populations
- Correlating syndromes with pathologic or chemical changes in tissues and fluids
- Correlating syndromes with increased or decreased concentrations of proteins or alterations in enzyme activity in the body

At that time, however, correlating disease states with amino acid substitutions in specific proteins or peptides was not possible, except for hemoglobin variants: these were well characterized.

Diseases are intellectual constructs, not reality. They are constructs whose definition can change depending on the speaker, the audience, and most importantly the historical era in which the disease is described. Names of diseases are useful ways to tie what is being said to the medical technology and assumptions of the era in which the statement about an illness is being made. However, the description and definition of a disease each can change as technology improves and understanding of the pathophysiological processes evolves.

Even the basic name of a disease can change as decades (and even centuries) pass. This is particularly true in the last century or two. What was called idiocy in the mid-nineteenth century became subdivided into terms such as amaurotic idiocy, which was later named the following: Tay Sachs' disease, Tay-Sachs disease, generalized gangliosidosis, generalized GM2 gangliosidosis, infantile type with McKusick classification 230500. The current disease name is severe deficiency of hexosaminidase A with infantile onset of symptoms.

The reality of medicine is the sick patient. The definition of a specific disease has less correlation to the real world than the construct of species has in biology. This is true even for genetic diseases, although genetic diseases superficially appear to be linked closely to a specific cause, a specific mutation. That this is not always true is discussed later in the article, under Polymorphism With and Without Disease.

Another critical issue in the definition of disease is that having a genetic defect that may result in a disease is not the same as having the disease. Having the defect means only having a propensity or a risk of developing the disease. Whether the problem is an enzyme deficiency in carbohydrate metabolism or the excessive triplet repeats that characterize the mutation for Huntington chorea does not matter. The biochemical or genetic abnormality is no more a definition of a disease state than a positive result on a purified–protein-derivative (PPD) skin test is for tuberculosis. The issue is not an academic one. In view of the desire of insurance carriers to avoid pre-existent illnesses, the issue of

when someone gets a disease is currently a matter of intense ethical and legal concern. One gets a disease when one has symptoms, not merely signs, that point to the disease, and not before that time.

Watson and Crick's work, and the work of both their acknowledged and unacknowledged collaborators in the 1950s, led to the unraveling of the genetic code, the recognition that the code of both DNA and RNA had to be read from a specific end of the macromolecule, the understanding that both DNA and RNA sequences are decoded in triplets, and the recognition that a triplet codes for only one amino acid. These triplets are called codons. (Later work showed that some codons do not code for an amino acid. Some mark the beginning or end of a peptide chain.) Molecular biologists do not know the function of some long sequences of codons called introns. Once we understood clearly how DNA codes for a protein, we recognized that an amino acid substitution in a protein must result from a change in the mRNA triplet as a result of a mutation in the corresponding DNA (the gene).

At first, clinical and biochemical investigations led to recognition of the consequences of altered protein products and to an understanding of how these might produce disease. The new molecular techniques led to a reverse approach. Alterations in DNA were traced to RNA changes. Science is only now on the verge of explaining some of these as changes in proteins. This approach from DNA back flourished in the 1980s and 1990s as various methods were developed that showed alterations in the structure of nucleic acid chains independent of other aspects of biochemistry or biology.

One technique, called restriction fragment length polymorphism (RFLP) analysis, includes cutting DNA into fragments by using one or more bacterial enzymes, each with a specific nucleic acid recognition site that directs where the DNA is to be cut. The lengths of the fragments then are measured on the basis of their migration within a gel when exposed to an electrical current. Another technique, polymerase chain reaction (PCR), converts traces of DNA into large amounts of identical material. Scientists can analyze the genetic material once it has been magnified this way. These techniques have totally changed research on inherited diseases. They are similar to the methods society currently uses to identify each of us as individuals (by DNA fingerprinting) and to infer how we and other species may have evolved (by analyses of nuclear and mitochondrial DNA in individuals, even individuals dead for millions of years, and in species and in populations of species).

Genetic mutations have been presumed to be the basis of inherited disease since the time of Mendel and Garrod. Even now, some diseases that have been recognized for 100-150 years have known genetic defects but the protein products are not well characterized. Chediak-Higashi syndrome is an example of such an inherited metabolic defect; Huntington chorea a much better-known example in inherited diseases of the CNS. Many genetic diseases of the nervous system can be diagnosed accurately by DNA analysis, and the pattern of inheritance can be demonstrated within families. The molecular tools sometimes allow us predict who is likely to develop the disease and who in the family can neither develop it nor pass it on to offspring. With recent advances in enzyme replacement therapy and gene therapy, we may someday be able to treat or perhaps even prevent some of these disorders even if we do not know how the genetic change gives rise to the disease.

Metabolism is the physiological and biochemical mechanisms by which foodstuffs are taken in by the body and converted from one form to another to provide energy for all the activities of the body. Metabolism includes the methods our cells use to build multitudes of specific molecules that the body uses for its myriad activities. Some of these are small hormones or neurotransmitters, others are large enzymes or constituents of cell structures that, with several long chains of lipid, make a key part of a membrane or, with long chains of several peptides, make up a single functional protein. Metabolism includes not only the mechanisms for building molecules, but also the degradation processes that enable cells to excrete waste products. In its broadest sense, metabolism encompasses virtually every biochemical pathway and biophysical mechanism in the body and the resultant physiologic activities.

In a more limited sense, inborn errors of metabolism can be defined as disorders of the mechanisms by which specific major foodstuffs are converted to energy or cellular and tissue building blocks and final products and the mechanisms by which foodstuffs and products are degraded to be excreted. These include mechanisms involving absorption and modification of vitamins and minerals; mechanisms for degrading molecules to provide energy or to be excreted; mechanisms for making acetyl-coenzyme A, nonessential amino acids, cholesterol, long-chain fatty acids, prostaglandins, and the complex lipids they lead to; mechanisms for making the proteins that are the structure of cells, inside and out, and that are the prime catalysts of cellular chemistry, enzymes; and mechanisms for neutralizing molecules that represent potential environmental toxins.

Biochemical and biophysical processes that are related closely but are not included in the term metabolism include the following:

- Mechanisms for recognizing certain molecules, especially the large macromolecules, by receptors on the outer membranes of specific cells
- Mechanisms by which the cells react to external or internal molecules by changing the cell membrane properties (eg, opening ion pores), phosphorylating proteins within the cytoplasm, or forming vesicles to engulf and ingest macromolecules, aggregates, and even viruses and bacteria
- Mechanisms for moving molecules, macromolecules, and subcellular organelles such as mitochondria from one part of the cell to another, as in the cytoplasmic flow on the railroads of actin in the axons of neurons
- Mechanisms for preserving cell structure through the cytoskeleton
- Mechanisms for changing cell shape or structure for the benefit of the organism (eg, amoeboid movement, beating of flagella, contraction of muscle fibers, exocytosis of digestive enzymes by the cells of the mucosa of the gut and of vesicles of neurotransmitters by the synaptic swellings at the end of axons, even the endocytosis of hormones)

The distinction between these processes and what traditionally has been considered metabolism is arbitrary since they are clearly interdependent cellular functions. Ingesting a macromolecule is not considered part of metabolism but breaking it down in the resultant lysosome is. The ever-expanding knowledge of the genetic basis of neurological disease will probably blur the distinction more. The distinction initially was defined on the basis of a limited understanding of inborn errors from a physiological rather than molecular perspective.

