

## ORIGINAL ARTICLE

# Screening Newborns for Inborn Errors of Metabolism by Tandem Mass Spectrometry

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## ABSTRACT

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**BACKGROUND**

The recent development of electrospray tandem mass spectrometry makes it possible to screen newborns for many rare inborn errors of metabolism, but the efficacy and outcomes of screening remain unknown. We examined the effect of the screening of newborns by tandem mass spectrometry on the rates of diagnosis of 31 disorders.

**METHODS**

We compared the rates of detection of 31 inborn errors affecting the metabolism of the urea cycle, amino acids, and organic acids and fatty-acid oxidation among 362,000 newborns screened by tandem mass spectrometry over a four-year period (April 1998 through March 2002) with the rates in six preceding four-year birth cohorts in New South Wales and the Australian Capital Territory, Australia, where screening, diagnostic, and clinical services were centralized.

**RESULTS**

The overall prevalence of disorders during the periods when clinical diagnosis was used did not vary between 1982 and 1998. In the cohort screened with tandem mass spectrometry, the prevalence of inborn errors, excluding phenylketonuria, was 15.7 per 100,000 births (95 percent confidence interval, 11.9 to 20.4), as compared with adjusted rates of 8.6 to 9.5 per 100,000 births in the four preceding four-year cohorts. Of the 57 cases diagnosed after the introduction of newborn screening, 15 were diagnosed clinically; 7 of the 15 newborns had a normal result on screening. The rate of detection was increased specifically for medium-chain acyl-coenzyme A dehydrogenase deficiency ( $P < 0.001$ ) and other disorders of fatty-acid oxidation ( $P = 0.007$ ), as compared with the 16-year period before the implementation of neonatal screening for these disorders.

**CONCLUSIONS**

More cases of inborn errors of metabolism are diagnosed by screening with tandem mass spectrometry than are diagnosed clinically. It is not yet clear which patients with disorders diagnosed by such screening would have become symptomatic if screening had not been performed.

**I**N MANY COUNTRIES, IT IS A ROUTINE COMPONENT of neonatal care to screen infants for congenital hypothyroidism, phenylketonuria, and a variable number of other disorders. The primary aim is the early detection and treatment of clinically important disorders in order to minimize morbidity and mortality in early childhood. Recently, with the development of electrospray tandem mass spectrometry, it has become possible to use a single test to screen for a wide range of very rare disorders that have not been screened for previously.

Formal evidence of the clinical effectiveness of newborn screening is lacking. The only randomized, controlled trials have been of screening for cystic fibrosis in the United States and the United Kingdom.<sup>1,2</sup> The clinical effectiveness of screening for phenylketonuria and hypothyroidism is generally accepted,<sup>3</sup> although no formal trials were ever conducted; there is also evidence to support screening for sickle cell disease in regions where it is prevalent.<sup>4</sup> One reason for the lack of randomized, controlled trials of screening is the rarity of these disorders; a very large number of infants would need to be enrolled in a trial for it to have sufficient power to assess the benefits of screening.<sup>5</sup> Another factor that has discouraged researchers from conducting randomized, controlled trials of screening for some disorders has been a strong conviction, based on clinical experience, that there is a benefit from early diagnosis. As a result, the usefulness of many screening tests remains uncertain.

Tandem mass spectrometry is used in many screening programs to analyze amino acids and acylcarnitines in blood to detect disorders of amino acids, organic acids, and fatty-acid metabolism. The ability to select which analytes to detect effectively permits screeners to choose which disorders to screen for. In Australia, the screening of newborns by tandem mass spectrometry was introduced in New South Wales and the Australian Capital Territory in early 1998, in South Australia in 1999, and in Victoria in 2002. The technology is being introduced widely in the United States, some European countries, and elsewhere.<sup>6-13</sup>

Among the disorders that may be diagnosed, some cause severe illness or death within the first few days of life, and newborn screening may serve only to suggest a diagnosis that might otherwise have been missed. However, most of the disorders are treatable if they are diagnosed early. With early diagnosis and appropriate treatment, some prob-

lems can be avoided; these include biochemical disturbances such as hyperammonemia in patients with urea-cycle disorders that present after the newborn period, severe metabolic acidosis in patients with disorders of organic acids, or hypoketotic hypoglycemia, cardiomyopathy, or rhabdomyolysis in patients with disorders of fatty-acid oxidation; if left untreated, these disorders may lead to brain damage, other organ damage, or death.

