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The frequency of 844ins68 mutation in the cystathionine β-synthase gene is not increased in patients with venous thrombosis

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Abstract

Background and Objectives. **A frequent mutation in the cystathionine β-synthase (CBS) gene (844ins68, a 68-bp insertion in the coding region of exon 8) was recently discovered. In the present study we investigated this mutation as a candidate risk factor for venous thrombosis.**

Design and Methods. **The prevalence of the 844ins68 CBS mutation was determined in 101 patients with objectively diagnosed deep venous thrombosis and in 101 healthy controls matched for age, sex and race. PCR amplification of a DNA fragment containing exon 8 of the CBS gene was employed to determine the genotypes. Additionally, *BsrI* restriction enzyme digestion of the PCR products was performed in all samples from carriers of the insertion, to test for concurrent presence of a second mutation (T833C) in the CBS gene.**

Results. **The insertion was found in 21 out of 101 patients (20.8%; allele frequency 0.109) and in 20 out of 101 controls (19.8%; allele frequency 0.114), yielding a relative risk for venous thrombosis related to the 844ins68 CBS mutation close to 1.0. In addition, the T833C CBS mutation was detected in all alleles carrying the 844ins68 CBS insertion, confirming the co-inheritance of the two mutations.**

Interpretation and Conclusions. **Our findings do not support the hypothesis that the 844ins68 mutation in the CBS gene is a genetic risk factor for venous thrombosis.**

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Key words: cystathionine β-synthase; homocysteine; insertion.

Cystathionine β-synthase (CBS) participates in the transulfuration pathway of homocysteine metabolism as a critical enzyme by converting homocysteine into cystathionine, with pyridoxal phosphate as a cofactor.[w1] Abnormalities of the CBS gene may result in enzyme deficiency and hyperhomocysteinemia, which is currently recognized as a risk factor for venous thrombosis, premature atherosclerotic vascular disease and neural tube defects (NTD).[1-6]

Recent studies have shown that a 68-bp insertion (844ins68) in the coding region of exon 8 of the CBS gene is a common mutation among normal individuals (prevalence of 11.7% in North Americans, 7.5% in Italians, 18.8% in the Irish population and 16.8% in the Dutch).[7-10] The 844ins68 variant is an exact duplication of the intron-exon boundary of exon 8, and was found to be associated in cis with an additional mutation in the CBS gene: a T to C transition at nucleotide position 833, which causes an Ile to Thr amino acid substitution.[8] Interestingly, the 844ins68 mutation creates an alternative splice site which rescues the wild-type CBS sequence from the mutated allele. Although the CBS insertion apparently does not result in enzyme activity impairment or hyperhomocysteinemia, mRNA data provided evidence that the allele carrying the insertion is poorly transcribed.[7,8]

So far, the 844ins68 CBS mutation has been investigated as a risk factor for arterial thrombotic disease in different studies on limited numbers of patients and controls, which yielded controversial results.[7,10,11] In contrast, no study has been conducted in order to investigate this mutation as a genetic risk factor for venous thrombotic disease. In the present study we investigated the 844ins68 CBS mutation as a risk factor for venous thrombosis by determining its prevalence among relatively young patients with objective diagnosis of deep venous thrombosis and in healthy controls.

Materials and Methods

Subjects

Blood samples were obtained after informed consent from 101 consecutive patients (42 men and 59 women; mean age, 36 years; age range, 1 to 50 years) with a first episode of deep venous thrombosis objectively confirmed by phlebography or ultrasonography (color duplex scan). None of the individuals included in the patient group had evidence of malignant

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disease. For each patient, one age-, sex- and race-matched healthy unrelated individual without a personal or family history of venous thrombosis was investigated as a control. All of the patients and the controls were Brazilians and inhabitants of the same geographic area (state of São Paulo, Brazil). The study was approved by the local Ethics Committee.

