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A revisit to the natural history of homocystinuria due to cystathionine β -synthase deficiency

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Abstract

We review the evidence that in Denmark and probably certain other European countries the number of individuals identified with homocystinuria due to homozygosity for the widespread c.833T>C (p.I278T) mutation in the gene that encodes cystathionine β -synthase (CBS) falls far short of the number of such individuals expected on the basis of the heterozygote frequency for this mutation found by molecular screening. We conclude that the predominant portion of such homozygotes may be clinically unaffected, or may be ascertained for thromboembolic events occurring no sooner than the third decade of life. If so, there was significant ascertainment bias in the time-to-event curves previously published describing the natural history of untreated CBS deficiency [Mudd et al, 1985] and these curves should be used with care.

Keywords

cystathionine β -synthase; deficiency; homocystinuria; I278T; ascertainment; bias

In this report we discuss the probability that the predominant portion of individuals homozygous for the c.833T>C (p.I278T) mutation in the cystathionine β -synthase (CBS) gene are not being identified. Especially strong evidence supporting this likelihood is provided by consideration of the situation in Denmark, a country that has two advantages in this regard: an estimate, based upon molecular screening of newborns, is available for the potential number of such c.833T>C (p.I278T) homozygotes; and there are only two centers that make the biochemical and molecular diagnoses of homocystinuria due to CBS deficiency.

Homocystinuria was first described in 1962 in a search among mentally retarded individuals [1], and the discovery of cystathionine β -synthase deficiency as a causative enzymatic deficiency followed shortly thereafter [2]. Characteristic phenotypic signs are mental retardation, ectopia lentis, thromboembolic episodes, and osteoporosis. Elevations of total homocysteine (tHcy) are almost always accompanied by elevations of methionine. Administration of pyridoxine (vitamin B6) lessens the metabolic abnormalities in some subjects, but not in all, determined chiefly by the underlying mutation(s) in CBS [3;4]. In an

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effort to define the natural history of the disorder, between 1981 and 1985 we conducted an international survey. Based on the clinical findings in more than 600 cases, we derived time-to-event curves for the major manifestations in untreated patients [5]. Most, 86%, had been ascertained on the basis of ectopia lentis as either the sole, or a contributory, abnormality, and the results indicated that optic lens dislocation would occur by age 6 in 50% of those with B6-nonresponsive disease and by age 10 in 50% of B6-responsive individuals. Although thromboembolic events were calculated to be likely to occur in 50% of the combined group of B6-nonresponsive and B6-responsive patients by age 29, only 16% of those ascertained had had a thromboembolic event, and the possibility was mentioned that “patients who present as young adults with thrombotic events and none of the other ‘typical’ features of CBS deficiency may be remaining undiagnosed”.

Since that time significant progress has been made in our knowledge of CBS deficiency. The *CBS* gene has been cloned and sequenced and more than 140 mutations have been identified (<http://www.uchsc.edu/cbs/cbsdata/cbsmain.htm>). Obstacles in biochemical diagnosis due to the protein-binding of homocysteine have been overcome, and the measurement of total homocysteine (tHcy) in plasma or serum is now widely available and increasingly included in thrombophilia screening of patients with thromboembolic episodes.

Incidence of CBS deficiency based on alternative criteria

Overall incidence in newborn screening

Using the criterion of elevated blood methionine, CBS deficiency has been detected in newborn screening programs in different countries at rates of 1:58,000 to 1:1,000,000; overall 1:344,000 [3]. In spite of the fact that these are undoubtedly underestimates because B6-responsive patients are usually missed by such newborn screening [4], CBS deficiency has generally been regarded as a rare disease.

Frequency of homozygosity for c.833T>C (p.I278T) predicted by frequency of heterozygosity

Within the last decade, molecular screening studies have produced estimates that the prevalence of homozygosity for the B6-responsive c.833T>C (p.I278T) mutation (which may account, world-wide, for some 21% of all CBS-inactivating alleles), may be considerably higher among Caucasians in several European countries: In 1999 Gaustadnes et al. tested for the presence of the *CBS* c.833T>C (p.I278T) mutation in 500 Danish newborns, and found 1.4% (95% CI 0.58–2.88) to be heterozygotes, corresponding to an incidence of predicted homozygosity of 1:20,400 (95% CI 1:4800–1:128,000) [6]. In Norway, screening of blood samples from 1133 newborns found the frequency of heterozygosity for c.833T>C (p.I278T) to be 0.62% (predicted frequency of homozygosity 1:104,000) [7]. In the Czech and Slovak Republics, among 1284 newborns screened 5 heterozygotes were identified, a frequency of 0.389%, giving a predicted frequency of homozygosity of 1:264,000 [8]. Among unrelated healthy adults, heterozygote frequencies of 1.5% were found in Germany (200 screened; predicted homozygote frequency, 1:17,800) [9]; and 0.4% in The Netherlands (500 screened; predicted homozygote frequency, 1:250,000) [10].

