

REDUCTION OF FALSE NEGATIVE RESULTS IN SCREENING OF NEWBORNS FOR HOMOCYSTINURIA

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ABSTRACT

Background Mental retardation and other medical problems (including ectopia lentis, osteoporosis, and thromboembolism) in patients who have homocystinuria as a result of a deficiency of cystathionine β -synthase can be prevented by the screening of newborns with measurement of blood methionine, followed by the early treatment of affected infants. Many infants with this disorder, however, are not identified by screening and have irreversible brain damage.

Methods We reviewed the results of neonatal screening for homocystinuria over a period of 32 years in New England. Additional specimens were requested for repeated analysis when blood methionine measurements were at or above the established cutoff level. Homocystinuria due to cystathionine β -synthase deficiency was confirmed by quantitative amino acid analyses.

Results For the first 23.5 years of the review period, the blood methionine cutoff value was 2 mg per deciliter (134 μ mol per liter). Among the 2.2 million infants screened during that period, 8 with homocystinuria were identified (1:275,000). In 1990, the cutoff value was reduced to 1 mg per deciliter (67 μ mol per liter). Among the 1.1 million infants screened in the subsequent 8.5 years, 7 with the disorder were identified (1:157,000). During the latter period, the specimens were collected from six of the seven infants when they were two days of age or less; five of the six had blood methionine concentrations below 2 mg per deciliter. Use of the reduced cutoff level increased the false positive rate from 0.006 percent to 0.03 percent.

Conclusions A cutoff level for blood methionine of 1 mg per deciliter in neonatal screening tests for homocystinuria should identify affected infants who have only slightly elevated concentrations of methionine and reduce the frequency of false negative results. (N Engl J Med 1999;341:1572-6.)

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HOMOCYSTINURIA due to a deficiency of cystathionine β -synthase, hereafter referred to simply as homocystinuria, is an inborn error of the metabolism of sulfur-containing amino acids that results in ectopia lentis, mental retardation, osteoporosis with bone deformities, thromboembolism, and psychiatric disturbances.¹ The major biochemical findings include high plasma homocyst(e)ine and methionine concentrations and low plasma cyst(e)ine concentrations. The gene for cystathionine β -synthase has been cloned and mapped to chromosome 21q22.3. Over 60 mu-

tations associated with homocystinuria have been found.²

Cystathionine β -synthase is pyridoxal phosphate (vitamin B₆)-dependent (Fig. 1). There are two forms of homocystinuria, which are differentiated on the basis of the biochemical response to treatment with vitamin B₆. Infants with vitamin B₆-responsive homocystinuria tend to be less severely affected and can be treated with high doses of vitamin B₆. Infants with vitamin B₆-nonresponsive homocystinuria are treated with a methionine-restricted diet supplemented with cystine. Infants given a diagnosis after the first few years of life or who are not fed according to the recommended diet are treated with betaine, a methyl donor that reduces plasma homocysteine concentrations by stimulating the methylation of homocysteine to methionine (Fig. 1). Infants in whom vitamin B₆ provides less than optimal control are also treated with betaine.³

The identification and prompt treatment of homocystinuria during the neonatal period can prevent or greatly reduce the severity of the clinical consequences. In Ireland, 18 of 21 persons between the ages of 2 and 23 years who had vitamin B₆-nonresponsive homocystinuria identified by neonatal screening and who were compliant with treatment had no complications. The remaining three patients were noncompliant with treatment, and ectopia lentis and osteoporosis developed. These patients also had low IQ scores.⁴ The experience in Manchester, England, was similar; 12 persons with vitamin B₆-nonresponsive homocystinuria identified by neonatal screening and treated continuously had a median IQ of 100, as compared with a median IQ of 58 for those with vitamin B₆-nonresponsive homocystinuria that was diagnosed after infancy.⁵ Consequently, the screening of newborns for this disorder is very beneficial.

