Expanded newborn screening: Lessons learned from MCAD deficiency

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Newborn screening has been a standard component of care in every Canadian province for decades. All provinces screen for phenylketonuria (PKU), an inborn error of amino acid metabolism, and congenital hypothyroidism (CH), and some have added additional tests applicable to their unique population. A new technology for newborn screening, tandem mass spectrometry (MS/MS) has recently been implemented in several Canadian provinces, allowing for the expansion of newborn screening to include an increasing number of rare metabolic disorders. In October 2000, Nova Scotia began to screen for medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, the most common fatty acid oxidation defect, with MS/MS technology. MCAD deficiency illustrates the potential of expanded newborn screening and the process undertaken when adding new tests to the newborn screen.

Newborn screening for PKU began in the 1960s after Dr Robert Guthrie (1) developed both a bacterial inhibition assay for the diagnosis of PKU and a filter paper method for collection, storage and transportation of samples, known as the newborn screening blotter. This endeavour would not have been as successful had the newborn screening blotter not been such an ideal method of sample collection. In the 1970s, Dussault (2) developed a screen for CH using the newborn screening blotter, which promoted the global adoption of these two screens in Canada. In the 1980s and 1990s, many centres throughout Europe and North America added additional tests to their newborn screens, but none became universally accepted. A significant barrier was that screening for these additional diseases required a different sample of blood or urine, making the addition of new tests costly, in part because of sample collection and storage. Finally, in the late 1990s, MS/MS was introduced (3-7) and has revolutionized the field, providing the ability, with a single sample, to screen newborns for a new and wide variety of rare metabolic diseases (Table 1).

Mass spectrometry uses a sophisticated device to separate ions (electrically charged molecules) based on their molecular mass and their charge. The MS/MS is capable of performing multiple rounds of mass spectrometry on ions fragmented from chemical compounds. MS/MS enables the accurate detection and quantification of minute amounts of compounds based upon their unique ion fragment 'signatures'. Used in newborn screening, MS/MS can detect more than 30 inborn errors of the metabolism (8). Newborn screening blotters are used to provide the sample, and multiple tests can be performed simultaneously, which allows expanded screening to be efficient and cost effective (9,10). The newborn period is one of intense catabolism, inducing

TABLE 1
Metabolic conditions detectable by expanded newborn screening

Amino acid disorders
Phenylketonuria
Homocystinuria
Tyrosinemia
Maple syrup urine disease
Nonketotic hyperglycinemia
Organic acidemias
Methylmalonic acidemia
Propionic acidemia
Isovaleric acidemia
Glutaric acidemia type 1
Ethylmalonic acidemia
Beta-ketothiolase deficiency
Malonic acidemia
3-methyl-crotonyl CoA carboxylase deficiency
Urea cycle defects
Argininosuccinic aciduria
Citrullinemia
Hyperornithinemia, hyperammonemia, homocitrullinemia syndrome
Argininemia
Fatty acid oxidation defects
Medium-chain acyl CoA dehydrogenase deficiency
Very long-chain acyl CoA dehydrogenase deficiency
Long-chain L-3-hydroxyl CoA dehydrogenase deficiency
Short-chain acyl CoA dehydrogenase deficiency
HMG CoA lyase deficiency
Glutaric aciduria type 2
Carnitine palmitoyl transferase 1 deficiency
Carnitine palmitoyl transferase 2 deficiency
Carnitine translocase deficiency
Carnitine transporter deficiency

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the production of abnormal metabolites in neonates with metabolic disease. As a result, the sensitivity of MS/MS screening is maximal in the first 72 h of life, and declines thereafter. The test is specific, and is as good as, or better than, the other PKU assays currently in use in Canada.

As indicated in Table 1, MS/MS can detect amino-acidopathies such as PKU; organic acidopathies, such as methylmalonic academia; urea cycle defects; and fatty acid oxidation defects, such as MCAD deficiency. However, expanded newborn screening does not rule out metabolic disease. MS/MS is not able to detect all metabolic diseases; for example, it does not detect mitochondrial disorders (that is, electron transport chain defects), and even of those diseases that it can detect, not every case of any identifiable disease will be detected with the expanded newborn screen (11). In particular, the test may not detect mildly affected individuals. However, the benefits are exciting. While the metabolic diseases individually are rare, as a group they constitute a clinically significant problem for paediatricians. MS/MS technology can more than double the number of newborns with metabolic disease detected by screening.

Provinces that implement newborn screening will have to decide for which conditions they will screen. There are various screening principles that have been proposed, with pros and cons of each set of principles (12,13). The Nova Scotia Newborn Screening Committee has focused on the following principles regarding the addition of new tests to the expanded newborn screen. The disorder should be clinically and biochemically well-defined. There must be a reliable means to confirm a diagnosis after receipt of a positive newborn screen. The condition should be associated with known significant morbidity and mortality. Finally, it is most important that effective treatment that improves the outcome be available.

These decisions are difficult given the limited numbers of patients, limited data on the natural history and treatment outcomes of these affected children, and the wide variability of expression of the disorders, with some affected individuals being more or less severely affected. Expanded screening does not have established 'cut-off' levels for a positive test, and these will vary somewhat between laboratories. Choosing appropriate 'cut-offs' is crucial, both to limit the number of false negatives (and therefore unidentified cases), and, more importantly, to limit the number of false positives, given the invasive testing required for diagnosis of, and complex therapy needed for, many metabolic disorders. The investigation of many of these disorders requires a skin biopsy for enzymatic confirmation, which may not necessarily be easy to interpret, leaving families with uncertainty and creating illness where it may not exist. Finally, given the number of physicians familiar with the diagnosis and treatment of these diseases, these limited resources make minimizing false positives crucial. Results of the screening test should be available before the expected onset of symptoms in affected children; therefore, turnaround time needs to be optimized. Fortunately, MS/MS allows screening for these rare diseases to be cost effective (9,10).

