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# Newborn screening of inherited metabolic disorders by tandem mass spectrometry: past, present and future

*Lo screening neonatale per le patologie metaboliche ereditarie con spettrometria tandem mass: passato, presente e futuro*

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**Key words:** *tandem mass spectrometry, inborn errors of metabolism, metabolic screening, newborn*

## Abstract

Inborn errors of metabolism are inherited biochemical disorders caused by lack of a functional enzyme, transmembrane transporter, or similar protein, which then results in blockage of the corresponding metabolic pathway. Taken individually, inborn errors of metabolism are rare. However, as a group these diseases are relatively frequent and they may account for most of neonatal mortality and need of health resources. The detection of genetic metabolic disorders should occur in a pre-symptomatic phase. Recently, the introduction of the tandem mass spectrometric methods for metabolite analysis has changed our ability to detect intermediates of metabolism in smaller samples and provides the means to detect a large number of metabolic disorders in a single analytical run. Screening panels now include a large number of disorders that may not meet all the criteria that have been used as a reference for years. The rationale behind inclusion or exclusion of a respective disorder is difficult to understand in most cases and it may impose an ethical dilemma. The current organization is an important tool of secondary preventive medicine, essential for children's healthcare, but the strong inhomogeneity of the regional models of screening applied today create in the Italian neonatal population macroscopic differences with regards to healthcare, which is in effect mainly diversified by the newborn's

place of birth, in possible violation of the universal criterion of the equality of all citizens. Carefully weighed arguments are urgently needed since patient organizations, opinion leaders and politicians are pressing to proceed with expansion of neonatal population screening.

## Riassunto

Gli errori congeniti del metabolismo sono malattie ereditarie causate dalla carenza di un enzima funzionale, trasportatore transmembrana o proteina, che si traduce poi in blocco della via metabolica corrispondente. Gli errori congeniti del metabolismo, presi singolarmente, sono rari. Tuttavia, come gruppo queste malattie sono relativamente frequenti e spesso caratterizzate da alta incidenza di mortalità neonatale. La diagnosi, pertanto, dovrebbe avvenire in una fase pre-sintomatica. Recentemente, l'introduzione di metodi di spettrometria di tandem massa per le analisi dei metaboliti ha cambiato la nostra capacità di rilevare metaboliti in piccoli campioni ed ha fornito i mezzi per rilevare un gran numero di malattie metaboliche in un'unica seduta analitica. I pannelli di screening includono, ad oggi, un gran numero di disturbi che non soddisfano i criteri utilizzati come riferimento per anni. Il razionale alla base dell'inclusione o dell'esclusione di un rispettivo disordine è difficile da definire nella maggior parte dei casi e può imporre un dilemma etico. L'attuale organizzazione è un importante strumento di medicina preventiva secondaria, indispensabile per la salute dei bambini, ma la forte disomogeneità dei modelli regionali di screening applicati oggi crea, nella popolazione italiana neonatale, macroscopiche differenze per quanto riguarda le cure, che sono effettivamente diversificate in base al luogo di nascita del neonato, in possibile violazione del criterio universale della parità di tutti i cittadini. Un approfondito dibattito in tal senso, è urgentemente necessario poiché le organizzazioni di pazienti, mezzi di informazione e parte dei rappresentanti politici pressano per l'espansione dello screening nella popolazione neonatale.

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## Scientific and historical background

The congenital disorders associated with growth retardation and/or intellectual disabilities may recognize different etiologies: environmental and genetic causes among which metabolic diseases, monogenic diseases,<sup>1</sup> syndromes, submicroscopic chromosomal aberrations. Recently, the introduction of modern techniques such as array-CGH<sup>2</sup> and tandem mass spectrometric methods for metabolite analysis has changed our ability to detect intermediates of metabolism in smaller samples and provides the means to detect a large number of metabolic disorders in a single analytical run.

Inborn errors of metabolism are permanent and inherited biochemical disorders, also sometimes known as inherited metabolic diseases. The term inborn errors of metabolism was coined in the early 1900s by Garrod,<sup>3</sup> who described the first alkaptonuria. In general, an inborn error of metabolism is caused by lack of a functional enzyme, transmembrane transporter, or similar protein, which then results in blockage of the corresponding metabolic pathway. There may be accumulation of metabolites prior to the metabolic block, and/or deficiency in the ultimate product(s) of the pathway. Both may provide a means of therapeutic intervention, either by restricting the supply of precursors to the pathway or by supplying a missing product, or both. Approximately half of all inborn errors of metabolism can be treated biochemically, although the success of such treatment is variable. Taken individually, inborn errors of metabolism are rare. However, as a group these diseases are relatively frequent and collectively their incidence may approach 1 in 800 to 2500 births.<sup>4</sup> Their collective importance is growing and they occupy a significant place in the practice of paediatrics and in general medicine, because clinical presentation may occur in any age group, from fetuses and newborn to adulthood. Neonatal onset is common because the early neonatal period is a time of prevalent catabolism.<sup>4</sup> Many important molecules, produced at birth, are likely involved in early-onset and late-onset diseases and their role in complex metabolic pathways is currently investigated.<sup>6,7</sup> Together with prematurity,<sup>8,9</sup> infections<sup>10-13</sup> and congenital malformations,<sup>14-16</sup> inborn errors of metabolism may account for most of neonatal morbidity/mortality and need of health resources. The detection of genetic metabolic disorders should occur very early in life, in a presymptomatic phase, before health, growth and neuromotor development could be affected by the metabolic error. In the early 1960s, Guthrie developed the first method applicable to whole population screening for phenylketonuria (PKU).<sup>17</sup> In this assay, he measured phenylalanine in blood spots on filter paper using a bacterial inhibition assay. Since it was instituted the first newborn screening program for PKU in 1962, it was already recognized that early detection of the disease and early introduction of a diet, poor in phenylalanine resulted in a significant decrease in morbidity as well as significant savings of medical costs.<sup>18</sup> The criteria for inclusion of a new disease test into a newborn screening program continue to evolve. Currently the disorders included in most newborn

