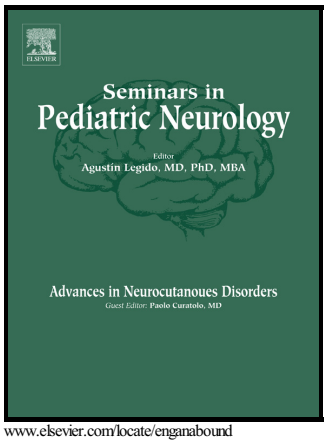


Neonatal Screening for Inherited Metabolic Diseases in 2016

Judit Garcia Villoria, Sonia Pajares, Rosa Lopez, José Luis Marin, Antonia Ribes

PII: S1071-9091(16)30031-6
DOI: http://dx.doi.org/10.1016/j.spen.2016.11.001
Reference: YSPEN605

To appear in: *Seminars in Pediatric Neurology*

Cite this article as: Judit Garcia Villoria, Sonia Pajares, Rosa Lopez, José Luis Marin and Antonia Ribes, Neonatal Screening for Inherited Metabolic Diseases in 2016, *Seminars in Pediatric Neurology,* http://dx.doi.org/10.1016/j.spen.2016.11.001

Neonatal screening for inherited metabolic diseases in 2016

Judit Garcia Villoria*, Sonia Pajares*, Rosa Lopez, José Luis Marin, Antonia Ribes**

Seccción de Errores Congénitos del Metabolismo-IBC, Servicio de Bioquímica y Genética Molecular, Hospital Clínic, CIBERER, IDIBAPS, Barcelona, Spain"

*Equal contribution

Professional affiliation:

****Corresponding author:**

Antonia Ribes, PhD

Sección de Errores Congénitos del Metabolismo-IBC

Servicio de Bioquímica y Genética Molecular

Hospital Clínic, CIBERER, IDIBAPS, Barcelona, Spain

c/ Mejia Lequerica s/n Helios III

08028 Barcelona, Spain

Tel: +0034 932279340

Fax: +0034 932275668.

Email: aribes@clinic.ub.es

Judit García-Villoria, BSc

Sección de Errores Congénitos del Metabolismo-IBC

Servicio de Bioquímica y Genética Molecular

Hospital Clínic, CIBERER, IDIBAPS, Barcelona, Spain

c/ Mejia Lequerica s/n Helios III

08028 Barcelona, Spain

Tel: +0034 932277585

Fax: +0034 932275668.

Sonia Pajares, BSc

Sección de Errores Congénitos del Metabolismo-IBC

Servicio de Bioquímica y Genética Molecular

Hospital Clínic, CIBERER, IDIBAPS, Barcelona, Spain

c/ Mejia Lequerica s/n Helios III

08028 Barcelona, Spain

Tel: +0034 932279342

Fax: +0034 932275668.

Rosa Maria López, PhD

Sección de Errores Congénitos del Metabolismo-IBC

Servicio de Bioquímica y Genética Molecular

Hospital Clínic, CIBERER, IDIBAPS, Barcelona, Spain

c/ Mejia Lequerica s/n Helios III

08028 Barcelona, Spain

Tel: +0034 932277226

Fax: +0034 932275668.

Jose Luís Marín, MD

Sección de Errores Congénitos del Metabolismo-IBC

Servicio de Bioquímica y Genética Molecular

Hospital Clínic, CIBERER, IDIBAPS, Barcelona, Spain

c/ Mejia Lequerica s/n Helios III

08028 Barcelona, Spain

Tel: +0034 932277582

Fax: +0034 932275668.

ABBREVIATIONS

BCKD	branched-chain α-ketoacid dehydrogenase complex
BKT	β-ketothiolase

C0	free carnitine
C2	acetylcarnitine
C3	propionylcarnitine
C5	isovalerylcarnitine
C5:1	tiglylglycine
C5DC	glutarylcarnitine
C5OH	3-hydroxy-isovalerylcarnitine
C6DC	3-methyl-glutarylcarnitine
C8	octanoylcarnitine
C16	palmitoylcarnitine
C16-OH	3-hydroxyhexadecanoylcarnitine
C17	heptadecanoic acid
C18-OH	3-hydroxyoctadecanoylcarnitine
C18:1-OH	3-hydroxyoctadecenoylcarnitine
CACT	carnitine acylcarnitine translocase
CBS	cystathionine β-synthase
CPT1	carnitine palmitoyltransferase I
CPT2	carnitine palmitoyltransferase II
CTD	carnitine transporter deficiency
DBS	dried Blood spots
FAO	fatty acids oxidation
GA-1	glutaric aciduria type 1
GAMT	guanidinoacetate methyltransferase
GALT	galactose uridyltransferase 1-phosphate
HCS	holocarboxylase synthase deficiency
HCY	homocystinuria
HMG	3-hydroxy-3-methylglutaric aciduria
IQ	intellectual quotient
IVA	isovaleric acidemia
LCHAD	loong chain acyl-CoA dehydrogenase
LSDs	lysosomal diseases
MAD	multiple acyl-CoA dehydrogenase
MCAD	medium chain acyl-CoA dehydrogenase
3-MCC	3-methylcrotonyl-glycinuria
2M3HBD	2-methyl-3-hydrobutyryl CoA dehydrogenase deficiency

MMA	methylmalonic aciduria
MPSI	mucopolysaccharidosis type I
MS/MS	spectrometry mass
MSUD	maple syrup disease
MTHFR	methylenetetrahydrofalate reductase
MTP	mitochondrial trifunctional protein
MUT	methylmalonyl-CoA mutase
NBS	newborn screening
OTC	ornithine transcarbamylase
PA	propionic acidemia
Phe	phenylalanine
PKU	phenylketonuria
TYR 1	tyrosinemia type 1
UCD	urea cycle disorder
VLCAD	very long chain acyl-CoA dehydrogenase
X-ALD	X-linked adrenoleukodytrophy

SUMMARY

The scope of newborn screening (NBS) progrmas is continuously expanding. NBS programs are secondary prevention interventions widely recognized internationally in the "field of Public Health. These interventions aimed at early detection of asymptomatic children affected by certain diseases, with the objective to establish a definitive diagnosis and apply the proper treatment to prevent further complications and sequelae and ensure a better quality of life.

The most significant event in the history of neonatal screening was the discovery of phenylketonuria (PKU) in 1934. This disease has been the paradigm of inherited metabolic diseases. The next paradigm was the introduction of tandem mass spectrometry in the NBS programs that makes possible the simultaneous measurement of several metabolites and consequently, the detection of several diseases in one blood spot and in an unique analysis.

We aim to review the current situation of neonatal screening in 2016 worldwide and show scientific evidence of the benefits for some diseases. We will also discuss future

challenges. It should be taken into account that any consideration to expand a NBS panel should involve a rigorous process of decision-making that balances benefits against the risks of harm.

INTRODUCTION

Inherited metabolic diseases are especially relevant due to the high morbidity and mortality, the high risk of recurrence in affected families, the possibility of therapeutic options and the potential identification of asymptomatic infants through the neonatal screening (NBS) programs

NBS programs are secondary prevention interventions widely recognized internationally in the "field of Public Health. These interventions aimed at early detection of asymptomatic infants affected by certain diseases, most of them rare diseases, with the objective to establish a definitive diagnosis and apply the proper treatment to prevent further complications and sequelae and ensure a better quality of life.

The most significant event in the history of neonatal screening was the discovery of phenylketonuria (PKU) by Dr. Asbjørn Folling in 1934[1]. PKU has been the paradigm of inherited metabolic diseases. It was also the first time that a biochemical explanation for the mental retardation was given. In addition, he developed a test for detecting the disease by adding ferric chloride to two affected brothers' urine. As a result of the reduction of iron, the urine turned green. He later showed it was due to accumulation of phenylpyruvic acid. In 1953 Dr. Bickel established an effective treatment for PKU[2]. He proposed that the general development of children with PKU would be practically normal if phenylalanine would be restricted in the diet from the neonatal period. This fact led to the prospective studies of disease detection in many countries, and in 1958 the first population screening program was carried out in the city of Cardiff using the Folling test.

The USA NBS programs began in the early 1960's, when Dr. Robert Guthrie described a simple screening test to measure phenylalanine in large populations in blood spots[3]. The benefits of newborn screening in children quickly attracted the population support because the early low-phenylalanine diet could prevent progression of intellectual and developmental disability. Since then, the large-scale screening became possible. The universal newborn screening program for PKU started in Massachusetts in 1963 and many countries quickly established their own NBS programs.

The next paradigm was the introduction of tandem mass spectrometry (MS/MS) in the NBS programs. Mass spectrometry is an analytical technique that allows the identification and quantification of compounds in a biological sample according to the mass/ charge ratio. This methodology was one of the triggering factors of the revision and expansion of NBS programs worldwide, that makes possible the simultaneous measurement of several metabolites and, consequently the detection of several diseases in one blood spot and in an unique analysis. Millington and Chace[4,5] were the pioneers in implementing MS/MS in NBS through the analysis of amino acids and acylcarnitines, allowing the identification of a number of aminoacid disorders, organic acidurias and mitochondrial fatty acid β-oxidation disorders. In addition, ratio measurements of one analyte respect to another are also possible, which improves the specificity of screening[6,7]. For that reason MS/MS is currently used in many NBS programs[8].

The role and scope of NBS is expanding. While traditional newborn screening was only concerned with few diseases associated with mental retardation, the programs now include disorders that can cause premature death, inherited metabolic diseases, hemoglobin diseases, lysosomal storage disorders, and others. It should be taken into account that any consideration to expand a NBS panel should involve a rigorous process of decision-making that balances benefits against the risks of harm[9].

We aim to review the current situation of neonatal screening in 2016 worldwide and show scientific evidence of the benefits for some diseases. We will also discuss future challenges.

Worldwide inherited metabolic diseases included in the neonatal screening (NBS) programs in 2016

After the introduction of MS/MS [4,5] in NBS laboratories some states in the USA began NBS for up to 30 different disorders. In response to a lack of national uniformity, the Newborn Screening Task Force of the American Academy of Pediatrics recommended the development of a national system to coordinate NBS standards and policies[10]. In 2003, the Secretary of Health and Human Services formed an Advisory Committee to provide recommendations on screening tests, technologies, policies, guidelines, and standards[11]. The Committee recommended screening for a panel of 29 core disorders and 25 secondary target disorders, the last obtained mainly by differential diagnosis of the core conditions. These disorders included organic acids, amino acids and mitochondrial fatty-acid oxidation disorders[12], but not all the states screen for all the

conditions (table 1). Since then, the Committee has evaluated a further 12 disorders and three have been added to the recommended NBS panel[11].

Canada has 15 regions and although individual territories have their own decision-making processes, all programs include cystic fibrosis and congenital hypothyroidism (not shown in table 1), as well as, medium chain acyl-CoA dehydrogenase (MCAD) deficiency and phenylketonuria (PKU)[13].

In 2009, the European Commission initiated a review of current NBS services and a network of experts to assess and recommend a core panel of disorders to be screened was nominated[14]. However, currently the variability in the content of screening programs in the European Union is very high, as there are programs that include up to 30 disorders and others include only two. In addition, the different conditions or diseases vary not only among the different European countries but also within different regions of a particular country. Therefore, there is an urgent need to have a consensus to the diseases that should be included in the 48 European countries[15].

In the Middle East and North Africa, comprising 21 countries, neither exist uniformity in the panels, and the NBS by MS/MS is selective or limited, while for congenital hypothyroidism there is a high coverage[13] (table 1).

The 20 countries of Latin America and the 24 countries of Asia Pacific neither have a consensus uniform panel (table 1) and except one or two countries, all of them screen for congenital hypothyroidism (data not shown).

Currently, developed countries worldwide have adopted NBS programs. Very recently Therrell et al[13] published an extensive review, country by country, that we grouped by geographic areas and have summarized in table 1.

