EXPANDED NEWBORN SCREENING BY MASS SPECTROMETRY: NEW TESTS, FUTURE PERSPECTIVES

Daniela Ombrone,[1,2] **Elisa Giocaliere,**[1] **Giulia Forni,**[1] **Sabrina Malvagia,**[1] **and Giancarlo la Marca**[1,2]*

[1]Newborn screening, Clinical Chemistry and Pharmacology Lab, Meyer Children's University Hospital, Viale Pieraccini 24, Florence 50139, Italy
[2]Department of Neurosciences, Psychology, Drug Research and Child Health, University of Florence, Viale Pieraccini 6, Florence 50139, Italy

Tandem mass spectrometry (MS/MS) has become a leading technology used in clinical chemistry and has shown to be particularly sensitive and specific when used in newborn screening (NBS) tests. The success of tandem mass spectrometry is due to important advances in hardware, software and clinical applications during the last 25 years. MS/MS permits a very rapid measurement of many metabolites in different biological specimens by using filter paper spots or directly on biological fluids. Its use in NBS give us the chance to identify possible treatable metabolic disorders even when asymptomatic and the benefits gained by this type of screening is now recognized worldwide. Today the use of MS/MS for second-tier tests and confirmatory testing is promising especially in the early detection of new disorders such as some lysosomal storage disorders, ADA and PNP SCIDs, X-adrenoleucodistrophy (X-ALD), Wilson disease, guanidinoacetate methyltransferase deficiency (GAMT), and Duchenne muscular dystrophy. The new challenge for the future will be reducing the false positive rate by using second-tier tests, avoiding false negative results by using new specific biomarkers and introducing new treatable disorders in NBS programs. © 2015 Wiley Periodicals, Inc. Rapid Commun. Mass Spectrom. 9999: XX–XX, 2015.

Keywords: newborn screening; tandem mass spectrometry; dried blood spot; lysosomal storage disorders; aevere combined immunodeficiences

I. INTRODUCTION

The use of mass spectrometry (MS) in clinical laboratories is very much increased on the outset of the 21st century. This development is obviously due to great advances in MS hardware, software and research in the last 25 years.

MS permits a very rapid measurement of many metabolites in different biological specimens using filter paper spots or directly in biological fluids. Because of its high sensitivity and specificity, this technique can be used for qualitative and quantitative analysis of many analytes, such as amino acids and acylcarnitines, organic acids, homocysteine, orotic acid, purines and pyrimidines, steroids, and vitamin D to name a few, with appropriate internal standards.

Newborn screening (NBS) is definitely one of the most successful applications of MS in clinical chemistry in the recent years. The measurement of amino acids and acylcarnitines enables the identification of many inborn errors of metabolism (IEM) few days after birth. If they are not diagnosed and early treated, most of them might cause mental and/or growth retardation, severe permanent sequelae and in some case death.

The history of NBS as population-based test started in 1960s when Robert Guthrie developed a simple and cheap bacterial inhibition assay on filter paper spot able to identify the phenylketonuria (PKU), the most frequent aminoacidopathia (Guthrie & Susi, 1963). The following development of electrospray tandem mass spectrometry (ESI-MS/MS) in more recent years (1990s) has permitted the introduction of this new technology in clinical chemistry laboratories, in particular for newborn screening purposes (Rashed et al., 1995).

Originally, fast atom bombardment (FAB) and thermospray (Yergey et al., 1984; Millington et al., 1991) were used to measure carnitine and acylcarnitines in plasma samples for the detection of fatty acid disorders.

Later on, advancements in MS/MS and research demonstrated that the analysis of acylcarnitines could be used to identify fatty acid oxidation defects in a neonatal population from dried blood spots. In fact, carnitine and its derivatives were ideal molecules for a MS/MS screening technique since they all presented a common product ion (85 m/z) containing a quaternary ammonium function. These structural characteristics enabled the detection of a panel of saturated, unsaturated, and hydroxylate acylcarnitines with high specificity and sensitivity.

More or less in the same years, FAB-MS/MS was also used for the first attempt to measure phenylalanine and tyrosine for PKU diagnosis (Chace et al., 1993). The procedure required esterification (as butyl esters) to enhance surface ionization in a glycerol matrix. The butylation "quenched" the negative charge on the acid moiety of phenylalanine increasing ionization efficiency and sensitivity.

During the first tests other amino acids were identified deducing a common neutral product, the butylformate, presenting with a mass of 102 Da. Since then, the common neutral loss of 102 Da is the basis for the MS/MS assay of several key butilated amino acids in newborn screening.

While butyl esterification assays are now routine, in the last 10 years new methods were developed able to detect amino acids and acylcarnitines as their native free acids (underivatized)

Daniela Ombrone and Elisa Giocaliere contributed equally.
*Correspondence to: Giancarlo la Marca, Newborn screening, Clinical Chemistry and Pharmacology Lab, Meyer Children's University Hospital, Viale Pieraccini 24, Florence 50139, Italy.
E-mail: g.lamarca@meyer.it, giancarlo.lamarca@unifi.it

in an attempt to simplify analytical operations and to minimize the use of butanol in chloridic acid.

Derivatized and underivatized methods with some modifications with respect to the original version are now worldwide used in clinical chemistry labs and we should consider that many millions of newborns are today screened for a variable number of diseases by meaning of that test.

But the high number of disorders covered in expanded newborn screening corresponded also to the increase in the recall rate (Tarini, Christakis, & Welch, 2006). False-positive screening results may cause unnecessary parental stress and influence the child–parent relationship (Gurian et al., 2006). On the other hand, a number of false-negative cases, have been reported by several newborn screening laboratories (Wilcken et al., 2003; Frazier et al., 2006).

In fact, some important metabolites in expanded newborn screening programs, markers of diseases, have proven to have poor specificity, causing a critical impact on the population due to the high number of recalls. The best way to decrease the recall rate, due to false positive results in the primary screening test, is represented by the introduction of the second-tier test, performed on the same NBS specimen with no necessity to additionally contact patient' family. Second-tier test result overrules the primary result.

Since for some metabolites there is an overlap between controls and patients ranges, second-tier test proved its superiority in discriminating the two populations thanks to the high specificity.

The choice of reference values for NBS test should be a good compromise between sensitivity and specificity; a good sensitivity could result in a high false positive rate while having high specificity could cause false negative results. Adjusting cutoff values for each metabolite should consider the availability of a second-tier test; if it is available, primary test cut-off should be reduced to capture presumptive positive cases (Chace & Hannon, 2010).

Near the proliferation of new second-tier tests, advances in computerization, automation, and sensitivity of analytical instruments have resulted in the expansion of potential new NBS tests in recent years. Furthermore, improvements in treatments, such as gene therapy, bone marrow transplantation, enzyme replacement, and chaperone therapy, have raised the status of some disorders as candidates for NBS. The availability of a effective treatment is the worldwide approved criterion to include a disorder in a newborn screening panel.

In the last years NBS tests have been proposed for lysosomal storage disorders, Duchenne muscular dystrophy, GAMT, peroxisomal disorders, SCIDs, and Wilson's disease.

While pilot projects have demonstrated the potential of these tests, it remains to be discussed how effectively they can be applied in most NBS centers and how effective the newer treatments are. Each new test appends an additional complexity to the NBS program and some of the therapies are costly and long-term, often lifelong.

II. SECOND-TIER TESTS IN EXPANDED NEWBORN SCREENING

Expanded NBS by tandem mass spectrometry is normally performed by a flow injection analysis; since it does not require prior chromatographic separation, potential interference is possible. Second-tier testing is performed on newborns with abnormal screening result. It is considered a second level test performed on the original blood spot, in order to find a target analyte under optimum operating conditions. The introduction of second-tier testing, in newborn screening programs, has allowed the reduction of the number of false positives that were not only the cause of unnecessary parental stress but also resulted in the increase of laboratory analyses and personnel costs due to repeat tests. However, second-tier tests cannot be used as primary screening tests because of the low-throughput assay and laboratory costs (Chace & Hannon, 2010).

There are two categories of second-tier tests: a test looking for the same analyte identified in NBS analyses or a test looking for additional diagnostic markers not detectable among NBS analytes (Fig. 1).

In the first one, second-tier tests will obtain a more high specificity than the NBS method, so a potential false-positive interference from NBS process may be excluded. Some authors consider these methods part of primary screening instead of a real second-tier test (Chace & Hannon, 2010). However, these tests meet fully the definition of second-tier tests, as they distinguish true from false positives and improve the positive predictive value of primary NBS results. Using this strategy, several two-tier tests have been developed such as succinylacetone (SUAC), a specific marker for Tyrosinemia I (OMIM 276700), recently included in the NBS panel (la Marca et al., 2008; Turgeon et al., 2008). Although, in our experience, false positive rates for SUAC are very low (la Marca et al., 2011), a sample contamination cannot be excluded. A newborn falsely suspected for Tyr I causes a high cost of hospitalization, without considering the parental stress involved. To overcome this problem, the specificity of some important diagnostic markers of diseases which require immediate hospitalization should have the highest possible specificity.

In the other case, second-tier tests identify diagnostic metabolites, not available by routine NBS, by strongly supporting the presence of disorder.

The identification of free methylmalonic or propionic acid on DBS, after an abnormal propionylcarnitine (C3) result, has been revealed one of the most effective second-tier tests. A high C3 (also $>10\,\mu mol/L$) is not a real diagnostic marker contrariwise the presence of free organic acids has a pathognostic importance. By using this second-tier test, the recall rate for C3 has been drastically reduced as previously reported (la Marca et al., 2007).

In the other cases, separation of isomers by second-tier testing can resolve multiple diagnostic possibilities. High levels of the branched-chain amino acids, revealed on NBS process, could be due to total parenteral nutrition or to maple syrup urine disease (MSUD, OMIM 615135). Alloisoleucine (allo-Ile) is the pathognomonic marker of MSUD, but it cannot be differentiated in flow injection from isobaric amino acids (leucine, isoleucine, and hydroxyproline; la Marca et al., 2005). Hydroxyprolinemia (OMIM 23700) is a benign condition for which recall is not recommended. A second-tier test has been shown to be able to identify each isobaric compound, permitting unequivocal interpretation of NBS results (Oglesbee et al., 2008).