Persons with homocystinuria, however, are often not identified during the neonatal period. The omission of screening for homocystinuria in most neonatal screening programs in which blood samples obtained by heel stick are used is a major reason for the lack of identification of affected infants,⁶ but the rate of false

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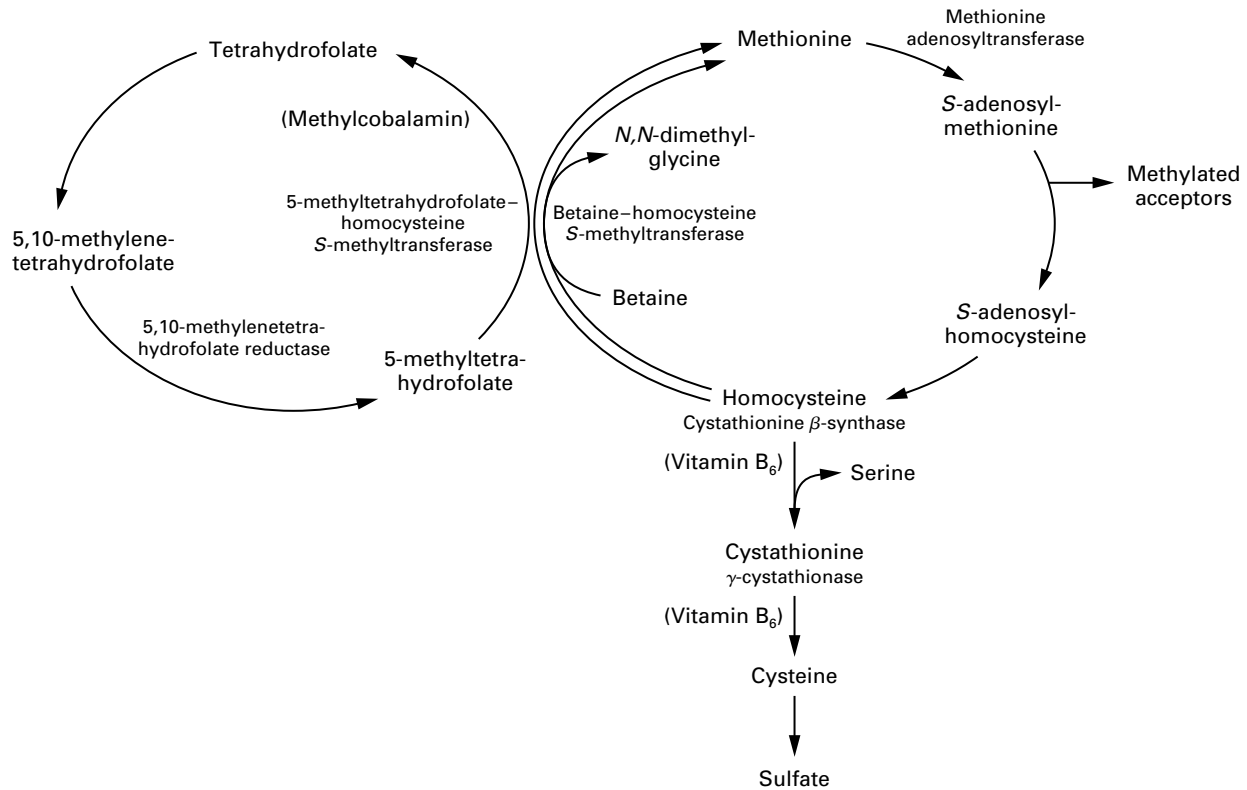


Figure 1. Metabolism of Methionine.

Cystathionine β -synthase and γ -cystathionase are vitamin B₆-dependent. Homocystinuria due to a deficiency of cystathionine β -synthase results in an accumulation of homocysteine, which is methylated to methionine by 5-methyltetrahydrofolate-homocysteine S-methyltransferase. Therapy with betaine results in further methylation of homocysteine by betaine-homocysteine S-methyltransferase. Vitamins required for enzyme activity are shown in parentheses.

negative results is an important contributor. Notable among those with false negative results are infants with vitamin B₆-responsive homocystinuria, who may account for 50 percent of affected infants¹ but who apparently do not have hypermethioninemia when the specimen is collected during neonatal screening.⁷

Another potential reason for false negative results in newborns undergoing screening for homocystinuria is that the blood methionine cutoff value is higher than the degree of hypermethioninemia in some affected infants. Neonates with homocystinuria may have blood methionine concentrations on screening that are lower than the cutoff value of 2 mg per deciliter (134 μ mol per liter) that is usually used to make a presumptive diagnosis of homocystinuria. We reviewed the experience with neonatal screening for homocystinuria in New England, during a period when first 2 mg per deciliter and then 1 mg per deciliter (67 μ mol per liter) was used as the cutoff value.