Core diseases and evidence of newborn screening benefits

Aminoacid disorders

Phenylketonuria (PKU), the paradigmatic aminoacid disorder, has been included in almost all the NBS programs worldwide (table 1) and is considered the first successful story of NBS. There is not any doubt that PKU must be included in the NBS programs. Concerning the other aminoacid disorders there are some variability among different geographic areas. All US states, except two, perform screening for all the core aminoacid disorders. In Canada the proportion is about 30%. In Europe, Middle East and North Africa, Latin America and Asia Pacific the proportion is around 50%, except that the tree later geographic areas do not screen for argininsuccinic aciduria (table 1).

Phenylketonuria

PKU, (OMIM# 261600) is one of the most common inborn errors of amino acid metabolism, and was the first disorder detected by NBS programs[3,16]. High levels of phenylalanine (Phe) can cause serious irreversible brain damage[17]. Phe measurement on DBS is an easy test that allows the early identification of PKU and also benign hyperphenylalaninemia (Fig.1).

Benefits of NBS. PKU has been the paradigm of inherited metabolic disorders. After more than five decades of NBS a number of studies have demonstrated that early detection and treatment at birth prevents neurocognitive impairment, and even these patients can accomplish an adequate apprenticeship[18]. Nevertheless, neurophysiological and neuropsychological impairments may still persist in treated PKU patients[19-21]. Patients who were late-diagnosed have irreversible neurological damage; however in many cases there may be partial reversibility of cognitive deficits [18, 22-23].

Homocystinurias

Homocystinuria due to cystathionine β-synthase (CBS) deficiency (MIM# 236200) is the second most common disorder of amino acid metabolism. In addition to CBS, there are other conditions that present with homocystinuria, like remethylation defects, methylenetetrahydrofalate reductase deficiency (MTHFR OMIM#236250) or cobalamin biosynthesis defects, cblE, cblG, cblD-HCY and cblX; or other cobalamin defects that in addition present with methylmalonic aciduria (cblC, cblF, cblJ and cblD-MMA-HCY); see “organic acid disorders”. All these disorders are detected though the analysis of the same primary markers on DBS (Fig.1). Moreover, the specific second-tier test homocysteine, methylmalonic acid and methylcitric acid allow for the differentiation between CBS and the remethylation defects, and the cobalamin metabolism defects[24] (Fig.1). However, it is important to adjust the analyte cut-off values adequately, since missed cases of false negatives of cblC and CBS have been described[25].

Benefits of NBS. Early treatment in CBS deficiency can prevent ocular[26] and vascular complications[27]. Morbidity is also prevented and some patients present normal intellectual quotient (IQ)[27,28]. Contrarily, late diagnosed and late-treated patients develop ocular and vascular problems[26-27, 29-30]. Similarly, in MTHFR deficiency there is also evidence of a clear clinical effect on early treatment preventing mortality and allowing normal psychomotor development[31]. In cblE and cblG patients a positive impact of early treatment has been observed[32,33], while in other cblE patients, the

overall impact of treatment on neurodevelopmental disabilities is moderate. The impact is negative or moderate on eye[34,35].

Screening for CBS deficiency is highly recommended, since a large group is pyridoxine responsive. NBS for severe MTHFR deficiency is also recommended since early betaine administration is associated with a positive outcome. For the cblD-Hcy, cblE and cblG defects, NBS experience is very limited, but can be considered due to the evidence described in some cases about the benefit of early treatment[24].

Maple syrup urine disease

Maple syrup disease (MSUD, OMIM# 248600) is one of the most severe amino acid disorders. It is caused by deficiency of the branched-chain α-ketoacid dehydrogenase complex (BCKD) leading to high levels of branched-chain amino acids (leucine, isoleucine and valine). In the classical MSUD, clinical onset usually occurs within the first days of life, with acute metabolic decompensation[36].

NBS detection consists on the measurement of the primary markers leucine, valine and isoleucine on DBS. The analysis of alloisoleucine as second-tier test is very useful[37] as it is pathognomonic of MSUD and allows the differential diagnosis from other conditions (Fig.1).

Benefits of NBS. Several studies have demonstrated beneficial effects of early detection by NBS. A correlation between high plasma leucine levels and neurological damage has been reported[38,39], since leucine is a potent neurotoxic metabolite[40-41]. IQ is directly correlated with the prolonged amino acid imbalances that are present in these patients, mainly during the first years of life, resulting in structural and functional neurologic abnormalities that have morbid long-term psychomotor consequences[42-43]. Therefore, a frequent amino acid monitoring is essential[44]. In late-diagnosed patients encephalopathy and cerebral edema are frequently present[44-45]. Evidence reflects the aggressiveness of MSUD. Therefore, early detection is recommended to prevent irreversible neurological damage.

Citrullinemia type I and Argininsuccinic aciduria

Citrullinemia type I is a urea cycle disorder (UCD) caused by argininosuccinate synthetase deficiency (OMIM#215700), that produces a severe hyperammonaemic encephalopathy resulting in neurological and mental impairment and even death, if the start of treatment is delayed. NBS is based on citrulline measurement on DBS. Increased citrulline is also present in argininosuccinic aciduria (MIM#207900), but the measurement of argininsuccinic acid permits the specific identification of this entity

(Fig.1). It is also important to take into account the possibility of other diagnosis presenting with high citruline levels, such as citrin deficiency or pyruvate carboxylase deficiency[46].

Benefits of NBS. Early detection represents an advantage for citrullinemia type I and argininsuccinic aciduria, and also for other UCD. Patients can present with early neonatal onset or late onset forms. Neonatal mortality in the severe forms has been reported at about 50 %[47]. Blood ammonia level during the first hyperamonaemic attack is correlated with neurodevelopmental outcome[48-49]. For that reason, it is important to diagnose UCD when the blood ammonia levels in patients are low[50]. Early detection of citrullinemia and also argininsuccinic aciduria in the first days of life by NBS could prevent irreversible neurological damage. Consequently, it should be considered the inclusion of these amino acid disorders, extensive to other UCDs, in NBS programs in order to benefit from their early diagnosis.

Tyrosinemia type I

Tyrosinemia type I (TYR1, OMIM#276700) is characterized by a severe hepatorenal dysfunction with high risk of hepatocellular carcinoma if treatment is not implemented[51]. Porphyria-like neurological crisis are also present while cardiomyopathy is less frequently observed[52-53]. This disease is caused by the deficiency of fumarylacetoacetase leading to an accumulation of tyrosine and succinylacetone. High levels of tyrosine overlap with tyrosine concentrations found in common benign transient tyrosinemia of the neonate, and other entities such as Tyrosinemia type II and Tyrosinemia type III[54]. Moreover, normal tyrosine levels can be found in the first few days of life[55], resulting in potential false negatives. Measurement of succinylacetone in DBS allows to identify TYR1 specifically. Therefore, succinylacetone should be used as primary marker for the detection of this disease[56] (Fig. 1).

Benefits of NBS. Up to now NBS of TYR1 have been unsuccessful in many countries due to its relative rarity, unproven cost-benefit value of early diagnosis and lack of availability of succinylacetone test[55]. Recently, several studies reported the outcome of patients diagnosed from NBS, compared with the outcome of patients diagnosed following clinical presentation. No evidence of liver or kidney disease was observed in screened patients, in contrast to patients who presented clinically that had poorer outcome, including chronic liver disease with liver transplant requirement in many of them[51,57-59].

Therefore, an early diagnosis and treatment in TYR1 is effective in preventing liver disease and the risk of hepatocellular carcinoma[55,59].

Organic acidurias.

The number of states and countries that perform screening for the organic acidurias is the same for all the conditions, except for methylmalonic acidurias. All the US states, except two, perform screening for organic acidurias. In Latin America and Asia Pacific the proportion is around 50%, in Europe 40%, in Canada only 20% and in Middle East and North Africa the screening for these conditions is limited or selective (table 1).

Propionic acidemia

Propionic acidemia (PA) (OMIM# 606054) is caused by deficiency of the mitochondrial enzyme propionyl-CoA carboxylase. NBS for PA, and related diseases of propionate metabolism, is performed in DBS through elevation of propionylcarnitine (C3) with high propionylcarnitine/acetylcarnitine ratio (C3/C2), C3/ palmitoylcarnitine(C16) and low methionine. Recently, heptadecanoic acid (C17) has been proposed as new biomarker to improve the analytical performance of this metabolism[60]. Specificity is highly improved by the measurement of methylcitrate, methylmalonate and total homocysteine in DBS as second tier test. The last test, allowing the differential diagnosis of PA from the isolated or combined methylmalonic acidemia with homocystinuria (Figure 2)

Benefits of NBS. Data on the benefit of NBS for PA are sparse. Whereas PA iswidely screened for in NBS programs, some NBS guidelines do not advocate screening for this disorder. Dionisi-Vici et al (2006)[61] reported that diagnosis through NBS is not associated with a milder clinical course or better neurocognitive outcome. In 2012, twenty PA patients diagnosed through NBS were compared to 35 patients diagnosed clinically[62]. This paper was followed by another study in 2013[63]. These authors concluded that poor intellectual development is still the rule in PA and although improved acute and long-term management have increased the survival rates within the last decades, the neurologic outcome of PA patients is still unsatisfactory even if NBS is performed.

Isolated methylmalonic acidemia

Isolated methylmalonic academia (MMA), is caused by complete or partial deficiency of the enzyme methylmalonyl-CoA mutase (*mut*^{0} or *mut*$^-$), a defect in the transport or

synthesis of its cofactor, adenosyl-cobalamin (*cblA*, *cblB*, or *cblD*-MMA), or deficiency of the enzyme methylmalonyl-CoA epimerase.

Benefits of NBS. The near-universal implementation of expanded newborn screening by MS/MS has afforded the early detection of patients with a wide spectrum of disorders such as isolated MMA, where long-term outcomes are still pending, and further multicentre longitudinal studies are
needed to assess the usefulness of newborn screening for these diseases[61].

Combined methylmalonic aciduria with homocystinnuria (cblC and cblD)

Both defects affect the synthesis of methylcobalamin, and of adenosylcobalamin. The cblC defect (OMIM#277400), is the most common disease[64]. Primary markers and second tier test for NBS are the same of PA (Fig. 2); also the same markers are useful for the detection of the other disorders including cblF and CblJ, but efficacy and feasibility of screening for cblD, cblF and CblJ is unknown[24].

Benefits of NBS. Despite identification by NBS and early treatment, 11/12 patients showed developmental delay, 8/9 brain pathologies, 10/11 muscular hypotonia, and 9/12 nystagmus at a mean age of 50 months[65]. Screening for the cblC defect should be considered since in the early-onset CblC defect response to treatment has been observed for non-neurological symptoms. For late-onset patients treatment is mostly beneficial, especially when initiated before irreversible organ damage has occurred.

Glutaric aciduria type I

Glutaric aciduria type I (GA-1, OMIM#231670) is due to glutaryl-CoA dehydrogenase deficiency. NBS is performed by the identification of elevated glutarylcarnitine (C5DC) and/or high ratios of C5DC/palmitoylcarnitine (C16), C5DC/octanoylcarnitine (C8) and other secondary markers. Specificity substantially increases by analyzing glutaric acid, 3-hidroxiglutaric acid and 2-hidroxiglutaric acid in DBS as a second tier test (Fig. 2). The same second tier test markers are useful to discriminate GA I from multiple acil-CoA dehydrogenase deficiency. C5DC increase may also reflect kidney failure or maternal GA I[66]. Frazier et al. (2006)[67] reported two false negatives that do not exceed the cutoffs values at the time of screening. As a consequence, these authors reduced their cutoffs.

Benefits of NBS. Kolker et al reported the follow-up of 38 patients diagnosed presymptomatically of GA-I by NBS and compared the neurological outcome with a cohort of 62 patients diagnosed by clinical symptoms[68]. In 89% of the screened

patients the onset of encephalopathic crisis has been prevented whereas acute encephalopathic crises or progressive neurologic impairment was common in the patients diagnosed clinically. Other authors support that, early diagnosis and treatment are essential for the good clinical evolution of GA-1 patients[69]. However, previous studies obtained less satisfactory results[70]. Therefore, with few exceptions, NBS allows patients to achieve an appropriate motor development, as opposed to unscreened patients that mostly develop dystonia and other serious movement disorders. Its inclusion in NBS is highly recommended.