We compared the rates and profile of diagnoses identified through newborn screening by tandem mass spectrometry with those during periods preceding the use of this technology in the population of New South Wales and the Australian Capital Territory (population, 6 million).

## METHODS

### DISORDERS

We studied 31 disorders that may be able to be diagnosed by tandem mass spectrometry in blood samples from newborns (Table 1). Phenylketonuria and pterin disorders were excluded from consideration, since they had been screened for by another method for many years. Also excluded were disorders known to be benign, maternal disorders such as maternal 3-methylcrotonyl-coenzyme A (CoA) carboxylase deficiency, and errors that are not inborn, such as vitamin B<sub>12</sub> deficiency.

### CLINICAL DIAGNOSIS

Since 1974, whenever patients in New South Wales and the Australian Capital Territory have had symptoms suggestive of an inborn error of metabolism, their cases have been investigated in our biochemical genetics laboratory, the only laboratory in the state that provides diagnostic testing for defects of amino acids, organic acids, and fatty-acid metabolism. Diagnostic tests have included analysis of urinary organic acids by gas chromatography (until 1991) or gas chromatography–mass spectrometry; analysis of urinary amino acids, initially by high-voltage electrophoresis; analysis of plasma amino acids by quantitative amino acid analysis; and analysis of plasma acylcarnitines by tandem mass spectrometry. Other biochemical genetic tests, including enzyme and molecular analyses, were performed as indicated. Since April 1998, the laboratory has also evaluated patients identified by routine newborn screening. We have maintained a data base of all patients with a confirmed diagnosis of an inborn error of metabolism.

**Table 1.** Numbers of Patients in the Birth Cohorts with Inborn Errors of Metabolism Diagnosed after Clinical Presentation (between April 1974 and March 1998) or during the Newborn-Screening Period (April 1998 to March 2002).\*

Disorder	Screening Year							Total
	1974–1978	1978–1982	1982–1986	1986–1990	1990–1994	1994–1998	1998–2002	
	<i>number of patients</i>							
<b>Urea cycle</b>								
Carbamyl phosphate synthetase deficiency	1	1	0	1	1	0	1	5
Ornithine transcarbamylase deficiency	2	3	5	4	4	3	3	24
Argininosuccinate synthase deficiency	2	0	0	2	2	1	0	7
Argininosuccinate lyase deficiency	2	1	1	1	0	0	2	7
Arginase deficiency	0	0	0	0	0	0	0	0
Citrullinemia, type II (citrin deficiency)	0	0	0	0	0	0	1	1
<b>Amino acid</b>								
Nonketotic hyperglycinemia	3	1	1	3	3	4	3	18
Cystathionine $\beta$ -synthase deficiency (homocystinuria)	5	0	2	5	1	0	2	15
Maple syrup urine disease	1	1	4	3	3	2	1	15
Tyrosinemia								
Type I	0	1	1	1	0	1	2	6
Type II	1	2	2	0	0	1	1	7
<b>Organic acid</b>								
Propionyl-CoA carboxylase deficiency	0	0	1	0	0	1	1	3
Methylmalonyl-CoA mutase deficiency plus cobalamin A and B defects	2	3	1	2	4	3	0	15
Cobalamin C defect	0	1	0	0	0	3	1	5
Isovalericacidemia	0	1	1	1	0	0	1	4
Glutaryl-CoA dehydrogenase deficiency	1	1	0	0	0	0	2	4
Holocarboxylase synthase deficiency	0	1	1	1	3	0	0	6
Biotinidase deficiency	1	0	1	0	0	1	0	3
Hydroxymethylglutaryl-CoA lyase deficiency	0	0	1	0	0	1	0	2
Methylglutaconicaciduria	1	1	0	0	1	0	1	4
3-Methylcrotonyl CoA carboxylase deficiency	0	0	0	0	0	0	3	3
3-Ketothiolase deficiency	0	0	0	0	1	0	3	4

We identified patients with any of the 31 disorders who received a diagnosis after clinical referral and who were born during the six four-year periods from April 1974 through March 1998 or during the first four years when screening was being performed, from April 1, 1998, through March 31, 2002. The diagnosis was confirmed in almost all of these patients by enzymatic or molecular genetic tests. In cases of argininosuccinate lyase deficiency

and three of five cases of short-chain acyl-CoA dehydrogenase deficiency detected by newborn screening, we relied on biochemical variables. To take into account a possible late age at the time of clinical diagnosis, we examined the age at diagnosis in all patients born between 1974 and 1998. For disorders diagnosed in any child at ages beyond 4.5 years (the interval between the end of the last period before screening began and the time of writing),

