BIRTH PREVALENCE OF HOMOCYSTINURIA

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Serious complications of homocystinuria caused by cystathionine β-synthase deficiency can be prevented by early intervention. We determined the prevalence of 6 specific mutations in 1133 newborn blood samples. Our results suggest that homocystinuria is more common than previously reported. Newborn screening for homocystinuria through mutation detection should be further considered. ***(J Pediatr 2004;144:830-2)***

The most common cause of homocystinuria is cystathionine β-synthase (CBS) deficiency, an autosomal recessive disorder of the transsulphuration pathway. CBS deficiency results in markedly elevated blood levels of homocysteine and methionine. Symptoms include thromboembolic events, mental retardation, psychiatric disorders, ectopia lentis, and skeletal abnormalities (osteoporosis and marfanoid stature).[1] The worldwide prevalence has been reported at ~1 in 300,000 births, but with higher prevalence in Ireland and New South Wales.[1,2] Notably, this figure is based on newborn screening results with the use of a bacterial inhibition assay for methionine, which only detects the more severe and pyridoxine nonresponsive variants of CBS deficiency.[1] In a Danish study, which used DNA sequencing to search for the pyridoxine responsive CBS 833T→C mutation, the estimated birth prevalence was ~1:20,000.[3] In Norway, routine homocysteine measurements are common, and, based on clinical diagnosis and biochemical data, we assume that the homocystinuria prevalence is relatively high. In this study, we searched for specific CBS mutations in blood samples from Norwegian newborn infants.

METHODS

From February to April 1999, ~5000 samples were randomly selected among ~12,000 capillary blood samples that were sent to the Rikshospitalet University Hospital, Oslo, for routine newborn screening of phenylketonuria and congenital hypothyroidism. Blood was collected into a gel separator tube, usually 3 to 5 days after birth. The tube was centrifuged locally and sent to the screening laboratory. Aliquots (50-100 μL) of packed blood cells were transferred into microtiter plates, which were stored at −20° C until analyses. The samples used in the study were unlinked and anonymous, and no data about the babies were available.

Genotyping for CBS mutations was performed in 1133 random samples. We have previously identified 6 different mutations among Norwegian families with CBS deficiency: 785C→T, 797G→A, 833T→C, 919 G→A, 959T→C, and 1105C→T.[4] Using a multiplex, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry method,[5] we searched for these mutations.

The prevalence of homocystinuria was calculated on the basis of the assumption that Hardy-Weinberg equilibrium exists and that babies with two mutated alleles will have homocystinuria. When the frequencies are "w" for the wild-type allele and "m" for the mutant allele, the frequencies will be w^2, 2wm, and m^2 for the homozygous wild-type, the heterozygotes, and the homozygous mutant genotype, respectively. Since we know the prevalence of w^2 (babies without CBS mutation) and 2wm (heterozygous babies), we can calculate the prevalence of m^2 (babies with both alleles mutated, ie, homocystinuria).

CBS Cystathionine β-synthase

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Supported by The Advanced Research Programme of Norway, the Norwegian Research Council, and the European Union (Demonstration Project Contract No. BMH4-CT98-3549).

There is no conflict of interest for any of the authors.

The funding sources had no direct influence on design, collection, and analyses of the data or the decision to submit the paper for publication.

Submitted for publication Jan 14, 2004; last revision received Feb 18, 2004; accepted Mar 2, 2004.

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0022-3476/$ - see front matter

10.1016/j.jpeds.2004.03.004

Table. Prevalence of mutations in the cystathionine β-synthase gene in 1133 blood samples from Norwegian newborn infants

Mutation	Pyridoxine response*	Heterozygous CBS deficiency	
		n	(%)
785C → T (T262M)	No	0	0.00
797G → A (R266K)	Yes	1	0.09
833T → C (I278T)	Yes	7	0.62
919G → A (G307S)	No	2	0.18
959T → C (V320A)	No	0	0.00
1105C → T (R369C)	Yes	18	1.59
Any of the 6 mutations		28	2.47

*Based on experience in patients with homocystinuria.[4,6]

RESULTS

The two most common mutations were 1105C→T and 833T→C (Table). Overall, CBS heterozygosity was observed in 2.47% (95% CI, 1.57-3.37) of the samples, yielding an estimated birth prevalence of homocystinuria (ie, homozygosity or compound heterozygosity) of ~1:6400. Compared with the Danish study,[3] the frequency of heterozygosity for the 833T→C was nonsignificantly lower in Norway (1.40% vs 0.62%, $P = .11$). We limited our investigation to 6 CBS mutations previously found in Norwegians,[4] but there are numerous other CBS mutations associated with homocystinuria.[6] Thus, our estimate is probably too low.

DISCUSSION

The estimated birth prevalence in this population is surprisingly high. A critical question is whether the observed frequency of CBS heterozygosity, based on genetic analysis, can be used to estimate the prevalence of clinical homocystinuria in the population.

The use of newborn samples avoids the problem of selection bias caused by early death in children with mutant alleles. However, we do not know whether CBS mutations in the mother or the fetus cause reduced reproductive fitness. This could lead to a lower prevalence of live-born babies with homocystinuria. Hence, the Hardy-Weinberg equilibrium may not exist.