Second tier steroid profiling by MS/MS can be also performed for congenital adrenal hyperplasia (CAH, OMIM 201910), for which NBS programs are fluorescence based. The false positive rate of CAH screening is much higher than other
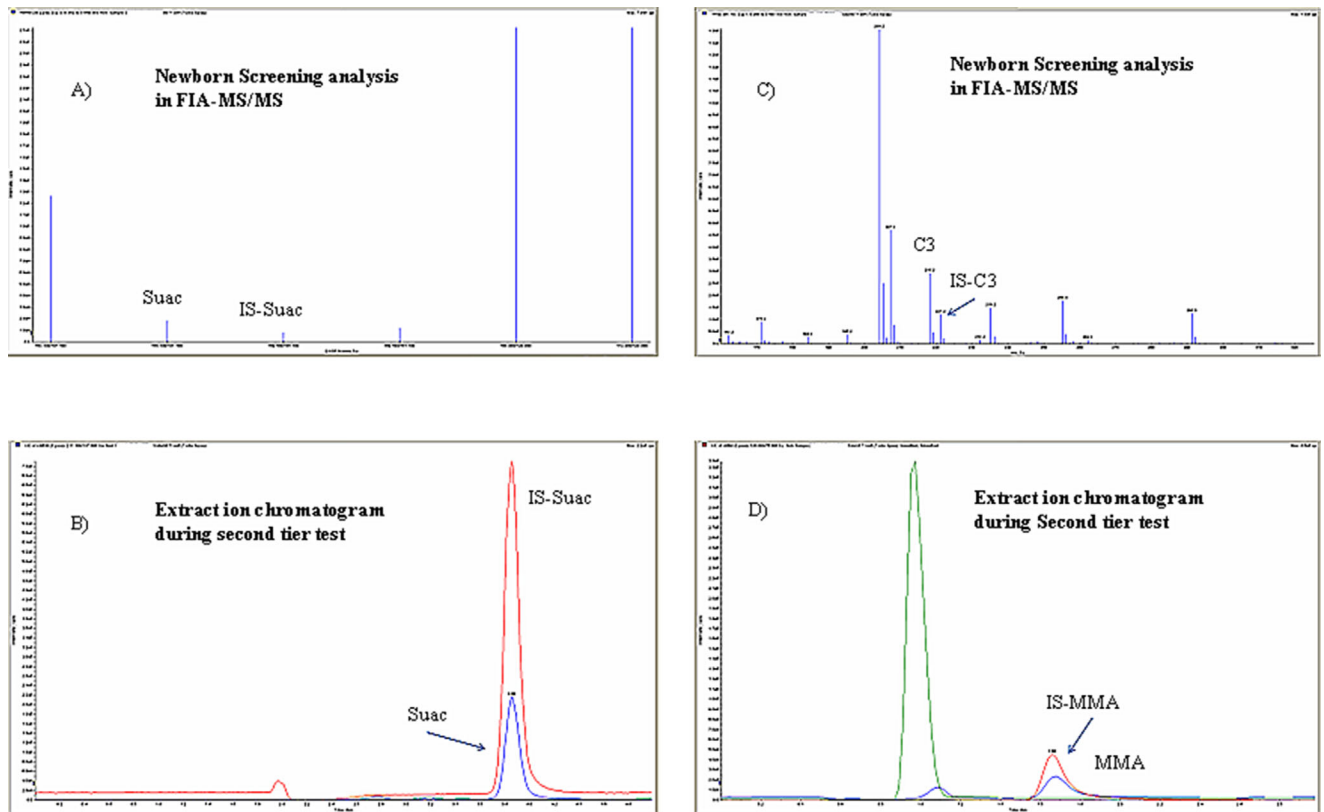
FIGURE 1. **A**: MS/MS analysis of Newborn Screening DBS from a patient affected by tyrosinemia type I. **B**: Extract ion chromatogram of m/z 155.1 > 137.1 and 159.1 > 141.1 for Suac and [$^{13}C_4$]-Suac during second-tier test. **C**: Partial acylcarnitine profile without derivatization in a newborn affected by methylmalonic aciduria Cbl C. **D**: Extract ion chromatogram of m/z 117 > 73 and 120 > 76 for MMA and [2H3]-MMA. Retection time was approximately 1.7 min.

screens and by using MS/MS in the second-tier tests, the specificity of NBS results improve (Schwarz et al., 2009), although not completely eliminating false-positive results but this benefit is opposed by the increased risk of false negatives as reported by Sarafoglou et al. (2012). These authors suggested that molecular testing as a second-tier test could have a major efficacy but cost effectiveness has not yet been evaluated.

However, MS/MS is a powerful technique that has very high analytical sensitivity and selectivity and so its poor effectiveness as second-tier test for CAH screening could be due to the lack of a consistent algorithm system, which determines the positive or negative results.

Many countries perform newborn screening to detect classic galactosemia (GALT deficiency, OMIM 230400) due to a deficiency of the galactose-1-phosphate uridyl transferase (GALT) enzyme. The first screening test can be performed either by fluorometric assay (Fujimoto et al., 2000) or by MS/MS (Jensen et al., 2001). In particular some false positive results by fluorimetric assay have been reported in DBS exposed to high temperature and humidity (Fujimoto et al., 2000; Ohlsson et al., 2012). In recent years, some second-tier tests able to reduce false positive rate have been developed, including multiplex enzyme testing by UPLC-MS/MS (Ko et al., 2011).

The combination of first and second-tier testings for many metabolic disorders can be considered the best working practice to achieve the most benefit out of newborn screening programs.

A list of MS-based second-tier tests is reported in Table 1.

III. SEVERE COMBINED IMMUNODEFICIENCY (SCID)

Severe combined immunodeficiency (SCID, OMIM 615615) is currently a genetically and clinically heterogeneous group of disorders belonging to primary immunodeficiency disorders (PIDDs) that result from thymic dysplasia and arrest in T lymphocyte maturation. There is also variable expression of B and natural killer (NK) cells, and patients are categorized into either SCID with absence of T lymphocytes but presence of B lymphocytes (T-B+ SCID) or SCID with absence of both T and B lymphocytes (T-B- SCID) (Somech & Etzioni, 2014). These diseases can be inherited either in an X-linked or an autosomal recessive manner.

Infants with SCID might be difficult to diagnose in the neonatal period because in most cases they are initially asymptomatic, (Geha et al., 2007; Slatter & Gennery, 2008), but a prompt identification and treatment may lead to less severe clinical symptoms and avoid fatal consequences.

Valid therapeutic options are represented from bone marrow transplantation (Slatter & Gennery, 2008; Gaspar et al., 2014) and enzyme replacement therapy. Recently, gene therapy has shown to be a highly effective treatment for infants with Adenosine Deaminase deficiency (ADA-SCID, OMIM 102700)

TABLE 1. Second tier tests performed by mass spectrometry

NBS MARKER	NBS ASSAY	MARKER II-TIER TEST	REFERENCES
Propionylcarnitne (C3)	TMS	free methylmalonic and propionic acids on blood	la Marca et al., 2007
Succinylacetone	TMS	Succinylacetone	la Marca et al., 2011
Methionine (↑) for homocystinuria Methionine (↓) for MTHFR	TMS	Homocysteine	Turgeon et al., 2010
17-Hydroxyprogesterone	immunoassay	17-hydroxyprogesterone, cortisol, androstenedione	Peter et al., 2008
Branched-chain amino acids	TMS	alloisoleucine, leucine, isoleucine, valine, OH-proline	Oglesbee et al., 2008
Isovalerylcarnitine (C5)	TMS	free isovaleric acid or isovalerylcarnitine separed from 2-methylbutyrylcarnitine and pivalic acid	Forni et al., 2010
Isobutyrylcarnitine (C4)	TMS	free ethylmalonic acid or isobutyrylcarnitine separed from butyrylcarnitne	Forni et al., 2010
Creatine and Guanidinoacetate	TMS	creatine and guanidinoacetate	Pasquali et al., 2014
Total galactose and/or GALT activity	immunoassay	Galactose-1-phosphate, UDP-galactose, and UDP-galactose	Ko et al., 2011

and X-linked SCID (X-SCID, OMIM 300400; Aiuti et al., 2009; Hacein-Bey-Abina et al., 2010; Gaspar et al., 2011a,b).

SCID shows all the characteristics required by neonatal screening programs and they were first introduced in 2008 in Wisconsin by T-cell receptor excision circles (TREC) assay and Massachusetts joined in 2009 and then several other states (Chase, Verbsky, & Routes, 2011).

TREC are DNA biomarkers of normal T cell development (Somech & Etzioni, 2014) and their quantification by real-time PCR on DBS currently represents the only test available to screen for SCID in routine NBS programs (Gaspar et al., 2014).

This test is shown to be a cost-effective test for SCID, due to the low cost of the TREC assay and to the extremely high costs associated with delayed identification and treatment (Chan et al., 2011).

The introduction of NBS for SCID using the TREC assay showed important advantages in early diagnosis and treatment of patients (Routes et al., 2009; Hale et al., 2010; Accetta et al., 2011); each state developed its own screening algorithm resulting in a great variability between areas (Chase, Verbsky, & Routes, 2011) .

High false-positive TREC assay rates are generated from infants born prematurely (Routes et al., 2009).

ADA-SCID represents the second most common form of SCID (Buckley, 2004; Booth et al., 2007; Gaspar et al., 2009); adenosine deaminase dysfunction causes accumulation of metabolites, including 2-deoxyadenosine (DAdo) and deoxyadenosine triphosphate, which inhibit the activity of ribonucleotide reductase and ultimately DNA synthesis. The incidence of ADA deficiency is estimated to be between one in 375,000 and one in 660,000 live births (Sauer & Aiuti, 2009) but it might be underestimated because of early death from infective diseases or disorders caused by toxic metabolites (Azzari, la Marca, & Resti, 2011).

Different ADA deficiency phenotypes have been described depending on the clinical onset and on the severity of the disease: the most severe form, presenting in infancy and usually resulting in early death, is "early onset" ADA-SCID; "delayed" onset concerning about 10–15% of patients; "later" onset characterized by less severe infections and "partial" ADA deficiency occurs in a subset of immunocompetent individuals (Arredondo-Vega et al., 1994). The incidence of late-onset ADA deficiency is also underestimated, even more than that of early onset ADA-SCID (Arredondo-Vega et al., 1998).

SCID NBS by TREC assay can identify newborns with early-onset ADA-SCID but it has been shown to be unable to identify newborns with delayed or late-onset ADA-SCID (la Marca et al., 2013).

In 2010, our group set up a MS/MS based method to detect ADA-SCID biomarkers, 2-deoxyadenosine and adenosine (Ado), on DBS from newborn screening cards. Results demonstrated that patients with ADA-SCID can be easily diagnosed at birth by using the existing underivatized acylcarnitine and aminoacid method (Azzari, la Marca, & Resti, 2011; Fig. 2). A two step extraction derivatized method is also possible (la Marca, 2014a). The method seemed to be promising and a pilot project for a population-based neonatal screening in Tuscany started in September 2010. The method resulted applicable also to premature infants whose mean levels of metabolites do not differ from those found in full-term babies. In 2011, the first diagnosis of ADA-SCID was made through expanded newborn screening including ADA metabolites quantification. The patient was confirmed by molecular analysis as a late onset phenotype. Normal TREC were detected on neonatal DBS (la Marca et al., 2014b).

At the beginning of 2013, Purine nucleoside phosphorylase deficiency (PNP, OMIM 613179) was also included in the MS/MS newborn screening panel of the Tuscany region (la Marca et al., 2014b).