**Table 1. (Continued.)**

Disorder	Screening Year							Total
	1974–1978	1978–1982	1982–1986	1986–1990	1990–1994	1994–1998	1998–2002	
	<i>number of patients</i>							
<b>Fatty acid</b>								
Short-chain acyl-CoA dehydrogenase deficiency	0	0	1	0	0	0	5†	6
Medium-chain acyl-CoA dehydrogenase deficiency	2	4	3	3	7+1	1+2	17	37
Very-long-chain acyl-CoA dehydrogenase deficiency	0	0	1	2	0	0	3	6
Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency	0	0	1	1	3	2	0	7
Multiple acyl-CoA dehydrogenase deficiency	0	0	1	0	0	0	0	1
Carnitine transporter defect	0	0	0	2	1	2	3	8
Carnitine palmitoyl transferase deficiency								
Type I	0	0	0	0	0	0	0	0
Type II	1	0	0	0	0	0	0	1
Carnitine acylcarnitine translocase deficiency	0	0	0	0	0	0	1‡	1
Unadjusted total	25	22	29	32	34+1	26+2	57	228
Adjustment for estimated missing data due to late age at diagnosis	1	1	1	1	2	6		
Adjusted total	26	23	30	33	36+1	32+2	57	

\* All periods began on April 1 and ended on March 31. A second number (following a plus sign) represents the number of patients given a diagnosis of medium-chain acyl-coenzyme A (CoA) dehydrogenase deficiency after the disorder had been diagnosed in a younger sibling through newborn screening. These cases are included in the unadjusted total for all the disorders combined but not in the total for the particular disorder.

† Three patients with probable cases of short-chain acyl-CoA dehydrogenase deficiency had elevated urinary ethylmalonic acid and elevated plasma butyrylcarnitine levels (3.53 to 5.06  $\mu\text{mol}$  per liter [reference range, 0.08 to 0.24]), but skin-fibroblast studies were declined.

‡ This patient had a presumed diagnosis only: there was a substantial elevation of the palmitoylcarnitine concentration (20.0  $\mu\text{mol}$  per liter); the patient subsequently died suddenly on day 3, but no tissue was available for testing.

we calculated the likely number of patients in the 1994–1998 birth cohort whose disorder remained undiagnosed. Similarly, for the 1990–1994 cohort, we calculated the likely number of patients whose disorder remained undiagnosed for 8.5 years, and so forth. For patients with cystathionine  $\beta$ -synthase deficiency, diagnoses were in some cases made in adulthood, and we estimated the expected number of diagnoses per four-year birth period on the basis of our data base of 51 patients with this disorder.

#### DIAGNOSIS DURING THE NEWBORN-SCREENING PERIOD

Between April 1998 and March 2002, blood samples obtained at 48 to 72 hours of life from all infants born in New South Wales or the Australian Capital Territory were tested by tandem mass spectrometry,

as previously described.<sup>11</sup> Samples were butyrylated. Spectra were initially interpreted with the use of NeoLynx software (Micromass). Amino acids and acylcarnitines were quantitated against dried-blood-spot calibrators. Only selected compounds were analyzed, so as to avoid the identification of benign disorders. The results were transferred to a central data base, where each result was checked against predefined algorithms and reference ranges (described previously<sup>11</sup>), including reference ranges for ratios and for second samples, when requested. Results were generally available within 24 hours. Confirmatory testing was performed by our biochemical genetics laboratory, as was the investigation of patients in this cohort who presented with suggestive symptoms but had not been identified by newborn screening as having a disorder.

**BIRTH COHORTS**

The numbers of infants born during the four-year periods were taken from data of the Australian Bureau of Statistics on birth registrations. The number of first tests performed in any year was more than the number of births registered, despite double checks for duplication. Routine periodic checks according to name and hospital of birth indicate that coverage is greater than 99 percent.

