Microsatellite polymorphism in the human platelet glycoprotein IIIa gene (GP3A) on chromosome 17

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Primers/Description: Two primers (GPIIIa-1, 5'-AGCCATTGC-TCCTAGTGGAG-3', and GPIIIa-2, 5'-GCTAAATCATCC-TTAGCCTTC-3') were used to amplify a 166–184 bp CT-repeat located in intron 6 of the human platelet glycoprotein IIIa gene (1). Accession numbers M32673 and J05427, from bp 3873–3902.

Frequency: Eight alleles were observed in 57 unrelated Caucasians. The observed heterozygosity was 61%.

Allele	bp	Frequency	Allele	bp	Frequency
B1	184	0.009	B2	182	0.009
B3	180	0.202	B4	178	0.421
B5	176	0.009	B6	170	0.052
B7	168	0.009	B8	166	0.289

Chromosomal Localization: *GP3A* was assigned to chromosome 17q21–23 (2, 3).

Mendelian Inheritance: Co-dominant inheritance was observed in 6 CEPH families with a total of 44 meioses.

Other Comments: The PCR was performed using ^{32}P-labeled GPIIIa-2 and unlabeled GPIIIa-1. DNA was initially denatured at 94°C for 6 min and followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 2 min, and extension at 72°C with a final extension step of 10 min. The PCR buffer contained 2.0 mM $MgCl_2$. The PCR products were analysed on a 5% denaturing polyacrylamide gel.

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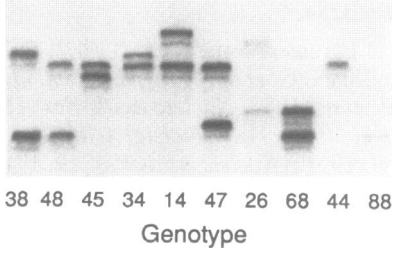

Figure 1. PCR amplification of CT-repeat DNA polymorphism in the human GP3A gene. The genotypes are noted at the bottom of the figure.

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PCR detection of a *BglII* polymorphism in intron I of the human p53 gene (TP53)

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The human p53 gene is thought to play a major role in tumor suppression (1). It contains a *BglII* polymorphism that is located in intron 1 (2).

We present a PCR method to analyze this *BglII* polymorphic. The precise localization of the *BglII* site as well as a sequence variation were determined by direct sequencing of PCR-amplified products.

Polymorphism: Using primers and PCR conditions as described below, *BglII* (A'GATCT) digestion of PCR products identifies two alleles: G1 = 158 bp, G2 = 128 bp and 30 bp. Direct sequencing located the polymorphic *BglII* site at position 8545.

PCR Primers: Sense oligomer: 5'-TTTAGGAGTGGGGGT-GGGAG-3' bp 8516–8535 (3) Antisense oligomer: 5'-GTAGA-GTTGAGGAAAGTGCTGG-3' bp 8652–8673 (3).

PCR Conditions: PCR's were carried out in a total volume of 100 µl containing: 500 ng of genomic DNA, 50 pmoles of each primer, 2 mM $MgCl_2$, 250 µM dNTPs, 50 mM KCl, 10 mM Tris–HCl (pH 8.4), 0.001% gelatine and 2.5 units Taq DNA polymerase (Life Technologies). Amplification conditions: 35 cycles 1 minute at 95°C, 1 minute at 59°C and 1 minute at 72°C in a Perkin-Elmer Cetus thermocycler® .

Frequency/Inheritance: In 57 unrelated caucasians. Allele G1 = 0.92, Allele G2 = 0.08. (PIC = 0.10) as in (4). Codominant segregation was confirmed in 2 two-generation families.

Chromosomal Localization: The human p53 gene is located at chromosome 17p13.1 (5).

Precise Localization by Direct Sequencing/Sequence Variation: Direct sequencing (6) of 9 individuals (4 homozygotes A1, 3 heterozygotes, 2 homozygotes A2 for the *BglII* polymorphism) revealed that in all alleles investigated an adenine residue was present at position 8545 instead of the reported thymidine (3). Supplemented with the presence in some alleles of a thymidine instead of the more common cytidine at position 8550 (3) this results in the additional *BglII* recognition site.

Acknowledgments: This work was supported by the Bekalis Foundation, the Maurits en Anna de Kock Foundation and the Ank van Vlissingen Foundation.

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