PNP deficiency is a rare form of SCID (Markert, 1991; Al-Saud et al., 2009; Somech et al., 2013); the enzyme catalyses the phosphorolysis of guanosine, deoxyguanosine, inosine, and
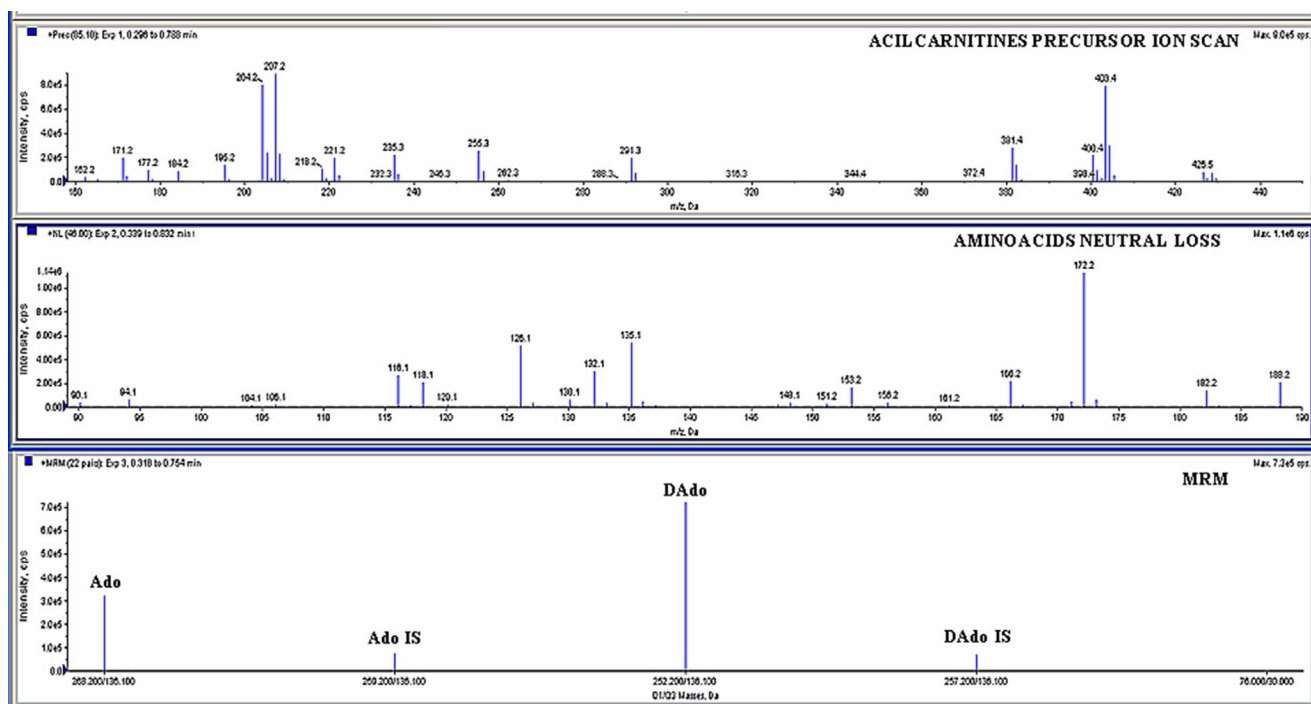
FIGURE 2. Newborn screening analysis by flow injection from a newborn confirmed to have ADA-SCID. (Top), unbutylated acylcarnitine profile acquired through a precursor ion scan of 85 Da. (Middle), amino acid profile obtained by neutral loss scan of 48 Da. (Bottom), simultaneous MRM acquisitions of m/z 268.2 > 136.1 (Ado), 169.2 > 136.1 ([$^{13}C_1$]-ado), 252.2 > 136.1 (DAdo), 257.2 > 136.1 ([$^{13}C_5$]-DAdo).

deoxyinosine to their respective purine bases and pentose-1-phosphates (Hershfield & Mitchell, 2001). Lack of PNP function determines an intracellular accumulation of deoxyguanosine triphosphate derived from deoxyguanosine, toxic to lymphoid cells and resulting in various neurologic abnormalities (Hershfield, 2004; Myers et al., 2004; Al-Saud et al., 2009). As for ADA-SCID, incidence and prevalence remain unknown because of misdiagnosis and insufficient available laboratory testing.

MS/MS can easily identify abnormal metabolites in DBS for PNP patients at birth as shown by retrospective studies on DBS samples from patients with genetically confirmed PNP deficiency.

When ADA and PNP SCID were added to the expanded newborn screening panel, a cost-effectiveness evaluation was performed. SCID metabolites inclusion was carried out without any additional operator intervention, sample preparation stages and any additional instrumentation beside that necessary for routine neonatal screening. Therefore, the cost was calculated for reagents only and rounded up to a few euro cents per newborn.

IV. LYSOSOMAL STORAGE DISORDERS

The lysosomal storage disorders (LSDs) are rare inherited diseases caused by a deficiency of one or more specific lysosomal enzymes, activator protein or membrane protein, resulting in deficient enzymatic activity. The defect causes the progressive accumulation of the substrate interfering with normal cellular activity and possibly resulting in cellular death.

LSDs represent a group of more than 50 different diseases (Filocamo & Morrone, 2011; la Marca, 2014c) and have a combined incidence of about 1 in 1,500–7,000 live births (Stone & Sidransky, 1999; Fletcher, 2006).

Most affected newborn seems to be healthy at birth but they can develop a wide range of clinical manifestations within the first few years of life. If left undetected and untreated, these diseases can lead to different clinical symptoms depending on the specific disorder. An early treatment could prevent these serious problems.

Thanks to the new effective therapeutic options available for some LSDs (Escolar et al., 2005; Urbanelli et al., 2011; Parenti et al., 2013; Ortolano et al., 2014) and to the development of new analytical methods to test enzyme activity on DBS specimens, these disorders have become strong candidates for inclusion in future mandatory screening panels.

Although in 2006 the American College of Medical Genetics (ACMG) Newborn Screening Expert Group declared LSDs as not appropriate for newborn screening because they did not meet the current evaluation criteria, more recently the Discretionary Advisory Committee on Heritable Disorders in Newborns and Children (DACHDNC) recommended to include Pompe disease to the Recommended Universal Screening Panel (RUSP) for all north American newborns (http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendations/correspondence/uniformpanel060313.pdf).

There are net evidences that presymptomatic diagnosis and early treatment for some LSDs can favorably modify the natural progression of the disease (Chien et al., 2009; Muenzer, 2014).

An additional argument for inclusion of LSDs in NBS programs is the relative prevalence of these conditions. The incidence of LSDs is not known but, surprisingly, pilot NBS programs for Fabry disease revealed an incidence of 1 in ~3,100

in Italy (Spada et al., 2006) and 1 in ~1,250 in Taiwan (Hwu et al., 2009) in male newborns.

Some countries already include some LSDs into their newborn screening panels, and local or pilot programs have been launched in others (Chien et al., 2008; Paciotti et al., 2012; Wittmann et al., 2012; Inoue et al., 2013; Liao et al., 2014).

The state of New York was the first state to perform population screening for the lysosomal disorder Krabbe disease starting in 2006 (Duffner et al., 2009).

In the Tuscany region of Italy, a prospective pilot project screening program for Pompe, Fabry, and Mucopolysaccharidosis type I has started in November 2014.

Going to technical procedures, the pioneering work of Chamoles et al. (2004) first established the possibility of assay lysosomal enzyme activity on DBS using fluorescence because many of these enzymes are still active in re-hydrated DBS. Fluorometric methods have been developed for many LSDs such as Pompe disease, Gaucher disease, Fabry disease, mucopolysaccharidosis (MPS), Hurler-like LSDs, Niemann-Pick A/B disease, Tay–Sachs disease and Sandhoff disease (Chamoles, Blanco & Gaggioli, 2001a,b; Chamoles et al., 2002a,b). However, the incorporation of a chromophore or fluorophore into the substrate caused false negative results and had limitations of specificity and limited capacity for multiplexing. In the last year, new technology for simultaneous screening of several enzyme activities related to lysosomal storage disorders from DBS was developed replacing the old methods enabling the assay of single enzyme activity (Li et al., 2004; Zhang et al., 2008).

Li et al. firstly developed a direct multiplex assay of lysosomal enzymes for Gaucher, Pompe, Krabbe, Fabry, and Niemann-Pick A/B diseases in DBS by flow injection analysis (FIA)-tandem mass spectrometry.

Later on, an expansion of simultaneously diagnosable disorders by MS has been reported (Orsini et al., 2012; Spacil et al., 2013).

Novel substrates, internal standards and assays have been developed over the years for testing enzyme activity of Krabbe, Pompe, Niemann-Pick A and B, Gaucher, Fabry, MPS-I, MPS-II, MPS-III, MPS-IVA, and MPS-VI (Gelb et al., 2006; Wang et al., 2007; Blanchard et al., 2008; Duffey et al., 2010a,b; Khaliq et al., 2011; Wolfe et al., 2011; Chennamaneni et al., 2014). Moreover in order to facilitate the introduction of LSD in newborn screening panels, the Center for Disease Control and Prevention distribute substrates and internal standards to interested newborn screening centres; QC programs are also available (De Jesus et al., 2009).

The substrates used are structurally closer to the natural substrates, the enzyme products are specific to each enzyme reaction and are quantified against internal standards with known concentrations for the measurements of enzyme activities (Li et al., 2004).

The multiplex assay developed by Li et al. was later refined by (Zhang et al. (2008) to make it more suitable for newborn screening laboratories. These methods required liquid or solid-phase extraction and subsequent FIA-MS/MS analysis to determine the concentration of each reaction product based on the intensity of internal standards.

la Marca et al. (2009) simplified Zhang's assay eliminating the liquid/liquid and solid-phase extraction steps by using an online trapping-and-cleanup liquid chromatography/mass spectrometry method. As an example a chromatogram from a LC-MS/MS combined six enzyme assay on DBS of a Pompe patient is reported in Figure 3.

Furthermore, the introduction of the separation of analytes by liquid chromatography (LC) coupled to the tandem mass spectrometer (LC-MS/MS) was crucial (la Marca et al., 2009; Kasper et al., 2010; Spacil et al., 2011). In fact it has been demonstrated that a too high declustering potential value in the mass spectrometer could cause a in source breakdown of the substrate with an undue appearance of the product (la Marca et al., 2009). Chromatographic separation allowed the distinction of the real enzymatic product from the one derived by the source breakdown of the substrate.

In the last years, new sample preparation methods reporting on a combination of substrates and internal standards into a single buffer, for a triplex assay (Duffey et al., 2010c; Scott et al., 2013) or a multiplex assay have been reported (Spacil et al., 2013).

Mechtler et al. (2012) have recently published on a short-incubation mass spectrometry-based protocol.

Metachromatic leukodystrophy (MLD) is a disorder caused by the deficiency of the enzyme arylsulfatase A (ASA) and resulting in the accumulation of 3-O-sulfogalactosyl ceramides (sulfatides) in tissues.

Recently, a noteworthy interest for newborn screening of MLD has arisen due to the development of potential therapies (Biffi et al., 2013).

However, application of direct assay of lysosomal enzyme in DBS seems to be difficult for MLD because trace amounts of ASA activity are sufficient to minimize disease severity (Renaud, 2012) and pseudodeficiency in ASA is a relatively frequent condition in the normal population (Emre et al., 2000; Lugowska et al., 2000).

Barcenas et al. (2014) in 2014 proposed a UHPLC/MS/MS method to quantify sulfatides in DBS and dried urine spots (DUS) for newborn screening of metachromatic leukodystrophy.

However, they show that analysis of sulfatides on DBS is problematic because their levels appear to be increased in some but not in all DBS patient samples, while there was a good separation between MLD and non-MLD samples using DUS. These results suggest that urine sample collection should be required for a reliable newborn screening program for metachromatic leukodystrophy.

Talking about neonatal screening for LSDs, some ethical considerations must be made.

Considerations should be made about the best strategy to treat presymptomatic individuals with positive screening results, the best way to inform parents of the potential outcomes of the affected individual and risks for future pregnancies.