Another factor is clinical penetrance, that is, whether homozygosity or compound heterozygosity for any pair of these mutations will lead to a clinically evident homocystinuria. We found that >90% of the mutated alleles in newborn infants are associated with a pyridoxine responsive phenotype. This proportion differs markedly from the widespread experience that only ~50% of patients respond to pyridoxine.[1,2,4,7] Pyridoxine responsiveness is usually associated with a less severe clinical disease.[1] The discrepancy between the genetic findings in newborn blood samples and that observed in patients[4,7] could suggest that many homocystinurics have a pyridoxine responsive variant with mild or no symptoms. Another possibility is that the diagnosis frequently is missed because the medical community is not fully aware of this disorder and its clinical manifestations.[1]

The high frequency of CBS heterozygosity in our study is mainly explained by the 1105C→T mutation. As far as we know, this mutation has only been reported in three homocystinuria patients: two from Norway (siblings)[4] and one from Australia.[7] All three were compound heterozygotes. Two of the patients had serious complications in the form of psychiatric disease and venous thrombosis. The third was free of symptoms but had a homocysteine level of 230 μmol/L.[4,7] Thus, we believe that 1105C→T, at least in combination with other CBS mutations, may cause a severe biochemical or clinical phenotype.

The importance of an optimal approach for diagnosing homocystinuria caused by CBS deficiency is related to the fact that treatment from infancy with pyridoxine, folic acid, and betaine reduces cardiovascular risk by 80% to 90%.[2] Development of other sequelae, including mental retardation, is also delayed.[8] Since treatment is effective, inexpensive, and has few side effects, newborn screening may be valuable in regions with a high prevalence of homocystinuria.

The introduction of routine screening should be according to certain principles,[9] which include identification of appropriate test(s). Current programs for homocystinuria screening, based on methionine measurements, often miss pyridoxine responsive variants of CBS deficiency and have a high rate of false-positive results.[1] Our findings indicate that novel high throughput techniques for mutation detection[10] may be a useful procedure to identify babies with mutations causing both mild and severe variants of homocystinuria. However, this approach will miss cases caused by unknown or rare CBS mutations and may include those with a genetic defect but normal biochemical and clinical phenotype. Thus, the optimal approach for detection of homocystinuria remains to be determined. Our data suggest that mutation analyses should be further evaluated and compared with metabolic screening and clinical assessment for the detection of homocystinuria in the newborn infant.

We thank the members of the Department of Pharmacology, University of Bergen, and Department of Pediatric Research, Rikshospitalet University Hospital, Oslo, for technical assistance.

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50 Years Ago in *The Journal of Pediatrics*

OXYGEN AND RETROLENTAL FIBROPLASIA (EDITORIAL)

J Pediatr 1954;44:488

Retinopathy of prematurity, a disease entity initially referred to as retrolental fibroplasia (RLF), describes a disorder occurring in premature, low-birth-weight infants. In this condition, there is abnormal development of blood vessels in the retina. Initially, the abnormal vessels develop in the retinal periphery. In advanced stages, the retina may completely detach and form a fibrovascular mass behind the crystalline lens, thus the term retrolental fibroplasia.

Terry first described RLF in 1942.[1] In 1952, The Journal published an editorial entitled "Anoxia and retrolental fibroplasia."[2] It pointed out that two points on RLF stood out: (1) it occurred in the more immature of the premature infants; and (2) the increased incidence had taken place with improved pediatric techniques that had lowered the mortality rate of premature infants. The editorial reviewed the work of Dr Thaddeus Szewczyk, an ophthalmologist from East Saint Louis, Illinois, who followed premature infants admitted to the hospital for treatment. Szewczyk's data indicated a relationship between the development of RLF and high exposure to oxygen in an incubator or by withdrawing oxygen too rapidly.[3]

In the January 1954 issue of The Journal, a second editorial on the topic of RLF stated "Rarely have we encountered an idea or suggestion of which pro and con sides were so violently taken."[4] The editorial reviewed various clinical and animal studies and concluded by stating, "There is a growing feeling that oxygen should not be given routinely to the premature infant but reserved for individualized cases of asphyxia, and further, it should be used in as low a concentration as possible and for as short a time as possible."

A third editorial on RLF that appeared in the April 1954 issue of The Journal reviewed additional clinical and experimental data.[5] The editorial also discusses statistics from the National Society for the Prevention of Blindness, which reported a 47 % increase in blindness in preschool children from 1943 until 1950, mostly due to RLF. The author concludes, "Evidence continues to accumulate that the oxygen concentration to which the small premature infant is exposed in the incubator is related to the development of retrolental fibroplasia."

In July 1954, a fourth editorial discusses various studies, including an epidemiologic survey of RLF in Maryland.[6] In this study, the various hospitals were graded as to quality, including resources such as incubators. The editor states that, "It was not surprising to learn the better the service the higher incidence of RLF."

Taken as a whole, the four editorials are fascinating in that they reveal the challenges the medical profession faces in recognizing the unintended consequences of new technology.

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YMPD725
10.1016/j.jpeds.2004.01.016

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