Clinical detection of c.833T>C (p.I278T) homozygotes

If c.833T>C (p.I278T) homozygotes have a normal life expectancy, among the Danish population of approximately 5.5 million, about 270 such individuals would be expected (95% CI 43–1148). However, we know of only ten c.833T>C (p.I278T) homozygous patients in Denmark (Table 1), far below the lower limit of the expected number, and, because, as mentioned above, in Denmark the biochemical and molecular diagnoses of CBS deficiency are performed only in the departments of two of the present authors, we can be certain that no

more have been identified. All were diagnosed as young adults at ages 21–32 years. A thromboembolic event prompted the diagnosis in 8 of the 10, and 2 were asymptomatic and diagnosed because of an affected sib.

There are several possible explanations for the discrepancy between the number of those identified in Denmark and the number expected:

- a. c.833T>C (p.I278T) homozygotes may die in utero or, at early ages without diagnoses. We know of no data to support this hypothesis and, given the relatively mild clinical histories of the 10 who have been identified and their relatively advanced ages at ascertainment (Table 1), early mortality appears to be extremely unlikely.
- b. As suggested by the findings in Table 1, c.833T>C (p.I278T) homozygotes may not be mentally retarded and only infrequently have dislocated optic lenses. However, some may have thromboembolic episodes, yet not be recognized as hyperhomocysteinemic. In order to evaluate this possibility, we asked Danish departments of clinical biochemistry performing thrombophilia screening about the number of such screening tests done, and the number of investigations showing plasma tHcy >100 $\mu\text{mol/l}$. Among 25,758 thrombophilia screenings with measurements of homocysteine, only 38 revealed plasma tHcy >100 $\mu\text{mol/l}$. Results of further work-up of these 38 patients were not available. Furthermore, among the 1512 patients referred for thrombophilia investigation to the Centre for Haemophilia and Thrombosis, Skejby Hospital, Aarhus, Denmark, between February 1995 and November 1998, in all of whom tHcy was assayed, only 28 had tHcy ≥ 30 $\mu\text{mol/l}$, 5 had tHcy ≥ 100 $\mu\text{mol/l}$, and, among these 5 patients only one was found to be homozygous for c.833T>C (p.I278T), while two were compound heterozygotes with c.833T>C (p.I278T) being one of the mutations [11]. Hyperhomocysteinemia due to c.833T>C (p.I278T) homozygosity thus seems to be a rare finding among patients with thromboembolic events, consistent with the possibility that many c.833T>C (p.I278T) homozygotes are indeed free of vascular disease.
- c. c.833T>C (p.I278T) homozygotes are vitamin B6-responsive. Our preliminary observations suggest that at least some may lower their plasma tHcy levels to less than 30 $\mu\text{mol/l}$ on an oral supplement of less than 5 mg pyridoxine/d, a dose close to the normal Danish dietary intake of 0.6–2.7 mg/d (<http://www.food.dtu.dk/Default.aspx?ID=8366>) and thus be in sufficient response to prevent adverse clinical effects.
- d. Most c.833T>C (p.I278T) homozygotes may be clinically normal, even if not in partial B6 response and in spite of plasma tHcy levels above the normal range. Investigation of additional siblings of individuals proven to be homozygotes for c.833T>C (p.I278T) with respect not only to the clinical histories of such sibs, but also to their plasma tHcy levels and CBS genotypes would further test this possibility – some clinically normal sibs may turn out to be c.833T>C (p.I278T) homozygotes (as are individuals G and H in Table 1). Extensive experience has shown that during treatment of CBS-deficient patients, although plasma tHcy is rarely, if ever, reduced to within the normal range, the incidence of thromboembolic episodes is markedly lowered – even for B6-nonresponsive patients for whom the ascertainment bias under discussion is likely to be less [12]. Furthermore, experiments with CBS KO mice have shown that the presence of a transgene carrying c.833T>C (p.I278T) prevents the neonatal lethality otherwise manifested in these KO mice, in spite of a mean serum tHcy concentration of 274 $\mu\text{mol/l}$ in those KO mice with the transgene [13].