Clinical Features and Differential Diagnosis

Inherited diseases affect virtually all parts of the nervous system. Many of these disorders also exist in sporadic forms that do not seem to have a primary genetic cause. In time, clinicians may decide to rename these conditions so that the genetic and nongenetic forms are differentiated clearly.

Examples of conditions that come in genetically determined and nongenetic types include dementias of the subtypes Alzheimer, Pick, frontotemporal, Parkinson disease, amyotrophic lateral sclerosis, and peripheral neuropathies. For each of these conditions, a small percentage of affected patients exhibit a clearly genetically determined cause and have pedigrees that demonstrate a classical Mendelian pattern of inheritance. For each condition, however, most patients appear to have an acquired defect, without evidence of a genetic predisposition. As time goes on, additional genetic factors may be found that predispose to development of disease in even apparent sporadic cases.

Scientists now speculate that, if such gene alterations exist, they may be expressed because of epigenetic factors, modifier genes, or environmental influences.

In the strictest sense, inherited disorders of metabolism encompass a narrow spectrum of conditions that have been defined on a biochemical basis. Broad categories include disorders of carbohydrate metabolism, disorders of amino acid metabolism, organic acidemias, lysosomal storage diseases, disorders of fatty acid metabolism, and mitochondrial disorders. Most, but not all, of these conditions are associated with some neurologic sequelae. In patients with inborn errors of metabolism, dysgenesis of the corpus callosum serves as a marker for other developmental defects within the nervous system.

Another useful way to categorize inborn errors of metabolism is by the neurologic subsystems most prominently affected. These are listed in Table 1 below. In addition to those listed here, some metabolic disorders produce acute changes in behavior or in the function of the forebrain, while others give rise to specific clinical features such as frontal bossing (eg, the mucopolysaccharidoses) or specific skin lesions (eg, Fabry disease, Refsum disease, ataxia telangiectasia). A few metabolic disorders primarily affect the liver, spleen, or heart and may be detected early by changes in these organs. The nervous system is affected late.

These particular features are dealt with specifically in the relevant articles of Medscape Reference. Readers also may refer to various modern textbooks of child neurology and neurology, such as Clarke's useful monograph

Table 1. Typical Neurologic Syndromes Associated with Known Inherited Metabolic Disorders

Syndrome	Inherited Metabolic Defects
Psychosis, irritability, mood disorder, hyperactivity, agitation, or hallucinations	Absorption of vitamin B-12, lysosomal defects (eg, Sanfilippo and Hunter diseases, GM2 gangliosidosis of late onset, neuronal ceroid lipofuscinosis, metachromatic leukodystrophy of late onset, Krabbe disease), peroxisomal defects (ie, adrenoleukodystrophy), Lesch-Nyhan syndrome (ie, defect of purine metabolism), Wilson disease (especially young onset), acute intermittent porphyria and other hepatic porphyrias, defects of the urea cycle, homocystinuria, cerebrotendinous xanthomatosis
Global mental retardation/developmental delay with progressive neurological signs; dementias	Lysosomal defects (early onset causes mental retardation, late onset causes dementia); disorders of amino acids, organic acids, carbohydrates (especially of pyruvate metabolism), copper metabolism (eg, Menkes disease, some cases of Wilson disease), peroxisomes (eg, adrenoleukodystrophy, Zellweger syndrome)
Seizures	Those of the disorders already mentioned that affect gray matter, especially peroxisomal defects (other than Refsum disease) and some lysosomal defects, pyridoxine-dependent defects (eg, glutamate decarboxylase deficiency); biotin-related defects (eg, biotinidase deficiency, branched chain amino acid defects); molybdenum cofactor deficiency; defects of pyruvate metabolism; defects of mitochondrial electron transport
Loss of vision	Those listed with seizures above
Optic atrophy	Leber hereditary optic atrophy (any of the several mitochondrial DNA mutations)
Retinitis pigmentosa	Defects of pyruvate metabolism, defects of mitochondrial electron transport chain, Refsum disease, abetalipoproteinemia, Hallervorden-Spatz disease
Cherry red spot (in macula)	Tay-Sach disease, sialidosis II (i.e lipomucopolysaccharidosis)
Extrapyramidal disorders (especially with rigidity, tremor, or chorea)	Wilson disease, Lesch-Nyhan syndrome, Segawa syndrome, some organic acid defects, defects of RBC glycolysis (eg, triosephosphate isomerase)
Ataxias	Defects of pyruvate metabolism, defects of amino acid metabolism (eg, Hartnup disease, maple syrup urine disease), defects of organic acid metabolism, defects of mitochondrial electron transport, defects of the urea cycle, lipoprotein disorders (abetalipoproteinemia, alpha-lipoproteinemia), peroxisomal defects (eg, Refsum disease), lysosomal disorders with predominantly gray matter effects
Pyramidal and cerebellar syndrome with or without peripheral neuropathy (white matter disease)	Peroxisomal (eg, adrenoleukodystrophy and its late onset variants, Zellweger syndrome, late stages of Refsum disease), Canavan disease (ie, aspartoacylase deficiency), Alexander disease (similar to Canavan disease, but biochemical defect not known), lysosomal defects (especially late onset variants)
Peripheral neuropathy with or without dysautonomia	Acute intermittent porphyria, peroxisome assembly defect, some cases of Refsum disease, some cases of abetalipoproteinemia, familial amyloidosis (not a metabolic defect in the strict sense cited above)
Myopathy	-
Episodic cramps and myoglobinuria related to exercise	Defects of glycolysis and of glycogenolysis; defects of oxidation of fatty acids, defects of carnitine and its derivatives
Ragged red diseases (i.e limb-girdle neuromuscular diseases with ragged red fibers seen on trichrome stain of frozen biopsied specimens)	Defects of mitochondria, especially of the electron transport chain and of proteins coded by mitochondrial DNA

Epidemiology and Statistics

Although individual inborn errors of metabolism are relatively rare conditions, as a group they represent a vast and diverse collection of diseases that are a significant cause of morbidity and mortality worldwide. Even though reports in the literature often quote a cumulative incidence varying between 1 in 1500 and 1 in 5000 live births, a recent retrospective study on an ethnically diverse population in the United Kingdom found this range to underestimate the real figure. This study placed the prevalence of inherited metabolic disorders at 1 in 784 live births. About 1000 inborn errors of metabolism are estimated to have been identified to date.

Most inborn errors of metabolism are inherited as autosomal recessive conditions. Some are due to mutations on the X chromosome and follow an X-linked recessive genetic pattern. Some mitochondrial disorders are due to proteins that are transported into mitochondria and function there, but that are coded for by ordinary nuclear DNA. These follow an autosomal recessive pattern. Many mitochondrial disorders have a unique form of inheritance with only maternal transmission. The mitochondrial DNA (which is circular, like that of a bacterium) all comes from the egg and hence from the mother. None of the mitochondria in the sperm is passed on to the zygote.

Autosomal conditions generally affect equal numbers of males and females. X-linked recessive conditions generally affect only males. These males may be related through unaffected carrier females. In some conditions the differential expression of an autosomal gene can lead to one gender having more severe symptoms than the other. For some X-linked conditions, carrier females may have symptoms and signs that are considerably milder than those in affected males.

Many inherited diseases were first described in one race or ethnic group. Examples are sickle cell disease among black Africans, Tay-Sachs, familial dysautonomia, and Canavan disease in Ashkenazi Jews, cystic fibrosis in northern Europeans, and the inherited predisposition to multiple sclerosis (MS) in descendants of the Vikings. Over time, however, most inborn errors of metabolism have been found to occur in almost all races and groups that have been studied. Many populations are now characterized by admixture of various gene pools. Spontaneous mutations can occur in any person within a population. The occurrence of a defect in a population can be influenced by "founder effect."