Furthermore, newborn screening could potentially identify adult-onset variants or variants of uncertain significance and some patients with these variants may never develop symptoms or require therapy. It must be also considered that effective therapies are available only for few LSDs.

In addition LSD newborn screening could identify, as previously described for ASA, enzymatic pseudodeficiency, in fact there are some individuals showing a reduced enzyme activity but they remain clinically healthy (Filocamo & Morrone, 2011).
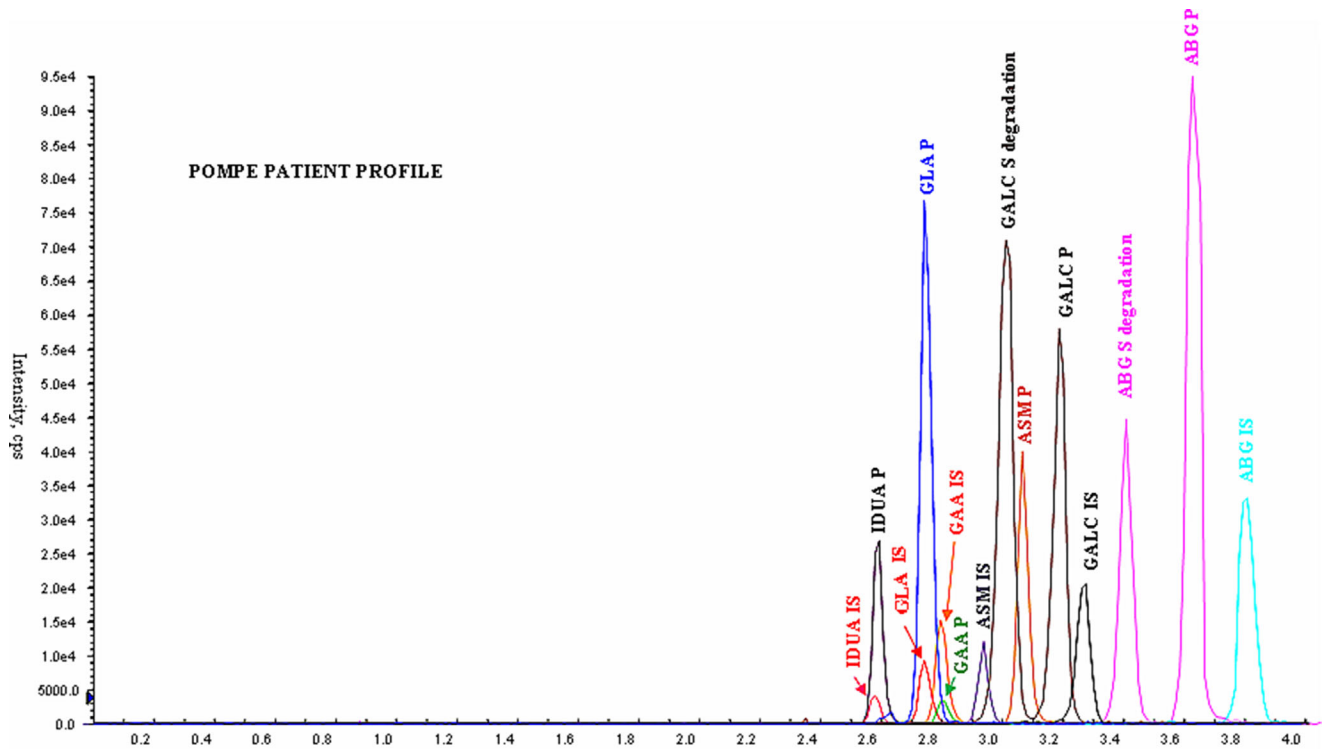
FIGURE 3. Lysosomal enzymatic screening on DBS from Pompe patient. Chromatograms of substrates, products, and internal standards for six lysosomal enzymes. The peak area corresponding to the transition m/z 498.4 > 398.3 for GAA product is equivalent to the area obtained from a blank sample (data not shown) resulting in a positive test for Pompe disease.

If a newborn screening panel would include Fabry disease, a complication could be the potential false negative of a female affected with Fabry disease. In fact, Fabry disease is an X-linked disorder and random X-inactivation results in highly variable activity of alpha galactosidase in female patients with Fabry disease. A negative newborn screening for Fabry disease in female should be interpreted with caution (Linthorst et al., 2005).

V. GUANIDINOACETATE METHYLTRANSFERASE DEFICIENCY

Guanidinoacetate methyltransferase (GAMT, OMIM 601240) deficiency is a rare creatine synthesis disorder resulting in a developmental delay and intellectual disability. It is characterized by a depletion cerebral creatine and an accumulation of guanidinoacetate (GAA) in the brain and body fluids. Early diagnosis and treatment with creatine, ornithine, and an arginine-restricted diet reduces the risk of neurological compromise (Gordon, 2010; El-Gharbawy et al., 2013). For this reason, GAMT deficiency might be a good candidate for newborn screening.

In the last years, some pilot projects have been started for newborn screening of GAMT deficiency (Mercimek-Mahmutoglu et al., 2012; Pasquali et al., 2014). GAA and creatine measurements can be included in the expanded newborn screening by using butyl esterification assays (Carducci et al., 2001). However, a high number of false positives is reported during newborn screening because of an overlap between the maximum value of GAA in healthy newborns and the lowest value in GAA patients. This problem can be solved by a second-tier test which allows to achieve the positive predictive value to 100% (Pasquali et al., 2014). A reliable method for the detection of Arginine: Glycine Amidino-transferase Deficiency (AGAT, OMIM 612718; low levels of both GAA and creatine) is still not present, given the variability of creatinine levels with the time of collection and the significant overlap of low levels with the normal population.

The detection of GAMT in the neonatal period has been demonstrated to improve the severe neurological outcome encouraging, with a minimal additional cost, the inclusion of GAMT deficiency within the routine newborn screening (El-Gharbawy et al., 2013).

VI. X-LINKED ADRENOLEUKODYSTROPHY

X-linked adrenoleukodystrophy (X-ALD) is a genetic disorder caused by mutations in ABCD1 gene on the X-chromosome resulting in the failure of peroxisomal oxidation of very long chain fatty acids (VLCFA; Ferrer, Aubourg, & Pujol, 2010; Kemp, Berger, & Aubourg, 2012). X-ALD is estimated to affect 1 in every 17,000 newborns (Bezman and Moser, 1998); however, the true prevalence can be known only from the newborn screening data.

The disease primarily affects the nervous system and the adrenal glands with onset of symptoms at variable ages (Moser, Mahmood, & Raymond, 2007). Phenotypes include rapidly progressive childhood, adolescent, and adult cerebral forms;

adrenomyeloneuropathy, which presents as slowly progressive paraparesis in adults and Addison disease without neurological manifestations.

X-ALD could be added to the list of disorders that could benefit from newborn screening because new therapies have been developed in the last years and they show encouraging promise for clinical outcomes (Moser, Raymond, & Dubey, 2005). In particular, hematopoietic stem-cell transplantation is the most effective treatment that is often recommended but is beneficial only if the patient has not already reached a stage with irreversible brain damage. Thus an early diagnosis is critical for improving the outcome of these patients.

The biochemical marker of XALD is the accumulation of saturated very long-chain fatty acids (VLCFA) in tissues and body fluids particularly of hexacosanoic acid (C26:0).

In 2006, an LC-MS/MS method has been developed to measure lyso-phosphatidylcholines (L-PC) C20:0, C22:0, C24:0 e C26:0 on dried blood spots, in which C26:0 and its ratios appeared the potential diagnostic marker for X-ALD. However, this method required a gradient analysis that had a chromatographic run time of 13 min/sample making it inconvenient for newborn screening (Hubbard et al., 2006). The method was slightly modified in 2009 (Hubbard et al., 2009) reducing run time analysis to 7 min/sample.

Recently, other methods have been proposed to make newborn screening feasible: in 2012, Sandlers et al. (2012) reported on a modified method from Hubbard 2009 for quantitation of L-PC 26:0 able to reduce both sample preparation and analysis time and to perform simultaneous extraction of acylcarnitines. In a prospective study, nearly 5,000 newborn blood spot samples were analysed by this method, providing evidence that it can be applied as a high throughput method for X-ALD newborn screening (Theda et al., 2014). In order to further improve the efficiency of newborn screening for X-ALD, Haynes and De Jesús (2012) developed a valve-switching system able to acquire acylcarnitines and amino acids in positive ion mode and L-PC 26:0 in negative ion mode to eliminate an interfering compound that could result in false positives.

An early diagnosis of X-ALD through newborn screening offers to these patients the best chance for treatment, thus an implementation of NBS programs could be desirable. Presently, heterozygous females, who are frequently symptomatic, remain an unresolved problem. Carrier females have a reduced L-PC 26:0 accumulation and that could lead to false negative in blood investigations (Berger & Gärtner, 2006).

Two peroxisomal storage disorders have been clinically diagnosed as X-ALD and Zellwegger syndrome (OMIM 214100) from 2004 to 2010 in Tuscany. A retrospective analysis of butylated acylcarnitines on their neonatal DBS has been performed by extending precursor ion scan of 85 m/z upto 600 Th (range was set from 200 to 600 m/z) and by adding acylcarnitine C23 as internal standard to quantify very long chain acylcarnitines. Their profiles revealed abnormal concentration of C16DC, C18DC, C24, and C26 acylcarnitines in the Zellweger syndrome neonatal DBS, as reported by Rizzo et al. (2003) and an increase of C24 and C26 (but not C16DC and C18 DC) acylcarnitines in X-ALD sample (Fig. 4). The ratio C26/C22 was also informative. Normal cutoff ranges for C16DC, C18DC, C22, C24, and C26 were than calculated on 20,000 NBS samples; no abnormal values of these markers were found (unpublished data).

Given the current poor data further evaluation should be done in order to develop a reliable, sensitive, and specific screening test for X-ALD patients.

VII. WILSON DISEASE

Wilson disease (WD; OMIM# 27790) is an autosomal recessive disorder caused by mutations in the gene encoding the copper transporting ATPase, ATP7B, that transports citosolic copper into the lumen of the trans-Golgi network for incorporation in ceruloplasmin (CP).

The enzyme defect reduces the incorporation of copper into ceruloplasmin causing copper accumulation mainly in the liver and in the central nervous system (deWilde et al., 2008) and a reduction in ceruloplasmin levels in the bloodstream.

WD incidence is reported to be about 1/30,000 live births but in some populations it is much more common (Figus et al., 1995; Zappu et al., 2008).

The pharmacological treatments are represented by copper chelators to promote its excretion from the body, zinc salts to reduce its absorption in the intestine and liver transpantation (Schilsky, 2009; Loudianos et al., 2014)

Due to the severity of the disease and to the existence of an effective therapy approach, an early diagnosis is fundamental.

Usually the diagnosis of WD is performed through the measurement of copper in liver tissue followed by molecular confirmation with genetic testing of ATP7B gene (Hahn, 2014). However level of ceruloplasmin in infants and newborns are considerably lower than in adults and this complicates a newborn screening programs due to a high rate of false positive rate.

A measurement of ceruloplasmin on DBS through ELISA assay, even if it is able to distinguish WD patients from healthy control individuals, is critical for a successful screening program because of the variation due to ceruplasmin antibody binding (Kroll et al., 2006).