screening programs essentially meet defined criteria, summarized in table 1.<sup>19</sup> Phenylketonuria (PKU) serves as an excellent example of a metabolic disorder that fulfills these criteria. The incidence of PKU is about 1 in 14.000 in USA, 1 in 10.000 in Italy. The phenotype is well defined. The natural history of the untreated disease is characterized by a severe neurodegenerative course, but early diagnosis and treatment significantly improve the outcome.<sup>20</sup> Based on the success of the screening for PKU, programs have been extended with the same benefit to other conditions (congenital hypothyroidism, congenital adrenal hyperplasia, etc.) during the later decades of the twentieth century.<sup>21</sup> More recently, newborn screening and genetic testing have expanded rapidly with the advent of multiplex and/or DNA technologies. Until the late 1990s, screening tests were relatively simple and inexpensive. Recently, the introduction of the tandem mass spectrometric methods for metabolite analysis has changed our ability to detect intermediates of metabolism in smaller samples and it has supplied greater efficiency with fewer tests.<sup>22</sup> Expanded newborn screening with Tandem Mass Spectrometry (MS/MS) provides the means to detect a large number of metabolic disorders in a single analytical run. Screening panels now include a large number of disorders that may not meet all the criteria that have been used as a reference for years.

## State of the art

In many countries, such expansion of newborn screening (NBS) programs has been driven by technological advances, public pressure (lobbying of advocacy groups), the entry of large private laboratories providing NBS services, and international recommendation with the aim of harmonization of national plans for rare diseases.<sup>23</sup> In 2005, the American College of Genetics and Genomics<sup>24</sup> recommended 29 core disorders for which they found evidence of benefit. The disorders chosen to be included in European newborn screening programs differ considerably. Given that all European populations and also largely the USA have a common genetic background, the reason for these differences cannot be explained by major differences in disease prevalence but only by different approaches to the estimation of risks and benefits. In the most restrictive European countries, only medium chain acyl dehydrogenase deficiency (MCADD) is screened in addition to PKU (UK, Switzerland). Twenty disorders are screened in NBS program of Austria.<sup>25</sup> In Germany, where extended screening started in 1999, health authorities decided in 2005 to limit the number of metabolic disorders to be detected by MS/MS to 10 and decided that all analytes that are not needed for this purpose have to be suppressed or deleted immediately after the analysis. The disorders that have been omitted include some conditions that are regarded as non-diseases or at least as biochemical abnormalities with doubtful pathological meaning. 3-Methylcrotonyl carboxylase deficiency, thought to be a rare disorder with severe neurological pathology,

Table 1

## SCREENING CRITERIA.

**Screening criteria**

have a significant incidence in the population screened  
 are clinically well defined with the untreated natural history characterized;  
 have a well-defined biochemical phenotype;  
 cause significant morbidity and/or mortality;  
 are treatable, where treatment improves outcome;  
 testing is safe, simple and sufficiently sensitive;  
 specific confirmatory testing is available;  
 testing, treatment and treatment outcome are cost effective with respect to non-treatment

had been detected in an appreciable number of newborns and in their clinically unaffected mothers.<sup>26</sup> Therefore, the disorder has been excluded from the German panel. The rationale behind inclusion or exclusion of a respective disorder is difficult to understand in most cases. In some cases this restriction may impose an ethical dilemma. The more global approach of screening for all of the potentially detectable conditions, while partially neglecting the classical screening criteria, burdens the health care system and the affected individuals. It was shown that false-positive results by expanded screening may cause disruption of family life through a combination of unnecessary hospitalizations, high parental stress, and parent-child relationship dysfunction.<sup>27</sup> Part of the decision concerning inclusion of certain disorders depends on organizational and timing differences among different countries. In the UK the recommended date for taking the blood sample is the 5th day of life, whereas in the USA, in Germany or in Italy the blood sample is obtained by the 2nd day of life with the result being available at the 3rd to 5th day of life, enabling early intervention. It seems clear that in the UK the most common organic acidurias, methylmalonic aciduria and propionic aciduria, may manifest their symptoms before the screening sample is taken or before the result will be available, without benefit of the NBS program to the patient.<sup>28</sup> Considering the complexity of decisions, it is hoped that screening policies will become standardized between European countries, and of course also between screening centres within one country, with regard to all aspects of analytical and organizational steps in the process of newborn screening. Increasing knowledge about the natural course of diseases and their variants, new treatment options, and analytical developments improving sensitivity and/or specificity of screening tests are some of the reasons for the necessity to re-assess expanded screening for additional