The association of some inherited disorders of metabolism with one race, especially with Ashkenazi Jews, has turned out in the past 30 years to be, in part, an artifact of medical history. Between 1945 and 1970, more research on these disorders was carried out in the northeastern United States than elsewhere in the world. With little effort needed to recover from World War II in the United States, medical research was highly regarded, encouraged, and funded. This research had a strong technical basis, using key new biochemical techniques. Many of the bright doctors doing the research were in the largest American city, New York. Many were Ashkenazi, and so were many of their patients.

Only when countries in the rest of the world became able to afford similar equipment and similar research did we learn that many inherited disorders of metabolism are very widespread, even though rare in each population. The known incidence of metabolic disorders is often higher in one population than another, but few diseases are confined to a single race.

Of the few inherited disorders of intermediary metabolism still known to occur exclusively in one race, pentosuria, the best example, appears to be unique to Ashkenazi Jews. It is not really a disease: it has no symptoms, and affected persons do not suffer. Instead, it is a biochemical curiosity with no known ill effects. The phenomenon is exceedingly rare, but it results from any one of 3 known mutations. (That many mutations in so rare and, seemingly, clinically insignificant a change suggest to the author that the disorder may give an evolutionary disadvantage to humans who carry the mutation.)

The US Secretary of Health and Human Services' Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children provides guidance to reduce the morbidity and mortality associated with heritable disorders, with a special emphasis on those conditions detectable through newborn screening. Although long-term follow-up is necessary to maximize the benefit of diagnosis through newborn screening, such care is variable and inconsistent. To begin to improve long-term follow-up, the Advisory Committee has identified its key features, including the assurance and provision of quality chronic disease management, condition-specific treatment, and age-appropriate preventive care throughout the lifespan of affected individuals. Four components are central to achieving long-term follow-up: care coordination through a medical home, evidence-based treatment, continuous quality improvement, and new knowledge discovery.

Morbidity and Mortality

Some inherited metabolic disorders are fatal in the first weeks or months of postnatal life, for example, severe defects in the conversion of pyruvate to acetyl coenzyme A (CoA), some urea cycle defects, and severe defects in the processing of fructose. Others are compatible with a very long life, for example, nonneuronopathic Gaucher disease, McArdle disease, and phenylketonuria (if treated with dietary restriction of phenylalanine). Many inborn errors of metabolism may exist that are entirely incompatible with life and that never result in a live-born infant. As advances occur in enzyme replacement therapy and, ultimately, we hope, in gene therapy, some inborn errors that cannot be treated today may become treatable in the future.

In the 1960s, inherited defects of the Krebs cycle were believed to be so deleterious that they would not be compatible with postnatal life. However, John Blass identified inherited abnormalities of the pyruvate dehydrogenase multi-enzyme complex in infants and children with neurological problems. Since then, geneticists and other physicians have hesitated to presume that any particular metabolic defect would necessarily or invariably be fatal.

Affected Enzymes and Pathways

The biochemical methods of the 1920s through the 1980s led to discoveries of defects in most of the known metabolic pathways. Some of the defects lead to neurological disorders, others to diseases confined to RBCs, liver, kidneys, or other organs. Most inborn errors have multisystem effects. For example, defects of pyruvate oxidation can affect the retina, the heart, and bone as well as the nervous system. Sickle cell anemia leads secondarily to strokes, as do defects of metabolism of the amino acid homocysteine. Other articles in Medscape Reference discuss specific inherited defects of amino acids, lipids, mucopolysaccharides, carbohydrate metabolism, and the breakdown of large molecules within lysosomes and peroxisomes.

Lysosomal storage disorder is a heterogeneous group of rare disorders characterized by abnormal accumulation of incompletely degraded substances in various tissues and organs. Manifestations generally include neurologic impairment, skeletal deformities, intellectual and cardiac abnormalities, and gastrointestinal problems. Ocular complications often cause severe reduction in visual acuity and can affect any part of the eye including cataract, vitreous degeneration, retinopathy, optic nerve swelling and atrophy, ocular hypertension, and glaucoma. Corneal opacification of varying severity is frequently seen. Most of these patients have poor vision due to various ocular complications that are often difficult to monitor and treat.

Inborn errors of metabolism in many instances are difficult or impossible to treat. The need to explore novel therapeutic modalities, including gene therapy, is compelling. Among available gene delivery systems,

recombinant adeno-associated virus (AAV) shows special promise for the treatment of metabolic disease. Despite the relatively low immunogenicity of AAV vectors, immune responses are also emerging as a factor requiring special attention as efforts accelerate toward human clinical translation. Trials targeting lipoprotein lipase deficiency are showing early evidence of efficacy.

Fabry disease is a progressive, multisystemic, potentially life-threatening disorder caused by a deficiency of [alpha]-galactosidase A. This deficiency results in accumulation of glycosphingolipids, particularly globotriaosylceramide (GL-3), in the lysosomes of various tissues. This accumulation is the underlying driver of disease progression. Agalsidase beta (Fabrazyme) is a recombinant human [alpha]-galactosidase A enzyme approved for intravenous use in the treatment of Fabry disease, thus providing an exogenous source of [alpha]-galactosidase A. This is effective and well tolerated in patients with Fabry disease, which represents an important advance in the treatment of Fabry disease.

Straightforward Diseases, Especially Recessively Inherited

Garrod's work in the 1920s led to the notion that a single genetic disease was likely due to a single inherited defect. This hypothesis led to fruitful work through the 1960s. By the 1970s, however, the complexity of genetic disorders became an issue in research. The first defects of amino acid metabolism that were elucidated followed the Garrod rule. These included phenylketonuria, maple syrup urine disease, homocystinuria as it first presented, and the first disorders of lysosomal metabolism. GM2 gangliosidosis led to Tay-Sachs disease, and specific defects in the breakdown of glycosaminoglycans (ie, mucopolysaccharidoses III) caused Sanfilippo disease.

Defects of other lysosomal enzymes led to the clinically different pictures of Hurler syndrome, Hunter syndrome, Morquio syndrome, and others. Since the mucopolysaccharides are degraded through an entirely different pathway than the globosides, the Garrod rule still held, despite the fact that lysosomes were involved in each case. Refsum disease, caused by lack of the enzyme catalyzing alpha-oxidation of the fatty acid phytanic acid, was the first peroxisomal defect to be discovered. This disease was distinct clinically from the next peroxisomal defect identified, adrenoleukodystrophy.

The defects of the enzymes that convert sugars to energy seemed to follow suit. Phosphorylase deficiency was a disorder of the muscles. Deficiency of fructose 1, 6-diphosphate dehydrogenase led to a disease of the brain and the liver. A single amino acid substitution can come about easily if one DNA base is changed in one codon at a specific site within the gene for that enzyme. Such a mutation is called a point mutation. (The deletion of a single base is also a point mutation but would result in a shift of the triplet reading frame and likely the production of a truncated protein with little or no activity.) By the late 1960s, many of the mutations of hemoglobin were known to be point mutations, and indirect evidence existed that this also was true for those few enzymes whose mutations could be studied in fine detail.

In the period from the 1960s to the early 1980s, research on the molecular basis of inherited disorders of metabolism assumed that these were largely due to a point mutation in the peptide chain of the relevant enzyme. A substitution in or near the active center of the enzyme seemed a ready explanation for a change in the binding of substrate or product and thus to a change in the enzyme's catalytic ability. The center is the catalytic cleft or region in the natural state of the folded protein, the region to which substrate(s) bind for catalysis to take place.