An antibody-independent method using LC-MS/MS to quantify ceruloplasmin on DBS was developed by deWilde et al. (2008). However this new approach requires a time consuming sample preparation step (8 hr) due to trypsin digestion and long chromatographic run for the separation of specific CP peptides (7.5 min).

Therefore, nowadays, due to the absence of an effective and efficient approach for NBS, Wilson disease is not included in newborn screening programs (Matern, Oglesbee, & Tortorelli, 2013).

VIII. SICKLE CELL DISEASE

Sickle cell disease (SCD) is an inherited disorder caused by a replacement of a glutamic acid to a valine at position six on the β-globin chain (Stuart & Nagel, 2004) resulting in the structural variant hemoglobin S (HbS). The mutation causes sickling of hemoglobin rather than reduced amount of β globin which causes β-thalassemia (Galanello & Origa, 2010).

In homozygous state, HbS (HbSS) is responsible for anemia and tissue damage due to the occlusions of blood vessels caused by the abnormal red cells (Odièvre et al., 2011).

Heterozygous state associated with normal hemoglobin A (HbAS) is phenotypically silent, while different disease phenotypes occur in compound heterozygotes in which HbS variant is
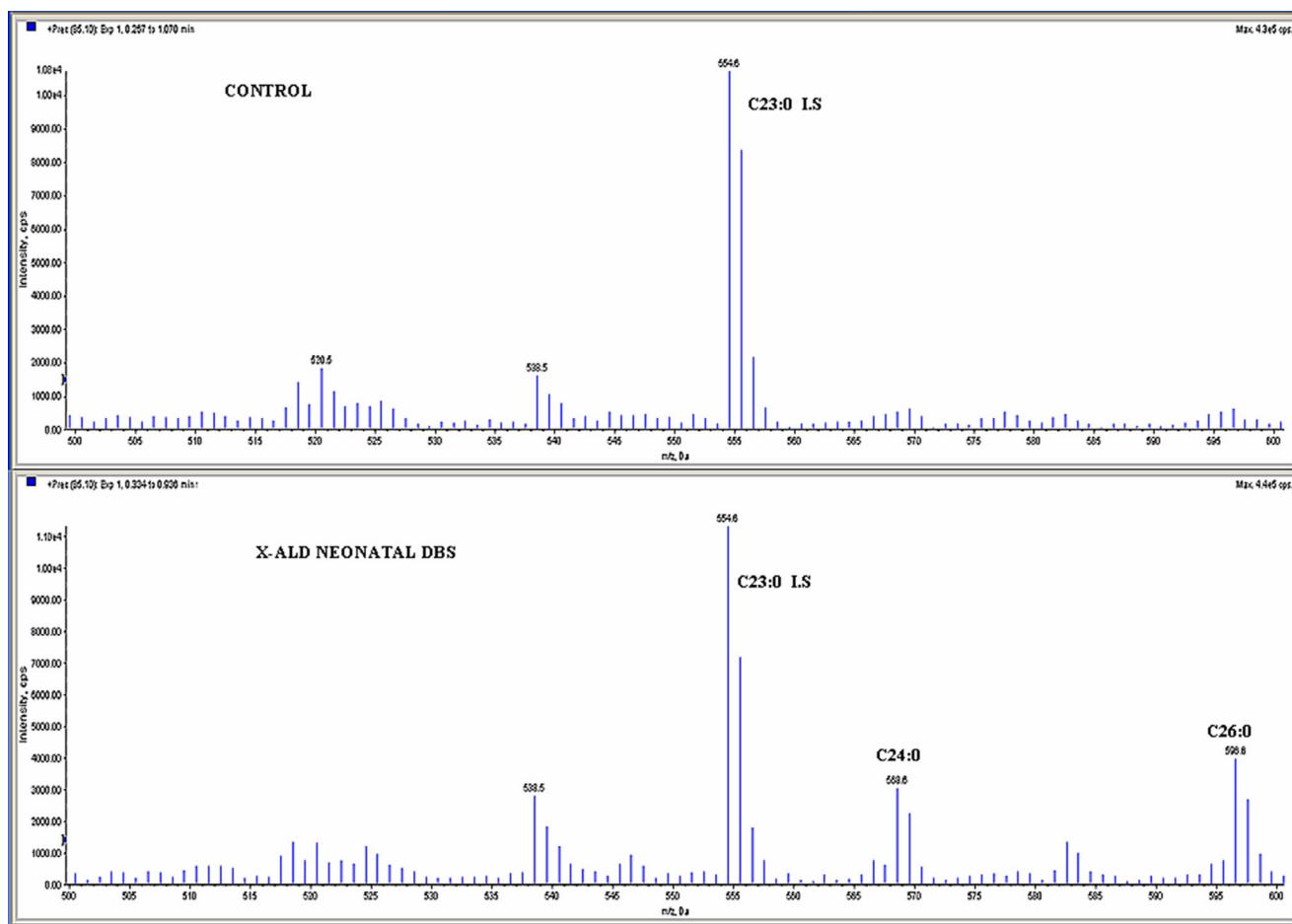
FIGURE 4. Partial precursor 85 full-scan analysis of acylcarnitines. The spectrum of X-ALD patient displayed most prominent ion signals consistent with C26:0 and C24:0 compared to the same signals in a control. C23:0 was added to the samples before extraction as an Internal Standard.

coinherited with another haemoglobin mutant (e.g., HbC, HbD Punjab/Los Angeles, or HbE).

SCD is associated with high mortality in early childhood and an early diagnosis by newborn screening could increase patient survival. Nowadays treatments help reduce the severity and frequency of complications of SCD; among them, some drugs that reduce HbS polymerization or stimulate HbF synthesis reactivation (Brugnara et al., 1996; Fathallah & Atweh, 2006; Migliaccio et al., 2008), such as hydroxyurea (Tang et al., 2005), have proved to be effective. Furthermore other important therapeutic options are represented by stem cell transplantation and transfusion programs (Styles & Vichinsky, 1994; Khoury & Abboud, 2011) and recently studies are focused on gene therapy (Sadelain et al., 2008).

Newborn screening programs for hemoglobin disorders can be performed on cord blood or on DBS by isoelectric focusing (IEF) or cation exchange HPLC (ceHPLC) HPLC–UV; these methods frequently produce results of difficult interpretation and data must be confirmed by second line testing using DNA sequencing or amino acid analysis by tandem mass spectrometry (Old, 2003).

Tandem mass spectrometry has recently been considered an alternative screening approach for the characterization of hamoglobin variants; different methods are used including the detection of the intact globin chains masses or analysis of tryptic peptide fragments (Boemer et al., 2008). Edwards et al. (2014) developed a method in which automated direct surface sampling of the DBS is coupled to top–down MS of the intact globin chains.

MS/MS for diagnosis of clinically significant Hb variants could be easily transferred for use in neonatal hemoglobinopathy screening due to the reasonable running cost, analytical sensitivity, and specificity and speed of analysis.

IX. CONCLUDING REMARKS

The NBS program is a complex process that is dependent on a multidisciplinary team which includes pediatricians, geneticists, dieticians as well as biologists, chemists, and technicians with expertise in mass spectrometry. The collaboration of all these professionals has contributed to the success of NBS for over 20 years and consequently to the beneficial impacts on public health care. Present trends in NBS are aimed at expanding testing panels in order to cover a wide range of conditions. Research including more testing in NBS and new therapeutic strategies has made noteworthy steps forward in this field.

However, the expansion of NBS raises some questions and the discussion of which disorders should be added to a NBS panel is still ongoing worldwide.

In any case, there are no doubts that the model of newborn screening and MS in preventive medicine may be exemplary in developing other clinical applications that go beyond metabolic disorders. Although there will be many challenges to the further expansion of MS in clinical chemistry, it is clear that this technique will play a fundamental role in the future.

ABBREVIATIONS

ADA	adenosine deaminase
Ado	adenosine
AGAT	arginine:glycine amidinotransferase
ASA	arylsulfatase A
CAH	congenital adrenal hyperplasia
CP	ceruloplasmin
C3	propionylcarnitine
DAdo	2-deoxyadenosine
DBS	dried blood spot
DUS	dried urine spot
ESI-MS/MS	electrospray tandem mass spectrometry
FAB	fast atom bombardment NBS newborn screening
FIA	flow injection analysis
GAA	guanidinoacetate
GAMT	guanidinoacetate methyltransferase
Hb	hemoglobin
IEF	isoelectric focusing
IEM	inborn errors of metabolism
L-PC	lyso-phosphatidylcholines
LSD	lysosomal storage disorders
MLD	metachromatic leukodystrophy
MPS	mucopolysaccharidosis
MSUD	maple syrup urine disease
NK	natural killer
PKU	phenylketonuria
PNP	purine nucleoside phosphorylase
SCD	sickle cell disease
SCID	severe combined immunodeficiency
SUAC	succinylacetone
TREC	T-cell receptor excision circles
VLCFA	very long chain fatty acids
X-ALD	X-linked adrenoleukodystrophy
WD	Wilson disease

ACKNOWLEDGMENTS

REFERENCES

Accetta D, Syverson G, Bonacci B, Reddy S, Bengtson C, Surfus J, Harbeck R, Huttenlocher A, Grossman W, Routes J, Verbsky J. 2011. Human phagocyte defect caused by a Rac2 mutation detected by means of neonatal screening for T cell lymphopenia. J Allergy Clin Immunol 127:535–538.

Aiuti A, Cattaneo F, Galimberti S, Benninghoff U, Cassani B, Callegaro L, Scaramuzza S, Andolfi G, Mirolo M, Brigida I, Tabucchi A, Carlucci F, Eibl M, Aker M, Slavin S, Al-Mousa H, Al Ghonaium A, Ferster A, Duppenthaler A, Notarangelo L, Wintergerst U, Buckley RH, Bregni M, Marktel S, Valsecchi MG, Rossi P, Ciceri F, Miniero R, Bordignon C, Roncarolo MG. 2009. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med 360:447–458.

Al-Saud B, Alsmadi O, Al-Muhsen S, Al-Ghonaium A, Al-Dhekri H, Arnaout R, Hershfield MS, Al-Mousa H. 2009. A novel mutation in purine nucleoside phosphorylase in a child with normal uric acid levels. Clin Biochem 42:1725–1727.

Arredondo-Vega FX, Santisteban I, Kelly S, Schlossman CM, Umetsu DT, Hershfield MS. 1994. Correct splicing despite mutation of the invariant first nucleotide of a 5-prime splice site: A possible basis for disparate clinical phenotypes in siblings with adenosine deaminase deficiency. Am J Hum Genet 54:820–830.

Arredondo-Vega FX, Santisteban I, Daniels S, Toutain S, Hershfield MS. 1998. Adenosine deaminase deficiency: Genotype-phenotype correlations based on expressed activity of 29 mutant alleles. Am J Hum Genet 63:1049–1059.

Azzari C, la Marca G, Resti M. 2011. Neonatal screening for severe combined immunodeficiency caused by an adenosine deaminase defect: A reliable and inexpensive method using tandem mass spectrometry. J Allergy Clin Immunol 127:1394–1399.

Barcenas M, Suhr TR, Scott CR, Turecek F, Gelb MH. 2014. Quantification of sulfatides in dried blood and urine spots from metachromatic leukodystrophy patients by liquid chromatography/electrospray tandem mass spectrometry. Clin Chim Acta 433:39–43.

