Two-Tier Approach to the Newborn Screening of Methylenetetrahydrofolate Reductase Deficiency and Other Remethylation Disorders with Tandem Mass Spectrometry

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Objective To validate a 2-tier approach for newborn screening (NBS) of remethylation defects.

Study design The original NBS dried blood spots of 5 patients with a proven diagnosis of a remethylation disorder and 1 patient with biochemical evidence of such disorder were analyzed retrospectively to determine disease ranges for methionine (Met; 4.7-8.1 μmol/L; 1 percentile of healthy population, 11.1 μmol/L), the methionine/phenylalanine ratio (Met/Phe; 0.09-0.16; 1 percentile of healthy population, 0.22), and total homocysteine (tHcy; 42-157 μmol/L; 99 percentile of normal population, 14.7 μmol/L). These preliminary disease ranges showed a sufficient degree of segregation from healthy population data, allowing the selection of cutoff values. A simple algorithm was then developed to reflex cases to a second-tier testing for tHcy, which has been applied prospectively for 14 months.

Results A total of 86 333 NBS samples were tested between January 2007 and March 2008, and 233 of them (0.27%) met the criteria for second-tier testing of tHcy. All cases revealed concentrations of tHcy <15 μmol/L and were considered unaffected. No false-negative results have been reported with a state-wide system based on 2 combined metabolic clinics and laboratories that cover the entire Minnesota population and border areas of neighboring states.

Conclusions Pending more conclusive evidence from the prospective identification of additional true-positive cases, NBS for remethylation disorders appears to be feasible with existing methodologies, with only a marginal increase of the laboratory workload. *(J Pediatr 2010;157:271-5)*.

The remethylation of homocysteine (Hcy) to methionine (Met) involves several enzymes, including 5,10-methylenetetrahydrofolate reductase (MTHFR, EC 1.5.1.20, MIM 236250), methionine synthase reductase (MTRR, EC 2.1.1.135, MIM 236270), and methionine synthase (MTR, EC 2.1.1.13, MIM 250940). The latter enzyme requires methylcobalamin as a cofactor. Decreased or absent activity of MTHFR, MTR, and MTRR results in isolated functional deficiency of methionine synthase (for the remaining of the text, the term "remethylation disorder" will be applied only for this subgroup). The clinical presentation of this group of disorders may vary considerably, with more than one-third of the patients presenting early in life with poor feeding, failure to thrive, and neurologic disease caused by demyelination or other brain abnormalities.[1] At least in cases of severe MTHFR deficiency, treatment leads to prevention of symptoms when initiated in the first months of life before irreversible central nervous system damage occurs.[2] The main biochemical findings in these disorders are hypo-methioninemia and hyper-homocystinemia without methylmalonic acidemia.

Disorders affecting both the remethylation of homocysteine and the activity of methylmalonyl-CoA mutase (defects of cobalamin [Cbl] metabolism such as Cbl C, D, and F complementation groups) are included in the secondary targets in the newborn screening panel recommended by the American College of Medical Genetics (ACMG),[3] in recognition of their role in the differential diagnosis of an elevated propionylcarnitine concentration. This is a common finding with overall poor specificity, unless a second-tier test is used for the determination of methylmalonic acid and total Hcy (tHcy) in the same blood spot.[4]

Remethylation defects other than Cbl C, Cbl D, and Cbl F are not screened for because of the perceived lack of appropriate primary markers detectable with current NBS methods, which do not include tHcy. Therefore, we investigated

ACMG	American College of Medical Genetics	MS/MS	Tandem mass spectrometry
Cbl	Cobalamin	MTHFR	5,10-methylenetetrahydrofolate reductase
DBS	Dried blood spots	NBS	Newborn screening
Hcy	Homocysteine	Phe	Phenylalanine
LC-MS/MS	Liquid chromatography–tandem mass spectrometry	tHcy	Total homocysteine
		TPN	Total parenteral nutrition
Met	Methionine	Xle	Leucine-isoleucine

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whether low concentrations of Met could be used as primary marker for MTHFR and other remethylation disorders when combined with a second-tier test to detect an elevated concentration of tHcy. The hypothesis for this approach originated from the retrospective analysis of the original newborn blood spots of 6 patients affected by this group of conditions, who consistently showed the expected phenotype of low Met and elevated tHcy concentrations. We report our observations during the prospective application of a low cutoff point for Met to >85 000 NBS samples. All samples with Met below the cutoff point were submitted for second-tier tHcy determination to assess the clinical performance of this approach and its impact on laboratory operation.