With the discovery of defects of oxidation of pyruvate to acetyl CoA in the late 1960s and early 1970s, the Garrod rule seemed no longer to be universally true. A defect of a certain severity in a specific pathway might be associated with a specific disease entity with a well-defined clinical picture, whereas defects of lesser severity in the same pathway or even in the same enzyme could sometimes be associated with different, less severe clinical entities. Thus, a severe defect of the pyruvate dehydrogenase complex often is associated with overwhelming lactic acidosis, mental deficiency, and severe hypotonia in infants; a more moderate defect is associated with ataxia and episodes of transient lactic acidosis in young children; even milder defects have been associated clearly with the onset of ataxia, areflexia, sensory loss, and abnormalities of heart and bone in adolescents.

Complex Diseases, Especially Autosomal Dominant

Even in the middle of the 20th century, the details of the autosomal dominant neurological diseases did not really fit the Garrod rule. For example, severity of both Huntington chorea and myotonic dystrophy seemed to vary depending on whether the carrier was the father or the mother. Earlier onset or juvenile Huntington disease, most commonly seen in adults of middle age, often was seen in the children who inherited the gene from their father, whereas the congenital form of myotonic dystrophy was seen mostly in children born to affected mothers. Influences in the womb alone—sometimes even called miasmas—were postulated to account for these discrepancies. Until the actual mutation was established, those who did not make the initial observation tended to disregard it. People also disregarded and even scoffed at the observation that these 2 conditions had a tendency to become worse and worse in successive generations. This tendency is the phenomenon of anticipation.

Thus, a grandfather may have developed symptoms of Huntington disease in his 50s; his children may have shown clear signs in their late 30s; and the grandchildren may have become symptomatic in their 20s. When this phenomenon first was described, clinicians incorrectly tried to attribute the earlier diagnosis of disease to improving clinical skills or ascertainment bias. Once the genetic basis was known (increasing numbers of triplet repeats in the mutant gene from generation to generation) anticipation became an established clinical fact in these diseases and in a number of other neurogenetic diseases that are due to triplicate codon repeats at the terminal end of the gene.

The mutations that lead to Huntington disease, myotonic dystrophy, Machado-Joseph disease, and a large number of other dominantly inherited neurological diseases are not point mutations after all. The mutations are in the number of triplets of cytosine, adenosine, guanine (CAG) at one end of the gene. In the case of Huntington disease, the normal allele has 18-20 CAG triplets. If more than 30 CAG triplets are present, the individual is a carrier of Huntington disease and has an approximately 90% chance of getting the disease if he or she lives to the age of 60 years or more.

The longer the chain of triplet repeats, the earlier the person is likely to have clinical signs and the more severe and rapid the clinical course is likely to be. The longer the stretch of triplet repeats, the more unstable this region becomes during meiosis and the more likely this stretch will expand even further in the next generation. In the case of HD, the gene from the father is more likely to result in very long chains of CAG and so in juvenile onset of HD than that transmitted through the mother. For myotonic dystrophy, the converse is true: the maternal gene is more likely to acquire excessive lengths of CAG.

While we think of patients with autosomal recessive disorders like Tay-Sachs or defects of pyruvate dehydroxygenase as being homozygous for a mutant gene, many are really compound heterozygotes. Each parent may carry a mutant allele, but the mutations may not be the same in both parents. The effect in the offspring still may be insufficient activity of the relevant enzyme, resulting in typical disease. The offspring is a compound heterozygote, not a true homozygote. This becomes important when genetic markers are used that detect one form of mutant gene but not other, less common mutations. A less common allele may be missed, giving the impression that one parent is not a carrier, or that

the (compound heterozygote) subject is merely a carrier, not affected with the disease.

Acute intermittent porphyria (AIP) is a rare metabolic disorder characterized by mutations of the porphobilinogen deaminase gene. It is presumed AIP is due to the neurotoxic effects of increased porphyrin precursors, although the underlying pathophysiology of porphyric neuropathy remains unclear.

Lin et al investigated the neurotoxic effect of porphyrins by undertaking excitability measurements (stimulus-response, threshold electrotonus, current-threshold relationship and recovery cycle) of peripheral motor axons in 20 patients with acute intermittent porphyria subjects. These measurements were combined with the results of genetic screening and biochemical and conventional nerve conduction studies. The authors proposed that porphyrin neurotoxicity causes a subclinical reduction in IH (the hyperpolarization-activated, cyclic nucleotide-dependent current) in acute porphyric episodes without clinical neuropathy (AIPWN) axons, whereas porphyric neuropathy may develop when reduced activity of the Na+/K+ pump results in membrane depolarization.

Polymorphism with and without Disease

Even in the 1960s, some amino acid substitutions that were clinically insignificant were recognized in hemoglobin molecules. These variant hemoglobin molecules migrated at an abnormal rate on the chromatographic papers (and in later years in gels) that were used to find such molecular changes. Instead of having clinically identifiable problems such as sickle cell disease, people with these harmless substitutions were healthy and lived healthy lives. Such innocent genetic changes were called polymorphisms. Similar harmless polymorphisms were discovered in other proteins as soon as analyses of their substructures became easy and work could be done with samples from various populations.

Diseases occurred only if the amino acid substitution in a particular protein or peptide was of a particular kind and at a particular place in the peptide chain. The polymorphism had to interfere with function and it had to interfere to such a degree that the body could not compensate readily. Only then would the gene change lead to disease.

The fact that the mutation leading to Wilson disease would be a harmless polymorphism on a planet with little or no copper was pointed out in the 1970s and 1980s. Refsum disease would not exist in a society that did not consume phytanic acid. Favism would not be recognized if fava beans did not exist. Hypokalemic periodic paralysis would not manifest itself if an affected individual never consumed a meal high in sugar. Individuals with mild forms of fructose intolerance would not have symptoms if they never ate foods containing fructose.

The relation between polymorphism and disease can be even more complex. In a number of instances, two or more things must go wrong for a disease to appear. Refsum disease often presents in children aged 7-12 years with acute onset of nerve deafness or blindness, with neuropathy or ataxia. A large excess of phytanic acid is present in tissues or body fluids. An exogenous disease or stressor precipitates episodes. Documented examples include appendicitis, a severe viral illness, surgery, or a fracture. Each episode is self-limited with partial resolution over weeks to months. However, the disease is progressive. The residual effects accumulate in adolescence and early adult life, until the disease takes on a progressively downhill course rather than the remitting and relapsing course of childhood.

The defect, an inability to oxidize phytanic acid at the alpha position, is present from conception. (Phytanic acid is a breakdown product of phytol, a major component of chlorophyll.) The disease appears only after an exogenous illness.

As such, Refsum disease is analogous to multiple sclerosis (MS). Blass and Steinberg pointed out a further analogy when they suggested that Refsum may represent an ideal scientific model for MS (D. Steinberg and J. P. Blass, personal communications, 1969-1971). The biochemical defect underlying Refsum disease appears to have been propagated by the western Vikings, just as the genetic defect predisposing to MS (in or near the genes for the human leukocyte antigen complex) was spread by both the eastern and the western Vikings to European populations. Often a marked improvement in the signs and symptoms of Refsum disease can be demonstrated when prevention of exogenous illness is combined with dietary restriction of phytol and phytanic acid. Not unexpectedly, eating a diet high in phytanic acid will again precipitate symptoms.

Unfortunately, no recognized dietary or preventive measures exist for MS, and only certain drugs related to immune phenomena, such as the ABC drugs, can decrease the risk of a relapse of MS.