Berger J, Gärtner J. 2006. X-linked adrenoleukodystrophy: Clinical, biochemical and pathogenetic aspects. Biochim Biophys Acta 1763:1721–1732.

Bezman L, Moser HW. 1998. Incidence of X-linked adrenoleukodystrophy and the relative frequency of its phenotypes. Am J Med Genet 76:415–419.

Biffi A, Montini E, Lorioli L, Cesani M, Fumagalli F, Plati T, Baldoli C, Martino S, Calabria A, Canale S, Benedicenti F, Vallanti G, Biasco L, Leo S, Kabbara N, Zanetti G, Rizzo WB, Mehta NA, Cicalese MP, Casiraghi M, Boelens JJ, Del Carro U, Dow DJ, Schmidt M, Assanelli A, Neduva V, Di Serio C, Stupka E, Gardner J, von Kalle C, Bordignon C, Ciceri F, Rovelli A, Roncarolo MG, Aiuti A, Sessa M, Naldini L. 2013. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science 341:1233158.

Blanchard S, Sadilek M, Scott CR, Turecek F, Gelb MH. 2008. Tandem mass spectrometry for the direct assay of lysosomal enzymes in dried blood spots: Application to screening newborns for mucopolysaccharidosis I. Clin Chem 54:2067–2070.

Boemer F, Ketelslegers O, Minon JM, Bours V, Schoos R. 2008. Newborn screening for sickle cell disease using tandem mass spectrometry. Clin Chem 54:2036–2041.

Booth C, Hershfield M, Notarangelo L, Buckley R, Hoenig M, Mahlaoui N, Cavazzana-Calvo M, Aiuti A, Gaspar HB. 2007. Management options for adenosine deaminase deficiency; proceedings of the EBMT satellite workshop (Hamburg, March 2006). Clin Immunol 123:139–147.

Brugnara C, Gee B, Armsby CC, Kurth S, Sakamoto M, Rifai N, Alper SL, Platt OS. 1996. Therapy with oral clotrimazole induces inhibition of the Gardos channel and reduction of erythrocyte dehydration in patients with sickle cell disease. J Clin Invest 97:1227–1234.

Buckley RH. 2004. The multiple causes of human SCID. J Clin Invest 114:1409–1411.

Carducci C, Birarelli M, Santagata P, Leuzzi V, Carducci C, Antonozzi I. 2001. Automated high-performance liquid chromatographic method for the determination of guanidinoacetic acid in dried blood spots: A tool for early diagnosis of guanidinoacetate methyltransferase deficiency. J Chromatogr B Biomed Sci Appl 755:343–348.

Chace DH, Millington DS, Terada N, Kahler SG, Roe CR, Hofman LF. 1993. Rapid diagnosis of phenylketonuria by quantitative analysis for phenylalanine and tyrosine in neonatal blood spots by tandem mass spectrometry. Clin Chem 39:66–71.

Chace DH, Hannon WH. 2010. Impact of second-tier testing on the effectiveness of newborn screening. Clin Chem 56:1653–1655.

Chamoles NA, Blanco M, Gaggioli D. 2001a. Fabry disease: Enzymatic diagnosis in dried blood spots on filter paper. Clin Chim Acta 308: 195–196.

Chamoles NA, Blanco M, Gaggioli D. 2001b. Diagnosis of alpha-L-iduronidase deficiency in dried blood spots on filter paper: the possibility of newborn diagnosis. Clin Chem 47:780–781.

Chamoles NA, Blanco M, Gaggioli D, Casentini C. 2002a. Tay-Sachs and Sandhoff diseases: Enzymatic diagnosis in dried blood spots on filter paper: retrospective diagnoses in newborn-screening cards. Clin Chim Acta 318:133–137.

Chamoles NA, Blanco M, Gaggioli D, Casentini C. 2002b. Gaucher and Niemann-Pick diseases- enzymatic diagnosis in dried blood spots on filter paper: retrospective diagnoses in newborn-screening cards. Clin Chim Acta 317:191–197.

Chamoles NA, Niizawa G, Blanco M, Gaggioli D, Casentini C. 2004. Glycogen storage disease type II: Enzymatic screening in dried blood spots on filter paper. Clin Chim Acta 347:97–102.

Chan K, Davis J, Pai SY, Bonilla FA, Puck JM, Apkon M. 2011. A Markov model to analyze cost-effectiveness of screening for severe combined immunodeficiency (SCID). Mol Genet Metab 104:383–389.

Chase NM, Verbsky JW, Routes JM. 2011. Newborn screening for SCID: Three years of experience. Ann NY Acad Sci 1238:99–105.

Chennamaneni NK, Kumar AB, Barcenas M, Spáčil Z, Scott CR, Tureček F, Gelb MH. 2014. Improved reagents for newborn screening of mucopolysaccharidosis types I, II, and VI by tandem mass spectrometry. Anal Chem 86:4508–4514.

Chien YH, Chiang SC, Zhang XK, Keutzer J, Lee NC, Huang AC, Chen CA, Wu MH, Huang PH, Tsai FJ, Chen YT, Hwu WL. 2008. Early detection of Pompe disease by newborn screening is feasible: Results from the Taiwan screening program. Pediatrics 122:e39–e45.

Chien YH, Lee NC, Thurberg BL, Chiang SC, Zhang XK, Keutzer J, Huang AC, Wu MH, Huang PH, Tsai FJ, Chen YT, Hwu WL. 2009. Pompe disease in infants: Improving the prognosis by newborn screening and early treatment. Pediatrics 124:e1116–e1125.

De Jesus VR, Zhang XK, Keutzer J, Bodamer OA, Mühl A, Orsini JJ, Caggana M, Vogt RF, Hannon WH. 2009. Development and evaluation of quality control dried blood spot materials in newborn screening for lysosomal storage disorders. Clin Chem 55:158–164.

deWilde A, Sadilkova K, Sadilek M, Vasta V, Hahn SH. 2008. Tryptic peptide analysis of ceruloplasmin in dried blood spots using liquid chromatography-tandem mass spectrometry: Application to newborn screening Clin Chem 54:1961–1968

Duffey TA, Sadilek M, Scott CR, Turecek F, Gelb MH. 2010a. Tandem mass spectrometry for the direct assay of lysosomal enzymes in dried blood spots: Application to screening newborns for mucopolysaccharidosis VI (Maroteaux-Lamy syndrome). Anal Chem 82:9587–9591.

Duffey TA, Khaliq T, Scott CR, Turecek F, Gelb MH. 2010b. Design and synthesis of substrates for newborn screening of Maroteaux-Lamy and Morquio A syndromes. Bioorg Med Chem Lett 20:5994–5996.

Duffey TA, Bellamy G, Elliott S, Fox AC, Glass M, Turecek F, Gelb MH, Scott CR. 2010c. A tandem mass spectrometry triplex assay for the detection of Fabry, Pompe, and mucopolysaccharidosis-I (Hurler). Clin Chem 56:1854–1861.

Duffner PK, Caggana M, Orsini JJ, Wenger DA, Patterson MC, Crosley CJ, Kurtzberg J, Arnold GL, Escolar ML, Adams DJ, Andriola MR, Aron AM, Ciafaloni E, Djukic A, Erbe RW, Galvin-Parton P, Helton LE, Kolodny EH, Kosofsky BE, Kronn DF, Kwon JM, Levy PA, Miller-Horn J, Naidich TP, Pellegrino JE, Provenzale JM, Rothman SJ, Wasserstein MP. 2009. Newborn screening for Krabbe disease: The New York State model. Pediatr Neurol 40:245–252.

Edwards RL, Griffiths P, Bunch J, Cooper HJ. 2014. Compound heterozygotes and beta-thalassemia: Top-down mass spectrometry for detection of hemoglobinopathies. Proteomics 14:1232–1238.

El-Gharbawy AH, Goldstein JL, Millington DS, Vaisnins AE, Schlune A, Barshop BA, Schulze A, Koeberl DD, Young SP. 2013. Elevation of guanidinoacetate in newborn dried blood spots and impact of early treatment in GAMT deficiency. Mol Genet Metab 109:215–217.

Emre S, Topçu M, Terzioğlu M, Renda Y. 2000. Arylsulfatase A pseudodeficiency incidence in Turkey. Turk J Pediatr 42:115–117.

Escolar ML, Poe MD, Martin HR, Kurtzberg J. 2005. Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. N Engl J Med 352:2069–2081.

Fathallah H, Atweh GF. 2006. Induction of fetal hemoglobin in the treatment of sickle cell disease. Hematol Am Soc Hematol Educ Program 1:58–62.

Ferrer I, Aubourg P, Pujol A. 2010. General aspects and neuropathology of X-linked adrenoleukodystrophy. Brain Pathol 20:817–830.

Figus A, Angius A, Loudianos G, Bertini C, Dessì V, Loi A, Deiana M, Lovicu M, Olla N, Sole G, De Virgiliis S, Lilliu F, Farci AMG, Nurchi A, Giacchino R, Barabino A, Marazzi M, Zancan L, Greggio NA, Marcellini M, Solinas A, Deplano A, Barbera C, Devoto M, Ozsoylu S, Kocak N, Akar N, Karayalcin S, Mokini V, Cullufi P, Balestrieri A, Cao A, Pirastu M. 1995. Molecular pathology and haplotype analysis of Wilson disease in mediterranean populations. Am J Hum Genet 57:1318–1324.

Filocamo M, Morrone A. 2011. Lysosomal storage disorders: Molecular basis and laboratory testing. Hum Genomics 5:156–169.

Fletcher JM. 2006. Screening for lysosomal storage disorders: A clinical perspective. J Inherit Metab Dis 29:405–408.

Forni S, Fu X, Palmer SE, Sweetman L. 2010. Rapid determination of C4-acylcarnitine and C5-acylcarnitine isomers in plasma and dried blood spots by UPLC-MS/MS as a second tier test following flow-injection MS/MS acylcarnitine profile analysis. Mol Genet Metab 101:25–32.

Frazier DM, Millington DS, McCandless SE, Koeberl DD, Weavil SD, Chaing SH, Muenzer J. The tandem mass spectrometry newborn screening experience in North Carolina: 1997-2005. J Inherit Metab Dis 29:76–85.

Fujimoto A, Okano Y, Miyagi T, Isshiki G, Oura T. 2000. Quantitative Beutler test for newborn mass screening of galactosemia using a fluorometric microplate reader. Clin Chem 46:806–810.

Galanello R, Origa R. 2010. Beta-thalassemia. Orphanet J Rare Dis 5:11.

Gaspar HB, Aiuti A, Porta F, Candotti F, Hershfield MS, Notarangelo LD. 2009. How I treat ADA deficiency. Blood 114:3524–3532.

Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Zhang F, Adams S, Bjorkegren E, Bayford J, Brown L, Davies EG, Veys P, Fairbanks L, Bordon V, Petropoulou T, Kinnon C, Thrasher AJ. 2011a. Hematopoietic stem cell gene therapy for adenosine deaminase-deficient severe combined immunodeficiency leads to long-term immunological recovery and metabolic correction. Sci Transl Med 3:97ra80.

Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Adams S, Howe SJ, Al Ghonaium A, Bayford J, Brown L, Davies EG, Kinnon C, Thrasher AJ. 2011b. Long-term persistence of a polyclonal T cell repertoire after gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med 3:97ra79.

Gaspar HB, Hammarström L, Mahlaoui N, Borte M, Borte S. 2014. The case for mandatory newborn screening for severe combined immunodeficiency (SCID). J Clin Immunol 34:393–397.

Geha RS, Notarangelo LD, Casanova JL, Chapel H, Conley ME, Fischer A, Hammarström L, Nonoyama S, Ochs HD, Puck JM, Roifman C, Seger R, Wedgwood J. 2007. Primary immunodeficiency diseases: An update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. J Allergy Clin Immunol 120:776–794.

Gelb MH, Turecek F, Scott CR, Chamoles NA. 2006. Direct multiplex assay of enzymes in dried blood spots by tandem mass spectrometry for the newborn screening of lysosomal storage disorders. J Inherit Metab Dis 29:397–404.

Gordon N. 2010. Guanidinoacetate methyltransferase deficiency (GAMT). Brain Dev 32:79–81.

Guthrie R, Susi A. 1963. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 32:338–343.

Gurian EA, Kinnamon DD, Henry JJ, Waisbren SE. 2006. Expanded newborn screening for biochemical disorders: The effect of a false-positive result. Pediatrics 117:1915–1921.

Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, Martinache C, Rieux-Laucat F, Latour S, Belohradsky BH, Leiva L, Sorensen R, Debré M, Casanova JL, Blanche S, Durandy A, Bushman FD, Fischer A, Cavazzana-Calvo M. 2010. Efficacy of gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 363:355–364.

Hale JE, Bonilla FA, Pai SY, Gerstel-Thompson JL, Notarangelo LD, Eaton RB, Comeau AM. 2010. Identification of an infant with severe combined immunodeficiency by newborn screening. J Allergy Clin Immunol 126:1073–1074.

Hahn SH. 2014. Population screening for Wilson's disease. Ann NY Acad Sci 1315:64–69.

Haynes CA, De Jesús VR. 2012. Improved analysis of C26:0-lysophosphatidylcholine in dried-blood spots via negative ion mode HPLC-ESI-MS/MS for X-linked adrenoleukodystrophy newborn screening. Clin Chim Acta 413:1217–1221.

Hershfield MS, Mitchell BS. 2001. Immunodeficiency diseases caused by adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill. pp. 2585–2625.

Hershfield M. 2004. Combined immune deficiencies due to purine enzyme defects. In: Stiehm ER, Ochs HD, Winkelstein JA, editors. Immunologic disorders in infants and children. Philadelphia: Elsevier. pp. 480–504.

Hubbard WC, Moser AB, Tortorelli S, Liu A, Jones D, Moser H. 2006. Combined liquid chromatography-tandem mass spectrometry as an analytical method for high throughput screening for X-linked adrenoleukodystrophy. Mol Genet Metab 89:185–187.

Hubbard WC, Moser AB, Liu AC, Jones RO, Steinberg SJ, Lorey F, Panny SR, Vogt RF Jr, Macaya D, Turgeon CT, Tortorelli S, Raymond GV. 2009. Newborn screening for X-linked adrenoleukodystrophy (X-ALD): validation of a combined liquid chromatography-tandem mass spectrometric (LC-MS/MS) method. Mol Genet Metab 97:212–220.

Hwu WL, Chien YH, Lee NC, Chiang SC, Dobrovolny R, Huang AC, Yeh HY, Chao MC, Lin SJ, Kitagawa T, Desnick RJ, Hsu LW. 2009. Newborn screening for Fabry disease in Taiwan reveals a high incidence of the later-onset GLA mutation c.936+919G>A (IVS4+919G>A). Hum Mutat 30:1397–1405.

Inoue T, Hattori K, Ihara K, Ishii A, Nakamura K, Hirose S. 2013. Newborn screening for Fabry disease in Japan: Prevalence and genotypes of Fabry disease in a pilot study. J Hum Genet 58:548–552.

Jensen UG, Brandt NJ, Christensen E, Skovby F, Nørgaard-Pedersen B, Simonsen H. 2001. Neonatal screening for galactosemia by quantitative analysis of hexose monophosphates using tandem mass spectrometry: A retrospective study Clin Chem 47 1364–1372.

Kasper DC, Herman J, De Jesus VR, Mechtler TP, Metz TF, Shushan B. 2010. The application of multiplexed, multi-dimensional ultra-high-performance liquid chromatography/tandem mass spectrometry to the high-throughput screening of lysosomal storage disorders in newborn dried bloodspots. Rapid Commun Mass Spectrom 24:986–994.

Kemp S, Berger J, Aubourg P. 2012. X-linked adrenoleukodystrophy: Clinical, metabolic, genetic and pathophysiological aspects. Biochim Biophys Acta 1822:1465–1474.

Khaliq T, Sadilek M, Scott CR, Turecek F, Gelb MH. 2011. Tandem mass spectrometry for the direct assay of lysosomal enzymes in dried blood spots: Application to screening newborns for mucopolysaccharidosis IVA. Clin Chem 57:128–131.

Khoury R, Abboud MR. 2011. Stem-cell transplantation in children and adults with sickle cell disease: An update. Expert Rev Hematol 4:343–351.

Ko DH, Jun SH, Park KU, Song SH, Kim JQ, Song J. 2011. Newborn screening for galactosemia by a second-tier multiplex enzyme assay using UPLC-MS/MS in dried blood spots. J Inherit Metab Dis 34:409–414.

Kroll CA, Ferber MJ, Dawson BD, Jacobson RM, Mensink KA, Lorey F, Sherwin J, Cunningham G, Rinaldo P, Matern D, Hahn SH. 2006. Retrospective determination of ceruloplasmin in newborn screening blood spots of patients with Wilson disease. Mol Genet Metab 89:134–138.

la Marca G, Malvagia S, Pasquini E, Donati MA, Gasperini S, Procopio E, Zammarchi E. 2005. Hyperhydroxyprolinaemia: A new case diagnosed during neonatal screening with tandem mass spectrometry. Rapid Commun Mass Spectrom 19:863–864.

la Marca G, Malvagia S, Pasquini E, Innocenti M, Donati MA, Zammarchi E. 2007. Rapid 2nd-tier test for measurement of 3-OH-propionic and methylmalonic acids on dried blood spots: Reducing the false-positive rate for propionylcarnitine during expanded newborn screening by liquid chromatography-tandem mass spectrometry. Clin Chem 53:1364–1369.

la Marca G, Malvagia S, Pasquini E, Innocenti M, Fernandez MR, Donati MA, Zammarchi E. 2008. The inclusion of succinylacetone as marker for tyrosinemia type I in expanded newborn screening programs. Rapid Commun Mass Spectrom 22:812–818.

la Marca G, Casetta B, Malvagia S, Guerrini R, Zammarchi E. 2009. New strategy for the screening of lysosomal storage disorders: The use of the online trapping-and-cleanup liquid chromatography/mass spectrometry. Anal Chem 81:6113–6121.

la Marca G, Malvagia S, Pasquini E, Cavicchi C, Morrone A, Ciani F, Funghini S, Villanelli F, Zammarchi E, Guerrini R. 2011. Newborn screening for tyrosinemia type I: Further evidence that succinylacetone determination on blood spot is essential. JIMD Rep 1:107–109.

la Marca G, Canessa C, Giocaliere E, Romano F, Duse M, Malvagia S, Lippi F, Funghini S, Bianchi L, Della Bona, Ombrone C, Moriondo D, Villanelli M, Speckmann F, Adams C, Gaspar S, Hershfield BH, Santisteban M, Fairbanks I, Ragusa L, Resti G, de Martino M, Guerrini M, Azzari R. 2013. Tandem mass spectrometry, but not T-cell receptor excision circle analysis, identifies newborns with late-onset adenosine deaminase deficiency. J Allergy Clin Immunol 131:1604–1610.

la Marca G. 2014a. Mass spectrometry in clinical chemistry: The case of newborn screening. J Pharm Biomed Anal 101:174–182.

la Marca G, Canessa C, Giocaliere E, Romano F, Malvagia S, Funghini S, Moriondo M, Valleriani C, Lippi F, Ombrone D, Della Bona ML, Speckmann C, Borte S, Brodszki N, Gennery AR, Weinacht K, Celmeli F, Pagel J, de Martino M, Guerrini R, Wittkowski H, Santisteban I, Bali P, Ikinciogullari A, Hershfield M, Notarangelo LD, Resti M, Azzari C. 2014b. Diagnosis of immunodeficiency caused by a purine nucleoside phosphorylase defect by using tandem mass spectrometry on dried blood spots. J Allergy Clin Immunol 134 155–159.

la Marca G. 2014c. Lysosomals. In: Blau N, Duran M, Gibson KM, Dionisi-Vici C, editors. Physician's guide to the diagnosis, treatment, and follow-up of inherited metabolic diseases. Berlin Heidelberg: Springer. pp. 785–793.

Li Y, Scott CR, Chamoles NA, Ghavami A, Pinto BM, Turecek F, Gelb MH. 2004. Direct multiplex assay of lysosomal enzymes in dried blood spots for newborn screening. Clin Chem 50:1785–1796.

Liao HC, Chiang CC, Niu DM, Wang CH, Kao SM, Tsai FJ, Huang YH, Liu HC, Huang CK, Gao HJ, Yang CF, Chan MJ, Lin WD, Chen YJ. 2014. Detecting multiple lysosomal storage diseases by tandem mass spectrometry—A national newborn screening program in Taiwan. Clin Chim Acta 431:80–86.

Linthorst GE, Vedder AC, Aerts JM, Hollak CE. 2005. Screening for Fabry disease using whole blood spots fails to identify one-third of female carriers. Clin Chim Acta 353:201–203.

Loudianos G, Lepori MB, Mameli E, Dessì V, Zappu A. 2014. Wilson's disease. Prilozi 35:93–98.

Lugowska A, Czartoryska B, Tylki-Szymańska A, Bisko M, Zimowski JG, Berger J, Molzer B. 2000. Prevalence of arylsulfatase A

pseudodeficiency allele in metachromatic leukodystrophy patients from Poland. Eur Neurol 44:104–107.

Markert ML. 1991. Purine nucleoside phosphorylase deficiency. Immunodefic Rev 3:45–81.

Matern D, Oglesbee D, Tortorelli S. 2013. Newborn screening for lysosomal storage disorders and other neuronopathic conditions. Dev Disabil Res Rev 17:247–253.

Mechtler TP, Metz TF, Müller HG, Ostermann K, Ratschmann R, De Jesus VR, Shushan B, Di Bussolo JM, Herman JL, Herkner KR, Kasper DC. 2012. Short-incubation mass spectrometry assay for lysosomal storage disorders in newborn and high-risk population screening. J Chromatogr B Analyt Technol Biomed Life Sci 908:9–17.

Mercimek-Mahmutoglu S, Sinclair G, van Dooren SJ, Kanhai W, Ashcraft P, Michel OJ, Nelson J, Betsalel OT, Sweetman L, Jakobs C, Salomons GS. 2012. Guanidinoacetate methyltransferase deficiency: First steps to newborn screening for a treatable neurometabolic disease. Mol Genet Metab 107:433–443.