Isovaleric acidemia

Isovaleric acidemia (IVA, OMIM#243500) is caused by a defect in leucine catabolism due to isovaleryl-CoA dehydrogenase. NBS can be performed by identification of elevated isovalerylcarnitine (C5), and high ratios C5/free carnitine(C0), C5/propionylcarnitine (C3), C5/acetylcarnitine (C2) and other secondary markers (Fig.2) However, isobaric acylcarnitines like 2-methylbutyrylcarnitine and pivaloylcarnitine can not be distinguished by the screening method[71].

Benefits of NBS. Grünert et al (2012) retrospectively analyzed clinical data of a large cohort of patients with IVA[72], 57% of them were diagnosed within the first weeks of life and 43% in childhood. Comparison of both groups showed that IQ was not related to the number metabolic crisis, but a significant inverse relationship between IQ and age of diagnosis was observed. Moreover, 82% of early diagnosed patients did not present learning disorders, compared with the 44% of patients diagnosed lately[72]. These authors concluded that mortality associated with neonatal manifestations is high, but survivors benefit from early diagnosis and treatment. The potential to avoid early mortality and to improve neurocognitive outcome reinforces IVA to be included in the for NBS programs.

3- methylcrotonyl-glycinuria

3- methylcrotonyl-glycinuria (3-MCC, OMIM#210200,210210) is a caused by the deficiency of the enzyme 3-methylcrotonyl-CoA carboxylase. NBS for 3-MCC can be identified by elevated 3-hidroxy-isovalerylcarnitine (C5OH) and high C5OH/carnitine (C0) and C5OH/octanoylcarnitine (C8) ratios. These acylcarnitines may also be markers for other diseases, and differential diagnosis should be done (Fig 2). High C5OH may also reflect maternal 3-MCC[73.]

Benefits of NBS. The California Newborn Screening Program's have reported 71 diagnosed with 3-MCC (8 cases severe, 19 cases mild-variants or heterozygote carriers- and 44 cases could not be classified). The authors concluded that a significant proportion of the 3-MCCD "confirmed" cases have a mild biochemical phenotype. These findings raise the concern that a significant number of individuals receiving treatment for 3-MCCD may not have a clinically significant condition[74]. Other authors[76] reported a cohort of 88 3-MCC patients, 53 identified by newborn screening, 26 diagnosed due to clinical symptoms or positive family history and 9 mothers, identified following the positive newborn screening result of their baby; 57% of patients were asymptomatic while 43% showed clinical symptoms, many of which were probably not related to MCC deficiency but due to ascertainment bias. However, 12 patients (5 of 53 identified by newborn screening) presented with acute metabolic decompensations. Their data confirm that MCC deficiency, despite low penetrance, may lead to a severe clinical phenotype resembling classical organic acidurias. However, neither the genotype nor the biochemical phenotype is helpful in predicting the clinical course.

3-Hidroxy-3-methyl glutaric aciduria

3-Hidroxy-3-methyl glutaric aciduria (HMG, OMIM#246450) is caused by the deficiency of 3-hydroxy-3-metilgutaril-CoA lyase (HMG-CoA lyase). This enzyme is involved in the metabolism of leucine. Two phenotypes have been described, the neonatal form in approximately 30% of the cases and late form that usually occurs between 3 and 11 months of age and affects approximately 60% of cases[76]-[77]. NBS for HMG can be performed by identification of elevated levels of 3-hidroxy-isovalerilycarnitine (C5OH), 3-methyl-glutarylcarnitine (C6DC) and high C5OH/carnitine (C0), C5OH/octanoylcarnitine (C8) and C5OH/acylcarnitine (C2). Therefore, diferential diagnosis with 3-MCC, 3-methylglutaconic aciduria (3MGA), 2-methyl-3-hidrobutyryl CoA dehydrogenase deficiency (2M3HBD) and beta-thiolase deficiency should be done[73].

Benefits of NBS. The available evidence is sparse. There are few cases of HMG detected trough the NBS programs. A report on practices for NBS programs for rare diseases in the European Union, reported explicitly that 87% of HMG cases are asymptomatic at the detection of the disease and treatment and usually starts at an average at 16 days of life[78]. There are studies that conclude the response to treatment is variable and in patients initiating treatment before the onset of the disease also develop metabolic crises of varying severity with brain damage and mental retardation.

Other studies consider that early medical intervention improves prognosis with normal growth and development. There is insufficient evidence of the benefits of NBS.

Beta-Ketothiolase deficiency

Beta-ketothiolase deficiency (BKT, OMIM#203750) is a disease that affects ketone bodies and isoleucine metabolism. NBS for BKT can be performed by identification of elevated tiglylglycine (C5:1) and /or not 3-hidroxy-isovalerycarnitine (C5OH) and high ratios C5:1/carnitine (C0), C5OH/C0, C5OH/octanoylcarnitine (C8). Therefore, differential diagnosis with other conditions should be done[79-80] (fig. 2).

Benefits of NBS. The available evidence is scarce and of poor quality. A report on practices of neonatal screening in the European Union, concluded that an average of 55% of BKT cases detected by NBS were asymptomatic at diagnosis[78]. Estrella J et al. 2014[25] believes that late diagnosis does not appear to seriously affect the patients. However, two deaths have been reported at 2 years of age due to an episode of ketoacidosis and, on the other hand, a favorable outcome of a patient with symptoms starting at 3 days of life[81] was reported. One and two false negatives in the NBS programs of North Carolina[67] and Australia[25] were detected, respectively. However, cutoff values were not changed since the rate of false positives increased to unacceptable values. In Minnesota, over a period of 9 years, two false negative were detected, both patients had undetectable levels of C5:1 and C5OH in the asymptomatic period and only showed the characteristic profile during periods of metabolic stress[82]. Consequently, early diagnosis of BKT deficiency is more complex than other disorders, and it seems this disease does not meet the full criteria to be included in the NBS programs[82], or at least there is insufficient evidence of the benefits of NBS.

Holocarboxylase synthase deficiency

Holocarboxylase synthase deficiency (HCS, OMIM#253270) causes multiple carboxylase deficiency. NBS for the HCS deficiency can be performed by identification of elevated 3-hidroxy-isovalerycarnitine (C5OH) and high ratios C5OH/carnitine (C0), C5OH/octanoylcarnitine (C8). Differential diagnosis with other conditions should be done[73].

Benefits of NBS. The exact prevalence of HCS is unknown, and patients with this condition usually starts symptoms at a few hours, days or weeks of birth. Due to the early appearance of symptoms there are some doubts about the benefits of its inclusion in the NBS programs, but there is no doubt of the benefits of biotin treatment.

HCS is common in the Faroe Islands, for that reason Lund AM et al 2007[83], assessed the feasibility of neonatal screening for HCS. Eight patients from five families were found and all patients responded to treatment with biotin, these authors concluded that the diagnosis through neonatal screening detection ensures good result. Another study performed in Hong Kong between 2005 and 2009[84] concluded that HCS deficiency is one of diseases that must be included in the NBS program of Hong Kong due to its high prevalence and to the good response to treatment.

Mitochondrial Fatty acid β-oxidation disorders

The core conditions of mitochondrial fatty acids oxidation (FAO) disorders are outlined in grey in fig.3, these conditions have been included in all NBS states of US. In Canada all regions perform screening for MCAD, while the other core conditions are only screened in 60% of the regions. All these diseases are included in the 76% of the countries from Middle East and North Africa and in the 50% of the countries from Europe, Latin America and Asia Pacific (table 1). The other FAO conditions are only included in 25 % of the US states and European countries, and in one region in Canada (table 1).

Medium chain acyl-CoA dehydrogenase deficiency deficiency

Medium chain acyl-CoA dehydrogenase deficiency (MCAD) deficiency (OMIM# 201450) is the most common fatty acid oxidation disorder. NBS is performed by the identification of a high level of the primary markers in DBS: C8 and C8/C2 ratio, among others (Fig. 3). Recently the ratio C8/ octenoylcarnitine (C8:1) has been described as an effective marker to reduce false positives, especially in very low birth weight infants[85].

Benefits of NBS. It is assumed that 50% of MCAD deficient patients have remained undiagnosed before the screening era. There is strong evidence that NBS reduces morbidity and mortality of deficient children. The benefit is particularly apparent in patients harboring the common mutation c.985A>G[86-91]. However, sudden death in few patients, despite their detection by NBS, has been described[88-89,92-93]. An elevation of C8 level greater than 6 µmol/L and homozygosity for the common c.985A>G mutation, or MCAD deficiency caused by nonsense or deletion mutations, represents a particular risk of sudden death [93-94]. An Australian study revealed that the risk of death among the patients diagnosed clinically was 14% compared with 4% in the screened cohort[95]. In addition, a few cases have been reported with an early onset of the disease at 12-72

hours after birth, before the neonatal screening result is obtained, in contrast it might be that some of the detected newborns will never develop clinical symptoms[90,96-97].

Very long chain acyl-CoA dehydrogenase deficiency

Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency is the most common defect of long-chain FAO disorders (OMIM#201475). The primary markers are elevation of tetradecenoylcarnitine (C14:1), tetradecadienylcarnitine (C14:2) and high ratios of C14:1/C2 and C14:1/C16 among others (Fig.3). It should be taken into account that long-chain acylcarnitines in DBS can normalize from the fourth day of life, and the diagnostic can be missed[98-99]. Some false positives with an increase of C14:1 are detected. A C14:1 level > 1 µmol/L strongly suggests VLCAD deficiency, whereas concentrations < or = 1 µmol/L do not allow a clear discrimination among affected patients, carriers, and healthy individuals. Further diagnostic evaluation, including enzyme and molecular analyses, is essential to identify the deficiency correctly[99-104].

Benefits of NBS. Implementation of NBS has significantly reduced morbidity and mortality of VLCAD deficiency, but NBS also identifies a great number of mildly affected patients that may never develop clinical symptoms[104]. A retrospective analysis of 242 newborns with elevated C14:1-acylcarnitine on NBS in California, Oregon, Washington, and Hawaii resulted in, 34 symptomatic positive cases, 18 asymptomatic positives, 112 false positives, 55 heterozygotes, 11 lost to follow-up, and 12 diagnosed of other disorders[105]. Retrospective analysis of 75 patients from Germany, Switzerland, Austria and the Netherlands revealed that dietary treatment is effective in many patients and can prevent acute metabolic derangements and prevent or reverse severe long-term complications such as cardiomyopathy. However, 38% of patients had intermittent muscle weakness and pain despite adhering to therapy[103]. Some false negatives have been reported, in some cases by normalitzation of the markers in the second requested sample[104] and in others because of normal results in the first analyzed sample, achieving the diagnosis in post-mortem samples[106].

Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and mitochondrial trifunctional protein deficiency

Long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, (OMIM#609016) and mitochondrial trifunctional protein (MTP) deficiency (OMIM # 609015) have the same primary markers in DBS: 3-hydroxyhexadecanoylcarnitine (C16-OH), 3-

hydroxyoctadecanoylcarnitine (C18-OH), 3-hydroxyoctadecenoylcarnitine (C18:1-OH) and a high ratio of C16-OH/C16 among others (Fig. 3).

Benefits of NBS. Generalized MTP deficiency has high early mortality rate[105]. In both disorders, neuropathy and retinopathy are progressive and irreversible despite treatment measures[104]. Several studies revealed that some patients developed satisfactorily while others, particularly MTP patients, did not survive[108-109]. Despite detection by NBS, some patients with MTP deficiency died few weeks after birth, or they were already severely ill at the time the NBS results[110-111].

