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OBM Genetics

Review

Newborn Screening for Inborn Errors of Metabolism

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Academic Editor: François Rousseau

Special Issue: Newborn Screening and Inherited Metabolic Disorders

OBM Genetics
2023, volume 7, issue 4
doi:10.21926/obm.genet.2304197

Received: June 14, 2023
Accepted: October 11, 2023
Published: October 18, 2023

Abstract

Newborn screening can now detect more than 50 disorders, providing early and often life-saving treatment. Inborn errors of metabolism account for the majority of these disorders. This review will consider the more common metabolic disorders identified on newborn screening, including history, technique and management of these disorders.

Keywords

Newborn screening; tandem mass spectrometry; diagnosis; management

1. Newborn Screening for Inborn Errors of Metabolism

Newborn screening (NBS) is a life-saving public health program that can detect 50 or more disorders in newborn infants, enabling early or pre-symptomatic treatment and thus saving lives. There can be no doubt that NBS saves lives and improves outcomes; the United States Center for Disease Control has stated that NBS expansion is one of the 10 great public health achievements in

the first decade of the 21st century [1]. The majority of disorders detected by NBS are inborn errors of metabolism. While NBS is a screening test and not a diagnostic test, infants who are screen-positive for metabolic disorders require urgent medical evaluation for diagnostic testing, and if necessary appropriate treatment. NBS is well established in most industrialized countries around the world, though the specific tests included on the screen are unique to each testing jurisdiction. This review will consider NBS for inborn errors of metabolism.

2. History

The story of newborn screening (NBS) begins with the story of phenylketonuria (PKU) and continues with the story of the power of family advocacy. In 1943, a mother of two children was determined to find the cause of their intellectual disability and unusual odor. Her search led her to Dr. Ivar Asborn Folling, who asked for a urine sample from the children and eventually received 20 liters of urine [2]. He identified the biochemical defect in the metabolism of the amino acid phenylalanine, and called it oligophrenia phenylpyruvica, or what we know of today as phenylketonuria or PKU [3]. A few decades later it was determined that the intellectual disability could be prevented by dietary restriction of phenylalanine, provided diet was initiated before cognitive damage occurred [4]. This presented a new problem - how to detect affected children and institute treatment before cognitive damage occurred?

A fortuitous sequence of events advanced the quest to improve earlier detection. Pediatricians could do a ferric chloride “diaper test” after two weeks of age visit to detect PKU early. However, this was not universally or equitably adopted. A cancer researcher, Dr. Robert Guthrie, had an interest in developmental disabilities because of his own disabled son, and developed a relatively easy and inexpensive method to detect elevated phenylalanine in a small spot of blood [5, 6]. Dr. Guthrie’s test used a bacterium that required phenylalanine to grow. The baby’s blood, obtained from a drop of blood from a heel prick and applied to a filter paper card, could be spotted on a plate infused with the bacteria, and a ring of bacterial growth around the sample with excessive phenylalanine would be visible, with the size of the ring predicting the amount of phenylalanine in the blood. This afforded a simple and inexpensive method to detect phenylketonuria in the first days to weeks of life, enabling early treatment and cognitive protection. The American president John F Kennedy also had a deep interest in intellectual disability because of his own intellectually disabled sister Rosemary Kennedy, and the US Children’s Bureau led a national trial of the “Guthrie test” [7]. Massachusetts was the first state to adopt mandatory newborn screening for PKU; after identifying 8 affected babies in the first months, it led to a heightened appreciation of PKU as one of the more common causes of intellectual disability and expansion of NBS [8].

Some experts, families and the Association for Retarded Children actively advocated for widespread adoption of mandatory newborn screening, eventually overcoming strong objections that it constituted “socialized medicine” [7]. With success in PKU, the “Guthrie test” was relatively easily adopted to other amino acid disorders such as homocystinuria (for methionine level), maple syrup urine disease (for leucine level), and others. Other disorders also amenable to testing from dried blood spots were added, including testing for hypothyroidism, galactosemia, congenital adrenal hyperplasia, and other disorders. The growth of testing panels created a need for consensus on which disorders were optimal candidates for NBS. The World Health Organization filled this void with the publication of Wilson and Jungner’s Principles and Practice of Screening for Disease [9],

which was considered the "gold standard" for NBS for nearly 40 years (Table 1). These criteria included that screening disorders should be relatively common and treatable if diagnosed before symptoms developed, and testing/treatment should be widely available and cost-effective.