**STATISTICAL ANALYSIS**

Fisher's exact test was used to compare the number of patients in the 1998–2002 birth cohort in whom a given disorder was diagnosed with the number of patients with a diagnosis of that disorder during previous years. Chi-square tests of linear trend were performed with the use of Epi Info software, version 6.<sup>14</sup>

**RESULTS**

The numbers of patients with the 31 target disorders born during the four-year periods before screening by tandem mass spectrometry was implemented and during the first four years of screening with this technique are shown in Table 1. The total number of births and the prevalence of each class of disorder are shown in Table 2. During the six four-year periods preceding the implementation

of screening with mass spectrometry, 22 to 34 cases were diagnosed per period, resulting in rates of 6.6 to 9.0 cases per 100,000 births. When a possible late age at diagnosis was taken into account, the expected final range for these cohorts is estimated to be 23 to 36 cases per period, with rates of 6.9 to 9.5 cases per 100,000 births. There was no trend toward increased overall rates of diagnosis of these disorders between 1982 and 1998 (Table 2). Specifically, there was no increase in the rate of diagnosis of disorders of fatty-acid metabolism over this period, although many of these disorders were described for the first time during these years.

During the four years in which tandem mass spectrometry was used routinely for newborn screening, we tested 362,000 newborns, of whom 560 (0.15 percent) required a second test or urgent follow-up because of an abnormal test result. During the screening period, 57 newborns were given a diagnosis of 1 of the 31 inborn errors of metabolism (15.7 diagnoses per 100,000 births [95 percent confidence interval, 11.9 to 20.4]). Of these cases, 48 were diagnosed by newborn screening, and 6 of those diagnosed by screening were also diagnosed clinically before or at the same time as the screening result became available. Two patients, siblings with ornithine transcarbamylase deficiency born to a mother with known risk, did not undergo newborn screening, since both screening and

**Table 2. Incidence of the 31 Disorders, According to Type of Disorder and Birth Cohort.\***

Variable	Screening Year							P Value for Comparison of 1982–1998 with 1998–2002
	1974–1978	1978–1982	1982–1986	1986–1990	1990–1994	1994–1998	1998–2002	
Urea-cycle disorders	7	5	6	8	7	4	7	0.70
Amino-acid disorders	10	5	10	12	7	8	9	0.15
Organic-acid disorders	5	8	6	4	9	9	12	0.11
Medium-chain acyl-CoA dehydrogenase deficiency	2	4	3	3	7+1	1+2	17	<0.001
Other fatty-acid-oxidation disorders	1	0	4	5	4	4	12	0.007
No. of births	336,000	331,000	349,000	360,000	378,000	367,000	362,000	
Incidence per 100,000 births	7.4	6.6	8.3	8.9	9.0	7.0	15.7	
Adjusted incidence per 100,000 births†	7.9	6.9	8.6	9.2	9.5	8.7		

\* A second number (following a plus sign) represents the number of patients given a diagnosis of medium-chain acyl-CoA dehydrogenase deficiency after the disorder had been diagnosed in a younger sibling through newborn screening.

† Data are adjusted for estimated missing data due to late age at diagnosis.

treatment for the affected sons were declined. Seven patients in whom disorders were later diagnosed clinically had a negative result on newborn screening.

The greatest increase in the rate of diagnosis was for medium-chain acyl-CoA dehydrogenase deficiency (Table 1). The overall positive predictive value of an abnormal screening test was 10 percent, but the positive predictive value varied among analytes. For example, an abnormal level of tyrosine had a positive predictive value of only 2 percent for the detection of type I or type II tyrosinemia. The overall specificity was high, with a false positive rate of only 0.15 percent. In some patients with either a positive or a negative result on newborn screening the diagnosis was made on clinical grounds (Table 3).

The incremental cost of tandem mass spectrometry within the laboratories where newborn screening and biochemical genetic testing were conducted was approximately \$0.70 (1.17 Australian dollars; amounts are reported here in U.S. dollars) per newborn screened. This amount included the cost of reagents, microtiter plates and other consumables, maintenance and depreciation of instruments, staffing, and confirmatory tests. The mean cost of confirmatory testing for infants who actually required it was \$217. The cost per relevant disorder detected (excluding phenylketonuria) was \$3,939. Including phenylketonuria, the cost was \$2,519. Data on costs incurred outside of the newborn-screening and biochemical genetics laboratories are not yet available.

