A highly informative CACA repeat polymorphism upstream of the human dystrophin gene (DMD)

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Source/Description: The polymorphic (CA)₁₄ repeat is located approximately 1.5 kb upstream of the first brain-type exon of the human dystrophin gene at Xp21 (1, 2).

CA Primer: 5'-TAGCTAAAATGTATGAGTA-3'

GT Primer: 5'-AATAGTGTTTTCCTAAGGG-3'

Frequency: Determined from 68 chromosomes of unrelated individuals. PIC: 0.78.

Allele (bp)	Frequency	Allele (bp)	Frequency
AG9: (88)	0.34	AG5: (100)	0.25
AG8: (94)	0.01	AG4: (102)	0.03
AG7: (96)	0.01	AG3: (104)	0.11
AG6: (98)	0.10	AG2: (106)	0.14
		AG1: (108)	0.01

Mendelian Inheritance: Co-dominant segregation was observed in seven informative families.

Other Comments: The PCR reaction (25 µl) contains 200 ng DNA, 1 µM of each primer, 3 units of Taq polymerase (Cetus), 0.2 mM each dNTP, 1.5 mM MgCl₂, 50 mM KCl and 10 mM Tris-HCl pH 8.3. Amplification conditions: 94°C (7 min), followed by 25 cycles of 94°C (1 min), 55°C (2 min), 72°C (3 min). Alleles may be observed either on a 12% polyacrylamide gel or, for some of them, on a 3% NuSieve (FMC®) + 1% agarose gel. The polymorphism is useful in determining the inheritance of DMD in individuals at risk (see Figure).

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References: 1) Nudel, U., *et al.* (1989) *Nature* **337**, 76–78. 2) Boyce, F.M. *et al.* (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1276–1280.

Note Added in Proof: This CA repeat corresponds to the 5'-DYS-II marker that was published by Feener *et al.* (*Am. J. Hum. Genet.* (1991), **48**, 621–627) while the present report was submitted.

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AatII polymorphism in von Willebrand factor gene at codon 471

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Source/Description: Published nucleotide sequences of the human von Willebrand factor gene show a nucleotide difference at the first base of codon 471 in comparison with other published sequences (1, 2, 3). A potential *AatII* recognition site is present when the base is G (GACGTC) but absent when it is A.

Polymorphism: Detected using the PCR and subsequent digestion of the product with *AatII*. PCR conditions: 50 mM KCl, 10 mM tris-HCl pH 8.5, 1.5 mM MgCl₂, 0.01% gelatin, 100 µM of each dNTP, 250 ng of genomic DNA, 1 U of *T.aq.* DNA polymerase and 0.5 µM of each primer A and primer B in a total volume of 100 µl. The following PCR regime: 94°C for 3 min; 55°C for addition of enzyme; 30 cycles of: 72°C for 1 min; 94°C for 30s and 55°C for 30s; 72°C for 10 min. PCR product (5 µl) was diluted to 20 µl with 15 µl of 1×*AatII* buffer (50 mM KCl, 10 mM tris-HCl pH 7.5, 10 mM MgCl₂ and 1 mM dithiothreitol) containing 2 U of *AatII*. The digest was incubated at 37°C for 3h and then analysed by PAGE using a 6% gel. *AatII* (–/–) individuals show one band at 229 bp, *AatII* (+/+) individuals show two bands at 154 bp and 75 bp, and *AatII* (+/–) individuals show all three bands.

Primers:

Primer A: 5' GTGCAGCAAAGTCACCCTGTCTT3',

Primer B: 5' TGCTAAGGGATGGGCTGTGCCA3',

Primers A and B correspond to nucleotides 9/60–82 and 9/267–288 respectively in Mancuso *et al.* (1).

Chromosomal Location: 12pter-p12; vWF gene; codon 471.

Mendelian Inheritance: Codominant inheritance observed in one family.

Frequency: Estimated from 40 unrelated caucasian individuals (80 chromosomes 12).

(+) = 0.45 (–) = 0.55 heterozygosity rate = 0.49

References: 1) Mancuso, D.J. *et al.* (1989) *J. Biol. Chem.* **264**, 19514–19527. 2) Bonthron *et al.* (1986) *Nucl. Acids Res.* **14**, 7125–7127. 3) Vewey *et al.* (1986) *Embo J.* **5**, 1839–1847.

A: undigested PCR product.
B: *AatII* digested PCR product.
m: pBR322 digested with *MspI*.