Methods

Two groups of samples were analyzed. The first group included dried blood spots (DBS) from 6 patients with a remethylation defect (Table). All the biochemical diagnoses were confirmed with complementation studies or enzyme assay with the exception of case 2 (further testing was refused). Molecular genetic analysis corroborated the Cbl D variant 1 diagnosis in case 4.[5] The patients' original NBS cards were obtained with informed parental consent and in compliance with the respective NBS program requirements. The samples had been stored for different periods and at different temperatures (Table).

The second group consists of 86 333 blood spot samples analyzed prospectively as part of Minnesota NBS program between January 2007 and March 2008. More than 95% of these specimens were collected between 24 and 48 hours after birth.

The determination of Met in all DBS was performed according to our routine procedure for amino acid and acylcarnitine analysis in DBS with tandem mass spectrometry (MS/MS) and completed within 24 hours of sample receipt.[6] Simultaneous determination of tHcy, methylmalonic acid, and methylcitric acid was achieved with liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis by using a modification of a method described earlier.[7]

Results

Retrospective analysis of the original NBS samples of the 5 patients with confirmed diagnosis and 1 patient with biochemical evidence of remethylation disorder revealed Met concentrations ranging from 4.7 to 8.1 μmol/L, all <1 percentile (11.1 μmol/L) of >400 000 NBS samples analyzed in our laboratory since 2004 (Table and Figure 1). The highest (ie, less informative) Met/Phe and Met/Leucine-isoleucine (Xle) values of the disease ranges were 0.16 and 0.07, respectively. The lowest (less informative) tHcy concentration was 42 μmol/L; therefore, tHcy concentration was significantly elevated in all 6 patients.

Comparison of disease and historical control ranges for Met, Met/Phe, and Met/Xle ratios was the foundation for the algorithm depicted in Figure 2. Better than the Met/Xle ratio, the Met/Phe ratio is critical to minimize the number of samples requiring the second-tier assay for tHcy after the finding of a Met value in the 8 to 11 μmol/L range (Figure 1). The algorithm was then applied prospectively to 86 333 NBS samples submitted for routine NBS between January 2007 and March 2008. A total of 233 samples (0.27%) were submitted for second-tier testing of tHcy (Figure 2). All samples yielded normal results for tHcy (<15 μmol/L).

Discussion

Several inborn errors of Cbl metabolism or remethylation of Hcy (deficiencies belonging to Cbl complementation groups C, D, E, F, and G or MTHFR) are characterized by low Met and elevated tHcy concentrations. Although patients affected with Cbl C, D, and F deficiencies also have elevated concentrations of methylmalonic acid because of additionally impaired production of adenosylcobalamin, MTHFR, Cbl E, G, and recently described Cbl D variant 1 deficiencies only affect synthesis of methylcobalamin or the activity of MTR, and therefore do not cause elevations of methylmalonic acid.[8]

Remethylation disorders frequently present in the neonatal period or in early infancy with poor feeding, failure to thrive, hypotonia, and seizures. Neurologic disturbances with white matter changes become prominent with disease progression.[1] In addition to hypo-methioninemia and hyperhomocystinemia, affected patients have megaloblastic anemia (with the exception of MTHFR deficiency, in which no hematologic abnormalities are observed). Different therapeutic approaches have been implemented, including Cbl, folic acid, betaine, and methionine supplementation.[9,10] Most of the patients respond to these therapies with improvement of the biochemical measures and partial to

Table. Retrospective biochemical findings in neonatal dried blood spots of 6 patients with remethylation defects

Case	Diagnosis	Method of diagnosis	Met (μmol/L)	tHcy (μmol/L)	Met/Phe ratio	Met/Xle* ratio	Length, temperature of storage
1	MTHFR	complementation	4.8	157	0.10	0.07	2 months, ambient t°
2	Probable remethylation disorder	metabolic markers	4.7	42	0.10	0.04	15 months, ambient t°
3	Cbl G	complementation	4.2	75	0.15	0.04	60 months,-20°C
4	Cbl D, (Variant 1)	complementation/ DNA	5.5	64	0.09	0.05	10 months, -20°C
5	MTHFR	complementation	5.9	74	0.12	0.06	33 months, -20°C
6	Cbl G	complementation	8.1	63	0.16	0.06	1 months, -20°C
	1-99 percentile of normal population		11-54	0.6-14.7	0.22-0.79	0.11-0.43	

*Xle indicates both leucine and isoleucine, because these analytes cannot be separated by the method currently used.