The signs and symptoms of acute intermittent porphyria probably result from the accumulation of 2 neurotoxic metabolites in the pathway of porphyrin synthesis, delta-amino levulinic acid and porphobilinogen (see Acute Intermittent Porphyria). The clinical disease may be associated with attacks of sensory neuropathy, ataxia, psychosis, and even coma, and occurs when a patient with the polymorphism or genetic predisposition ingests a drug or eats food that precipitously increases the synthesis of porphyrins. These precipitants include phenytoin, strawberries, or any one of the long list of potentially harmful substances outlined in the article on porphyrias. With a sudden demand on this metabolic pathway and up-regulation of enzymes to synthesize porphyrins combined with the inherited partial deficiency of uroporphyrinogen I synthetase, the toxic metabolites accumulate and cause damage toneural tissues, resulting in clinical symptoms.

Mercury poisoning typically produces clinically disabling symptoms only in approximately 10% of a population uniformly exposed to low but toxic doses of the metal. Some patients with frank mercury poisoning have pes cavus or mild kyphoscoliosis associated with their neuropathies. This observation led Raymond Adams to ask whether an inherited defect might be present in these patients, a defect that, were it a little more severe, might lead to an inherited neuropathy or an inherited myelopathy (R. D. Adams, personal communication, 1969). In the end, remember what Sir Francis Bacon said: "Inquire carefully into the origin of things."

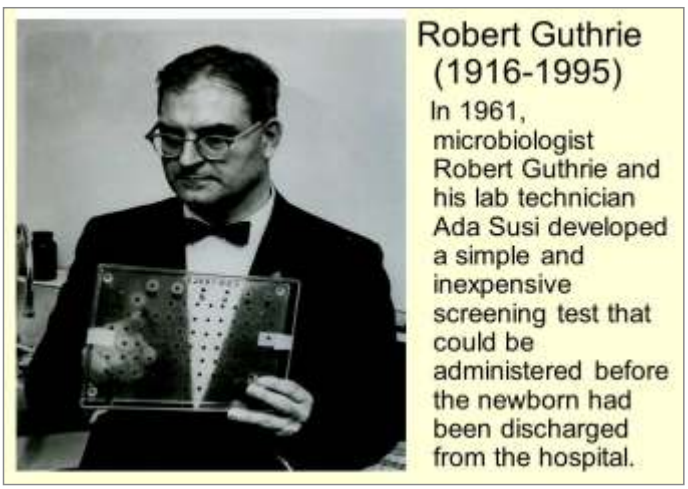
Other Resources

Children Living with Inherited Metabolic Diseases (CLIMB) was established in 1981 as an international health agency to provide support to children with inherited metabolic conditions, their families, and their healthcare professionals. To understand the utmost importance of the timely identification and management of inherited metabolic diseases, one has to remember a figure provided on the organization's Web site: Metabolic conditions affect 1 in 500 families in the United Kingdom. Inherited metabolic diseases: a guide to 100 conditions emerges from an effort coordinated by CLIMB as an important resource aiming to provide specialist information on metabolic diseases geared toward the nonspecialist.

A growing group of inborn errors of metabolism include inherited neurometabolic disorders causing central or peripheral nervous system dysfunction. It is increasingly recognized that motor neuron disease (MND), especially the amyotrophic lateral sclerosis variety, is one such entity. A broad range of rare inherited metabolic disorders can present with dystonia. Several neurometabolic disorders are treatable causes of dystonia. While careful phenotyping is the first step towards the diagnosis of the underlying condition, subsequent targeted treatment is further supported by imaging, biochemical diagnostics, and the availability of modern diagnostic techniques such as next-generation sequencing.

NEWBORN SCREENING

Newborn screening (NBS) is a public health program of screening in infants shortly after birth for conditions that are treatable, but not clinically evident in the newborn period. The goal is to identify infants at risk for these conditions early enough to confirm the diagnosis and provide intervention that will alter the clinical course of the disease and prevent or ameliorate the clinical manifestations. NBS started with the discovery that the amino acid disorder phenylketonuria (PKU) could be treated by dietary adjustment, and that early intervention was required for the best outcome. Infants with PKU appear normal at birth, but are unable to metabolize the essential amino acid phenylalanine, resulting in irreversible intellectual disability. In the 1960s, Robert Guthrie developed a simple method using a bacterial inhibition assay that could detect high levels of phenylalanine in blood shortly after a baby was born. Guthrie also pioneered the collection of blood on filter paper which could be easily transported, recognizing the need for a simple system if the screening was going to be done on a large scale. Newborn screening around the world is still done using similar filter paper. NBS was first introduced as a public health program in the United States in the early 1960s, and has expanded to countries around the world.



Screening programs are often run by state or national governing bodies with the goal of screening all infants born in the jurisdiction for a defined panel of treatable disorders. The number of diseases screened for is set by each jurisdiction, and can vary greatly. Most NBS tests are done by measuring metabolites or enzyme activity in whole blood samples collected on filter paper. Bedside tests for hearing loss using automated auditory brainstem response and congenital heart defects using pulse oximetry are included in some NBS programs. Infants who screen positive undergo further testing to determine if they are truly affected with a disease or if the test result was a false positive. Follow-up testing is typically coordinated between geneticists and the infant's pediatrician or primary care physician.

History

Robert Guthrie is given much of the credit for pioneering the earliest screening for phenylketonuria in the late 1960s using a bacterial inhibition assay (BIA) to measure phenylalanine levels in blood samples obtained by pricking a newborn baby's heel on the second day of life on filter paper. Congenital hypothyroidism was the second disease widely added in the 1970s. Guthrie and colleagues also developed bacterial inhibition assays for the detection of maple syrup urine disease and classic galactosemia. The development of tandem mass spectrometry (MS/MS) screening in the early 1990s led to a large expansion of potentially detectable congenital metabolic diseases that can be identified by characteristic patterns of amino acids and acylcarnitines. In many regions, Guthrie's BIA has been replaced by MS/MS profiles, however the filter paper he developed is still used worldwide, and has allowed for the screening of millions of infants around the world each year.

In the United States, the American College of Medical Genetics recommended a uniform panel of diseases that all infants born in every state should be screened for. They also developed an evidence-based review process for the addition of conditions in the future. The implementation of this panel across the United States meant all babies born would be screened for the same number of conditions. This recommendation is not binding for individual states, and some states may screen for disorders that are not included on this list of recommended disorders. Prior to this, babies born in different states had received different levels of screening. On April 24, 2008, President George W. Bush signed into law the Newborn Screening Saves Lives Act of 2007. This act was enacted to increase awareness among parents, health professionals, and the public on testing newborns to identify certain disorders. It also sought to improve, expand, and enhance current newborn screening programs at the state level.

Inclusion of disorders

Newborn screening programs initially used screening criteria based largely on criteria established by JMG Wilson and F. Jungner in 1968. Although not specifically about newborn population screening programs, their publication, Principles and practice of screening for disease proposed ten criteria that screening programs should meet before being used as a public health measure. Newborn screening programs are administered in each jurisdiction, with additions and removals from the panel typically reviewed by a panel of experts. The four criteria from the publication that were relied upon when making decisions for early newborn screening programs were:

1. having an acceptable treatment protocol in place that changes the outcome for patients diagnosed early with the disease
2. an understanding of the condition's natural history
3. an understanding about who will be treated as a patient
4. a screening test that is reliable for both affected and unaffected patients and is acceptable to the public

As diagnostic techniques have progressed, debates have arisen as to how screening programs should adapt. Tandem mass spectrometry has greatly expanded the potential number of diseases that can be detected, even without satisfying all of the other criteria used for making screening decisions. Duchenne muscular dystrophy is a disease that has been added to screening programs in several jurisdictions around the world, despite the lack of evidence as to whether early detection improves the clinical outcome for a patient.

