Familial Thrombophilia Associated With Homozygosity for the Cystathionine \( \beta \)-Synthase 833T→C Mutation

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Abstract—Severe hyperhomocysteinemia due to cystathionine \( \beta \)-synthase (CBS) deficiency is a strong risk factor for premature cardiovascular disease. Among untreated patients, \( \approx 50\% \) have suffered a thromboembolic event by 30 years of age. We report on 3 sisters with severe hyperhomocysteinemia due to homozygosity for the CBS 833T→C mutation. These patients, who displayed no other known thrombophilic predisposition, had suffered single or multiple venous thrombosis before CBS deficiency was diagnosed relatively late in life. In this family, homozygosity for the 833T→C mutation was associated with a mild phenotype with respect to other sequelae of CBS deficiency. Consequently, our results indicate that most cases with this genotype may remain undiagnosed. Investigated family members heterozygous for the 833T→C mutation displayed normal total homocysteine in plasma (tHcy) levels, even when they were homozygous for the methylenetetrahydrofolate reductase 677C→T polymorphism. The prevalence of homozygosity for the 833T→C mutation has previously been estimated at no less than 1:20 500 in our population. Because a reduction of the severely elevated levels of tHcy in CBS deficiency reduces cardiovascular risk and because homozygosity for the 833T→C mutation is more prevalent than previously thought, our results emphasize the importance of measuring tHcy routinely in thrombophilia screening. (Arterioscler Thromb Vasc Biol. 2000;20:1392-1395.)

Key Words: venous thrombosis ■ severe hyperhomocysteinemia ■ cystathionine \( \beta \)-synthase deficiency ■ family history ■ mutation analysis

An elevated plasma level of homocysteine is an independent risk factor for arterial as well as venous thrombosis.\(^1\),\(^2\) Mild or moderate hyperhomocysteinemia (an increased level of total homocysteine in plasma [tHcy] up to 30 \( \mu \)mol/L and 30 to 100 \( \mu \)mol/L, respectively) may result from a relative deficiency of folic acid, vitamin \( B_{12} \), or vitamin \( B_6 \) or from homozygosity for a common polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene (677C→T).\(^3\) Severe hyperhomocysteinemia (tHcy >100 \( \mu \)mol/L) is most often caused by cystathionine \( \beta \)-synthase (CBS, EC 4.2.1.22) deficiency.\(^5\) This autosomal recessive disease is the most common inborn error of sulfur amino acid metabolism, with an estimated incidence of \( \approx 1:200,000 \) worldwide, ranging from 1:20 500 to 1:1 000 000 in different populations.\(^6\),\(^7\) The clinical manifestations include premature atherosclerosis and early thromboembolism, ectopia lentis, mental retardation, other neuropsychiatric manifestations, and skeletal abnormalities, including osteoporosis. Vascular complications constitute the major cause of death.\(^5\) Overall, 50% of patients with untreated CBS deficiency have suffered a thromboembolic event by 30 years of age.\(^8\) Vascular occlusion can occur in any vessel at any age, with the majority of such occurrences involving peripheral veins (51%) and complicated by pulmonary embolism in one fourth of those cases.\(^5\) Because most countries do not systematically screen newborns for homocystinuria, the majority of cases of CBS deficiency have been diagnosed on the basis of the patients’ phenotypical features.

In transsulfuration of homocysteine, CBS catalyzes the condensation of homocysteine with serine, forming cystathionine in a pyridoxal 5'-phosphate (the active form of vitamin \( B_6 \))-dependent reaction. High-dose administration of vitamin \( B_6 \) in CBS deficiency may lower homocysteine, and patients are classified as pyridoxine responsive or nonresponsive according to their response pattern after pyridoxine supplementation. Alternatively, remethylation of homocysteine is stimulated by folic acid and betaine treatment. Early detection and lowering of a severely increased tHcy may prevent complications.\(^5\) In CBS-deficient patients, a total of 92 mutations have been detected in the CBS gene so far, with the majority being missense mutations.\(^9\)

We have recently shown that 1.4% of Danish newborns (n=500) are heterozygous for the geographically widespread 833T→C mutation,\(^10\) indicating a prevalence of homozygosity for this mutation at 1 in 20 500.\(^7\) In the present study, we report on 3 sisters with CBS deficiency and severe hyperhomocysteinemia due to homozygosity for this mutation. CBS deficiency was diagnosed late in life (ages 54 to 58 years) after at least 1 thromboembolic event.
Methods

Thrombophilia Screening Methods

Determinations of antithrombin, protein C, protein S, and plasminogen activities were performed by use of citrated platelet-poor plasma as previously described.11 Other analyses consisted of determinations of activated partial thromboplastin time, prothrombin time, P-fibrinogen, and thrombin time and assays for anticardiolipin antibodies and lupus anticoagulant.