METHODS

Capillary-blood specimens obtained by heel stick from neonates born in hospitals in New England (excluding those in Connecticut) at or just before the time of discharge were blotted on

filter paper (Schleicher and Schuell, Keene, N.H.), air-dried, and mailed or delivered to the New England Newborn Screening Program. From 1966 until 1968, the specimens were tested by paper chromatography, according to the method of Efron et al.,⁸ as well as for phenylalanine and leucine by bacterial inhibition assays developed by Guthrie⁹; from 1968 through 1998 the Guthrie assay for methionine⁹ was used. The blood methionine concentration was estimated by comparing the diameters of the bacterial growth around specimen disks with the diameters around reference disks containing methionine concentrations of 1, 2, 4, and 12 mg per deciliter (67, 134, 268, and 805 μ mol per liter).

Until May 1990, the blood methionine cutoff level for requesting an additional specimen for repeated analysis was 2 mg per deciliter. Thereafter, the cutoff value was 1 mg per deciliter. Additional testing was requested for infants with values at or above the cutoff value. If the methionine concentration in the repeated analysis was 2 mg per deciliter or higher, the infant was referred to a pediatric metabolic center. If the methionine concentration in the repeated analysis was below 2 mg per deciliter, the initial result was considered to have been falsely positive. Confirmatory testing included quantitative plasma and urinary amino acid analyses with an amino acid analyzer (Beckman Instruments, Palo Alto, Calif.) and measurements of plasma total homocysteine.¹⁰

Informed consent and approval by an institutional review committee are not required by the New England Newborn Screening Program in the case of disorders (such as homocystinuria) for which screening is either specifically mandated by law or regulation or for which there is an implication in the law or regulation that such screening is required. In each state, a newborn-screen-

ing advisory committee serves to advise and make recommendations about neonatal screening to the department of public health.

RESULTS

From October 1966 through December 1998, the 32-year period of this study, approximately 3.3 million infants were screened for hypermethioninemia. Of these, 16 (1:206,000) were known to have homocystinuria. Fifteen of them were identified by neonatal screening. The infant not identified by neonatal screening had a normal methionine concentration (<1 mg per deciliter) at the age of four days, when the blood specimen was collected. He was given the diagnosis at the age of four months when he was found to have macrocytic anemia and pancytopenia due to a dietary folate deficiency.¹¹

During the first period of the study, October 1966 through May 1990, the cutoff blood methionine value for a presumptive diagnosis of hypermethioninemia was 2 mg per deciliter. The 8 infants with homocystinuria identified among the 2.2 million screened during this 23.5-year period (Table 1) represent a frequency of 1:275,000. In June 1990, an additional specimen was requested from an infant whose blood methionine concentration in the specimen collected at two days of age was estimated to be only 1 mg per deciliter. Repeated testing was undertaken, however, because the original specimen was recognized to be an "early specimen" and was the only one on the bacterial-assay plate with a methionine concentration as high as 1 mg per deciliter. The methionine concentration in the second specimen from this infant was greater than 12 mg per deciliter, and the diagnosis of homocystinuria was subsequently confirmed.

On the basis of this experience, the cutoff value for a presumptive diagnosis of hypermethioninemia was lowered to 1 mg per deciliter in June 1990. Thereafter, 7 infants with homocystinuria were identified among 1.1 million tested, a frequency of 1:157,000. This was 1.8 times the frequency at the cutoff value of 2 mg per deciliter.