Carnitine transporter deficiency

Carnitine transporter deficiency (CTD, OMIM#212140) is identified in NBS by the extremely low levels free carnitine (C0) and other primary markers particularly low C16, octadecenoylcarnitine (C18:1) and C3 (Fig. 3). False positives have been detected. In some cases low C0 is secondary to malnutrition, organic acidemias or other FAO disorders. Mothers with this condition have been detected because their infants have low levels of carnitine at birth[112-113].

Benefits of NBS. In a study of 6 patients, 4 were asymptomatic, 1 showed hypoglycaemia and 1 showed movement disorders from 2 years of age, but their **clinical** symptoms disappeared 3 months later increasing the dose of carnitine administration[114]. In another cohort of 11 patients, although some of them developed symptoms they responded favorably to supplementation with carnitine[83].

Carnitine palmitoyltransferase I deficiency

NBS of carnitine palmitoyltransferase I deficiency (CPT1, OMIM#255120) is performed by the identification of high levels of C0 and high ratio C0/(C16+C18), together a decrease of C16 among others (Fig. 3). Some false positives can be detected as C0 levels can be high due to sepsis[115].

Benefits of NBS. CPT 1 deficiency may often be benign, although early presentation with hypoketotic hypoglycaemia also occurs[105]. Patients with a prevalent mutation in Alaska natives c.1436C>T can be missed by NBS[116].

Carnitine palmitoyltransferase II deficiency and of carnitine acylcarnitine translocase *deficiency*

The deficiency of carnitine palmitoyltransferase II (CPT2,OMIM#255110, 600649, 608836) and of carnitine acylcarnitine translocase (CACT, OMIM # 212138) have the same increased primary markers in DBS, high C16 and high (C16+C18:1)/C2 ratio

among others (Fig. 3). Specific enzyme testing in lymphocytes or fibroblasts or molecular analysis is necessary to achieve the differential diagnosis.

Benefits of NBS. After NBS identification and early treatment some patients remain asymptomatic. However, CACT and neonatal CPT2 deficiencies have an extremely high neonatal mortality rate, and some cases can die or present symptoms before the NBS result is obtained[117-118].

It is important to advice that the acylcarnitine profile in DBS of some patients can be normal and CPT2 deficiency can be missed[119].Some false negatives have been reported, especially in for the late-onset form[120-122].

Multiple acyl-CoA dehydrogenase (MAD) deficiency or glutaric aciduria type II

MAD deficiency (OMIM#231680) is identified by an generalized increase of acylcarnitines (Fig. 3). Specificity is highly improved by the measurement of 2-hydroxyglutaric acid, glutáric acid and ethylmalonic acid in DBS as second tier test.

Benefits of NBS. Some patients remain asymptomatic after NBS identification and early treatment. However, some patients with the neonatal form have a severe presentation and premature death[123].

Other inherited metabolic disorders

Biotinidase deficiency

Biotinidase deficiency (OMIM#253260) an autosomal recessive inherited metabolic disease of biotin metabolism. The disease is detected in the NBS programs through the analysis of biotinidase activity[124.]

Benefits of NBS. Among the reported benefits of NBS we would like to remark the cohort of patients reported by Weber et al (2004)[125] , and that reported by Couce et al (2011)[126]. In the firts one, the authors compared the clinical evolution of patients with profound biotinidase deficiency detected by NBS, with a group of patients diagnosed through the clinical symptoms. The median follow-up was 6years. All 25 cases diagnosed by NBS and treated with biotin showed normal psicomotor development. Patients with profund biotinidase deficiency (<1%) not detected by NBS, but treated later with biotin showed delay in the adquisition of several ítems in adition to sensorineurol hearing loss . In the second serie[126] the follow-up of 15 cases detected by NBS were compared with 6 cases diagnosed after the onset of symptoms. All of the children but one, of the first group remained asymptomatic for more than 10 years. Of

the 6 patients diagnosed clinically, 3 had sensorineural hearing loss (2 with partial biotinidase deficiency), and the other 3 patients had a variety of clinical symptoms. Therefore, biotinidase deficiency meets most of the criteria for inclusion in NBS programs: a) the affected child exhibits no symptoms at birth, b) the disease causes severe neurological damage c) the treatment of these patients is the oral administration of pharmacological doses biotin, which prevents the onset of symptoms and normal development of the patient. d) a simple and inexpensive test[126-127].

Classic Galactosemia

Galactosemia (OMIM#230400) is an inherited metabolic disease characterized by the inability to metabolize galactose into glucose due to a deficiency of galactose uridyltransferase 1-phosphate (GALT). NBS is performed by measuring galactose and galactose-1-phosphate is analyzed in DBS by fluorimetry or by tandem MS/MS[128]. *Benefits of NBS*. There is no doubt that NBS identification and early treatment (based on the restriction of dietary galactose) reduces morbidity and mortality and possible disabilities[129.] According to the results of an international survey of 371 patients in Europe and the US, 91% of infants treated at birth, as a result of an affected brother, showed no neonatal symptoms[130]. However, the benefits of NBS are still matter of debate and controversy[131]. The main arguments used against NBS is that most infants already show the characteristic symptoms of galactosemia and develop complications before screening results are obtained. On the other hand it seems that long term outcome of patients diagnosed clinically and those diagnosed by NBS do not differ significantly. Hughes et al (2009)[132] compared a group of patients (n=14) that does not comply strictly with the diet, and a group that achieves strict consumption of galactose (n = 8). These authors observed[132] that long-term complications do not differ between the two groups. Thefore, there is still a need for evidence-based recommendations to better standardize treatment for this disorder[133].

Future diseases to be included in the NBS programs

Lysosomal diseases (LSDs)

Currently there is a great interest in the inclusion of LSDs in the NBS programs. In the past, although there was a good evidence of a better outcomes when treatment was initiated early in life, there was no proven high-throughput screening test available. Nowadays, several screening tests have been established and have been shown that the prevalence of LSDs is more frequent than previously expected.

- Pompe disease (OMIM#232300) has been included as core condition in the uniform screening panel in USA. Pompe disease is screened in Taiwan, Austria and Hungary[134-136]. The long-term beneficial effects of an early treatment in patients with Pompe disease have been recently published[137-138].
- Krabbe disease (OMIM# 245200) is included in the NBS programs of New York,I llinois, New Mexico, New Jersey, Arizona, and Pennsylvania[134], but reports on the beneficial effects of NBS are still pending.
- Fabry disease (OMIM#301500) is screened in Missouri[135], and pilot studies have been conducted in Taiwan, China, Austria, Hungary and Washington[134,136].
- Mucopolysaccharidosis type I (MPS I) (OMIM#607014) is screened in Missouri and Illinois. Pilot studies are at present running in Washington, Austria, Hungary, and Taiwan[134,135].
- Gaucher disease (OMIM# 606463) is included in the NBS programs of Missouri, Illinois and Korea[134-136].
- NBS pilot studies for Niemann Pick type A and B have been conducted in Austria, Hungary, and[134].

Recently, benefits of the NBS for LSDs discussed is discussed in a recent report[139]. Pompe disease has been considered the most appropriate, and Krabbe the least. MPSI and MPSII were overall considered favorably, but MPSI ranked higher, due to a perception of better efficacy of the therapeutic options. Fabry and Gaucher diseases were viewed less favorably due to the ethical problems in the late forms of both diseases.

X-linked adrenoleukodytrophy (X-ALD)

Since hematopoietic cell therapy (HCT) is an available intervention for X-ALD (OMIM#300100), if diagnosed and treated before the onset of clinical symptoms, there has been a strong interest in improving pre-symptomatic detection. The new tests in DBS removed a major barrier to the implementation of newborn screening for X-ALD[140-141]. This disease was included recently in the New York and California programs[142].

Ornithine transcarbamylase (OTC) deficiency

OTC deficiency (OMIM# 311250) is the most frequent urea cycle defects. In the severe cases even extremely early diagnosis does not seem to improve prognosis very much, but the newborns suffering from less severe deficiencies may have a better outcome if

diagnosed ant treated during the first days of life. OTC deficiency is identified in NBS by a low citrulline levels in DBS, however this is not a reliable marker for OTC[143]. Today new methods have been described to measure orotic acid in DBS to improve the detection[144].

Wilson disease

Wilson's disease (OMIM#277900) is an autosomal recessive disorder of copper transport. Early detection of Wilson's disease can prevent lifelong neurological disabilities and/or cirrhosis. Currently, there are no effective biomarkers for NBS of Wilson disease, but new methods are ongoing to be developped[145].

Guanidinoacetate methyltransferase (GAMT) deficiency

Guanidinoacetate methyltransferase (GAMT,OMIM#601240) deficiency is a creatine biosynthesis disorder. Intellectual disability is not reversed by treatment once it is instaured, but presymptomatic treatment is predicted to improve outcomes, prompting interest in NBS[146]. Improvement in the indentification of GAMT in DBS are now beeing developed[147].

Conclusion.

We have reviewed the inherited metabolic diseases included in worldwide neonatal screening (NBS) programs in 2016. Evidences of benefits are also reported, as well as future directions of the NBS programs. We conclude with a paragraph from a report of Raffle and Gray 2007[9] that is still useful nowadays: “All screening programs do harm. Some do good as well and, of these, some do more good than harm at reasonable cost”.

REFERENCES

1. Folling A: Uber Ausscheindung von Phenylbrenztraubesaure in den Han Stoffwechselanomalie in Verbindung mit imbezilliat. Hoppe Seyler Z. Physiiol Cem 227:192;1934.
2. Bickel H, Gerrad J, Hickmans EM: Influence of phenylalanine intake on phenylketonuria. Lancet 2: 812-3;1953.
3. Guthrie R, Susi A: A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 32:338–43;1963.
4. Millington DS, Kodo N, Norwood DL, Roe CR: Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. J Inherit Metab Dis 13:321-4;1990.
5. Chace DH, DiPerna JC, Naylor EW: Laboratory integration and utilization of tandem mass spectrometry in neonatal screening: a model for clinical mass spectrometry in the next millennium. Acta Paediatr Suppl 88:45-7;1999.

6. Rinaldo P, Zafari S, Tortorelli S, Matern D:
   Making the case for objective performance metrics in newborn screening by tandem m ass spectrometry. Ment Retard Dev Disabil Res Rev 12:255-61;2006.
7. Schulze A, Kohlmueller D, Mayatepek E: Sensitivity of electrospray-
   tandem mass spectrometry using the phenylalanine/tyrosine-ratio
   for differential diagnosis of hyperphenylalaninemia in neonates. Clin Chim Acta 283:15-20;1999.
8. Sahai I, Marsden D: Newborn screening. Crit Rev Clin Lab Sci 46:55-82; 2009.
9. Raffle A, Gray M. Screening: Evidence and Practice. Oxford: Oxford University Press. 2007
10. Watson MS, Mann MY, Lloyd-Puryear MA, Rinaldo P, Howell R: Newborn Screening: Towards a Uniform Screening Panel and System. Genet Med 8:12S–252S;2006.
11. 21. US Department of Health and Human Services. Discretionary Advisory Committee on Heritable Disorders in Newborns and Children. Available at: http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/index.html. Accessed April 1, 2015.
12. American College of Medical Genetics Newborn Screening Expert Group. Newborn screening: toward a uniform screening panel and system-executive summary. Pediatrics 117(5 Pt 2):S296-307;2006.
13. Therrell BL, Padilla CD, Loeber JG, et al:
    Current status of newborn screening worldwide: 2015. Semin Perinatol 39:171-87;2015.
14. Cornel MC, Rigter T, Weinreich SS, et al: A framework to start the debate on neonatal screening policies in the EU: an Expert Opinion Document. Eur J Hum Genet 22:12–7;2014.
15. Holmes D: Europe plays catch-up on neonatal screening as US skips ahead. Nat Med 18:1596;2012.
16. Guthrie R: Screening for “inborn errors of metabolism” in the newborn infant – A Multiple test program. Birth Defects Original Article Series IV 92-98;1962.
17. Scriver CR: The PAH gene, phenylketonuria, and a paradigm shift. Human Mutation 28: 831–845;2007.
18. Pers S, Gautschi M, Nuoffer JM, Schwarz HP, Christ E:
    Integration of adult patients with phenylketonuria into professional life:
    long-term follow-up of 27 patients in a single centre in Switzerland. Swiss Med Wkly 23;144:w14074;2014.
19. Huijbregts SC, de Sonneville LM, van Spronsen FJ, Licht R, Sergeant JA: The neuropsychological profile of early and continuously treated phenylketonuria: orienting, vigilance, and maintenance versus manipulation-functions of working
    memory. Neurosci Biobehav Rev 26:697-712;2002.
20. Channon S, Cassina C, Lee P: Executive functioning, memory, and learning in phenylketonuria. Neuropsychology 18:613-20;2004.
21. Schindeler S, Ghosh-Jerath S, Thompson S, et al: The effects of large neutral amino acid supplements in PKU: An MRS and neuropsychological study. Mol Genet Metab 91:48-54;2007.
22. Grosse SD: Late-
    treated phenylketonuria and partial reversibility of intellectual impairment. Child Dev 81:200-11;2010.
23. Berry SA, Brown C, Grant M, et al:
    Newborn screening 50 years later: access issues faced by adults with PKU. Genet Med 15:591-9;2013.
24. Huemer M, Kožich V, Rinaldo P, et al: Newborn screening for homocystinurias and methylation disorders: systematic review and proposed guidelines. J Inherit Metab Dis 38:1007-19;2015.