Determination of tHcy

tHcy was measured after a protein-free morning meal by using gas chromatography–mass spectrometry and stable isotope dilution. Samples were collected in tubes containing heparin as anticoagulant and sodium fluoride, which was added to a final concentration of 4g/L,12 and plasma was separated by centrifugation within 2 hours. To exclude hyperhomocysteinemia due to cobalamin deficiency, methylmalonic acid in plasma was measured by gas chromatography–mass spectrometry.13 Furthermore, creatinine was determined in plasma with a Vitros 700 RC analyzer.

Genetic Analyses

Genomic DNA was isolated from EDTA-stabilized blood by using a Puregene DNA Isolation Kit (Genta Systems, Inc). CBS genotypes and haplotypes were analyzed by polymerase chain reaction amplification and direct sequencing of genomic DNA containing the entire coding region of the CBS gene, including adjacent intron-exon boundaries as previously described.14 Analyses of MTHFR 677 genotypes, factor V 1691 genotypes, and prothrombin 20210 genotypes were determined with respect to 2 common polymorphisms in exons 6 (699C→T) and 10 (1080T→C), respectively. The 833T→C allele was always associated with the 699C variant and the 1080T variant, whereas the 833C allele cosegregated with the 699T variant and the 1080C variant, in agreement with the neutral splice variant that does not appear to cause hyperhomocysteinemia.7 No other nongenetic causes of hyperhomocysteinemia were identified in this patient. Additionally, all siblings of the proposita (patient II:4), 5 sisters and 2 brothers, were investigated (Figure). Two sisters were homozygous for the 833T→C mutation (patients II:6 and II:7). Both had severe hyperhomocysteinemia (tHcy 246 and 206 μmol/L, respectively) and had suffered DVT. No other tested thrombophilic risk factor was present. The 2 brothers and 3 of the sisters (aged 53 to 63 years) were heterozygous for the 833T→C mutation. None had suffered a thromboembolic event. All 5 unaffected siblings had tHcy levels within the normal range for their age and sex according to Rasmussen et al.18 Two had the MTHFR T/T genotype (Figure). The 4 children of patients II:4 and II:7 were also investigated, although 1 had his genotype determined but otherwise abstained from participation in the study. Plasma tHcy was normal in each of the children (aged 27 to 37 years) in whom it was measured. None of the probands displayed other known risk factors.

Haplotypes of the CBS 833T allele and the 833C allele were determined with respect to 2 common polymorphisms in exons 6 (699C→T) and 10 (1080T→C), respectively. The 833T allele was always associated with the 699C variant and the 1080T variant, whereas the 833C allele cosegregated with the 699T variant and the 1080C variant, in agreement with the haplotypes determined in 2 other Danish CBS-deficient patients with the 833T→C mutation.14

The proposita’s mother had died of cancer at 68 years of age. Terminally, a thromboembolic event had occurred. The father died from pancreatitis at 73 years of age with no history of thromboembolic events. The mental states of the patients were normal. No material was available for genetic analysis.

Further Clinical Investigations

None of the 3 patients had reduced bone mineral content as determined by dual-energy x-ray absorptiometric scanning. Ophthalmologic examination revealed no ectopia lentis or other visual problems. The mental states of the patients were normal.