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#### DISCUSSION

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It is now possible to screen rapidly, simultaneously, and inexpensively for a number of very rare disorders with the use of tandem mass spectrometry, but the yield and usefulness of testing have not been clear. It is difficult to conduct a randomized, controlled trial of such screening because of the rarity of the individual disorders (necessitating the use of a very large sample) and because the strong belief of many proponents in the benefit of early diagnosis by tandem mass spectrometry has led to a public campaign in the United States and elsewhere for universal screening.<sup>16</sup>

Our study was designed to assess the diagnostic potential of this technology. To evaluate the technology fully, studies of the clinical effectiveness of screening and a detailed cost analysis will be necessary, but we do not yet have data to address these questions.

Our study design has potential drawbacks that we have attempted to counter. Although we used historical controls, the rate of clinical diagnosis for the 31 disorders had remained stable between 1982 and 1998, before tandem mass spectrometry was introduced for routine screening. Our centralized services and a high level of awareness of inborn errors of metabolism among pediatricians make it likely that we would be aware of patients in our region who have these disorders. Because of a high level of cooperation among state referral centers, children with such disorders diagnosed at any of the other five diagnostic centers in Australia would be routinely brought to our attention, and diagnosis outside of Australia would be very unlikely.

We have tried to account for the presence of children in the four-year birth cohorts from 1974 to 1998 that probably have undiagnosed cases by correcting for the expected number of cases remaining undiagnosed at the end of each four-year period. Although it is difficult to apply the same methods to the screened cohort, and this could affect the apparent sensitivity of the screening method, any such correction would not alter the overall conclusion that the target disorders have been diagnosed in more patients during the screening period than during the periods before screening. We cannot systematically account for patients who die with undiagnosed disorders. Although all coroners' offices have a protocol for the collection and investigation of samples in cases of possible metabolic disease, we do not have data on whether such investigations were routinely performed.

The two disorders that were clearly more frequently diagnosed by screening than clinically were medium-chain and short-chain acyl-CoA dehydrogenase deficiencies. Probably the rates of diagnosis of very-long-chain acyl-CoA dehydrogenase, 3-ketothiolase, and 3-methylcrotonyl CoA carboxylase deficiencies were also increased, although none of these increases was individually significant. During the past 24 years, only one case each of short-chain acyl-CoA dehydrogenase deficiency and 3-ketothiolase deficiency, three cases of very-long-chain acyl-CoA dehydrogenase deficiency, and no cases of 3-methylcrotonyl CoA carboxylase deficiency had been diagnosed clinically. It appears likely that some patients with these disorders could be at very low risk of ever having symptoms,<sup>17</sup> and short-chain acyl-CoA dehydrogenase deficiency and 3-methylcrotonyl CoA carboxylase deficiency may be largely benign disorders. There is no clear evidence yet to indicate whether early detection of