That the situation in Denmark just discussed may be more general is suggested by the experience in the Czech Republic. Sokolová and colleagues found that the observed frequency

of the c.833T>C (p.I278T) allele among homocystinuric patients ascertained by selective biochemical screening was about 11 times lower than that predicted based upon the c.833T>C (p.I278T) allele frequency found in molecular screening of 1284 unselected newborns. They discussed the possibility that mildly affected individuals carrying the c.833T>C (p.I278T) mutation were not being detected owing to mild clinical phenotypes and later onset of symptoms [8]. Magner and colleagues recently reported that, between 1980 and 2009, 20 patients with CBS deficiency were diagnosed in the Czech Republic. The proportion of these patients with a vascular manifestation as the reason for the first clinical presentation was 0/7 between 1980–1989 and 0/6 between 1990–1999, but increased to 5/7 between 2000–2009. Plasma tHcy measurement had become routinely available only in the last decade, as demonstrated by a five-fold increase in the total number of such assays in 2003–2007 compared to 1998–2002. These data were interpreted as suggesting that the change in phenotypic presentation was due to ascertainment of mildly affected adult patients (M. Magner, L. Krupova, T. Honzik, J. Zeman, and V. Kozich, 7th International Conference on Homocysteine Metabolism, Prague, Czech Republic, 21–25 June 2009, abstract).

Taken together, the observations outlined above suggest a wide discrepancy between the expected and the detected number of c.833T>C (p.I278T) homozygous patients in Denmark, and probably in other countries. After consideration of the possible explanations of this discrepancy, our tentative conclusion is that a large proportion of c.833T>C (p.I278T) homozygotes may be clinically normal. This possibility has also been put forth not only by Sokolová et al [8] (as mentioned above), but also by Linnebank and coworkers [9]. If so, the time-to-event curves for major manifestations presented in the international survey may be affected by ascertainment bias, at least for those with clinically less severe forms of CBS deficiency such as c.833T>C (p.I278T) homozygosity [5]. A more accurate picture of the natural history for c.833T>C (p.I278T) homozygotes might be obtained if all such homozygotes in large birth cohorts, for example from the 1970's and 1980's, were identified by molecular screening of stored newborn blood spots, and the clinical history of each homozygote determined. Such an undertaking is technically feasible, but prohibited by the current ethical standards and regulations for neonatal screening.

Ascertainment bias

Does ascertainment bias exist for other of the most common forms of CBS deficiency in addition to c.833T>C (p.I278T) homozygosity? Another mutation, c.1105C>T (p.R369C) [14], has been reported to be very common with heterozygote frequencies in newborns of 1.6 % among Norwegians [7], 1.0% among North Americans [14], and 1.0% among Czechs [15]. Homozygotes for this mutation may have B6-responsive homocystinuria with a mild phenotype or no symptoms [7;15], and strong ascertainment bias may well occur for them, although at the moment definitive proof of this possibility has not been presented. We consider that ascertainment bias is likely to be far less of a problem for B6-nonresponsive forms such as homozygosity for c.919G>A (p.G307S) (the mutation accounting for some 71% of those in Irish homocystinuria patients [16]), c.1006C>T (p.R336C) [17], the mutation found in the Qatari population in the homozygous state in 1:2100 newborns among 12,603 screened [18], or c.572C>T (p.T191M), the mutation highly prevalent in Spain, Portugal, and South America [19].

Concluding remarks

If further evidence confirms the presence worldwide of a large number of patients with CBS deficiency, but no, or only late onset clinical manifestations, or with symptoms limited to the vascular system, it will be time to modify our concept of this sort of homocystinuria. We

therefore urge caution when using previously published data based on patient groups that may well have been subject to significant ascertainment bias [5].

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Table 1

Clinical characteristics of Danish c.833T>C (p.I278T) homozygotes

Patient	Sibs ^a	Age at diagnosis(years)	Plasma tHcy at diagnosis(μmol/l)	Abnormalities before diagnosis		
				Eye	Vascular	CNS
A	B, C	23	181	-	+	-
B	A, C	21	246	-	+	-
C	A, B	21	206	-	+	-
D		24	not assayed	-	+	-
E		26	446	-	+	+
F	G, H	21	335	-	+	-
G	F, H	32	179	-	-	-
H	F, G	29	227	-	-	-
I		23	250	-	+	-
J		27	243	+	+	-

^aPatients A, B, and C are siblings, as are patients F, G, and H