DdeI polymorphism in intron 5 of the ATIII gene

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Description: Primers 5'-GAATTCCTCTGTGGATTGAAG-CCA-3' and 5'-TGCATGCCTTAACACTGGAAACAGGC-3' in introns 4 and 5 respectively of the ATIII gene were designed to amplify a 229 bp genomic DNA fragment containing exon 5. PCRs were performed in 100 μ l reaction volumes containing 1 μ g genomic DNA, 100 pmoles of each primer, 200 μ M of each dNTP in 50 mM KCl, 10 mM Tris HCl, pH 8.8, 1.5 mM MgCl₂, gelatin 200 μ g/ml and 2 U *Thermus aquaticus* polymerase (Cetus). Samples were subjected to 35 cycles of amplification consisting of 3 steps (94°C for 1 sec, 60°C for 1 sec and 76°C extension for 15 sec). On a further cycle the extension time was increased to 10 min.

Polymorphism: During sequence analysis of exon 5 and flanking intervening sequences in suspected ATIII variants, we noted the occurrence of either, or both G and C nucleotides at position 160 in segment 5 of the ATIII sequence (1). The variation occurs at a DdeI cutting site, thus digestion of the 229 bp amplified DNA with DdeI yields a constant fragment of 102 bp and either a single fragment of 127 bp (–) or two fragments of 53 bp (+) and 74 bp (+) (Fig. 1).

Frequency: Estimated from 41 unrelated individuals: Allele + (site present), 82.9%; Allele – (site absent), 17.1%.

Mendelian Inheritance: Co-dominant segregation was shown in two nuclear families and one 4-generation family.

Clinical Relevance: Although it occurs at a lower frequency than the polymorphic PstI site previously identified in exon 4 of the ATIII gene (2), this polymorphism may be of some additional use in segregation studies in families with ATIII deficiency causing thrombosis.

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References: 1) Bock, S.C. *et al.* (1988) *Biochemistry* **27**, 6171–6178. 2) Prochownik, E.V. *et al.* (1983) *New Engl. J. Med.* **308**, 1549–1552.

Figure 1. Detection of a polymorphic DdeI site in the human ATIII gene. Genomic DNA from individuals with (+) or without (–) the DdeI cutting site was amplified as described. Following digestion with DdeI samples were electrophoresed in 2% agarose. M: size markers.

BclI RFLP in fucosidase pseudogene (FUCA1P)

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Source/Description: Probe AF3E2.4 is a 2.4 kb genomic clone from the fucosidase pseudogene FUCA1P (1) isolated from a bacteriophage lambda library and subcloned into the EcoRI site of pAT153.

Polymorphism: BclI detects a single two-allele polymorphism in FUCA1P. As one allelic fragment comigrates with an invariant band, the RFLP is better detected after double digestion with BclI/PstI. This reveals allelic fragments of 12.0 (B₁) and 8.1 kb (B₂).

Frequency: Estimated from 59 unrelated European Caucasoids:

B₁ : 0.58

B₂ : 0.42

Not Polymorphic For: AvaII, BamHI, BglII, BglIII, EcoRI, EcoRV, HincII, HindIII, KpnI, MboI, MspI, PstI, PvuII, RsaI, SacI, SstI, StuI, TaqI, XbaI, XmnI (N = 9).

Chromosomal Localization: FUCA1P was assigned to chromosome 2 by somatic cell hybrids (1).

Mendelian Inheritance: Co-dominant segregation was observed in 3 families (13 individuals).

Probe Availability: AF3E2.4 is available from B.Carritt.

Other Comments: The BclI RFLP at FUCA1P is also recognized by AF3, a cDNA clone from the fucosidase structural gene FUCA1 localized on chromosome 1p34.1–1p36. AF3 also recognizes a PvuII and a BglII RFLP (2), which are not detected by AF3E2.4.

References: 1) Carritt and Welch (1987) *Hum. Genet.* **75**, 248–250. 2) Darby, J.K. (1988) *Am. J. Hum. Genet.* **43**, 749–755.

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