**Table 3. Presentation and Results on Newborn Screening in Patients with a Clinically Diagnosed Disorder.\***

Patient No.	Disorder	Presentation	Result on Newborn Screening†	Comments
<b>Positive result on newborn screening</b>				
1	Glutaryl-CoA dehydrogenase deficiency	Hypotonia at 10 mo; MRI results suggestive of disorder	Retrospectively, glutarylcarnitine, 3.2 $\mu\text{mol/liter}$ on day 3 (cutoff, 0.5 $\mu\text{mol/liter}$ )	First 2 wk of screening and analyte not included in panel
2	Propionyl-CoA carboxylase deficiency	Metabolic acidosis on day 4	Propionylcarnitine, 16.0 $\mu\text{mol/liter}$ (cutoff, 10 $\mu\text{mol/liter}$ ); result available on day 7	
3	Ornithine transcarbamylase deficiency	Prenatal diagnosis; treatment for 12 hr	Citrulline, 4 $\mu\text{mol/liter}$ before treatment started (lower limit, <5 $\mu\text{mol/liter}$ )	
4	Nonketotic hyperglycinemia	Hypotonia, seizures; diagnosis at 24 hr	Glycine, 1210 $\mu\text{mol/liter}$ (cutoff, 1100 $\mu\text{mol/liter}$ )	
5	Nonketotic hyperglycinemia	Hypotonia, seizures; diagnosis at 72 hr	Glycine, 1230 $\mu\text{mol/liter}$ (cutoff, 1100 $\mu\text{mol/liter}$ )	
6	Argininosuccinate synthase deficiency	Hyperammonemia; diagnosed at 72 hr	Citrulline, 230 $\mu\text{mol/liter}$ (upper cutoff, 75 $\mu\text{mol/liter}$ )	
<b>Negative result on newborn screening</b>				
7	Tyrosinemia, type I	Liver failure at 2 mo	Tyrosine 226 at 95th percentile (cutoff, 500 $\mu\text{mol/liter}$ )	No alteration of the cutoff planned
8	Tyrosinemia, type II	Diagnosed interstate at 9 mo	Tyrosine 220 at 94th percentile (cutoff, 500 $\mu\text{mol/liter}$ )	No alteration of the cutoff planned
9	Glutaryl-CoA dehydrogenase deficiency	Hypotonia from birth; gradually increasing dystonia; diagnosed at 10 mo	Glutarylcarnitine, 1.2 $\mu\text{mol/liter}$ ; repeated sample, 0.6 $\mu\text{mol/liter}$ (cutoff at the time, 0.8 $\mu\text{mol/liter}$ )	Protocol altered to recommend immediate clinical and biochemical review, without request for a second sample; cutoff lowered to 0.5 $\mu\text{mol/liter}$
10	Cobalamin C defect	Hypotonia and failure to thrive at 5 wk	Propionylcarnitine, 7.5 $\mu\text{mol/liter}$ , 95th percentile; acetylcarnitine, 24.6 $\mu\text{mol/liter}$ ; methionine, 19 $\mu\text{mol/liter}$ (cutoff, 5 $\mu\text{mol/liter}$ )	Retrospectively found the ratio of propionylcarnitine to acetylcarnitine elevated (0.3; cutoff, 0.25); now this ratio used as primary variable for diagnosis‡
11	Very-long-chain acyl-CoA dehydrogenase deficiency	Hypoglycemia at 12 mo	Initial sample: tetradecanoylcarnitine, 1.3 $\mu\text{mol/liter}$ ; tetradecenoylcarnitine, 2.0 $\mu\text{mol/liter}$ (cutoff for both at the time, 2.0 $\mu\text{mol/liter}$ ); repeated sample on day 14: tetradecanoylcarnitine, 0.4 $\mu\text{mol/liter}$ ; tetradecenoylcarnitine, 1.3 $\mu\text{mol/liter}$	Cutoff levels have been modified to 1.5 $\mu\text{mol/liter}$ for both and to 0.9 $\mu\text{mol/liter}$ for repeated samples
12	Nonketotic hyperglycinemia	Hypotonia, seizures on day 1	Glycine, 560 $\mu\text{mol/liter}$ of whole blood (cutoff, 1100 $\mu\text{mol/liter}$ )	No alteration of the cutoff planned
13	3-Ketothiolase deficiency	Severe ketosis at 15 mo	2- or 3-Hydroxy-3-methylbutyrylcarnitine, 1.20 $\mu\text{mol/liter}$ of whole blood (cutoff, 1.50 $\mu\text{mol/liter}$ )	No alteration of the cutoff planned

\* MRI denotes magnetic resonance imaging.

† Values are in micromoles per liter of whole blood.

‡ Change in protocol is according to Chace et al.<sup>15</sup>

short-chain acyl-CoA dehydrogenase deficiency would be clinically useful. All programs of newborn screening by tandem mass spectrometry have resulted in the diagnosis of cases of 3-methylcrotonyl CoA carboxylase deficiency, which was previously reported extremely rarely,<sup>12</sup> and have led to the diagnosis of maternal cases of this deficiency.<sup>18</sup>

After phenylketonuria, medium-chain acyl-CoA dehydrogenase deficiency was the most common disorder detected, with a prevalence of 1 per 10,000 births to 1 per 20,000 births; it is potentially lethal. Before screening was implemented, 25 percent of children with diagnosed cases died, usually during the first episode of decompensation, and an additional 30 to 40 percent had some developmental delay.<sup>19</sup> Some patients died during the newborn period.<sup>20</sup> From family studies, it was known that many patients with medium-chain acyl-CoA dehydrogenase deficiency remain healthy,<sup>21</sup> and population studies of the common mutation have indicated that a substantial proportion of cases remain undiagnosed. However, in a recent study in the United Kingdom, symptoms were common in patients whose disorder was diagnosed by retrospective screening of stored newborn samples but had escaped clinical diagnosis.<sup>22</sup>

In another report, some of the cases of medium-chain acyl-CoA dehydrogenase deficiency that had been diagnosed through newborn screening by tandem mass spectrometry in the United States have involved an unusual mutation that had not previously been seen in clinically diagnosed cases.<sup>23</sup> Two of our patients carried this mutation and may not be at risk for the development of symptoms. However, it was not possible to determine with certainty whether any of our patients were indeed at low or no risk of a dangerous decompensation, since they all had a demonstrable functional (biochemical) abnormality.<sup>24</sup> All such patients require careful follow-up and a management plan, including avoidance of fasting and maintenance of caloric intake during episodes of infection. With appropriate management, there appears to be a negligible risk of serious decompensation or death after diagnosis,<sup>19</sup> although formal studies are lacking.