Table 1 shows the data on the 15 infants with homocystinuria identified by neonatal screening over the 32-year period. For the infants identified before June 1990, the blood specimen used for the screening test was collected at the age of three days or more, whereas for all the infants except one identified in June 1990 or later the specimen was collected at the age of two days or less. The earlier collection of specimens beginning in June 1990 is consistent with the trend toward earlier hospital discharge in the United States that began in the 1980s.¹² It is evident that the degree of hypermethioninemia is a reflection of the time of collection of specimens. The blood methionine concentration was 3 mg per deciliter (201 μ mol per liter) or higher in all but one infant tested from 1966 through May 1990 but was less than 2 mg per decili-

TABLE 1. INFANTS WITH HOMOCYSTINURIA IDENTIFIED BY NEONATAL SCREENING IN NEW ENGLAND, 1966 THROUGH 1998.

PERIOD OF SCREENING AND PATIENT No.	YEAR OF BIRTH	AGE AT TIME OF SPECIMEN COLLECTION	BLOOD METHIONINE*		
			INITIAL	FOLLOW-UP	
			mg/dl		
October 1966– May 1990					
1	1967	5½		10†	
2‡	1968	5	35	<2	>12
3	1971	3		10	
4	1976	3		7	
5	1977	3		12	
6	1980	3		3	
7	1981	8		>20	
8	1982	3		8	
June 1990– December 1998§					
9	1990	2	19	1	>12
10	1991	2	16	1	8
11	1991	2	22	1	>12
12	1991	3		4	
13	1995	2		3	
14	1997	1½	14	1	12
15	1998	2	15	1.5	8

*To convert the values for methionine to micromoles per liter, divide by 0.0149.

†This result was obtained by paper chromatography.

‡Homocystinuria was detected on routine follow-up screening; this infant was the only one identified who had vitamin B₆-responsive homocystinuria.

§Beginning in June 1990, repeated testing of infants with initial methionine concentrations of 1 mg per deciliter (67 μ mol per liter) was implemented.

ter in five of the six infants tested in June 1990 and thereafter from whom specimens were collected no more than two days after birth. These five infants would not have been identified at the previous methionine cutoff level of 2 mg per deciliter.

The infant who had a blood methionine concentration of less than 2 mg per deciliter despite the fact that the blood specimen was collected at the age of five days was the only infant with vitamin B₆-responsive homocystinuria among those identified by the screening test. This infant was born during the period when there was routine follow-up screening practiced in Massachusetts.¹³ The initial neonatal screening result for this infant was considered normal, but by the age of five weeks, when the routine second specimen was collected, the infant had marked hypermethioninemia.⁷

Lowering the cutoff value for methionine from 2 mg per deciliter to 1 mg per deciliter increased the false positive rate from 0.006 percent to an average of 0.03 percent during the eight years in which the

lower cutoff value was used (annual range, 0.01 to 0.07 percent). For every 10,000 infants screened, a repeated analysis was requested for 3 who did not have hypermethioninemia, representing a false positive rate of 0.03 percent. Thus, approximately 40 such analyses would be requested among the 135,000 infants screened annually in the New England Newborn Screening Program.

DISCUSSION

These results over 32 years of neonatal screening for homocystinuria, with elevated levels of methionine as the identifier, indicate that the cutoff value for methionine needs to be at least as low as 1 mg per deciliter in order for the number of false negative results to be reduced. Lowering the cutoff value from 2 mg per deciliter to 1 mg per deciliter increased the frequency of infants correctly identified as having homocystinuria from 1:275,000 to 1:157,000. At the original cutoff value of 2 mg per deciliter, five of the seven infants identified in June 1990 and thereafter would have been missed.