In October 2000, Nova Scotia became the first province in Canada to implement neonatal screening for MCAD deficiency. The program uses electrospray ionisation MS/MS to screen for PKU, and simultaneously, MCAD deficiency. Samples in Nova Scotia are collected in newborns who are 16 h of age and older, with a median age at testing of 30 h. Phenylalanine levels are detected by MS/MS, and acylcarnitine species are used to screen for MCAD deficiency. As of the beginning of 2004, Saskatchewan and British Columbia have been offering MCAD testing as a part of their routine newborn screening, and Prince Edward Island has agreed to join the Nova Scotia MS/MS screening program for PKU and MCAD deficiency. Saskatchewan also tests for many of the other inborn errors that can be detected with this new technology.

MCAD deficiency (OMIM 201450) is the most common fatty oxidation defect and is inherited in an autosomal recessive fashion. The MCAD enzyme metabolizes C6-C10 fatty acyl-CoA molecules in the β-oxidation pathway, the use of which becomes critical at times of fasting stress. This pathway is used to produce ketones and energy from fat for use by the tissues of the body, in particular liver, muscle, heart and brain, when glucose is unavailable. A common mutation has been identified, termed A985G, which is homozygous in 80% and accounts for 90% of the MCAD alleles ascertained through symptomatic individuals (14). It is common in the northern European population. The manifestations of MCAD deficiency are typically precipitated by a febrile illness with fasting and/or vomiting. The manifestations of MCAD deficiency include hypoketotic hypoglycaemia, lethargy, hepatic dysfunction, Reye syndrome and seizures. If prompt infusion of intravenous dextrose is not administered, the child may develop a life-threatening coma and may die (15). Episodes of metabolic decompensation tend to occur between six and 18 months of age. They can be prevented (16-19) primarily through the education of families and physicians regarding the need to increase carbohydrate intake at times of metabolic stress, and to seek medical attention promptly if this is not possible for intravenous intervention before the onset of hypoglycemia or other symptoms of MCAD deficiency. Some physicians treat children with MCAD deficiency with l-carnitine, which often becomes depleted. Whether this prevents decompensation episodes is controversial.

Unfortunately, MCAD deficiency is clinically silent before life-threatening symptoms become apparent. It is estimated that 20% to 25% of children affected with MCAD deficiency die (17,18), usually during their first episode of decompensation. After an episode of decompensation, approximately 20% of survivors had global developmental delay, and 37% overall had abnormal development (17). Other significant outcomes included attention deficit/hyperactivity disorder, cerebral palsy, failure to thrive, seizure disorders and complete aphasia. Clearly, episodes of decompensation produce significant mortality and long-term morbidity. However, if the diagnosis is

established before the onset of symptoms, the prognosis is excellent, and most episodes of decompensation can be avoided (17). It should also be noted that 25% of children with MCAD deficiency never experience a decompensation, because it takes both the genetic tendency combined with a metabolic stress to express the condition.

MCAD deficiency was chosen as the first addition to the newborn screen in Nova Scotia for several important reasons. First, MCAD deficiency is the most commonly inherited defect of fatty acid oxidation in humans (15), and has a higher frequency in those of northern European descent, which is the background of much of this province's population. As well, the carrier frequency for the common A985G MCAD mutation for Nova Scotians is one in 68 (20), giving an incidence of MCAD deficiency in Nova Scotia of at least one in 18,500. Secondly, there is effective treatment available for the condition. Thirdly, there is significant morbidity and mortality associated with the condition (17,19) which can be significantly reduced or eliminated by preventing metabolic decompensation through presymptomatic diagnosis (18).

Since newborn screening for MCAD deficiency was implemented in Nova Scotia, two presymptomatic newborns have been detected. One child was homozygous for the A985G mutation, with no family history of MCAD deficiency, sudden death in infants/children or consanguinity. The family was of northern European decent. The second child was a compound heterozygote, with one MCAD allele containing the A985G mutation and another that was identified in a research laboratory. Neither of these children developed clinical manifestations of MCAD deficiency, although one has required use of the illness management protocol on several occasions during febrile illnesses, without the need for hospitalization. One had a younger sibling born after the diagnosis, who was not affected. The birth rate in Nova Scotia is about 8500 per year, and two children affected with MCAD deficiency in a three-and-one-half year period is consistent with the expected incidence of this disorder in our population.

In summary, the technology for expanded newborn screening is in place in several provinces in Canada. Newborn screening blotters collected for PKU and CH can now be tested for a variety of inborn errors of metabolism, such as MCAD deficiency, enabling the detection of more metabolic diseases presymptomatically, and allowing improvement in the outcome for the children and families affected by these rare disorders. The decision of which diseases to add to the newborn screen is a complex one, involving consideration of the natural history of the disease and the response to treatment, limited resources and patient satisfaction. In the near future, additional metabolic diseases will be added to Nova Scotia's expanded newborn screen in a stepwise fashion, in accordance with the screening criteria outlined here, in an effort to further reduce the burden of metabolic disease in the province.

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