disorders. Timely and aggressive treatment may change the outcome in some diseases which were previously classified as non-treatable conditions, thus justifying the inclusion of, in example glutaric aciduria type I, as a screening disease.<sup>29</sup> Common projects for quality control and assessment, as well as expert medical judgement, are urgently needed at a European level and should result in standardization of the screening panels and of the spectrum of analysed metabolites, as well as in suggestions for the sizes of screening laboratories, analytical procedures, follow-up management and proficiency and quality testing. In Italy neonatal screening program has been mandatory for congenital hypothyroidism, cystic fibrosis, and phenylketonuria since 1992. Moreover, on the basis of regional laws other screening programs for endocrinopathies and inborn errors of metabolism are performed in some Italian Regions. This implies that every year about 550.000 dried blood spot (DBS) cards from all Italian newborns are collected and analyzed in the 32 Screening Centers active in our country. A recent survey performed by the Italian Society for the Study of Metabolic Diseases and Neonatal Screening (SIMMESN) revealed a significant dishomogeneity among screening laboratories in the management of residual DBS in terms of time of retention, requirement of parental consent and use of the samples, and this dishomogeneity created important ethical issues. In order to achieve an appropriate balance between respecting the rights of newborns and their parents versus the potential for public good deriving from the use of DBS for research purposes, the Joint Group National Committee of Bioethics - National Committee for Biosafety Biotechnologies and Life Sciences have recommended developing a National Network of Regional Newborn Screening Repositories for collection of residual DBS, coordinated by the Italian National Institute of Health.<sup>30</sup>

Table 2

ITALIAN REGIONS IN WHICH A PROGRAM OF EXPANDED NEWBORN SCREENING IS ACTIVE  
 OR ASSESSMENT OF FEASIBILITY PROJECTS IS ONGOING.

Region	Description of expanded newborn screening programme
Tuscany	regional programme of expanded newborn screening, in line with the specific legislative decree of the Tuscany region.
Liguria	pilot regional programme of expanded newborn screening, with the collection of the parents' informed consent, approved by the regional healthcare authorities.
Lazio	pilot subregional programme of expanded newborn screening.
Veneto, Emilia Romagna, Sicily	dissemination of regional deliberations for the activation in a near future of programmes of expanded newborn screening.
Lombardy, Marche, Campania	there are currently assessments of projects of feasibility, not yet approved or activated by the competent regional Authorities.

## Future perspectives

As highlighted in the annual reports of the Italian Society of Neonatal Screening (SISN), in our Country there are 32 active Centres of Neonatal Screening, with a very diversified pool of patients according to area size (Sub-regional, Regional, Inter-regional) and number of samples (from < 5,000 born/year to > 100,000 born/year), 22 of them at the moment carrying out different regional programs, according to the local organization, defined by the competent regional Authority. It is clear that the current organization is an important tool of secondary preventive medicine, essential for children's healthcare, but the strong inhomogeneity of the regional models of screening applied today, for the profound differences in the composition of the panel of screened pathologies, create in the Italian neonatal population (in possible violation of the universal criterion of the equality of all citizens) macroscopic differences with regards to healthcare, which is in effect mainly diversified by the newborn's place of birth. It is also important to stress how the careful reading of the scientific documentation issued by the SISN-SISMME Society highlights also a considerable further inhomogeneity due to the organization and technical management of each single regional screening program, with numerous indicators (catchment area, cut-off values, recall index, analytical strategies of selection, efficiency index, etc.) presenting a discretionary variability that is not always justified by scientific evidence or by strictly economy management reasons.<sup>31</sup> Carefully weighed arguments are urgently needed since patient organizations, opinion leaders and politicians are pressing to proceed with expansion of neonatal population screening. In addition to disorders of amino acid, organic acid and fatty acid oxidation metabolism, MS/MS may help in screening newborns for other inborn errors of metabolism. Recently, Li et al.<sup>32</sup> demonstrated the utility of MS/MS in measuring the lysosomal enzymes responsible for Gaucher, Fabry, Neimann-Pick A/B, Pompe and Krabbe diseases. Additional groups of disorders including lysosomal storage disorders<sup>33</sup> and X-linked adrenoleukodystrophy<sup>34</sup> are awaiting implementation in diagnosis and treatment. The national health care systems are especially independent within the EU, thus a common political solution is not expected and does not seem desirable. The annual regional European meetings of the International Society of Newborn Screening could provide the first informal platform to organize Europe in respect of a voluntary basis.

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