25. Estrella J, Wilcken B, Carpenter K, Bhattacharya K, Tchan M, Wiley V: Expanded newborn screening in New South Wales: missed cases. J Inherit Metab Dis 37:881-7;2014.
26. Taylor RH, Burke J, O'Keefe M, Beighi B, Naughton E: Ophthalmic abnormalities in homocystinuria: the value of screening. Eye (Lond) 12 (Pt 3a):427-30;1998.
27. Yap S, Naughten E: Homocystinuria due to cystathionine b-synthase deficiency in Ireland: 25 years experience of a newborn screened and treated population with reference to clinical outcome and biochemical control. J Inherit Metab Dis 21:738–747;1998.
28. Walter JH, Wraith JE, White FJ, Bridge C, Till J: Strategies for the treatment of cystathionine beta-synthase deficiency: the experience of the willink biochemical genetics unit over the past 30 years. Eur J Pediatr 57(Suppl 2):S71–76;1998.
29. Burke JP, O'Keefe M, Bowell R, Naughten ER: Ocular complications in homocystinuria-early and late treated. Br J Ophthalmol 73:427-31;1989.
30. Mulvihill A, Yap S, O'Keefe M, Howard PM, Naughten ER: Ocular findings among patients with late-diagnosed or poorly controlled homocystinuria compared with a screened, well-controlled population. JAAPOS 5:311–315;2001.
31. Diekman EF, de Koning TJ, Verhoeven-Duif NM, Rovers MM, van Hasselt PM: Survival and psychomotor development with early betaine treatment in patients with severe methylenetetrahydrofolate reductase deficiency. JAMA Neurol 71:188-94;2014.
32. Müller P, Horneff G, Hennermann JB: A rare inborn error of intracellular processing of cobalamine presenting with microcephalus and megaloblastic anemia: a report of 3 children. Klin Padiatr 219:361-7;2007.
33. Schiff M, Benoist JF, Tilea B, Royer N, Giraudier S, Ogier de Baulny H: Isolated remethylation disorders: do our treatments benefit patients? J Inherit Metab Dis 34:137-45;2011.
34. Zavadáková P, Fowler B, Suormala T, et al: cblE type of homocystinuria due to methionine synthase reductase deficiency: functional correction by mini gene expression. Hum Mutat 25:239-47;2005.
35. Huemer M, Bürer C, Ješina P, et al: Clinical onset and course, response to treatment and outcome in 24 patients with the cblE or cblG remethylation defect complemented by genetic and in vitro enzyme study data. J Inherit Metab Dis 38:957-67;2015.
36. Saudubray J, Charpentier C. Clinical phenotypes: Diagno-sis/algorithms. In: Scriver CR, Beaudet AL, Sly WS, Valle D,editors. The metabolic and molecular bases of inherited diseases. New York: McGraw-Hill; 1995. p. 327-400.
37. Oglesbee D, Sanders KA, Lacey JM, et al: Second-tier test for quantification of alloisoleucine and branched-chain amino acids in dried blood spots to improve newborn screening for maple syrup urine disease (MSUD). Clin Chem 54:542-9;2008.
38. Chuang DT, Shih VE. Maple syrup urine disease (branched chain ketoaciduria). In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular basis of inherited disease. 8th ed. New York: McGraw-Hill; 2001. p. 1971e2006.
39. Funchal C, Tramontina F, Quincozes dos Santos A, et al: Effect of the branched-chain alpha-keto acids accumulating in maple syrup urine disease on S100B release from glial cells. J Neurol Sci 260:87e94;2007.
40. Langenbeck U, Wendel U, Mench-Hoinowski A, et al: Correlations between branched-chain amino acids and branched-chain alpha-keto acids in blood in maple syrup urine disease. Clin Chim Acta 88:283e91;1978.
41. Yudkoff M, Daikhin Y, Nissim I, et al: Brain amino acid requirements and toxicity: the example of leucine. J Nutr 135:1531Se8S;2005.
42. Carecchio M, Schneider SA, Chan H, et al: Movement disorders in adult surviving patients with map le syrup urine disease. Mov Disord 26:1324e8;2011.

43. Muelly ER, Moore GJ, Bunce SC, et al: Biochemical correlates of neuropsychiatric illness in maple syrup urine disease. J Clin Invest 123:1809-20;2013.
44. Couce ML, Ramos F, Bueno MA, et al: Evolution of maple syrup urine disease in patients diagnosed by newborn screening versus late diagnosis. Eur J Paediatr Neurol 19:652-9;2015.
45. Herber S, Schwartz IV, Nalin T, et al: Maple syrup urine disease in Brazil: a panorama of the last two decades. J Pediatr (Rio J) 91:292-8;2015.
46. Habarou F, Brassier A, Rioe M, et al: Pyruvate carboxylase deficiency: An underestimated cause of lactic acidosis. Molecular Genetics and Metabolism Reports 2 25–31;2015.
47. Häberle J, Boddaert N, Burlina A, et al: Suggested guidelines for the diagnosis and management of urea cycle disorders. Orphanet J Rare Dis 29;7:32;2012.
48. Uchino T, Endo F, Matsuda I: Neurodevelopmental outcome of long-term therapy of urea cycle disordersin Japan. J Inherit Metab Dis 21 Suppl 1:151-9;1998.
49. Bachmann C: Outcome and survival of 88 patients with urea cycle disorders: a retrospective evaluation. Eur J Pediatr 162:410-6;2003.
50. Kido J, Nakamura K, Mitsubuchi H, et al: Long-term outcome and intervention of urea cycle disorders in Japan. J Inherit Metab Dis 35:777-85;2012.
51. Dehner LP, Snover DC, Sharp HL, Ascher N, Nakhleh R, Day DL: Hereditary tyrosinemia type I (chronic form): pathologic findings in the liver. Hum Pathol 20:149–58;1989.
52. Chakrapani A, Gissen P, McKiernan P: Disorders of Tyrosine Metabolism. In: Saudubray J-M, van den Berghe G, Walter JH. Heidelberg, editors. Inborn Metabolic Diseases. 5th edition. Berlin: Springer; 2012. 275–276. Chapter 18.
53. de Laet C, Dionisi-Vici C, Leonard JV, et al: Recommendations for the management of tyrosinaemia type 1. Orphanet J Rare Dis 11;8:8;2013.
54. Heylen E, Scherer G, Vincent MF, Marie S, Fischer J, Nassogne MC: Tyrosinemia Type III detected via neonatal screening: management and outcome. Mol Genet Metab 107:605-7;2012.
55. Hadžić N, Vara R: Neonatal screening for hereditary tyrosinaemia: are we there yet? Arch Dis Child 100:720-1;2015.
56. De Jesús VR, Adam BW, Mandel D, Cuthbert CD, Matern D: Succinylacetone as primary marker to detect tyrosinemia type I in newborns and its measurement by newbornscreening programs. Mol Genet Metab 113:67-75;2014.
57. Mitchell GA, Grompe M, Lambert M, Tanguay RM. Hypertyrosinemia. In: Scriver CRBALSWSVD, editors. The Metabolic and molecular bases of inherited disease. New York: McGraw-Hill; 2001:1777–805.
58. McKiernan PJ: Nitisinone for the treatment of hereditary tyrosinemia type I. Expert Opin Orphan Drugs 1:491–7;2013.
59. McKiernan PJ, Preece MA, Chakrapani A: Outcome of children with hereditary tyrosinaemia following newborn screening. Arch Dis Child 100:738-41;2015.
60. Malvagia S, Haynes CA, Grisotto L, et al: Heptadecanoylcarnitine (C17) a novel candidate biomarker for newborn screening of propionic and methylmalonic acidemias. Clin Chim Acta 23;450:342-8; 2015.
61. Dionisi-Vici C, Deodato F, Röschinger W, Rhead W, Wilcken B: 'Classical' organic acidurias, propionic aciduria, methylmalonic aciduria and isovalerica ciduria: long-term outcome and effects of expanded newborn screening using tandem massspectro metry. J Inherit Metab Dis 29:383-9;2006.
62. Grünert SC, Müllerleile S, de Silva L, et al: Propionic acidemia: neonatal versus selective metabolic screening. J Inherit Metab Dis 35:41-9;2012.

63. Grünert SC, Müllerleile S, De Silva L, et al: Propionic acidemia: clinical course and outcome in 55 pediatric and adolescent patients. Orphanet J Rare Dis 10;8:6;2013.
64. Lerner-Ellis JP, Anastasio N, Liu J, et al: Spectrum of mutations in MMACHC, allelic expression, and evidence for genotype-phenotype correlations. Hum Mutat 30:1072-81;2009.
65. Weisfeld-Adams JD, Bender HA, Miley-Åkerstedt A, et al: Neurologic and neurodevelopmental phenotypes in young children with early-treated combined methylmalonic acidemia and homocystinuria, cobalamin C type. Mol Genet Metab 110:241-7;2013.
66. Lindner M, Ho S, Fang-Hoffmman J, Hoffmann GF, Kölker S: Neonatal screening for glutaric aciduria type I: Strategies to proceed. J Inherit Metab Dis 29:378–382;2006.
67. Frazier DS, Millington SE, McCandless DD et al: The tandem mass spectrometry newborn screening experience in North Carolina: 1997–2005. J Inherit Metab Dis 29: 76–85;2006.
68. Kölker S, Gabarde SF, NiKolas Boy et al: Decline of Acute Encephalopathic Crises in Children with Glutaryl-CoA Dehydrogenase Deficiency Identified by Newborn Screening in Germany. Pediatric Research 62:357-63;2007.
69. Couce ML, López-Suárez O, Bóveda MD, et al: Glutaric aciduria type I: outcome of patients with early- versus late-diagnosis. Eur J Paediatr Neurol 17:383-9. 5;2013.
70. Strauss KA, Puffenberger EG, Robinson DL, Morton DM: Type I Glutaric Aciduria, Part 1: Natural History of 77 Patients. American Journal of Medical Genetics Part C (Semin. Med. Genet.) 121C:38–52;2003.
71. Ensenauer R, Fingerhut R, Maier EM et al : Newborn Screening for Isovaleric Acidemia Using Tandem Mass Spectrometry: Data from 1.6 Million Newborns. Clinical Chemistry 57:4 623–626;2011.
72. Grünert S, Wendel U, Lindner M et al: Clinical and neurocognitive outcome in symptomatic isovaleric academia. Orphanet Journal of Rare Diseases 7:9;2012.
73. Schulze A, Lindner M, Kohlmüller D, Olgemöller K, Mayatepek E, Hoffmann GF: Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications. Pediatrics 111(6 Pt 1):1399- 406;2003.
74. Lam C, Carter JM, Cederbaum SD, et al.: Analysis of cases of 3-methylcrotonyl CoA carboxylase deficiency (3-MCCD) in the California newborn screening program reported in the state database. Molecular Genetics and Metabolism 110:477–483;2013.
75. Grünert SC, Stucki M, Morscher Rj, et al.: 3-methylcrotonyl-CoA carboxylase deficiency: Clinical, biochemical, enzymatic and molecular studies in 88 individuals. Orphanet Journal of Rare Diseases 7:31;2012.
76. Sass JO. Inborn errors of ketogenesis and ketone body utilization. J. Inherit. Metab. Dis. 2012;35:23-8.
77. http://www.orpha.net/consor/cgi-bin/OC_Exp.php?Lng=GB&Expert=20. Accessed April 24, 2016
78. Report on the practices of newborn screening for rare disorders implemented in Member States of the European Union, Candidate, Potential Candidate and EFTA Countries. EU Tender “Evaluation of population newborn screening practices for rare disorders in Member States of the European Union”. Available at: http://ec.europa.eu/chafea/documents/news/Report_NBS_Current_Practices_20120108_FINAL.pdf. Accessed April 21, 2016.
79. Catanzano F, Ombrone D, Di Stefano C, et al.: The first case of mitochondrial acetoacetyl-CoA thiolase deficiency identified by expanded newborn metabolic screening in Italy: the importance of an integrated diagnostic approach. J Inherit Metab Dis 33 Suppl 3:S91-4;2010.
80. Law CY, Lam CW, Ching CK, et al.: NMR- based urinalysis for beta-ketothiolase deficiency. Clinica Chimica Acta 438:222-5;2015.
81. Fukao T, Scriver CR, Kondo N, t2 Collaborative Working Group: The clinical phenotype and outcome of mitochondrial acetoacetyl-