Table 1 Jungner and Wilson vs ACMG criteria for disorder selection for NBS.

Wilson and Jungner (1968)	ACMG 2006
A suitable test is available	Evidence-based screening test available
An accepted treatment is available	Efficacious treatment available
Condition natural history understood	Condition natural history understood
Condition is an important health problem	Condition is clinically significant
Facilities for diagnosis and treatment are available	Condition may not meet criteria alone but is in the differential diagnosis of another core condition
A latent or early symptomatic stage exists	
Test acceptable to the population	
An agreed-on policy of whom to treat	
Test acceptable to population	
Cost of diagnosis and treatment economically balanced	

3. The TMS Technical Revolution

In the 1990's the new technology of tandem mass spectrometry (TMS) revolutionized NBS for metabolic disorders [10]. Instead of the limited number of metabolic disorders identified by the Guthrie bacterial inhibition assay, TMS could diagnose more than 40 inborn errors of metabolism from the blood spot, including both amino acid disorders as well as disorders of organic acids and fatty acid oxidation. But this leap in technology came with a new host of problems. The new technology required expensive equipment along with rare expertise to manage testing and interpret results. Many of these disorders did not meet the traditional Wilson and Jungner screening criteria. Though the metabolites were recognizable by TMS, some disorders were untreatable, some were likely not cost-effective to screen for, and some were of unclear medical significance. In many states the metabolic centers were concerned about capacity to manage the influx of patients who would require diagnostic and follow-up testing, and the follow-up DNA or other testing to differentiate affected from unaffected infants was not always covered by the state screening program or the patient's medical insurance. Commercial NBS programs began to compete with state screening programs in selling an expanded screen, and eventually TMS technology was initiated by all 50 states. In an effort to update the Wilson and Jungner criteria to the new technology, in 2006 the American College of Medical Genetics (ACMG) proposed a series of new criteria for screening suitability, identifying primary "target" disorders, as well as "secondary" disorders that are identified in the process for screening for target disorders [11] (Table 1). The new criteria recognized the ambiguity of identifying disorders not meeting other criteria for NBS but identified in the process of screening for target disorders. In the United States of America, NBS programs are administered by individual states, with the adoption of expanded screening on a state-by-state basis. In 2003 the federal Department of Health and Human Services convened the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC), which included a charge to maintain a

Recommended Uniform Screening Panel (RUSP) of primary and secondary disorders for inclusion on NBS panels the United States of America. The ACHDNC conducts a rigorous evidence-based review of proposed disorders to determine if that disorder is suitable for the RUSP addition as a target disorder. These criteria for inclusion as primary target disorders include adequate sensitivity/specificity for the screening and efficacious therapy. The original panel identified 26 primary target and 25 secondary disorders [11]. The ACHDNC is advisory in nature, and states are under no obligation to adhere to its recommendations.

4. Further Expansion of the RUSP

To date, parent advocacy groups and others have proposed a number of new disorders for evidence-based review for addition to the RUSP. The ACHDNC has recommended a number of these for universal adoption, such that currently there are 37 primary and 26 secondary target disorders [12]. Many of these disorders are lysosomal storage disorders, treatable with enzyme replacement therapy (ERT), including Pompe Disease, Fabry Disease and Mucopolysaccharidosis Types 1 and 2. Others recent disorders include Spinal Muscular Atrophy (now treatable with gene therapy or medication) and Guanidinoacetate methyltransferase (GAMT) deficiency.

While the RUSP serves as a scientific guide at the federal level, in the United States regulation of NBS lies with individual states and can become an emotional or political issue as well as a scientific issue. Families have often successfully pressed for expansion of the screening panel in their individual states. As one example, based on a publication in the New England Journal of Medicine suggesting early bone marrow hematopoietic stem cell transplantation (HSCT) might treat or palliate the effects of Infantile Onset Krabbe Disease [13], star football player and father of a child affected with Krabbe Disease, Jim Kelly advocated for the New York State governor to issue an executive order mandating adding Krabbe Disease to the New York state newborn screen. Screening began in 2006 and has been controversial. Through their efforts, to date ten states in the United States have made the controversial decision to add KD. Others have decided against adding to their screen [14]. In the ensuing 17 years since screening began in New York, the ability to discern a true positive diagnosis among screen-positive infants is improving [15], although the disorder was not approved for the RUSP on re-application in 2023.