Pyridoxine Responsiveness

tHcy levels decreased from 181.0 to 63.7 μmol/L, from 246.0 to 42.1 μmol/L, and from 206.0 to 23.9 μmol/L on folic acid (5 mg/wk) plus pyridoxine (250 mg/d) treatment for 6 weeks. By increasing the dose of pyridoxine to 500 mg/d, tHcy levels further decreased to 19.0, 14.4, and 17.7 μmol/L, respectively. Betaine (3 g/d) was also tested in the treatment. Only patient II:6, who had the highest initial level of tHcy, is at present treated with betaine. The pyridoxine responsiveness
of all 3 sisters is in agreement with previous reports on patients homozygous for the 833T→C mutation.10,19

**Discussion**

CBS deficiency is detected by means of either newborn screening or, most often, its clinical features. The severity of the disease varies strongly; generally, an early diagnosis and treatment may prohibit the development of severe clinical manifestations or the deterioration of preexisting sequelae.20 The 833T→C mutation is one of the most prevalent mutations causing CBS deficiency, accounting for almost 25% of all homocystinuria alleles detected so far.9 It is present in Caucasian populations as well as among North Americans.9,21

In the present study, we report on a family in which 3 sisters were homozygous for the 833T→C mutation. The phenotype conferred by this genotype was mild with respect to known sequelae of CBS deficiency, with thromboembolicisms considered separately. Because of the lack of a typical homocystinuria phenotype, CBS deficiency was diagnosed relatively late in life in these patients. Considering the mild phenotype reported in the present study for the 833T→C mutation and because of the surprisingly high prevalence of the mutation among Danish newborns, we anticipate that numerous individuals homozygous for this, or other, mild CBS mutations remain undiagnosed.

One patient (II:6) had the MTHFR T/T genotype. It is noteworthy that her tHcy level was considerably more elevated than those of her sisters who had the MTHFR C/T genotype. We speculate that this may be explained by a synergistic effect of the MTHFR T/T genotype and a deficiency in CBS, with the latter resulting in an elevated level of S-adenosylmethionine,22 which further results in an inhibition of MTHFR. The reduced specific activity of MTHFR caused by the T/T genotype may further decrease the amount of homocysteine subjected to remethylation, thus resulting in a substantially higher accumulation of homocysteine. A similar influence of the MTHFR T/T genotype on tHcy levels in CBS-deficient patients was recently reported by Kluitjmans et al.23 However, the number of patients was too small to reach statistical significance.

No other known thrombophilic risk factor was present in the 3 patients. Hence, our data do not support the hypothesis of Mandel et al24 that the factor V Leiden mutation or another thrombotic risk factor should be present for thrombosis to occur in CBS-deficient patients. This postulate has also been refuted by other investigators.23,25,26

Our study of this limited number of individuals does not point to heterozygosity for the 833T→C mutation as being a risk factor for hyperhomocysteinemia or thrombosis, even in the presence of the MTHFR T/T genotype. However, postmethylone-loading tHcy levels were not recorded.

In this family, there were no signs of deleterious effects involving the central nervous system that were due to CBS deficiency, nor did the affected members have skeletal manifestations or the deterioration of preexisting sequelae.5 Shih et al19 previously reported on mild clinical features in 2 patients homozygous for the 833T→C mutation. The patients, who were diagnosed in early adulthood, had ectopia lentis and reduced bone mineralization but no thromboembolic events. Expression studies of the 833T→C mutation performed in *Escherichia coli* revealed a low specific activity (<10% of normal values) and instability of the polypeptide.19

In CBS deficiency and in a number of other monogenic diseases, variation in phenotypes between patients with the same genotype is observed. No well-defined correlation between genotype and phenotype has been established in CBS deficiency.9,27 However, it appears that homozygosity for the 919G→A (G307S) mutation correlates with pyridoxine nonresponsiveness,28 whereas homozygosity for the 833T→C mutation correlates with pyridoxine responsiveness, in agreement with treatment results in our study family.10,19 Yet exceptions from this rule exist.29 Genetic and/or environmental susceptibility factors not yet identified may influence the phenotypic expression of a given genotype. Defects in, for example, the “protein quality control” system, which is responsible for the cellular handling of mutated and misfolded proteins,30 might be factors influencing the observed phenotypical differences in CBS deficiency. Defects in these control systems (eg, in proteases or chaperones) may alter the buffering capacity of the systems, thereby modulating the phenotype caused by other, in this case CBS, mutations.

The present data indicate that individuals with untreated CBS deficiency due to homozygosity for the 833T→C mutation are at high risk of DVT. Our findings strongly emphasize the importance of including tHcy in the routine thrombophilia program. Furthermore, hyperhomocysteinemia should be suspected in cases of severe myopia in childhood and in other clinical conditions associated with known sequelae of homocystinuria to ensure an early detection of this relatively frequent, and treatable, inborn error of metabolism. Eventually, considerations on the perspective of national screening programs are advocated.

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**References**


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