Targeted disorders

Newborn screening is intended as a public health program to identify infants with treatable conditions before they present clinically, or suffer

irreversible damage. Phenylketonuria (PKU) was the first disorder targeted for newborn screening, being implemented in a small number of hospitals and quickly expanding across the United States and the rest of the world. After the success of newborn screening for PKU (39 infants were identified and treated in the first two years of screening, with no false negative results), Guthrie and others looked for other disorders that could be identified and treated in infants, eventually developing bacterial inhibition assays to identify classic galactosemia and maple syrup urine disease.

Newborn screening has expanded since the introduction of PKU testing in the 1960s, but can vary greatly between countries. In 2011, the United States screened for 54 conditions, Germany for 12, the United Kingdom for 2 (PKU and medium chain acyl-CoA dehydrogenase deficiency (MCADD)), while France and Hong Kong only screened for one condition (PKU and congenital hypothyroidism, respectively). The conditions included in newborn screening programs around the world vary greatly, based on the legal requirements for screening programs, prevalence of certain diseases within a population, political pressure, and the availability of resources for both testing and follow-up of identified patients.

Amino acid disorders

Newborn screening originated with an amino acid disorder, phenylketonuria (PKU), which can be easily treated by dietary modifications, but causes severe mental retardation if not identified and treated early. Robert Guthrie introduced the newborn screening test for PKU in the early 1960s. With the knowledge that PKU could be detected before symptoms were evident, and treatment initiated, screening was quickly adopted around the world. Ireland was the first country in the world to introduce a nationwide screening programme in February 1966 , Austria started screening the same year and England in 1968.

Fatty acid oxidation disorders

With the advent of tandem mass spectrometry as a screening tool, several fatty acid oxidation disorders were targeted for inclusion in newborn screening programs. Medium chain acyl-CoA dehydrogenase deficiency (MCADD), which had been implicated in several cases of sudden infant death syndrome was one of the first conditions targeted for inclusion. MCADD was the first condition added when the United Kingdom expanded their screening program from PKU only. Population based studies in Germany, the United States and Australia put the combined incidence of fatty acid oxidation disorders at 1:9300 among Caucasians. The United States screens for all known fatty acid oxidation disorders, either as primary or secondary targets, while other countries screen for a subset of these.

The introduction of screening for fatty acid oxidation disorders has been shown to have reduced morbidity and mortality associated with the conditions, particularly MCADD. An Australian study found a 74% reduction in episodes of severe metabolic decompensation or death among individuals identified by newborn screening as having MCADD versus those who presented clinically prior to screening. Studies in the Netherlands and United Kingdom found improvements in outcome at a reduced cost when infants were identified before presenting clinically.

Newborn screening programs have also expanded the information base available about some rare conditions. Prior to its inclusion in newborn screening, short-chain acyl-CoA dehydrogenase deficiency (SCADD) was thought to be life-threatening. Most patients identified via newborn screening as having this enzyme deficiency were asymptomatic, to the extent that SCADD was removed from screening panels in a number of regions. Without the cohort of patients identified by newborn screening, this clinical phenotype would likely not have been identified.

Endocrinopathies

The most commonly included disorders of the endocrine system are congenital hypothyroidism (CH) and congenital adrenal hyperplasia (CAH). Testing for both disorders can be done using blood samples collected on the standard newborn screening card. Screening for CH is done by measuring thyroxin (T4), thyrotropin (TSH) or a combination of both analytes. Elevated 17α-hydroxyprogesterone (17α-OHP) is the primary marker used when screening for CAH, most commonly done using enzyme-linked immunosorbant assays, with many programs using a second tier tandem mass spectrometry test to reduce the number of false positive results. Careful analysis of screening results for CAH may also identify cases of congenital adrenal hypoplasia, which presents with extremely low levels of 17α-OHP.

CH was added to many newborn screening programs in the 1970s, often as the second condition included after PKU. The most common cause of CH is dysgenesis of the thyroid gland After many years of newborn screening, the incidence of CH worldwide had been estimated at 1:3600 births, with no obvious increases in specific ethnic groups. Recent data from certain regions have showed an increase, with New York reporting an incidence of 1:1700. Reasons for the apparent increase in incidence have been studied, but no explanation has been found.

Classic CAH, the disorder targeted by newborn screening programs, is caused by a deficiency of the enzyme steroid 21-hydroxylase, and comes in two forms - simple virilizing and a salt-wasting form. Incidence of CAH can vary greatly between populations. The highest reported incidence rates are among the Yupic Eskimos of Alaska (1:280) and on the French island of Réunion (1:2100).

Hemoglobinopathies

Sickle cells in human blood: both normal red blood cells and sickle-shaped cells are present

Any condition that results in the production of abnormal hemoglobin is included under the broad category of hemoglobinopathies. Worldwide, it is estimated that 7% of the population may carry a hemoglobinopathy with clinical significance. The most well known condition in this group is sickle cell disease. Newborn screening for a large number of hemoglobinopathies is done by detecting abnormal patterns using isoelectric focusing, which can detect many different types of abnormal hemoglobins. In the United States, newborn screening for sickle cell disease was recommended for all infants in 1987, however it was not implemented in all 50 states until 2006.

Early identification of individuals with sickle cell disease and other hemoglobinopathies allows treatment to be initiated in a timely fashion. Penicillin has been used in children with sickle cell disease, and blood transfusions are used for patients identified with severe thalassemia.

Organic acidemias

Most jurisdictions did not start screening for any of the organic acidemias before tandem mass spectrometry significantly expanded the list of disorders detectable by newborn screening. Quebec has run a voluntary second-tier screening program since 1971 using urine samples collected at three weeks of age to screen for an expanded list of organic

acidemias using a thin layer chromatography method. Newborn screening using tandem mass spectrometry can detect several organic acidemias, including propionic acidemia, methylmalonic acidemia and isovaleric acidemia.

Cystic fibrosis

Cystic fibrosis (CF) was first added to newborn screening programs in New Zealand and regions of Australia in 1981, by measuring immunoreactive trypsinogen (IRT) in dried blood spots. After the CFTR gene was identified, Australia introduced a two tier testing program to reduce the number of false positives. Samples with an elevated IRT value were then analyzed with molecular methods to identify the presence of disease causing mutations before being reported back to parents and health care providers. CF is included in the core panel of conditions recommended for inclusion in all 50 states, Texas was the last state to implement their screening program for CF in 2010. Alberta was the first Canadian province to implement CF screening in 2007. Quebec, New Brunswick, Nova Scotia, Newfoundland and Prince Edward Island do not include CF in their screening programs. The United Kingdom as well as many European Union countries screen for CF as well. Switzerland is one of the latest countries to add CF to their newborn screening menu, doing so in January 2011.

Urea cycle disorders

Disorders of the distal urea cycle, such as citrullinemia, argininosuccinic aciduria and argininemia are included in newborn screening programs in many jurisdictions that using tandem mass spectrometry to identify key amino acids. Proximal urea cycle defects, such as ornithine transcarbamylase deficiency and carbamoyl phosphate synthetase deficiency are not included in newborn screening panels because they are not reliably detected using current technology, and also because severely affected infants will present with clinical symptoms before newborn screening results are available. Some regions claim to screen for HHH syndrome (hyperammonemia, hyperornithinemia, homocitrullinuria) based on the detection of elevated ornithine levels in the newborn screening dried blood spot, but other sources have shown that affected individuals do not have elevated ornithine at birth.

