Hypervariable polymorphism in the APOC3 gene

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Source/Description: A search for repetitive elements within the APOA1/APOC3/APOA4 cluster revealed a $(C_a(T_b))_c$ sequence with some variation in the third intron of APOC3, with *a* ranging from 1 to 3, *b* from 1 to 10, and *c* from 50 to 54 (1, 2). Polymorphisms of this repeat was readily detected by performing PCR (3) with oligonucleotides flanking the repetitive sequence, and examining the amplified sequences on DNA sequencing gels.

Primer Sequences:
Oligo 1 = GCATTCCTCCCAGGTCCCTC
Oligo 2 = AGCCGAGATGGCACCACTGC

Frequency: This was estimated in 30 unrelated Caucasian English individuals, in whom 24 alleles could be distinguished:

	Allele (nt)	Frequency		Allele (nt)	Frequency
K1	359	0.0166	K13	344	0.0833
K2	358	0.0333	K14	343	0.0666
K3	356	0.0166	K15	342	0.05
K4	354	0.0166	K16	341	0.0166
K5	353	0.0166	K17	340	0.0333
K6	352	0.0666	K18	339	0.05
K7	351	0.0333	K19	336	0.05
K8	350	0.0333	K20	334	0.05
K9	348	0.0666	K21	332	0.05
K10	347	0.0333	K22	331	0.05
K11	346	0.0333	K23	328	0.1
K12	345	0.0166	K24	327	0.01

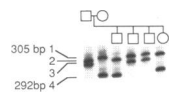
Only one of the 30 individuals was homozygous. The heterozygosity index was 0.95, and the polymorphic information content 0.94. Further alleles e.g. 317 nt have also been noted (Figure). A constant band measuring 340 nt was seen in 4 of 30 individuals.

Chromosomal Localisation: APOA1 and APOC3 genes have been localised to the q23 to q24 region of chromosome 11 (4).

Mendelian Inheritance: Co-dominant segregation was demonstrated in 13 pedigrees.

Other Comments: The PCR reaction was performed on genomic DNA (3) using end labelled oligo OLIGO 1 (~0.13 ng/μl) and unlabelled OLIGO 1 and 2 (0.5 ng/μl of each), 1.4 mmol $MgCl_2$, and 0.25 μl of PerfectMatch® (Stratagene) in a 25 μl reaction volume. DNA was denatured at 94°C for 5 min, followed by 30 one min cycles of denaturing at 94°C, annealing at 64°C, and extension at 72°C, with a final extension step of 9 min. The PCR products were sized on a 6% denaturing polyacrylamide gel by simultaneously running the dideoxy chain termination reaction products of phage M13mp18 (Sequenase 2.0).

References: 1) Protter,A.A., Levy-Wilson,B., Miller,J., Bencen,G., White,T. and Seilhamer,J.J. (1984) *DNA* **3**, 449–456. 2) Shelley,C.S., Sharpe,C.R., Baralle,F.E. and Shoulders,C.C. (1985) *J. Mol. Biol.* **186**, 4351. 3) Saiki,R.K., Galfand,D.J., Stoffel,S. *et al.* (1988) *Science* **239**, 487–491. 4) Junien,C. and McBride,O.W. (1989) *Cytogenet. Cell. Genet.* **51**, 226–258.



Biallelic ApaI polymorphism of the human p53 gene (TP53)

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Source and Description of Clone: php53c1 is a recombinant cDNA clone that encodes total human p53 cDNA (1).

Polymorphism: When exons 7, 8 and 9 of the human p53 gene are PCR amplified using oligos 5'GTGTTATCTCCTA-GGTTGGC and 5'AGACTTAGTACCTGAAGGGT a 780 bp fragment results which, when cut with ApaI gives
J1 allele, 2 fragments of 604 bp and 176 bp
J2 allele, 1 fragment of 780 bp
ApaI (GGGCCC) identifies a 2 allele polymorphism in total genomic DNA. The A1 allele has two bands at approximately 6.3 and 5.3 kb. A2 allele has 1 band at about 9 kb. There is a constant less intense band at 1 kb.

Allele Frequency: The allele frequencies are A1: 0.946 and A2: 0.054. Heterozygosity was studied in 60 female breast cancer patients (A2 heterozygous in 6 or 10%) and in 23 unrelated placental, tonsil and blood leucocyte DNAs (A2 heterozygous in 3 or 13%), giving an overall observed heterozygosity (A1/A2) of 11%. The expected frequencies would be A1/A1 = 89.5%; A1/A2 = 10.3%; A2/A2 = 0.3%.

Chromosomal Location: The human p53 locus has been localized to the short arm of chromosome 17 (17q13.1) (2).

Probe Availability: Reference 1.

Other Comments: No problems on RFLP analysis under normal stringent conditions nor with PCR amplification followed by restriction digestion and kinase labelling. Because the ApaI polymorphism is within the p53 gene (intron 7 where GGGCCC→GGGTCC), when both alleles are constitutively present it can be used to detect loss of a p53 allele in tumour material.

Not detected by the ApaI enzyme is a second change which occurs in the A2 allele. Twenty bases 3' to the C→T change is a T→G change. In the seven sequenced samples both changes are found concurrently.

References: 1) Zakut-Houri *et al.* (1985) *EMBO J.* **4**, 1251–1255. 2) Isobe *et al.* (1986) *Nature* **320**, 84–85.