Migliaccio AR, Rotili D, Nebbioso A, Atweh G, Mai A. 2008. Histone deacetylase inhibitors and hemoglobin F induction in beta-thalassemia. Int J Biochem Cell Biol 40:2341–2347.

Millington DS, Kodo N, Terada N, Roe D, Chace DH. 1991. The analysis of diagnostic markers of genetic disorders in human blood and urine using tandem mass spectrometry with liquid secondary ion mass spectrometry. Int J Mass Spectrom Ion Process 111:211–228.

Moser HW, Mahmood A, Raymond GV. 2007. X-linked adrenoleukodystrophy. Nat Clin Pract Neurol 3:140–151.

Moser HW, Raymond GV, Dubey P. 2005. Adrenoleukodystrophy: New approaches to a neurodegenerative disease. JAMA 294:3131–3134.

Muenzer J. 2014. Early initiation of enzyme replacement therapy for the mucopolysaccharidoses. Mol Genet Metab 111:63–72.

Myers LA, Hershfield MS, Neale WT, Escolar M, Kurtzberg J. 2004. Purine nucleoside phosphorylase deficiency (PNP-def) presenting with lymphopenia and developmental delay: Successful correction with umbilical cord blood transplantation. J Pediatr 145:710–712.

Odièvre MH, Verger E, Silva-Pinto AC, Elion J. 2011. Pathophysiological insights in sickle cell disease. Indian J Med Res 134:532–537.

Oglesbee D, Sanders KA, Lacey JM, Magera MJ, Casetta B, Strauss KA, Tortorelli S, Rinaldo P, Matern D. 2008. 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 54:542–549.

Ohlsson A, Guthenberg C, von Döbeln U. 2012. Galactosemia screening with low false-positive recall rate: The Swedish experience. JIMD Rep 2:113–117.

Old JM. 2003. Screening and genetic diagnosis of haemoglobin disorders. Blood Rev 17:43–53.

Orsini JJ, Martin MM, Showers AL, Bodamer OA, Zhang XK, Gelb MH, Caggana M. 2012. Lysosomal storage disorder 4+1 multiplex assay for newborn screening using tandem mass spectrometry: Application to a small-scale population study for five lysosomal storage disorders. Clin Chim Acta 413:1270–1273.

Ortolano S, Viéitez I, Navarro C, Spuch C. 2014. Treatment of lysosomal storage diseases: Recent patents and future strategies. Recent Pat Endocr Metab Immune Drug Discov 8:9–25.

Paciotti S, Persichetti E, Pagliardini S, Deganuto M, Rosano C, Balducci C, Codini M, Filocamo M, Menghini AR, Pagliardini V, Pasqui S, Bembi B, Dardis A, Beccari T. 2012. First pilot newborn screening for four lysosomal storage diseases in an Italian region: Identification and analysis of a putative causative mutation in the GBA gene. Clin Chim Acta 413:1827–1831.

Parenti G, Pignata C, Vajro P, Salerno M. 2013. New strategies for the treatment of lysosomal storage diseases. Int J Mol Med 31:11–20.

Pasquali M, Schwarz E, Jensen M, Yuzyuk T, DeBiase I, Randall H, Longo N. 2014. Feasibility of newborn screening for guanidinoacetate methyltransferase (GAMT) deficiency. J Inherit Metab Dis 37: 231–236.

Peter M, Janzen N, Sander S, Korsch E, Riepe FG, Sander J. 2008. A case of 11beta-hydroxylase deficiency detected in a newborn screening program by second-tier LC-MS/MS. Horm Res 69:253–256.

Rashed MS, Ozand PT, Bucknall MP, Little D. 1995. Diagnosis of inborn errors of metabolism from blood spots by acylcarnitines and amino acids profiling using automated electrospray tandem mass spectrometry. Pediatr Res 38:324–331.

Renaud DL. 2012. Lysosomal disorders associated with leukoencephalopathy. Semin Neurol 32:51–54.

Rizzo C, Boenzi S, Wanders RJ, Duran M, Caruso U, Dionisi-Vici C. 2003. Characteristic acylcarnitine profiles in inherited defects of peroxisome biogenesis: A novel tool for screening diagnosis using tandem mass spectrometry. Pediatr Res 53:1013–1018.

Routes JM, Grossman WJ, Verbsky J, Laessig RH, Hoffman GL, Brokopp CD, Baker MW. 2009. Statewide newborn screening for severe T cell lymphopenia. JAMA 302:2465–2470.

Sadelain M, Boulad F, Lisowki L, Moi P, Riviere I. 2008. Stem cell engineering for the treatment of severe hemoglobinopathies. Curr Mol Med 8:690–697.

Sandlers Y, Moser AB, Hubbard WC, Kratz LE, Jones RO, Raymond GV. 2012. Combined extraction of acyl carnitines and 26:0 lysophosphatidylcholine from dried blood spots: Prospective newborn screening for X-linked adrenoleukodystrophy Mol Genet Metab 105;416–420.

Sarafoglou K, Banks K, Gaviglio A, Hietala A, McCann M, Thomas W. 2012. Comparison of one-tier and two-tier newborn screening metrics for congenital adrenal hyperplasia. Pediatrics 130:1261–1268.

Sauer AV, Aiuti A. 2009. New insights into the pathogenesis of adenosine deaminase severe combined immunodeficiency and progress in gene therapy. Curr Opin Allergy Clin Immunol 9: 496–502.

Schilsky ML. 2009. Wilson disease: Current status and the future. Biochimie 91:1278–1281.

Schwarz E, Liu A, Randall H, Haslip C, Keune F, Murray M, Longo N, Pasquali M. 2009. Use of steroid profiling by UPLC-MS/MS as a second tier test in newborn screening for congenital adrenal hyperplasia: The Utah experience. Pediatr Res 66:230–235.

Scott CR, Elliott S, Buroker N, Thomas LI, Keutzer J, Glass M, Gelb MH, Turecek F. 2013. Identification of infants at risk for developing Fabry, Pompe, or mucopolysaccharidosis-I from newborn blood spots by tandem mass spectrometry. J Pediatr 163:498–503.

Slatter MA, Gennery AR. 2008. Clinical immunology review series: An approach to the patient with recurrent infections in childhood. Clin Exp Immunol 152:389–396.

Somech R, Lev A, Grisaru-Soen G, Shiran SI, Simon AJ, Grunebaum E. 2013. Purine nucleoside phosphorylase deficiency presenting as severe combined immune deficiency. Immunol Res 56:150–154.

Somech R, Etzioni A. 2014. A call to include severe combined immunodeficiency in newborn screening program. Rambam Maimonides Med J 5.

Spáčil Z, Elliott S, Reeber SL, Gelb MH, Scott CR, Tureček F. 2011. Comparative triplex tandem mass spectrometry assays of lysosomal enzyme activities in dried blood spots using fast liquid chromatography: Application to newborn screening of Pompe, Fabry, and Hurler diseases Anal Chem 83 4822–4828.

Spacil Z, Tatipaka H, Barcenas M, Scott CR, Turecek F, Gelb MH. 2013. High-throughput assay of 9 lysosomal enzymes for newborn screening. Clin Chem 59:502–511.

Spada M, Pagliardini S, Yasuda M, Tukel T, Thiagarajan G, Sakuraba H, Ponzone A, Desnick RJ. 2006. High incidence of later-onset fabry disease revealed by newborn screening. Am J Hum Genet 79:31–40.

Stone DL, Sidransky E. 1999. Hydrops fetalis: lysosomal storage disorders in extremis. Adv Pediatr 46:409–440.

Stuart MJ, Nagel RL. 2004. Sickle-cell disease. Lancet 364:1343–1360.

Styles LA, Vichinsky E. 1994. Effects of a long-term transfusion regimen on sickle cell-related illnesses. J Pediatr 125:909–911.

Tang DC, Zhu J, Liu W, Chin K, Sun J, Chen L, Hanover JA, Rodgers GP. 2005. The hydroxyurea-induced small GTP-binding protein SAR modulates gamma-globin gene expression in human erythroid cells. Blood 106:3256–3263.

Tarini BA, Christakis DA, Welch HG. 2006. State newborn screening in the tandem mass spectrometry era: More tests, more false-positive results. Pediatrics 118:448–456.

Theda C, Gibbons K, Defor TE, Donohue PK, Golden WC, Kline AD, Gulamali-Majid F, Panny SR, Hubbard WC, Jones RO, Liu AK, Moser AB, Raymond GV. 2014. Newborn screening for X-linked adrenoleukodystrophy: Further evidence high throughput screening is feasible. Mol Genet Metab 111:55–57.

Turgeon C, Magera MJ, Allard P, Tortorelli S, Gavrilov D, Oglesbee D, Raymond K, Rinaldo P, Matern D. 2008. Combined newborn screening for succinylacetone, amino acids, and acylcarnitines in dried blood spots. Clin Chem 54:657–664.

Turgeon CT, Magera MJ, Cuthbert CD, Loken PR, Gavrilov DK, Tortorelli S, Raymond KM, Oglesbee D, Rinaldo P, Matern D. 2010. Determination of total homocysteine, methylmalonic acid, and 2-methylcitric acid in dried blood spots by tandem mass spectrometry. Clin Chem 56:1686–1695.

Urbanelli L, Magini A, Polchi A, Polidoro M, Emiliani C. 2011. Recent developments in therapeutic approaches for lysosomal storage diseases. Recent Pat CNS Drug Discov 6:1–19.

Wang D, Wood T, Sadilek M, Scott CR, Turecek F, Gelb MH. 2007. Tandem mass spectrometry for the direct assay of enzymes in dried blood spots: Application to newborn screening for mucopolysaccharidosis II (Hunter disease). Clin Chem 53:137–140.

Wilcken B, Wiley V, Hammond J, Carpenter K. 2003. Screening newborns for inborn errors of metabolism by tandem mass spectrometry. N Engl J Med 348:2304–2312.

Wittmann J, Karg E, Turi S, Legnini E, Wittmann G, Giese AK, Lukas J, Gölnitz U, Klingenhäger M, Bodamer O, Mühl A, Rolfs A. 2012. Newborn screening for lysosomal storage disorders in hungary. JIMD Rep 6:117–125.

Wolfe BJ, Blanchard S, Sadilek M, Scott CR, Turecek F, Gelb MH. 2011. Tandem mass spectrometry for the direct assay of lysosomal enzymes in dried blood spots: Application to screening newborns for mucopolysaccharidosis II (Hunter Syndrome). Anal Chem 83:1152–1156.

Yergey AL, Liberato DJ, Millington DS. 1984. Thermospray liquid chromatography/mass spectrometry for the analysis of l-carnitine and its short-chain acyl derivatives. Anal Biochem 139:278–283.

Zappu A, Magli O, Lepori MB, Dessì V, Diana S, Incollu S, Kanavakis E, Nicolaidou P, Manolaki N, Fretzayas A, De Virgiliis S, Cao A, Loudianos G. 2008. High incidence and allelic homogeneity of Wilson disease in 2 isolated populations: A prerequisite for efficient disease prevention programs. J Pediatr Gastroenterol Nutr 47:334–338.

Zhang XK, Elbin CS, Chuang WL, Cooper SK, Marashio CA, Beauregard C, Keutzer JM. 2008. Multiplex enzyme assay screening of dried blood spots for lysosomal storage disorders by using tandem mass spectrometry. Clin Chem 54:1725–1728.