Lysosomal storage disorders

Lysosomal storage disorders are not included in newborn screening programs with high frequency. As a group, they are heterogenous, with screening only being feasible for a small fraction of the approximately 40 identified disorders. The arguments for their inclusion in newborn screening programs center around the advantage of early treatment (when treatment is available), avoiding a diagnostic odyssey for families and providing information for family planning to couples who have an affected child. The arguments against including these disorders, as a group or individually center around the difficulties with reliably identifying individuals who will be affected with a severe form of the disorder, the relatively unproven nature of the treatment methods, and the high cost / high risk associated with some treatment options.

New York State started a pilot study to screen for Krabbe disease in 2006, largely due to the efforts of Jim Kelly, whose son, Hunter, was affected with the disease. A pilot screening program for four lysosomal storage diseases (Gaucher disease, Pompe disease, Fabry disease and Niemann-Pick disease was undertaken using anonymised dried blood spots was completed in Austria in 2010. Their data showed an increased incidence from what was expected in the population, and also a number of late onset forms of disease, which are not typically the target for newborn screening programs.

Hearing loss

Undiagnosed hearing loss in a child can have serious effects on many developmental areas, including language, social interactions, emotions, cognitive ability, academic performance and vocational skills, any combination of which can have negative impacts on the quality of life. The serious impacts of a late diagnosis, combined with the high incidence (estimated at 1 - 3 per 1000 live births, and as high as 4% for neonatal intensive care unit patients) have been the driving forces behind screening programs designed to identify infants with hearing loss as early as possible. Early identification allows these patients and their families to access the necessary resources to help them maximize their developmental outcomes.

Newborn hearing testing is done at the bedside using transiently evoked otoacoustic emissions, automated auditory brainstem responses, or a combination of both techniques. Hearing screening programs have found the initial testing to cost between $10.20 and $23.37 per baby, depending on the technology used. As these are screening tests only, false positive results will occur. False positive results could be due to user error, a fussy baby, environmental noise in the testing room, or fluid or congestion in the outer/middle ear of the baby. A review of hearing screening programs found varied initial referral rates (screen positive results) from 0.6% to 16.7%. The highest overall incidence of hearing loss detection was 0.517%. A significant proportion of screen positive infants were lost to follow-up, before a diagnosis could be confirmed or ruled out in all screening programs.

Congenital heart defects

In some cases, critical congenital heart defects (CCHD) are not identified by prenatal ultrasound or postnatal physical examination. Pulse oximetry has been recently added as a bedside screening test for CCHD at 24 to 48 hours after birth. However, not all heart problems can be detected by this method, which relies only on blood oxygen levels.

When a baby tests positive, urgent subsequent examination, such as echocardiography, is undergone to determine the cause of low oxygen levels. Babies diagnosed with CCHD are then seen by cardiologists.

Severe combined immunodeficiency

Severe combined immunodeficiency (SCID) caused by T-cell deficiency is a disorder that was recently added to newborn screening programs in some regions of the United States. Wisconsin was the first state to add SCID to their mandatory screening panel in 2008, and it was recommended for inclusion in all states' panels in 2010. Since December 2018 all US´states perform SCID screening. As the first country in Europe, Norway started nationwide SCID screening January 2018 . Identification of infants with SCID is done by detecting T-cell receptor excision circles (TRECs) using real-time polymerase chain reaction (qPCR). TRECs are decreased in infants affected with SCID.

SCID has not been added to newborn screening in a wide scale for several reasons. It requires technology that is not currently used in most newborn screening labs, as PCR is not used for any other assays included in screening programs. Follow-up and treatment of affected infants also requires skilled immunologists, which may not be available in all regions. Treatment for SCID is a stem cell transplant, which cannot be done in all centers.

Other conditions

Duchenne muscular dystrophy (DMD) is an X-linked disorder caused by defective production of dystrophin. Many jurisdictions around the world have screened for, or attempted to screen for DMD using elevated levels of creatine kinase measured in dried blood spots. Because universal newborn screening for DMD has not been undertaken, affected individuals often have a significant delay in diagnosis. As treatment options for DMD become more and more effective, interest in adding a newborn screening test increases. At various times since 1978, DMD has been included (often as a pilot study on a small subset of the population) in newborn screening programs in Edinburgh, Germany, Canada, France, Wales, Cyprus, Belgium and the United States. In 2012, Belgium was the only country that continued to screen for DMD using creatine kinase levels.

As treatments improve, newborn screening becomes a possibility for disorders that could benefit from early intervention, but none was previously available. Adrenoleukodystrophy (ALD), a peroxisomal disease that has a variable clinical presentation is one of the disorders that has become a target for those seeking to identify patients early. ALD can present in several different forms, some of which do not present until adulthood, making it a difficult choice for countries to add to screening programs. The most successful treatment option is a stem cell transplant, a procedure that carries a significant risk.

Techniques

Sample collection

Newborn screening tests are most commonly done from whole blood samples collected on specially designed filter paper, originally designed by Robert Guthrie. The filter paper is often attached to a form containing required information about the infant and parents. This includes date and time of birth, date and time of sample collection, the infant's weight and gestational age. The form will also have information about whether the baby has had a blood transfusion and any additional nutrition the baby may have received (total parenteral nutrition). Most newborn screening cards also include contact information for the infant's physician in cases where follow up screening or treatment is needed. The Canadian province of Quebec performs newborn screening on whole blood samples collected as in most other jurisdictions, and also runs a voluntary urine screening program where parents collect a sample at 21 days of age and submit it to a provincial laboratory for an additional panel of conditions.

Newborn screening samples are collected from the infant between 24 hours and 7 days after birth, and it is recommended that the infant has fed at least once. Individual jurisdictions will often have more specific requirements, with some states accepting samples collected at 12 hours, and others recommending to wait until 48 hours of life or later. Each laboratory will have its own criteria on when a sample is acceptable, or if another would need to be collected. Samples can be collected at the hospital, or by midwives. Samples are transported daily to the laboratory responsible for testing. In the United States and Canada, newborn screening is mandatory, with an option for parents to opt out of the screening in writing if they desire. In many regions, NBS is mandatory, with an option for parents to opt out in writing if they choose not to have their infant screened. In most of Europe, newborn screening is done with the consent of the parents. Proponents of mandatory screening claim that the test is for the benefit of the child, and that parents should not be able to opt out on their behalf. In regions that favour informed consent for the procedure, they report no increase in costs, no decrease in the number of children screened and no cases of included diseases in children who did not undergo screening.

Laboratory testing

Because newborn screening programs test for a number of different conditions, a number of different laboratorial methodologies are used, as well as bedside testing for hearing loss using evoked auditory potentials and congenital heart defects using pulse oximetry. Newborn screening started out using simple bacterial inhibition assays to screen for a single disorder, starting with phenylketonuria in the early 1960s. With this testing methodology, newborn screening required one test to detect one condition. As mass spectrometry became more widely available, the technology allowed rapid determination of a number of acylcarnitines and amino acids from a single dried blood spot. This increased the number of conditions that could be detected by newborn screening. Enzyme assays are used to screen for galactosemia and biotinidase deficiency. Immunoassays measure thyroid hormones for the diagnosis of congenital hypothyroidism and 17α-hydroxyprogesterone for the diagnosis of congenital adrenal hyperplasia. Molecular techniques are used for the diagnosis of cystic fibrosis and severe combined immunodeficiency.