CoA thiolase deficiency (beta-ketothiolase or T2 deficiency) in 26 enzymatically proved and mutation-defined patients. Mol Genet Metab 72(2):109-14;2001.
82. Sarafoglou K, Matern D, Redlinger-Grosse K, et al.: Siblings with mitochondrial acetoacetyl-CoA thiolase deficiency not identified by newborn screening. Pediatrics 128(1):e246-50;2011.
83. Lund AM, Joensen F, Hougaard DM, et al.: Carnitine transporter and holocarboxylase synthetase deficiencies in The Faroe Islands. J Inherit Metab Dis 30:341–349;2007.
84. Hencher LCH, Mak CM, Lam CW, et al.: Analysis of inborn errors of metabolism: disease spectrum for expanded newborn screening in Hong Kong. Chinese Medical Journal 124:983-989;2011.
85. Hall PL, Wittenauer A, Hagar A: Newborn screening for medium chain acyl-CoA dehydrogenase deficiency: performance improvement by monitoring a new ratio. Mol Genet Metab 113:274-7;2014.
86. Pandor A, Eastham J, Berverley C, et al.: Clinical effectiveness and cost-effectiveness of neonatal screening for inborn errors of metabolism using tandem mass spectrometry: a systematic review. Health Technol Assess 8:1–152;2004.
87. Nennstiel-Ratzel U, Arenz S, Maier EM, et al.: Reduced incidence of severe metabolic crisis or death in children with medium chain acyl-CoA dehydrogenase deficiency homozygous for c.985A>G identified by neonatal screening. Mol Genet Metab 85:157-9;2005.
88. Maier EM, Liebl B, Röschinger W, et al.: Population spectrum of *ACADM* genotypes correlated to biochemical phenotypes in newborn screening formedium-chain acyl-CoA dehydrogenase deficiency. Hum Mutat 25:443-52;2005.
89. Rhead WJ: Newborn screening for medium-chain acyl-CoA dehydrogenase deficiency: A global perspective. J Inherit Metab Dis 29:370–377;2006.
90. Wilcken B, Haas M, Joy P, et al.: Outcome of neonatal screening for medium-chain acyl-CoA dehydrogenase deficiency in Australia: a cohort study. Lancet 369:37-42;2007.
91. Schatz UA, Ensenauer R: The clinical manifestation of MCAD deficiency: challenges towards adulthood in the screened population. J Inherit Metab Dis 33:513-20;2010.
92. Ziadeh R, Hoffman EP, Finegold DN, et al.: Medium chain acyl-CoA dehydrogenase deficiency in Pennsylvania: neonatal screening shows high incidence and unexpected mutation frequencies. Pediatr Res 37:675-8;1995.
93. Yusupov R, Finegold DN, Naylor EW, Sahai I, Waisbren S, Levy HL: Sudden death in medium chain acyl-coenzyme a dehydrogenase deficiency (MCADD) despite newborn screening. Mol Genet Metab 101:33-9;2010.
94. Arnold GL, Saavedra-Matiz CA, Galvin-Parton PA, et al.: Lack of genotype-phenotype correlations and outcome in MCAD deficiency diagnosed by newborn screening in New York State. Mol Genet Metab 99:263-8;2010.
95. Wilcken B, Haas M, Joy P, et al.: Expanded newborn screening: outcome in screened and unscreened patients at age 6 years. Pediatr 124: e241–e248;2009.
96. Rice G, Brazelton T 3rd, Maginot K, Srinivasan S, Hollman G, Wolff JA: Medium chain acyl-coenzyme A dehydrogenase deficiency in a neonate. N Engl J Med 357:1781;2007.
97. Yusuf K, Jirapradittha J, Amin HJ, Yu W, Hasan SU: Neonatal ventricular tachyarrhythmias in medium chain acyl-CoA dehydrogenase deficiency. Neonatology 98:260-4;2010.
98. Schymik I, Liebig M, Mueller M, et al.: Pitfalls of neonatal screening for very-long-chain acyl-CoA dehydrogenase deficiency using tandem mass spectrometry. J Pediatr 149:128-30;2006.
99. Boneh A, Andresen BS, Gregersen N, et al.: VLCAD deficiency: pitfalls in newborn screening and confirmation of diagnosis by mutation analysis. Mol Genet Metab 88:166-70;2006.

100. Liebig M, Schymik I, Mueller M, et al.: Neonatal screening for very long-chain acyl-coA dehydrogenase deficiency: enzymatic and molecular evaluation of neonates with elevated C14:1-carnitine levels. Pediatrics 118:1065-9;2006.
101. Tajima G, Sakura N, Shirao K, et al.: Development of a new enzymatic diagnosis method for very-long-chain Acyl-CoA dehydrogenase deficiency by detecting 2-hexadecenoyl-CoA production and its application in tandem mass spectrometry-based selective screening and newborn screening in Japan. Pediatr Res 64:667-72;2008.
102. ter Veld F, Mueller M, Kramer S, et al.: A novel tandem mass spectrometry method for rapid confirmation of medium- and very long-chain acyl-CoA dehydrogenase deficiency in newborns. PLoS One 4:e6449;2009.
103. Spiekerkoetter U, Lindner M, Santer R, et al.: Management and outcome in 75 individuals with long-chain fatty acid oxidation defects: results from a workshop. J Inherit Metab Dis 32:488-97;2009.
104. Spiekerkoetter U: Mitochondrial fatty acid oxidation disorders: clinical presentation of long-chain fatty acid oxidation defects before and after newborn screening. J Inherit Metab Dis 33:527-32;2010.
105. Wilcken B: Fatty acid oxidation disorders: outcome and long-term prognosis. J Inherit Metab Dis 33:501-6;2010.
106. Merritt JL 2nd, Vedal S, Abdenur JE, et al.: Infants suspected to have very-long chain acyl-CoA dehydrogenase deficiency from newborn screening. Mol Genet Metab 111:484-92;2014.
107. Ficicioglu C, Coughlin CR 2nd, Bennett MJ, Yudkoff M: J Pediatr 156:492-4;2010. Very long-chain acyl-CoA dehydrogenase deficiency in a patient with normal newborn screening by tandem mass spectrometry.
108. Sander J, Sander S, Steuerwald U, et al.: Neonatal screening for defects of the mitochondrial trifunctional protein. Mol Genet Metab 85:108-14;2005.
109. Sperk A, Mueller M, Spiekerkoetter U: Outcome in six patients with mitochondrial trifunctional protein disorders identified by newborn screening. Mol Genet Metab 101:205-7;2010.
110. Das AM, Illsinger S, Lücke T, et al.: Isolated mitochondrial long-chain ketoacyl-CoA thiolase deficiency resulting from mutations in the HADHB gene. Clin Chem 52:530-4;2006.
111. Diekman EF, Boelen CC, Prinsen BH, et al.: Necrotizing enterocolitis and respiratory distress syndrome as first clinical presentation of mitochondrial trifunctional protein deficiency. JIMD Rep 7:1-6;2013.
112. Lee NC, Tang NL, Chien YH, et al.: Diagnoses of newborns and mothers with carnitine uptake defects through newborn screening. Mol Genet Metab 100:46-50;2010.
113. Longo N, Frigeni M, Pasquali M: Carnitine transport and fatty acid oxidation. Biochim Biophys Acta pii: S0167-4889(16)30013-1;2016.
114. Han LS, Ye J, Qiu WJ, et al.: Primary carnitine deficiency in 17 patients: diagnosis, treatment and follow up. Zhonghua Er Ke Za Zhi 50:405-9;2012.
115. Sim KG, Wiley V, Carpenter K, Wilcken B: Carnitine palmitoyltransferase I deficiency in neonate identified by dried blood spot free carnitine and acylcarnitine profile. J Inherit Metab Dis 24:51-9;2001.
116. Gessner BD, Gillingham MB, Johnson MA, et al.: Prevalence and distribution of the c.1436C→T sequence variant of carnitine palmitoyltransferase 1A among Alaska Native infants. J Pediatr 158:124-9;2011.
117. Albers S, Marsden D, Quackenbush E, Stark AR, Levy HL, Irons M: Detection of neonatal carnitine palmitoyltransferase II deficiency by expanded newborn screening with tandem mass spectrometry. Pediatrics 107:E103;2001.

118. Wilson C, Kerruish NJ, Wilcken B, Wiltshire E, Bendikson K, Webster D: Diagnosis of disorders of intermediary metabolism in New Zealand before and after expanded newborn screening: 2004-2009. N Z Med J 125:42-50;2012.

119. de Sain-van der Velden MG, Diekman EF, Jans JJ, et al.: Differences between acylcarnitine profiles in plasma and bloodspots. Mol Genet Metab 110:116-21;2013.

120. Kobayashi H, Hasegawa Y, Endo M, Purevsuren J, Yamaguchi S: A retrospective ESI-MS/MS analysis of newborn blood spots from 18 symptomatic patients with organic acid and fatty acid oxidation disorders diagnosed either in infancy or in childhood. J Inherit Metab Dis 30:606;2007.

121. Illsinger S, Lücke T, Peter M, et al.: Carnitine-palmitoyltransferase 2 deficiency: novel mutations and relevance of newborn screening. Am J Med Genet A 146A:2925-8;2008.

122. Edmondson AC, Salant J, Ierardi-Curto LA, Ficicioglu C: Missed Newborn Screening Case of Carnitine Palmitoyltransferase-II Deficiency. JIMD Rep Apr 12; 2016. [Epub ahead of print].

123. Singla M, Guzman G, Griffin AJ, Bharati S: Cardiomyopathy in multiple Acyl-CoA dehydrogenase deficiency: a clinico-pathological correlation and review of literature. Pediatr Cardiol 29:446-51;2008.

124. Wolf B: Clinical issues and frequent questions about biotinidase deficiency. Mol Genet Metab 100:6-13;2010.

125. Weber P, Scholl S, Baumgartner ER: Outcome in patients with profound biotinidase deficiency: relevance of newborn screening. Dev Med Child Neurol 46:481-4;2004.