For certain disorders, false negative test results, obtained primarily during the first two years of the screening program, have led us to alter the cutoff point used in our protocols for the definition of the disorder (Table 3). These alterations have resulted in an overall increase in the false positive rate of ap-

proximately 0.01 percent, but a review of the results obtained before the change in cutoff value did not reveal any other likely missed cases.

The ultimate sensitivity of testing by tandem mass spectrometry will depend on what false positive rate will be tolerated, both overall and for each disorder. This tolerable level may, in turn, be predicated on the perceived seriousness of each disorder and the urgency of early treatment. Our study so far indicates a high sensitivity for most disorders in the target group but also defines some disorders in which sensitivity is likely to be low. Sensitivity was apparently 100 percent for cases of phenylketonuria and pterin disorders (data not shown) and is likely to be close to 100 percent for medium-chain acyl-CoA dehydrogenase deficiency.<sup>24</sup> There are clearly diagnostic problems for type I tyrosinemia unless another assay for succinylacetone is added<sup>25</sup>; given the rarity of this disorder in our population, we have elected not to add such an assay. It is unlikely that pyridoxine-responsive homocystinuria can be detected without a high false positive rate by the current strategy of measuring methionine.<sup>26</sup>

New South Wales has a mixed population, largely derived from the United Kingdom and Ireland, but with substantial contribution from southern Europe, the Middle East, and Asia. We believe that these results are likely to be mirrored in many other parts of the world.

The screening of newborns by tandem mass spectrometry detects more cases than are diagnosed after clinical presentation, but the excess cases seem to be confined to a small number of disorders. The long-term outcomes and costs associated with neonatal screening for these disorders require further study. However, the diagnosis of cases that might never come to clinical attention should not be used as an argument against this expanded program of newborn screening. With the possible exceptions of short-chain acyl-CoA dehydrogenase deficiency and 3-methylcrotonyl CoA carboxylase deficiency, the disorders in question can all lead to substantial morbidity and mortality. It is important that follow-up tests be performed by experienced biochemical genetics laboratories and that clinical care be provided by physicians experienced in the management of inborn errors of metabolism, so that the benefit of early detection can be maximized and the risk of harm and unnecessary worry minimized.