5. Process

NBS is initiated by spotting a drop of blood from an infant's heel onto a special filter paper card. The card is dried and sent to a laboratory specific to the screening jurisdiction. A standard sized punch is obtained from the card and blood eluted from the punch with an assumption of how much blood is in the spot (hence concerns with under or over-saturation of the filter paper). As a number of metabolic disorders are cleared through the placenta prenatally, cord blood is not a suitable specimen for NBS as the abnormal metabolites require time to build up; thus screening should take place no earlier than 24, 36 or 48 hours of age, depending on regional recommendations**.** Some programs include a second screen at two weeks for enhanced detection of some disorders that may have better sensitivity later. Most programs utilize a two-tier reporting system: mild abnormalities are typically reported to the physician of record requesting a repeat screen, while emergent abnormalities are called to the physician and/or nearest treatment center. Mild abnormalities can be caused by immaturity, under or over saturation of the filter paper card, carrier status, overlap

between the normal and affected state metabolites, insufficient time for diagnostic abnormal metabolites to accumulate, and other reasons.

Screening techniques vary by laboratory and by the disorder in question. Tandem mass spectrometry detects more than 40 inborn errors of metabolism including phenylketonuria, some forms of homocystinuria, some organic acidemias such as methylmalonic. propionic or isovaleric acidemia, some (but not all) urea cycle disorders, and many fatty acid oxidation disorders. Some tests are enzyme assays, for example for galactosemia (galactose-1-phosphate uridyl transferase, aka GALT)) or biotinidase. The GALT enzyme is temperature sensitive and excess heating of the sample can damage the sample. Others are non-invasive tests not requiring the filter paper spot, for example hearing testing or pulse oximetry (for cyanotic heart disorders). Non-metabolic disorders include primary immune deficiencies, congenital adrenal hyperplasia, hypothyroidism, and others.

6. Accuracy of NBS and Diagnosis

As a screening test, it is important that the screen should have a high sensitivity and specificity, as well as an acceptable positive predictive value (PPV). In the pre-TMS era, the PPV for metabolic NBS ranged from 0.5% to 6% [16]. The PPV has been improved by both refining of the TMS process (including metabolite ratios) and by the addition of second tier testing for many disorders, such as evaluating secondary metabolites or DNA testing. Using these adjuncts has greatly improved PPV; for example the 2019 Michigan state report notes a PPV for metabolic disorders in the range of 11.6% for fatty acid oxidation defects to 75% for classic and Duarte galactosemia [17]. However, evaluating these statistics requires an understanding of potential pitfalls in diagnosis. In some cases, patients might manifest borderline metabolite concentrations or enzyme activities that can overlap with carrier status, be consistent with a subclinical/asymptomatic manifestation, or possibly reflect significant clinical disease. When metabolite testing is inconclusive, and when secondary analysis is unable to clarify (for example if DNA testing identifies variants of uncertain significance), it can be very difficult to ascertain with certainty if the patient is affected or unaffected with the disease in question.

7. Inborn Errors of Metabolism

A variety of metabolic disorders are detectable on NBS, including disorders of amino acid metabolism, organic acid metabolism, fatty acid oxidation, some carbohydrate disorders, lysosomal disorders, and others (Table 2). This list is not exhaustive but represents the most common metabolic disorders listed on the RUSP that are identified on NBS. For the complete list and more details on these disorders, refer to https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp screened disorders (accessed June 1, 2023). Various sites are available that provide information for parents, for example https://www.babysfirsttest.org/?# (accessed June 1, 2023). For information on specific disorders, consider GeneReviews [18].

Table 2 The more common primary target disorders from the RUSP as of January 2023. Specific metabolites are as per: https://www.acmg.net/ACMG/Medical-Genetics-Practice-Resources/ACT_Sheets_and_Algorithms.aspx. A complete list with information about each disorder is available at https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp.