Early collection of the specimen used for neonatal screening increases the importance of a lower cutoff blood methionine value. In the five infants who would have been missed at the higher cutoff value, the specimen was collected at two days of age or earlier. Blood methionine concentrations seem to rise slowly in infants with homocystinuria, perhaps because hypermethioninemia is a secondary feature of the disorder. Specifically, although the sequence of metabolic events that causes hypermethioninemia in persons with homocystinuria begins with blocking of the conversion of homocysteine to cystathionine, hypermethioninemia is directly dependent on the subsequent methylation of homocysteine to methionine (Fig. 1). This is unlike the sequence that occurs with phenylketonuria, in which hyperphenylalaninemia is the primary consequence of the enzyme defect and develops rapidly.¹⁴

Even with the lower cutoff value, it is not possible to identify all neonates with homocystinuria on the basis of screening for hypermethioninemia. Naughten et al.¹⁵ have estimated that at least one in every five affected infants is missed. The actual false negative rate is probably even higher. For instance, it is likely that infants with the vitamin B₆-responsive form of homocystinuria are missed, since almost no such infants are identified by neonatal screening, although about 50 percent of those with homocystinuria whose condition is identified on the basis of clinical complications have the vitamin B₆-responsive form.¹ The only infant with vitamin B₆-responsive homocystinuria whom we identified by neonatal screening had a blood methionine concentration of less than 2 mg per deciliter on the initial screening test at five days of age and was identified only by routine second screening at the age of five weeks.⁷ In the study by Naugh-

ten et al., the only infant with vitamin B₆-responsive homocystinuria among 25 affected infants from Ireland was missed by neonatal screening, whereas 21 of the remaining 24 infants had vitamin B₆-nonresponsive homocystinuria and were identified by screening.¹⁵ Thus, it is likely that few infants with vitamin B₆-responsive homocystinuria have hypermethioninemia at the time of neonatal screening.^{7,16}

False negative results are not limited to affected infants who have vitamin B₆-responsive homocystinuria. Watanabe et al.¹⁷ reported a normal screening result at the age of five days for an infant with the vitamin B₆-nonresponsive form of homocystinuria. The only infant we know to have been missed in New England also had vitamin B₆-nonresponsive homocystinuria.¹¹ On the basis of the prevalence of a mutation in the gene for cystathionine β -synthase among newborn infants, Gaustadnes et al.¹⁸ estimated the incidence of homocystinuria in Denmark to be 1:20,500. This is several times higher than any frequency reported on the basis of neonatal screening.¹

Increasing the sensitivity of screening results in decreased specificity, and thus more false positive results, because it enlarges the area of overlap between true positive results (those that indicate actual disease) and false positive ones.¹⁹ When we lowered the cutoff value for methionine from 2 mg per deciliter to 1 mg per deciliter, the false positive rate increased from 0.006 percent to an annual average of 0.03 percent. However, the latter rate results in only about 40 unnecessary requests for repeated analysis per year in New England. In other neonatal-screening programs for homocystinuria, the false positive rates have also been low, ranging from 0.001 percent among 86,000 neonates screened in Indiana to 0.04 percent among 112,000 neonates screened in Georgia and 0.06 percent among 271,000 neonates screened in New York State.²⁰ As in New England, a lower cutoff value for methionine in these programs tended to be associated with a higher false positive rate. The cutoff value was 2 mg per deciliter in Indiana and Georgia and 1.5 mg per deciliter (100 μ mol per liter) in New York. Nevertheless, all these rates are considerably lower than the false positive rates associated with neonatal screening for other disorders — 0.2 to 0.5 percent for congenital adrenal hyperplasia,²¹ 0.5 to 0.6 percent for congenital hypothyroidism,²² and 0.1 percent for phenylketonuria.²⁰

