

Dinucleotide repeat polymorphism in a gene on chromosome 20 encoding a G-protein coupled receptor (D20S32e)

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Primers/Description: Two primers (GPR112-1, GTTACATGG-AGAGATGGATC, and GPR112-2, TGTAGATCATATGAA-CTCAG) were used to amplify a 115–125 bp TG repeat-rich region (D20S32e) located in a gene encoding a member of the G-protein coupled family of receptors; the ligand for this receptor is unknown.

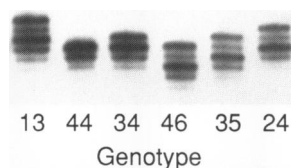
Frequency: Six alleles were observed in 23 unrelated Caucasians. The heterozygosity was 43%.

| Allele (bp) | Frequency |
|-------------|-----------|
| A1 125 | 0.02 |
| A2 123 | 0.09 |
| A3 121 | 0.05 |
| A4 119 | 0.81 |
| A5 117 | 0.05 |
| A6 115 | 0.02 |

Chromosomal Localization: GPR112 was assigned to chromosome 20 using DNA templates prepared from a panel of somatic cell hybrids (Bios, New Haven, CT).

Mendelian Inheritance: Co-dominant inheritance was observed in a five-generation pedigree in which 113 individuals were typed.

Other Comments: The PCR was performed using ³²P-labeled GPR112-1 and unlabeled GPR112-2 for 30 cycles: denaturation at 94°C for 1 min; annealing for 55°C for 1.5 min; and extension at 72°C for 3 min. The PCR products were analyzed on a 5% denaturing polyacrylamide gel (Figure).



A NheI RFLP in the human antithrombin III gene (1q23-q25) (AT3)

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Probe Description: The 1.5 kb EcoRI/HIII fragment of pAT3c (1) was used as a probe, and contains human antithrombin cDNA sequences extending from 46 bp 5' to the initiating methionine codon through 88 bp 3' to the stop codon.

Polymorphism: A single two-allele NheI RFLP is present near the middle of the human AT3 locus. Due to the very large size of the allelic fragment in which the polymorphic NheI site is absent, double digestion with NheI and SpeI is recommended. NheI/SpeI digestion generates a 20 kb fragment from the N(-) allele and a 9/11 kb doublet from the N(+) allele.

Frequency: Estimated from 11 unrelated individuals:
N(+) allele = 0.64
N(-) allele = 0.36

Chromosomal Localization: 1q23-q25.1.

Mendelian Inheritance: Codominant segregation was observed in two families (10 individuals). See Fig. 1 for NheI/SpeI blot of one family.

Probe Availability: pAT3c is available from S.C.Bock.

Other Comments: i) The polymorphic NheI site is located approximately 100 bp downstream from the 3' end of AT3 exon 4 (Fig. 2). It was mapped based on information from NheI/PstI blots on genomic DNAs of known NheI and PstI (ref. 2 in (2)) RFLP haplotypes. ii) NheI is a somewhat expensive restriction enzyme, but can be used at low concentrations for extended incubation times.

Acknowledgements: We thank Drs. M. Hultin and U. Albigdaard for supplying the family shown in Fig. 1. Supported by USPHS HL-30712 and American Heart Association Established Investigatorship # 88-0261.

References: 1) Bock, S.C. and Levitan, D.J. (1983) *Nucl. Acids Res.* **11**, 8569–8582. 2) Daly, M.E. and Perry, D.J. (1990) *Nucl. Acids Res.* **18**, 5583.

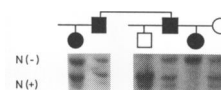


Figure 1: Inheritance of NheI RFLP in a family with AT3 deficiency. Solid symbols indicate AT3 deficient individuals.

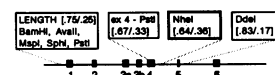


Figure 2: Position of polymorphic NheI site in the human antithrombin III locus. The positions and allele frequencies [] of the previously reported length (1), exon 4–PstI (ref. 2 in (2)), and DdeI (2) RFLPs are also noted.

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