Disorder	***Major Screening Metabolite****	Approved Treatment
Amino Acid Metabolism		
PKU	Phenylalanine	Dietary phenylalanine restriction
Homocystinuria	Methionine	Dietary methionine restriction
Maple Syrup Urine Disease	Leucine	Dietary leucine restriction, fasting prevention
Tyrosinemia Type 1	***Succinylacetone*** ***(Tyrosine in some programs)***	Nitisinone Dietary tyrosine restriction
Organic Acid Metabolism		
Propionic Acidemia	*Propionylcarnitine* (C3)	Dietary amino acid[1] restriction, carnitine, fasting prevention
Methylmalonic Acidemia	***Propionylcarnitine (C3),*** ***Methylmalonyl/succinylcarnitine*** (C4DC)	Dietary amino acid restriction[1], carnitine, fasting prevention (Vitamin B12)
Isovaleric Acidemia	***Isovaleryl/2-methylbutyrylcarnitine*** (C5)	Dietary leucine restriction, carnitine, glycine, fasting prevention
3-MCC	**3-hydroxyisovaleryl-/2-methyl-3-hydroxyacylcarnitine (C5OH)**	(Leucine restriction, fasting prevention, carnitine) **
Multiple Carboxylase Deficiency	***Propionyl carnitine (C3),*** ***3-hydroxyisovaleryl-/2-methyl-3-hydroxy acylcarnitine*** (C5OH)	High dose biotin, fasting prevention
Glutaric Acidemia	***Glutarylcarnitine*** (C5DC)	Amino acid restriction[2], fasting prevention, carnitine
Biotinidase Deficiency	Biotinidase activity	High dose biotin
Urea Cycle Defects		
Argininosuccinate Synthase Deficiency (Citrullinemia Type 1)	Citrulline	Protein restriction, ammonia scavenging medications, arginine, fasting prevention
Argininosuccinate Lyase Deficiency	Citrulline (Argininosuccinate)	Protein restriction, ammonia scavenging medications, arginine, fasting prevention
Fatty Acid Oxidation Disorders		
Carnitine Deficiency	Free carnitine (C0)	Carnitine
MCAD	***Hexanoyl carnitine (C6,)*** ***Octanoyl carnitine (C8),*** ***Decanoyl carnitine (C10)*** ***(Ratio of C8/C2)***	Fasting prevention

VLCAD	***Tetradecenoylcarnitine*** (C14:1)	MCT[3], fasting prevention, long chain fat restriction
LCHAD/TFP	***3-OH-hexadecanoylcarnitine (C16:1-OH), 3-Hydroxyoleoylcarnitine (C18:1-OH)***	MCT[3], fasting prevention, long chain fat restriction
Carbohydrate Disorders		
Galactosemia	GALT activity (Galactose 1-Phosphate) (Galactose)	Dietary galactose restriction
Lysosomal Storage Disorders		
Pompe Disease	Enzyme Activity	Enzyme replacement
Mucopolysaccharidosis Type 1	Enzyme Activity	Enzyme replacement (HSCT) [4]
Mucopolysaccharidosis Type 2	Enzyme Activity	Enzyme replacement
Other		
Guanidinoacetate Methyltransferase Deficiency	Guanidinoacetate (GAA) (Creatine, Creatine/GAA)	Creatine, ornithine supplements, na benzoate

*In addition to the major metabolites, many programs use ratios to improve specificity rather than only single metabolite levels, for example C3/C2 (ratio of propionylcarnitine/acetylcarnitine) for propionic acidemia

**Need for treatment is controversial at this time

[1] Valine, Methionine, Isoleucine, Threonine

[2]Tryptophan, Lysine

[3] Medium Chain Triglycerides

[4]Hematopoietic Stem Cell Transplant

8. Amino Acid Disorders

Phenylketonuria – PKU is due to deficiency of the enzyme phenylalanine hydroxylase (or in rare cases deficiency of the biopterin cofactor) [19]. Affected infants will have rising phenylalanine levels beginning in the days after birth. Unless treated with a phenylalanine restricted diet, the patient will develop intellectual disability and a number of other problems. Some patients may respond (to varying degrees) to administration of the biopterin cofactor. Enzyme replacement with subcutaneous injections of phenylalanine ammonium lyase is currently approved for adults in many countries and other treatments are under study.