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REFERENCES

1. Mudd SH, Levy HL, Skovby F. Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited disease*. 7th ed. Vol. 1. New York: McGraw-Hill, 1995:1279-327.
2. Kraus JP. Biochemistry and molecular genetics of cystathionine β -synthase deficiency. *Eur J Pediatr* 1998;157:Suppl 2:S50-S53.
3. Wilcken DE, Wilcken B. The natural history of vascular disease in homocystinuria and the effects of treatment. *J Inher Metab Dis* 1997;20:295-300.
4. Yap S, Naughten E. Homocystinuria due to cystathionine β -synthase deficiency in Ireland: 25 years' experience of a newborn screened and treated population with reference to clinical outcome and biochemical control. *J Inher Metab Dis* 1998;21:738-47.
5. Walter JH, Wraith JE, White FJ, Bridge C, Till J. Strategies for the treatment of cystathionine β -synthase deficiency: the experience of the Willink Biochemical Genetics Unit over the past 30 years. *Eur J Pediatr* 1998;157:Suppl 2:S71-S76.
6. Walraven C, Sorrentino JE, Levy HL, Grady GE. Early newborn specimen: survey of practices among newborn screening programs in the United States. *Screening* 1995;4:1-8.
7. Levy HL, Shih VE, MacCready RA. Screening for homocystinuria in the newborn and mentally retarded population. In: Carson NAJ, Raine DN, eds. *Inherited disorders of sulphur metabolism*. Edinburgh, Scotland: Churchill Livingstone, 1971:235-44.
8. Efron ML, Young D, Moser HW, MacCready RA. A simple chromatographic screening test for the detection of disorders of amino acid metabolism. *N Engl J Med* 1964;270:1378-83.
9. Guthrie R. Screening for "inborn errors of metabolism" in the newborn infant — a multiple test program. In: Bergsma D, ed. *Human genetics*. Vol. 4. No. 6 of Birth defects original article series. New York: National Foundation-March of Dimes, 1968:92-8.
10. Brattstrom LE, Israelsson B, Jeppsson JO, Hultberg BL. Folic acid — an innocuous means to reduce plasma homocysteine. *Scand J Clin Lab Invest* 1988;48:215-21.
11. Wagstaff J, Korson M, Kraus JP, Levy HL. Severe folate deficiency and pancytopenia in a nutritionally deprived infant and homocystinuria caused by cystathionine beta-synthase deficiency. *J Pediatr* 1991;118:569-72.
12. Cunningham CG, Lorey F, Arnopp J, Patterson M, Currier R. Early discharge trends and their effect on PKU screening. In: Pass KA, Levy HL, eds. *Early hospital discharge: impact on newborn screening*. Atlanta: Council of Regional Networks for Genetic Services, 1995:31-56.
13. Levy HL, Shih VE, Karolkewicz V, MacCready RA. Screening for phenylketonuria. *Lancet* 1970;2:522-3.
14. Doherty LB, Rohr FJ, Levy HL. Detection of phenylketonuria in the very early newborn blood specimen. *Pediatrics* 1991;87:240-4.
15. Naughten ER, Yap S, Mayne PD. Newborn screening for homocystinuria: Irish and world experience. *Eur J Pediatr* 1998;157:Suppl 2:S84-S87.
16. Whiteman PD, Clayton BE, Ersser RS, Lilly P, Seakins JWT. Changing incidence of neonatal hypermethioninaemia: implications for the detection of homocystinuria. *Arch Dis Child* 1979;54:593-8.
17. Watanabe T, Ito M, Naito E, Yokota I, Matsuda J, Kuroda Y. Two siblings with vitamin B₆-nonresponsive cystathionine β -synthase deficiency and differing blood methionine levels during the neonatal period. *J Med Invest* 1997;44:95-7.
18. Gaustadnes M, Ingerslev J, Rütiger N. Prevalence of congenital homocystinuria in Denmark. *N Engl J Med* 1999;340:1513.
19. Morrison AS. *Screening in chronic disease*. 2nd ed. Vol. 19 of Monographs in epidemiology and biostatistics. New York: Oxford University Press, 1992.
20. Illinois Department of Public Health. *Newborn screening: an overview of newborn screening programs in the United States, Canada, Puerto Rico and the Virgin Islands*. Springfield, Ill.: Council of Regional Networks for Genetic Services, 1996.
21. Pang S, Clark A. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: newborn screening and its relationship to the diagnosis and treatment of the disorder. *Screening* 1993;2:105-39.
22. Verkerk PH, Buitendijk SE, Verloove-Vanhorick SP. Congenital hypothyroidism screening and the cutoff for thyrotropin measurement: recommendations from the Netherlands. *Am J Public Health* 1993;83:868-71.

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