126. Couce ML, Pérez-Cerdá C, García Silva MT, et al.: Clinical and genetic findings in patients with biotinidase deficiency detected through newborn screening or selective screening for hearing loss or inherited metabolic disease. Med Clin (Barc) 137:500-3;2011.

127. Wolf B: The neurology of biotinidase deficiency Mol Genet Metab. 104:27-34;2011.

128. Jensen UG, Brandt NJ, Christensen E, Skovby F, Nørgaard-Pedersen B, Simonsen H:
Neonatal screening for galactosemia by quantitative analysis of hexose monophosphat es using tandem massspectrometry: a retrospective study. Clin Chem 47:1364-72;2001.

129. Milánkovics I, Schuler A, Kámory E, et al.:
Molecular and clinical analysis of patients with classic and Duarte galactosemia in west ern Hungary. Wien Klin Wochenschr 122:95-102;2010.

130. Waggoner DD, Buist NR, Donnell GN: Long-term prognosis in galactosaemia: results of a survey of 350 cases. J Inherit Metab Dis 13:802-18;1990.

131. Varela L, Paz L, Atienza G: Cribado neonatal de la galactosemia clásica. Revisión sistemática. Informes de Evaluación de Tecnologías Sanitarias; 2014.

132. Hughes J, Ryan S, Lambert D, et al.: Outcomes of siblings with classical galactosemia. J Pediatr 154:721-6;2009.

133. Van Calcar SC, Bernstein LE, Rohr FJ, Scaman CH, Yannicelli S, Berry GT: A re-evaluation of life-long severe galactose restriction for
the nutrition management of classicgalactosemia. Mol Genet Metab 112:191-7;2014.

134. Matern D, Gavrilov D, Oglesbee D, Raymond K, Rinaldo P, Tortorelli S: Newborn screening for lysosomal storage disorders. Semin Perinatol 39:206-16;2015.

135. Hopkins PV, Campbell C, Klug T, Rogers S, Raburn-Miller J, Kiesling J: Lysosomal storage disorder screening implementation: findings from the first six months of full population pilot testing in Missouri. J Pediatr 166:172-7;2015.
136. TecCho SE, Kwak JR, Lee H, Seo DH, Song J: Triplex tandem mass spectrometry assays for the screening of 3 lysosomal storage disorders in a Korean population. Clin Chim Acta 454:20-7;2016.
137. Andersson HC: Newborn screening + enzyme replacement therapy = improved lysosomal storagedisorder: outcomes in infantile-onset Pompe disease. J Pediatr 166:800-1;2015.
138. Chien YH, Lee NC, Chen CA, et al.: Long-term prognosis of patients with infantile-onset Pompe disease diagnosed by newborn screening and treated since birth. J Pediatr 166:985-91.e1-2;2015.
139. Lisi EC, McCandless SE: Newborn Screening for Lysosomal Storage Disorders: Views of Genetic Healthcare Providers. J Genet Couns 25:373-84;2016.
140. Theda C, Gibbons K, Defor TE, et al.: Newborn screening for X-linked adrenoleukodystrophy: further evidence high throughput screening is feasible. Mol Genet Metab 111:55-7;2014.
141. Turgeon CT, Moser AB, Mørkrid L, et al.: Streamlined determination of lysophosphatidylcholines in dried blood spots for newborn screening of X-linked adrenoleukodystrophy. Mol Genet Metab 114:46-50;2015.
142. Vogel BH, Bradley SE, Adams DJ, et al.: Newborn screening for X-linked adrenoleukodystrophy in New York State: diagnostic protocol, surveillance protocol and treatment guidelines. Mol Genet Metab 114:599-603;2015.
143. Cavicchi C, Malvagia S, la Marca G, et al.: Hypocitrullinemia in expanded newborn screening by LC-MS/MS is not a reliable marker for ornithine transcarbamylase deficiency. J Pharm Biomed Anal 49:1292-5;2009.
144. Janzen N, Terhardt M, Sander S, et al.: Towards newborn screening for ornithine transcarbamylase deficiency: fast non-chromatographic orotic acid quantification from dried blood spots by tandem mass spectrometry. Clin Chim Acta 430:28-32; 2014.
145. Hahn SH: Population screening for Wilson's disease. Ann N Y Acad Sci 1315:64-9;2014.
146. El-Gharbawy AH, Goldstein JL, Millington DS, et al.: Elevation of guanidinoacetate in newborn dried blood spots and impact of early treatment in GAMT deficiency. Mol Genet Metab 109:215-7;2013.
147. Pasquali M, Schwarz E, Jensen M, et al.: Feasibility of newborn screening for guanidinoacetate methyltransferase (GAMT) deficiency. J Inherit Metab Dis 37:231-6;2014.

Figure 1. Diagnostic algorithm of amino acid disorders in NBS program.

Allo-ile: Alloisoleucine; ASA: argininsuccinic acid; ASL: argininsuccinic acid lyase deficiency; BIOPT: biopterin defects; CBS: cystathionine-β-synthase; Cit: citrulline; CIT I: citrullinemia type I; CIT II: citrullinemia type II; H-MET: hypermethioninemias; H-PHE: hyperphenylalaninemia; Met: methionine; MMA: methylmalonic acid; MSUD: maple syrup urine disease; PC: pyruvate carboxylase; Phe: phenylalanine; PKU: phenylketonuria; RMD: remethylation defects; Suac; succinylacetone; tHCY: total

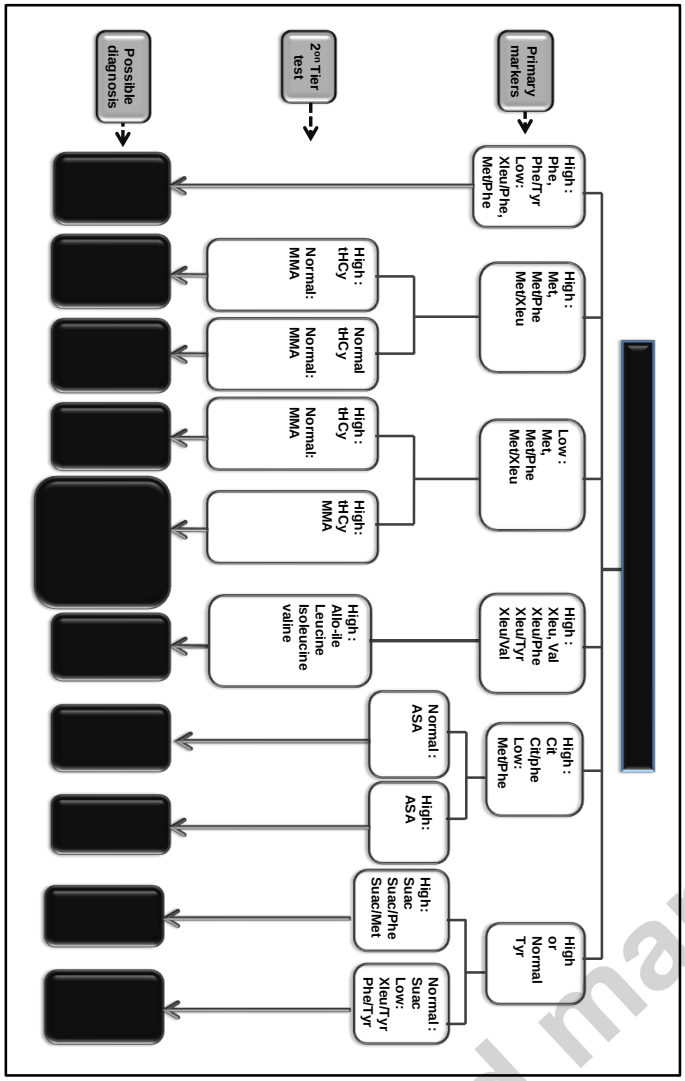
homocysteine; Tyr: tyrosine; TYR I: tyrosinemia type I; TYR II: tyrosinemia type II; TYR III: tyrosinemia type III; Val: valine; XLeu: leucine/isoleucine.

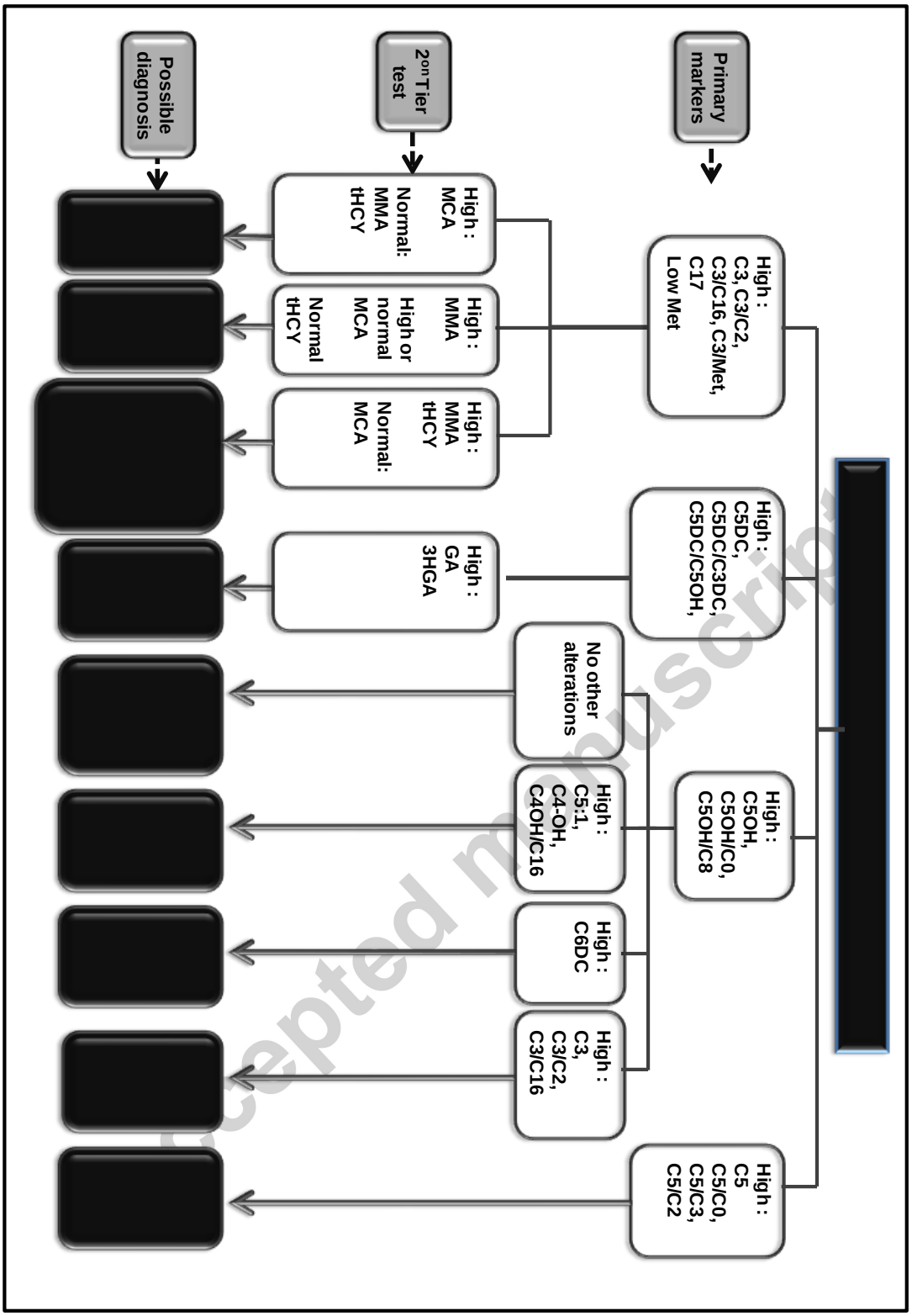
Figure 2. Diagnostic algorithm of organic acidurias in NBS program.