Homocystinuria – Classical HCU is due to deficiency of the enzyme cystathionine beta synthase, which is involved in the metabolism of methionine [20]. Because homocysteine is not stable on the NBS assay, the screen assays for the precursor methionine. Treatment is by a methionine restricted diet, and some patients respond (to varying degrees) to supplementation with the cofactor vitamin B6. If untreated, patients have varying degrees of intellectual disability, early development of vascular and clotting disorders, lens dislocation and can have a Marfanoid-like phenotype. Of note, non-classical homocystinuria due to vitamin B12 processing defects presents with low (not elevated) methionine; in some cases this is detectable on NBS if it also results in elevation of methylmalonic acid.

Maple Syrup Urine Disease – MSUD is caused by deficiency of branched chain ketoacid dehydrogenase with accumulation of leucine, valine and isoleucine [21]. The pathognomonic metabolite of MSUD is allo-isoleucine which requires chromatographic separation using liquid chromatography in a second-tier test. Leucine accumulation appears responsible for the major effects, and without dietary leucine restriction affected patients develop neurologic deterioration including intellectual disability, seizures and potentially death. Treatment of hyperleucine coma typically requires emergent dialysis; chronic management includes dietary leucine restriction and in rare cases the cofactor thiamine. Some patients are opting for liver transplantation.

Tyrosinemia Type 1 – This disorder is caused by deficiency of fumarylacetoacetate hydrolase, a later step in tyrosine metabolism [22]. Fumarylacetoacetate, which builds up in front of the metabolic block, is subsequently converted to succinylacetone, which is toxic to liver and kidney. Tyrosine levels are less significantly elevated than in Type 2, and if the NBS program does not measure succinylacetone (not all do), the patient can be missed on NBS. Affected patients can develop liver failure, liver adenomas (which can progress to hepatocarcinoma), and renal tubular acidosis. Treatment is by administration of nitisinone, which blocks tyrosine metabolism at an earlier step (preventing the formation of succinylacetone). This then necessitates dietary restriction of tyrosine. Some patients are treated with orthotopic liver transplantation.

9. Organic Acid Disorders

Propionic or Methylmalonic Acidemia – PA or MMA are due to defects in consecutive steps in the metabolism of valine, odd chain fatty acids, methionine, isoleucine and threonine (acronym VOMIT) most commonly due to deficiency of the enzyme propionyl CoA carboxylase or methylmalonyl CoA mutase, respectively [23, 24]. Affected patients present with varying degrees of anion gap acidosis, hyperammonemia (from secondary impairment of the urea cycle), bone marrow suppression (from secondary effects on bone marrow), along with varying degrees of intellectual disability, renal failure (MMA), cardiomyopathy (PA), or in some cases death. Emergent treatment for neonatal coma involves dialysis. Chronic management includes dietary restriction of the offending amino acids, as well as supplementation with B12 (some cases of MMA are responsive), and other supplements. Some patients benefit from orthotopic liver transplantation, which appears to largely prevent acidotic crises but does not provide complete cure. Some patients with MMA have disease caused by the deficiency in processing B12, the cofactor for methylmalonyl CoA dehydrogenase. Some of the B12 complementation group defects also result in elevated homocysteine.

Isovaleric acidemia – IVA is due to deficiency of the enzyme isovaleryl CoA dehydrogenase, an enzyme required in leucine metabolism [25]. Severity is variable, but can include anion gap acidosis, hyperammonemia, seizures, failure or thrive, or death. Ongoing management includes dietary leucine restriction with carnitine and/or glycine. One particular variant, 932C>T (A282V) has been identified frequently on NBS; clinical significance is unclear, but evidence is accumulating that this particular variant may be benign.

3-Methyl crotonyl CoA dehydrogenase deficiency – 3-MCC deficiency is due to a defect in the 3-methylcrotonyl CoA dehydrogenase enzyme [26]. Originally this disorder was believed to cause acidosis, hypoglycemia, hyperammonemia, intellectual disability and a variety of problems. A few publications have described metabolic abnormalities in affected patients. However, the majority of

infants identified by NBS appear asymptomatic. Some jurisdictions have removed this disorder from their screening list.

Multiple carboxylase deficiency (holocarboxylase synthetase deficiency) is due to a defect in three biotin-dependent enzymes: propionyl CoA carboxylase, 3-methycrotonyl CoA carboxylase, and pyruvate carboxylase [27]. Affected patients can present with metabolites attributable to all 3 enzyme systems, including anion gap ketoacidosis, hyperammonemia, and lactic acidosis. High dose biotin is the mainstay of treatment.

