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Antithrombin III Milano 2: a single base substitution in the thrombin binding domain detected with PCR and direct genomic sequencing

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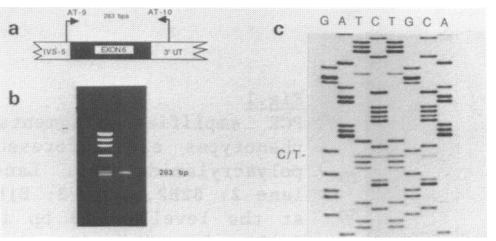
Mutations affecting the antithrombin III [ATIII] gene represent one of the commonest causes of an inherited thrombotic tendency. ATIII deficiency may be classified as either type I, with decreased plasma concentration of the protein, or type II where the protein is quantitatively normal but a variant form can be identified in the plasma (1). ATIII Milano 2 is a type IIb variant; the abnormal protein has impaired thrombin inhibitory activity (2). Thrombin inhibition involves the cleavage of the Arg393-Ser394 [P_1-P_1'] bond of the ATIII molecule (3), this region being encoded within exon 6 of the gene. We have amplified exon 6 of the ATIII gene from a patient with ATIII Milano 2, using the polymerase chain reaction [PCR] (4). The PCR product was purified from agarose gel by electroelution and each strand of the double stranded template was directly sequenced [Sequenase; USB] using the same primers as utilised in the PCR [Figure 1]. The nucleotide sequence shows the presence of a T in addition to the normal C in the second position of codon 394. This predicts a mutation from serine to leucine (TCG->TTG), identical to the previously reported variant ATIII Denver (5), which also has impaired protease inhibitory activity. The serine residue in the P_1' site is conserved in some of the other members of the serine protease inhibitor family (6). The mutation identified in ATIII Milano 2 emphasises the importance of the P_1' residue in the thrombin inactivation activity of ATIII.



1a. Exon 6 showing the position of the oligonucleotide primers (AT-9 and AT-10) used for amplification by PCR and sequencing. AT-9 5'-CTGCAGGTAAATGAAGAAGGC-3'. AT-10 5'-TGCAGAGTCCATTTATAATGTG-3'.
1b. Agarose gel demonstrating 283 bp fragment resulting from PCR using primers AT-9 and AT-10.
1c. Part of the nucleotide sequence of exon 6 demonstating an additional T in the second position of codon 394.

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