BKT: beta-ketothiolase deficiency; BTD: biotinidase deficiency; C0: free carnitine; C2: acetylcarnitine; C3:propionylcarnitine; C3DC: malonylcarnitine; C4OH: 3-hydroxy butyryl***carnitine;*** **C5: isovalerylcarnitine; C5:1:** tiglyl/3-methylcrotonylcarnitine**; C5DC: glutarylcarnitine; C5OH: 3-Hydroxyisovalerylcarnitine**; C6DC: methylglutarilcarnitine; C8: octanoylcarnitine; **C16: hexadecanoylcarnitine; C17: heptadecanoylcarnitine**; GA: glutaric acid; GA1: glutaric aciduria type 1; 3HGA: 3-hydroxyglutaric acid; ***HMG: 3-***hydroxy-3-methylglutaric aciduria; HSD10: 17β-***hydroxysteroid dehydrogenase*** type ***10 deficiency;*** IVA: isovaleric aciduria; 2MBG: **2-methylbutyrylglycinuria**; MCA: methylcitric acid; 3MCC: 3-methylcrotonylglicinuria; ***MCD:*** multiple carboxylase deficiency; Met: methionine; MGA: 3-methylglutaconic aciduria; MMA: methylmalonic acid; PA: propionic aciduria; tHCY: total homocysteine.

Figure 3. Diagnostic algorithm of fatty acid β-oxidation disorders in NBS program.

C0= free carnitine; C2: acetylcarnitine; C3: **propyonilcarnitine; C5: isovalerylcarnitine;** C6: hexanoylcarnitine; C8: octanoylcarnitine; C8:1: octenoylcarnitine; C10: decanoylcarnitine; C10:1: decenoylcarnitine; C12: dodecanoylcarnitine; C12:1: dodecenoylcarnitine; C14: tetradecanoylcarnitine; C14:1: tetradecenoylcarnitine; C14:2: tetradecadienylcarnitine; **C16:** hexadecanoylcarnitine**;** C16-OH: 3-hydroxyhexadecanoylcarnitine; C16:1-OH: 3-hydroxyhexadecenoylcarnitine; C18: octadecanoylcarnitine; C18:1 octadecenoylcarnitine; C18:2: octadecadienylcarnitine; C18:1-OH: 3-hydroxyoctadecenoylcarnitine; C18-OH: 3-hydroxyoctadecanoylcarnitine; CACT: carnitine-acylcarnitine translocase deficiency; CPT1: **carnitine palmitoyl transferase 1A deficiency**; CPT2: **carnitine palmitoyltransferase 2 deficiency**; CTD: carnitine transporter deficiency; GA1: glutaric aciduria type 1; GA2: **multiple acyl-CoA dehydrogenase deficiency**LCHAD: **long-chain 3-hydroxyacyl-CoA dehydrogenase** deficiency; MCAD: medium-chain acyl-Co A dehydrogenase deficiency; MTP: mitochondrial trifunctional protein defect; VLCAD: **very long chain acyl-CoA dehydrogenase deficiency.**





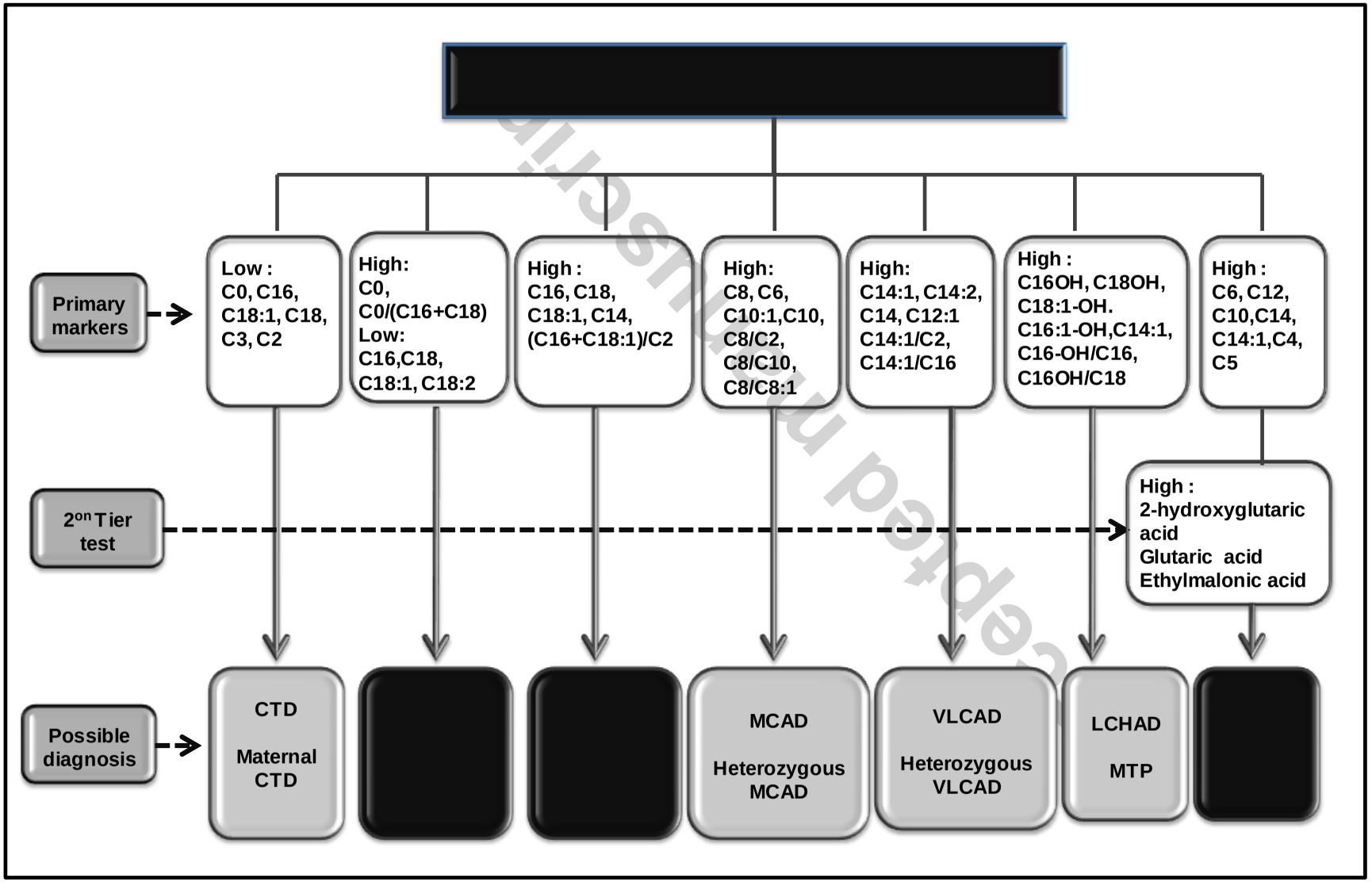


Table 3. Newborn screening panel of inherited metabolic diseases in the different geographic areas worldwide in 2016

Disease	**United States** number of states/total number	**Canada** number of states/ total number	**Europe** number of countries/total number	**Middle East and North Africa** number of countries/total number	**Latin America** number of countries/ total number	**Asia Pacific** number of countries/total number
Amino acid disorders						
Phenylketonuria (PKU)	51/51	15/15	41/48	16/21 but limited or selective	15/20	12/24
Homocystinuria (HCY)	49/51	5/15 †††	28/48	16/21 but limited or selective	9/20	11/24
Maple syrup urine disease (MSUD)	49/51	5/15 †††	28/48	16/21 but limited or selective	5/20	12/24
Citrullinemia type I (CIT I)	49/51	5/15 †††	28/48	16/21 but limited or ,selective or in less population	9/20	12/24
Argininsuccinic aciduria (ASA)	49/51	5/15 †††	28/48	0/21	0/20	0/24
Tyrosinemia type I (TYR I)	49/51	5/15 †††	28/48	16/21 but limited or selective	9/20	12/24
Other secondary conditions ***	13/51 all conditions 36/51 not all conditions	1/15	8/48	0/21	0/20	0/24
Organic acid disorders						
Propionic acidemia (PA)	49/51	4/15 †	20/48	16/21 but limited or selective	9/20	12/24
Methylmalonic acidemia (MUT,CblA, CblB)	49/51	4/15 †	20/48	16/21 but limited or selective	9/20	12/24
Methylmalonic acidemia with homocystinuria (CblC, CblD)	21/51	1/15 †	10/48	0/21	0/20	0/24
Glutaric acidemia type I (GA I)	49/51	4/15 †	20/48	16/21 but limited or selective	9/20	12/24
3-methylcrotonyl-glycinuria (3-MCC)	49/51	4/15 †	20/48	16/21 but limited or selective	9/20	12/24
3-hydroxy-3-methyl glutaric aciduria (HMG)	49/51	4/15 †	20/48	16/21 but limited or selective	9/20	12/24
Holocarboxylase synthase deficiency	49/51	4/15 †	20/48	16/21 but limited or	9/20	12/24

(MCD)				selective		
B-Ketothiolase deficiency (BKT)	49/51	4/15 †	20/48	16/21 but limited or selective	9/20	12/24
Isovaleric academia (IVA)	49/51	4/15 †	20/48	16/21 but limited or selective	9/20	12/24
Other secondary conditions *	21/51 all conditions 25/51 not all conditions	1/15 †	10/48	0/21	0/20	0/24
Fatty acid β-oxidation disorders						
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)	51/51	15/15	23/48	16/21 but limited or selective	9/20	12/24
Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	51/51	9/15††	23/48	16/21 but limited or selective	9/20	12/24
Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)	51/51	9/15 ††	23/48	16/21 but limited or selective	9/20	12/24
Trifunctional protein deficiency (TFP)	51/51	9/15 ††	23/48	16/21 but limited or selective	9/20	12/24
Carnitine transport defect (CTD)	51/51	9/15 ††	23/48	16/21 but limited or selective	9/20	12/24
Carnitine palmitoyltransferase I deficiency (CPT I)	11/51	1/15	13/48	0/21	0/20	0/24
Carnitine palmitoyltransferase II deficiency (CPT II)	11/51	1/15	13/48	0/21	0/20	0/24
Carnitine-acylcarnitine translocase deficiency (CACT)	11/51	1/15	13/48	0/21	0/20	0/24
Glutaric acidemia type II (GA II)	11/51	1/15	13/48	0/21	0/20	0/24
Other secondary conditions **	11/51 all conditions 38/51 not all conditions	1/15	13/48	0/21	0/20	0/24
Other disorders						
Biotinidase deficiency (BIOT)	51/51	7/15	14/48	0/21	10/20	0/24
Classic Galactosemia (GALT)	51/51	8/15	13/48	0/21	14/20	9/24
Glycogen storage disease type II (Pompe)	5/51	0/15	0/48	0/21	0/20	0/24
Mucoplysaccharidosis type 1 (MPS 1)	2/51	0/15	0/48	0/21	0/20	0/24
X-linked adrenoleukodystrophy (X-ALD)	3/51	0/15	0/48	0/21	0/20	0/24
Others ****	3/51	0/15	3/48	0/21	3/20	11/24

* Malonic acidemia; isobutyrylglycinuria; 2-methylbutyrylglycinuria; 3-methylglutaconic acidurias; 2-methyl-3-hydroxybutyric acidurias.
** Short-chain acyl-CoA dehydrogenase deficiency; Medium/Short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency; Medium-chain ketoacyl-CoA thiolase deficiency; 2,4-dienoyl-CoA reductase deficiency.
*** Argininemia; Citrullinemia type II; Hypermethioninemia; Bening hyperphenylalaninemia; Biopterin defects; Tyrosinemia type II and III;
**** Galactoepimerase deficiency; Galactokinase deficiency; T-cell related lymphocyte deficiencies; Glucose-6-phosphate dehydrogenase deficiency; Other lysosomal diseases.
† There are 11 states that screen for some organic acidurias without specifying.
††There are 6 states that screen for some fatty acid oxidation disorders without specifying.
††† There are 9 states that screen for some amino acid disorders without specifying.