Reporting results

The goal is to report the results within a short period of time. If screens are normal, a paper report is sent to the submitting hospital and parents rarely hear about it. If an abnormality is detected, employees of the agency, usually nurses, begin to try to reach the physician, hospital, and/or nursery by telephone. They are persistent until they can arrange an evaluation of the infant by an appropriate specialist physician (depending on the disease). The specialist will attempt to confirm the diagnosis by repeating the tests by a different method or laboratory, or by performing other corroboratory or disproving tests. The confirmatory test varies depending on the positive results on the initial screen. Confirmatory testing can include analyte specific assays to confirm any

elevations detected, functional studies to determine enzyme activity, and genetic testing to identify disease-causing mutations. In some cases, a positive newborn screen can also trigger testing on other family members, such as siblings who did not undergo newborn screening for the same condition or the baby's mother, as some maternal conditions can be identified through results on the baby's newborn screen. Depending on the likelihood of the diagnosis and the risk of delay, the specialist will initiate treatment and provide information to the family. Performance of the program is reviewed regularly and strenuous efforts are made to maintain a system that catches every infant with these diagnoses. Guidelines for newborn screening and follow up have been published by the Pediatric societies worldwide.

Laboratory performance

Newborn screening programs participate in quality control programs as in any other laboratory, with some notable exceptions. Much of the success of newborn screening programs is dependent on the filter paper used for the collection of the samples. Initial studies using Robert Guthrie's test for PKU reported high false positive rates that were attributed to a poorly selected type of filter paper. This source of variation has been eliminated in most newborn screening programs through standardization of approved sources of filter paper for use in newborn screening programs. In most regions, the newborn screening card (which contains demographic information as well as attached filter paper for blood collection) is supplied by the organization carrying out the testing, to remove variations from this source.

Society and culture

Controversies

Newborn screening tests have become a subject of political controversy in the last decade. In 2003, two California babies, Zachary Wyvill and Zachary Black, were both born with glutaric acidemia type I. Wyvill's birth hospital only tested for the four diseases mandated by state law, while Black was born at a hospital that was participating in an expanded testing pilot program. Black's disease was treated with diet and vitamins; Wyvill's disease went undetected for over six months, and during that time the damage from the enzyme deficiency became irreversible. Birth-defects lobbyists pushing for broader and more universal standards for newborn testing cite this as an example of how much of an impact testing can have.

Instituting MS/MS screening often requires a sizable up front expenditure. When states choose to run their own programs the initial costs for equipment, training and new staff can be significant. Moreover, MS/MS gives only the screening result and not the confirmatory result. The same has to be further done by higher technologies or procedure like GC/MS, Enzyme Assays or DNA Tests. This in effect adds more cost burden and makes physicians lose precious time. To avoid at least a portion of the up front costs, some states such as Overseas have chosen to contract with private labs for expanded screening. Others have chosen to form Regional Partnerships sharing both costs and resources.

But for many states, screening is an integrated part of the department of health which can not or will not be easily replaced. Thus the initial expenditures can be difficult for states with tight budgets to justify. Screening fees have also increased in recent years as health care costs rise and as more states add MS/MS screening to their programs. Amount spent for these programs may reduce resources available to other potentially lifesaving programs. It has been recommended that one disorder, Short Chain Acyl-coenzyme A Dehydrogenase Deficiency, or SCAD, be eliminated from screening programs, due to a "spurious association between SCAD and symptoms. However, recent studies suggest that expanded screening is cost effective and articles published in Pediatrics'. Advocates are quick to point out studies such as these when trying to convince state legislatures to mandate expanded screening.

Expanded newborn screening is also opposed by among some health care providers, who are concerned that effective follow-up and treatment may not be available, that false positive screening tests may cause harm, and issues of informed consent. A recent study by Genetic Alliance and partners suggests that communication between health care providers and parents may be key in minimizing the potential harm when a false positive test occurs. The results from this study also reveal that parents found newborn screening to be a beneficial and necessary tool to prevent treatable diseases. To address the false positive issue, researchers from the University of Maryland, Baltimore and Genetic Alliance established a check-list to assist health care providers communicate with parents about a screen-positive result.

Controversy has also erupted in some countries over collection and storage of blood or DNA samples by government agencies during the routine newborn blood screen. It was revealed that in Texas the state had collected and stored blood and DNA samples on millions of newborns without the parents' knowledge or consent. These samples were then used by the state for genetic experiments and to set up a database to catalog all of the samples/newborns. Samples obtained without parents' consent were destroyed.

Bioethics

As additional tests are discussed for addition to the panels, issues arise. Many question if the expanded testing still falls under the requirements necessary to justify the additional tests. Many of the new diseases being tested for are rare and have no known treatment, while some of the diseases need not be treated until later in life. This raises more issues, such as: if there is no available treatment for the disease should we test for it at all? And if we do, what do we tell the families of those with children bearing one of the untreatable diseases? Studies show that the more rare the disease is and the more diseases being tested for, the more likely the tests are to produce false-positives. This is an issue because the newborn period is a crucial time for the parents to bond with the child, and it has been noted that ten percent of parents whose children were diagnosed with a false-positive still worried that their child was fragile and/or sickly even though they were not, potentially preventing the parent-child bond forming as it would have otherwise. As a result, some parents may begin to opt out of having their newborns screened. Many parents are also concerned about what happens with their infant's blood samples after screening. The samples were originally taken to test for preventable diseases, but with the advance in genomic sequencing technologies many samples are being kept for DNA identification and research, increasing the possibility that more children will be opted out of newborn screening from parents who see the kept samples as a form of research done on their child.

BOUQUET

IN LIGHTER VEIN

WISDOM WHISPERS

BRAIN TEASERS

1. A routine blood test carried out on babies a few days after birth to detect the condition phenylketonuria goes by the name of

A. Lester
B. Hansen
C. Guthrie
D. Ronald Ross

2. Which test is done to screen urine to detect basic Inborn Errors of Metabolism

A. Ferric Chloride test
B. Ammonium Hydroxide test
C. Benedict's Test
D. Benzidine test

3. Which is the most definitive treatment established for Inherited Metabolic Disorders

A. Gene therapy
B. Plasmapheresis
C. Organ transplants
D. Blood transfusions

4. Ninhydrin paper chromatography test detects

A. Abnormal Amino Acid patterns
B. Abnormal Carbohydrate patterns
C. Abnormal Haemoglobin patterns
D. Abnormal Enzymatic patterns

Answers : 1: C, 2: A, 3: A, 4: A

BornSafe™

Microwell based new born screening tests

Tulip has always led the way in the development of *in vitro* diagnostics products. Screening of inborn error of metabolism (IEM) and disorders due to genetic abnormalities in new borns is the first step to decrease mortality and morbidity as well as to reduce the financial and social burden these disorders cause to families and societies. These assays work on dried blood spots as samples and can be run on vertical photometers at visual wavelengths. The designing of the kit ensures that these assays can be easily integrated into small and large volume laboratories. The assays are clinically validated and the calibration is traceable to international standards, (e.g. CDC, ISNS).

- **New-born Screening Test Parameters**
  n-TSH
  17-OHP
  G6PD
  T-Galactose
  PKU
  MSUD
  Biotinidase
- **Dried Blood Spot Collection Cards (Whatman 903™)**
- **Instruments & Accessories**
  Absorbance Microplate Reader
  Orbital Microplate Shaker
  Incubator
  DBS Puncher
  Pipette Set
  DBS Card Drying Stand
  Universal Reagent Reservoir

Presentation: 96 Tests

Printed and published by D.G. Tripathi, Edited by Dr. Ramnik Sood, M.D. (Path.) for and on behalf of Tulip Diagnostics Private Ltd., Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403 202, INDIA.
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