Glutaric acidemia type 1: this disorder is due to a defect in the enzyme glutaryl CoA dehydrogenase, required in the metabolic degradation of tryptophan, lysine, and hydroxylysine [28]. During periods of catabolism affected patients can develop injuries to the basal ganglia, resulting in movement disorder, intellectual disability, and macrocephaly among other findings. The mainstay of treatment is prevention of fasting with dietary restriction of the offending amino acids.

Biotinidase deficiency is due to inability to recycle biotin [29]. As biotin is an important cofactor for a number of enzymes, deficiency can result in a wide range of problems including abnormalities of skin and hair, vision and hearing loss and neurologic abnormalities. Treatment is by taking supra-physiologic doses of biotin.

10. Fatty Acid Oxidation Disorders

Carnitine deficiency – Carnitine is required to esterify long chain fat and allow it to pass into the mitochondria for oxidation [30]. Primary carnitine deficiency is due to a defect in carnitine transport and results in very low plasma carnitine levels. It can be asymptomatic, but can also present with sudden death from arrhythmia. Treatment is by administering high dose carnitine.

Disorders of beta-oxidation – Once inside the mitochondria, fatty acids undergo successive cycles of beta-oxidation, a process in which each cycle which removes two carbons from the fat, which become acetyl-CoA and enter the Krebs cycle, or ketone bodies which can be exported to other tissues. The first step is an acyl CoA dehydrogenase that acts on the CoA esterified fat (fatty acyl-CoA) by removing hydrogen and creating a double bond at the beta carbon (hence, the process of beta oxidation). This step has chain-length specificity, with separate enzymes for short chain (4 carbon fats), medium chain (6-10 carbon fats) and long chain fatty acids (12-18 carbon fats). It has been estimated that disorders of fatty acid oxidation may be involved in up to 5% of cases of sudden infant death syndrome [31] Disorders of short-chain acyl CoA dehydrogenase (SCADD) are now controversial and some programs have recommended removing this disorder from their screen [32]. Medium-chain acyl CoA dehydrogenase deficiency (MCADD) is the most common fatty-acid oxidation disorder [33]. Affected patients demonstrate fasting intolerance, and can develop hypoketotic hypoglycemia and death if allowed to become catabolic. Treatment is by fasting prevention. Very long chain acyl CoA dehydrogenase deficiency (VLCADD) can present with severe neonatal hypoketotic hypoglycemia, cardiomyopathy and Reye's syndrome-like findings and can be fatal, or can present later with fasting intolerance or myopathy [34]. Treatment involves supplementation with medium chain triglycerides or triheptanoin (a seven-carbon fat), as well as fasting prevention. The third step of the beta-oxidation cycle is another dehydrogenase, long-chain hydroxy acyl CoA dehydrogenase, or LCHADD. In some cases the LCHAD enzyme itself is deficient, in other cases it is the trifunctional carrier protein that is deficient (trifunctional protein deficiency,

or TFPD) [35]. This can also present with variable severity including hypoketotic hypoglycemia or cardiomyopathy. Treatment is similar to VLCADD.

11. Urea Cycle Disorders

When amino acids are metabolized for energy, waste ammonia is produced. This ammonia is toxic and must be de-toxified through the urea cycle [36]. In the *proximal* steps of the urea cycle, carbamyl phosphate synthase (CPS) works with n-acetylglutamate (created by n-acetylglutamate synthase- aka NAGS) to create carbamoyl phosphate. Carbamoyl phosphate combines with ornithine to make citrulline by the enzyme ornithine transcarbamylase (OTC). In the *distal* part of the cycle, citrulline is then converted to argininosuccinate, then arginine. Arginine splits off two nitrogen molecules as urea to create more ornithine, continuing the cycle. For the distal defects, accumulation of citrulline, argininosuccinate or arginine can identify which step of the urea cycle is impaired. However NBS for proximal cycle defect (CPS, NAGS, OTC) is more difficult, and low citrulline alone lacks sensitivity and specificity. Studies are underway to improve NBS for proximal urea cycle defects but at present NBS does not reliably identify these. Treatment of urea cycle disorders includes protein restriction and use of “scavenger” medications which help to remove ammonia and re-supply arginine or citrulline into the cycle.