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## REFERENCES

1. Chatfield S, Owen G, Ryley HC, et al. Neonatal screening for cystic fibrosis in Wales and the West Midlands: clinical assessment after five years of screening. *Arch Dis Child* 1991;66:29-33.
2. Farrell PM, Kosorok MR, Rock MJ, et al. Early diagnosis of cystic fibrosis through neonatal screening prevents severe malnutrition and improves long-term growth. *Pediatrics* 2001;107:1-13.
3. U.S. Congress, Office of Technology Assessment. Healthy children: investing in the future. Washington, D.C.: Government Printing Office, 1988. (Publication no. OTA-H-345.)
4. Lees CM, Davies S, Dezateux C. Neonatal screening for sickle cell disease. *Cochrane Database Syst Rev* 2000;2:CD001913.
5. Wilcken B. Rare diseases and the assessment of intervention: what sorts of clinical trials can we use? *J Inherit Metab Dis* 2001;24:291-8.
6. Albers S, Marsden D, Quackenbush E, Stark AR, Levy HL, Irons M. Detection of neonatal carnitine palmitoyltransferase II deficiency by expanded newborn screening with tandem mass spectrometry. *Pediatrics* 2001;107:1417. abstract.
7. Ranieri E, Gerace R, Bartlett B, Barnard K, Fletcher JM. The introduction of tandem mass spectrometry into the South Australian Neonatal Screening Programme: benefits and costs. *J Inherit Metab Dis* 2000;23:Suppl 1:3. abstract.
8. Roscher A, Liebl B, Fingerhut R, Olgemöller B. Prospective study of MS-MS newborn screening in Bavaria, Germany: interim results. *J Inherit Metab Dis* 2000;23:Suppl 1:4. abstract.
9. Wilcken B, Wiley V, Carpenter K. Two years of routine newborn screening by tandem mass spectrometry (MSMS) in New South Wales, Australia. *J Inherit Metab Dis* 2000;23:Suppl 1:4. abstract.
10. Wilcken B, Wiley V, Sim KG, Carpenter K. Carnitine transporter defect diagnosed by newborn screening with electrospray tandem mass spectrometry. *J Pediatr* 2001;138:581-4.
11. Wiley V, Carpenter K, Wilcken B. Newborn screening with tandem mass spectrometry: 12 months' experience in NSW Australia. *Acta Paediatr Suppl* 1999;88:45-51.
12. Zytovicz TH, Fitzgerald EF, Marsden D, et al. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001;47:1945-55.
13. Wood JC, Magera MJ, Rinaldo P, Seashore MR, Strauss AW, Friedman A. Diagnosis of very long chain acyl-dehydrogenase deficiency from an infant's newborn screening card. *Pediatrics* 2001;108:173-4. abstract.
14. Dean AG, Dean JA, Coulombier D, et al. Epi Info, version 6: a word processing, database, and statistics program for epidemiology on microcomputers. Atlanta: Centers for Disease Control and Prevention, 1994.
15. Chace DH, DiPerna JC, Kalas TA, Johnson RW, Naylor EW. Rapid diagnosis of methylmalonic and propionic acidemias: quantitative tandem mass spectrometric analysis of propionylcarnitine in filter-paper blood specimens obtained from newborns. *Clin Chem* 2001;47:2040-4.
16. Marshall E. Fast technology drives new world of newborn screening. *Science* 2001;294:2272-4. [Erratum, *Science* 2002;295:2370.]
17. Rhead WJ, Allain D, Van Calcar S, et al. Short chain acyl-CoA dehydrogenase (SCAD) and 3-methylcrotonyl-CoA carboxylase (MCC) deficiencies: tandem mass spectrometry newborn screening detects many clinically benign cases. *J Inherit Metab Dis* 2002;25:Suppl 1:4. abstract.
18. Gibson KM, Bennett MJ, Naylor EW, Morton DH. 3-Methylcrotonyl-coenzyme A carboxylase deficiency in Amish/Mennonite adults identified by detection of increased acylcarnitines in blood spots of their children. *J Pediatr* 1998;132:519-23.
19. Iafolla AK, Thompson RJ Jr, Roe CR. Medium-chain acyl-coenzyme A dehydrogenase deficiency: clinical course in 120 affected children. *J Pediatr* 1994;124:409-15.
20. Leung KC, Hammond JW, Chabra S, Carpenter KH, Potter M, Wilcken B. A fatal neonatal case of medium-chain acyl-coenzyme A dehydrogenase deficiency with homozygous A→G985 transition. *J Pediatr* 1992;121:965-8.
21. Heptinstall LE, Till J, Wraith JE, Besley GT. Common MCAD mutation in a healthy parent of two affected siblings. *J Inherit Metab Dis* 1995;18:638-9.
22. Pourfarzam M, Morris A, Appleton M, Craft A, Bartlett K. Neonatal screening for medium-chain acyl-CoA dehydrogenase deficiency. *Lancet* 2001;358:1063-4.
23. Andresen BS, Dobrowolski SE, O'Reilly L, et al. The mutational spectrum in the MCAD gene of newborns identified by prospective tandem MS screening for "diagnostic" acyl-carnitines in blood spots differs from that observed in clinically affected patients. *J Inherit Metab Dis* 2000;23:Suppl 1:12. abstract.
24. Carpenter K, Wiley V, Sim KG, Heath D, Wilcken B. Evaluation of newborn screening for medium chain acyl-CoA dehydrogenase deficiency in 275 000 babies. *Arch Dis Child Fetal Neonatal Ed* 2001;85:F105-F109.
25. Schulze A, Frommhold D, Hoffmann GF, Mayatepek E. Spectrophotometric microassay for delta-aminolevulinic acid dehydratase in dried-blood spots as confirmation for hereditary tyrosinemia type I. *Clin Chem* 2001;47:1424-9.
26. Peterschmitt MJ, Simmons JR, Levy HL. Reduction of false negative results in screening of newborns for homocystinuria. *N Engl J Med* 1999;341:1572-6.

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