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## The G<sup>1456</sup> to T Mutation in the Thrombomodulin Gene Is Not Frequent in Patients With Venous Thrombosis

To the Editor:

Thrombomodulin is a major component of the protein C anticoagulant pathway.<sup>1</sup> Biochemical detection of thrombomodulin defects are hampered by its location in the endothelial cell. The characterization of molecular alterations in the thrombomodulin gene of selected patient populations represents an alternative diagnostic approach. Recently, a point mutation G<sup>1456</sup> to T in the thrombomodulin gene of a 45-year-old man with venous thrombosis and that of his son was detected.<sup>2</sup> The mutation predicts an Asp<sup>468</sup> to Tyr amino acid substitution and creates a novel restriction site for *Rsa* I digestion in the gene. To assess the frequency of this mutation in patients with venous thrombosis, we screened 100 Italian patients consecutively referred to the Thrombosis Center for a documented episode of venous thrombosis and 100 age- and sex-matched asymptomatic controls. The most frequent thrombotic episode was deep vein thrombosis of the lower limbs (54%), followed by superficial thrombophlebitis (21%), pulmonary embolism (14%), cerebral vein thrombosis (7%), and visceral vein thrombosis (4%). Characteristics of the patients are shown in Table 1.

The G<sup>1456</sup> to T mutation was searched for by amplification and restriction analysis. Oligonucleotide primers employed were as originally described by Ohlin and Marlar,<sup>2</sup> ie, 5'-CGGTACCTTCGAGTGCATCT-3' (TM 23) and 5'-ACGGCCGGAGGAGTCAAGGT-3' (TM 24B). The original polymerase chain reaction (PCR) method was used with minor modifications (50  $\mu$ L reaction mixture, with annealing at 65°C). In addition, a DNA fragment containing the G<sup>1456</sup> to T mutation was constructed as a positive control. Briefly, two sets of oligonucleotides carrying the mutation of interest (5'-CAGGTGCCAGATGTTTTGCA-3' and 5'-TGTCGCCACCGT-ACACCTTGCCGGA-3'; 5'-CGGCAAGGTGTACGGTGGCGA-

CAGC-3' and 5'-TGGACGGAGCCAGGCTCCTG-3') were used to produce two PCR products with overlapping sequences at the mutagenized end. After the first round of PCR, 1  $\mu$ L of 1:100 dilution of the PCR products were used to amplify, in a second round of PCR, a 153-bp fragment using the TM 23 and TM 24B primers. The *Rsa* I digestion pattern of the mutagenized PCR products showed the introduction of a G to T substitution at nucleotide 1456. Soluble thrombomodulin levels were also measured in patients and controls by enzyme-linked immunosorbent assay (Asserachrom Thrombomodulin; Diagnostica Stago, Asnières, France), because the reported patient with the mutation had soluble thrombomodulin levels less than the normal laboratory range.<sup>2</sup>

We could not find the G<sup>1456</sup> to T mutation in any of the patients and controls; two of the patients and four control individuals had soluble thrombomodulin levels less than the normal laboratory range (mean  $\pm$  2 SD calculated from 100 determinations in healthy subjects; 7 ng/mL). We conclude that the G<sup>1456</sup> to T thrombomodulin gene mutation is not frequently associated with venous thrombosis in Italy and that it does not account for a significant proportion of the thrombotic episodes that remain still unexplained by the known causes of hereditary thrombophilia.<sup>3</sup> The low levels of soluble thrombomodulin antigen found in two patients and four control individuals may be due to acquired causes such as an increased turnover or to a different and as yet unidentified genetic defect in the thrombomodulin gene.

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**Table 1. Characteristics of 100 Patients With Venous Thrombosis**

Median age (range) of patients*	44 (17-73)
Sex (Males/Females)	40/60
Median age (range) at first thrombotic episode	35 (12-65)
Patients with 1 thrombotic episode* (%)	54
Patients with 2 thrombotic episodes* (%)	26
Patients with 3 or more thrombotic episodes* (%)	20
Median (range) soluble thrombomodulin (ng/mL)	
Patients	16 (5-62)
Controls	16 (3-47)

\* At time of referral.

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