12. Carbohydrate Disorders

Galactosemia is caused by inability to metabolize galactose into glucose by the enzyme galactose-1-phosphate uridyl transferase (GALT) [37]. The buildup of galactose and particularly galactose-1-phosphate leads to liver disease, cataracts, failure to thrive and in some cases gram-negative neonatal sepsis. Treatment is by dietary restriction of galactose.

13. Lysosomal Storage Disorders

Several lysosomal storage disorders are now on the RUSP, including Mucopolysaccharidosis Types 1 and 2, and Pompe disease. More information is available on these disorder in this issue (https://www.lidsen.com/journals/genetics/genetics-07-03-194). Enzyme replacement therapy is available for all three, and HCST can improve outcome in the severe form of Mucopolysaccharidosis type 1 (Hurler Syndrome).

14. Other

Guanidine methyltransferase (GAMT) deficiency is a defect in the synthesis of creatine [38]. Creatine is important for energy production. Deficiency is associated with developmental delay/intellectual disability, seizures, speech delay, and often behavioral manifestations.

15. Management of the Screen Positive Infant

On receiving a report of an abnormal screen, the physician should immediately verify the infant is well and follow the instruction from the screening report. If instructions are to repeat the screen this should be done expeditiously. If the instructions state to refer the patient to the nearest treatment center for diagnostic testing or treatment, this should be done emergently. The referring

physician should not begin any special formulas unless/until directed by the metabolic treatment center, but should be prepared for a potentially sick infant needing emergent support. Attempting to begin dietary restriction before confirmatory testing is obtained can mask the disorder and cause metabolites to appear normal on diagnostic testing. In an emergency, samples for plasma amino acids, plasma acylcarnitine, and urine organic acids or other recommended tests can be obtained, and treatment initiated before results are returned as indicated.

The American College of Medical Genetics has published information about NBS for physicians as ACT sheets and diagnostic algorithms. (https://www.acmg.net/ACMG/Medical-Genetics-Practice-Resources/ACT_Sheets_and_Algorithms.aspx accessed June 1, 2023). These can help the physician, but consultation with a metabolic referral center should be arranged as soon as possible. Altered mental status, hypo or hypertonia, abnormal deep tendon reflexes, clonus, vomiting, seizures, persistent hypoglycemia, anion gap acidosis, liver dysfunction or ketosis are among the more coming signs/symptoms of a metabolic crisis. Most disorders of amino or organic acids, fatty acid oxidation or urea cycle defects are made worse by fasting. In general, an initial first step in managing these affected infants in crisis is to prevent catabolism by delivering intravenous glucose (with appropriate electrolytes) at 8-10 mg/kg/min (approximately D10 with saline at 1.5 maintenance rate). Unless a disorder of fatty acid oxidation is suspected, intralipid can be started to support anabolism. Protein should not be restricted past 24 hours of treatment, as negative nitrogen balance will worsen many of these conditions; protein should be restarted, typically at 0.5 gm/kg/day and titrated upwards as tolerated. For some amino acid, organic acid or urea cycle disorders, emergent hemodialysis may be needed to remove the offending metabolites. For some, carnitine is indicated (100 mg/kg/day orally or 50 mg/kg/day intravenous). Many disorders have had clinical practice guidelines published, and additional resources can be found on GeneReviews [18] or The Metabolic and Molecular Bases of Inherited Disease [39], or on websites such as the New England Consortium of Metabolic Programs (https://www.newenglandconsortium.org/acute-illness).

There are currently 26 secondary disorders listed on the RUSP. These disorders do not meet criteria for screening by themselves, but are often identified during screening for the primary target disorders. A list of these disorders is available at: https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp. Some of these can present with metabolic crises (for example Glutaric Acidemia Type 2) and require emergent management. The clinical significance of some others is uncertain, for example Short Chain Acyl-CoA dehydrogenase deficiency.

As new disorders are identified and as screening tests and treatments improve, more disorders will be added to the RUSP. Pilot studies of genomic screening using DNA analysis are ongoing, and as variants are better understood, this may change the nature of NBS to a genetic rather than metabolite or enzyme based test [40].

Author Contributions

The author did all the research work of this study.

Competing Interests

The author has declared that no competing interests exist.

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