Methods

Genomic DNA was extracted from peripheral leukocytes employing standard methods.[12] DNA analysis was carried out by PCR amplification of a DNA fragment containing exon 8 of the CBS gene as previously described.[6] The PCR products were eletrophoresed on a 2% agarose gel and photographed under UV light after ethidium bromide staining. The resulting fragments were 252-bp in the presence and 184-bp in the absence of the 844ins68 CBS insertion. Digestion of the PCR products with BsrI restriction enzyme was performed in all samples from carriers of the insertion, to test for concurrent presence of the T833C CBS mutation.[7] Differences in allele frequencies and genotype distribution between patients and controls were assessed by the χ^2 test and a p value of 0.05 was taken as statistically significant.

Results

Genotype distribution of the 844ins68 CBS mutation in patients with venous thrombosis and healthy controls is shown in Table 1. Twenty heterozygotes (19.8%) and 1 homozygote (1%) for the CBS insertion were found among 101 patients with verified venous thrombosis (allele frequency 0.109). In the control group, 17 heterozygotes (16.8%) and 3 homozygotes (3%) for the mutation were observed (allele frequency 0.114). These data yield an odds ratio for venous thrombosis associated with the CBS insertion close to 1.0, or a neutral relative risk for venous thrombosis linked to the mutant CBS allele. BsrI restriction enzyme digestion revealed that the T833C CBS mutation was present in all alleles carrying the 844ins68 CBS insertion.

Table 1. Genotype distribution of the 844ins68 mutation in the CBS gene in patients with venous thrombosis and in healthy controls.

CBS genotype	*Patients (n=101)*	*Controls (n=101)*
N/N	80 (79.2%)	81 (80.2%)
N/I	20 (19.8%)	17 (16.8%)
I/I	1 (1%)	3 (3%)

None of the differences was statistically significant (p values > 0.05). N/N: normal genotype, N/I: heterozygous for the CBS insertion, I/I: homozygous for the CBS insertion.

Discussion

The description of new mutations in the CBS gene has stimulated the design of studies assessing the role of the specific genetic abnormality in vascular thrombosis and NTD.[7,9-11] Recently, an insertion in the coding region of exon 8 of the CBS gene was identified as a frequent mutation in different populations in which its prevalence was investigated.[7-11] Previous results for *BsrI* restriction enzyme analysis in carriers of the CBS insertion suggested that an additional mutation in the CBS gene (T833C) co-segregated in *cis* with the 844ins68 CBS insertion,[7,14] an association also observed in all 68-bp mutated alleles identified in the present study. Taken together, these findings confirm the close association of both mutations and therefore the pattern of co-inheritance of the double T833C/844ins68 CBS mutation.

The 844ins68 CBS mutation apparently does not result in impaired enzyme activity or hyperhomocysteinemia, but mRNA data provided evidence that the allele carrying the insertion is poorly transcribed.[7,8] The CBS insertion was investigated as a risk factor for arterial vascular disease in previous studies and controversial results were reported.[7,10,11] The role of this genetic variation in arterial thrombosis, therefore, remains unclear. To our knowledge, the CBS insertion has never previously been investigated as a risk factor for venous thrombosis.

In the present study we determined the prevalence of the CBS insertion in patients with verified venous thrombosis and in healthy controls. We investigated a selected population of relatively young patients, in whom any influence of genetic risk factors for vascular thrombosis would be expected to be more easily detected than in older patients. We observed that the CBS insertion is highly prevalent in the Brazilian population, reaching frequencies similar to those previously reported for other populations.[9,10] If the CBS insertion was indeed a risk factor for venous thrombosis, a higher prevalence of this mutation would be expected in the group of thrombosis patients in comparison with the control group. However, the mutation was found in a statistically identical prevalence among controls and patients, yielding a (neutral) relative risk for venous thrombosis linked to the insertion close to 1.0. This finding diminishes the likelihood that the CBS insertion is a risk factor for venous thrombotic disease.

The identification of genetic abnormalities which are of clinical significance and should be screened for in thrombotic patients is currently considered an important step for the management of venous thromboembolism.[3] The findings from the present study do not support the hypothesis that the 844ins68 mutation in the CBS gene is a risk factor for venous thrombosis, indicating that screening for this genetic variation is probably not recommended for patients suffering from venous thrombotic disease.

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Contributions and Acknowledgments

RF was the principal investigator involved in the design of the study, analysis of the data, and interpretation. He wrote the paper with MZ, who was responsible for the general supervision of the investigation and its funding. All of the other authors played a part in the design and execution of the study.

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