A new TaqI polymorphism in the p53 gene

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Polymorphism: Point mutation (A→G) at codon 213 of p53 cDNA (1) that abolishes a TaqI restriction site (CGA→CGG, arg→arg). The polymorphism was found by direct sequencing of the amplified exon 6 fragment showing an abnormal SSCP (2) pattern. The PCR procedure used to obtain amplified fragments corresponding to p53 exon 6 was performed as previously described (Gaidano *et al.*, 3). Briefly, we performed thirty cycles of denaturation (94°C), annealing (63°C), and extension (72°C) on an automated heat-block (DNA Thermal-Cycler; Perkin-Elmer Cetus) using the following primers:
5′-ACAGGGCTGGTTGCCCAGGGT-3′ (5′ primer).
5′-AGTTGCAAACCAGACCTCAG-3′ (3′ primer).

Allele Frequency: 10.8% in Italian population, as determined by TaqI restriction enzyme analysis of the p53 exon 6 amplified fragments. We analyzed 51 normal subjects (102 chromosomes), where 9 heterozygous and 1 homozygous individuals were found. The polymorphism CGA→CGG at codon 213 here reported, has been ascertained to be responsible for the abnormal SSCP pattern previously observed in approximately 2% of lymphoma samples (3).

Mendelian Inheritance: Co-dominant inheritance as determined by analyzing the segregation of the mutated allele in two informative families.

Chromosome Location: P53 has been mapped to 17p13.1.

Other Comments: This polymorphism may be detected by SSCP (Single Strand Conformation Polymorphism) analysis performed on amplified products containing the p53 exon 6 sequences.

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References: 1) Buchman,V.L. *et al.* (1988) *Gene* **70**, 245–252. 2) Orita,M. *et al.* (1989) *PNAS* **86**, 2766–2770. 3) Gaidano,G.L. *et al.* (1991) *PNAS* **88**, 5413–5417.

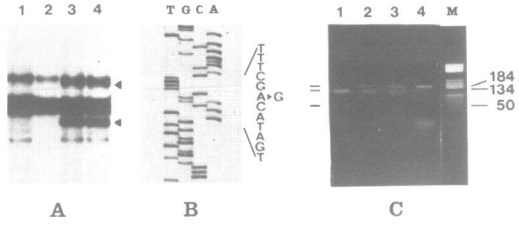

Figure 1. Panel A: SSCP analysis. Abnormal bands are indicated by arrows. Panel B: Direct sequence showing the A→G substitution. Panel C: TaqI digestion of amplified products. Lane 1 and 4 patients homozygous for 134 and 184 alleles; lane 2 and 3 patients heterozygous.

PCR detection of a neutral CGA/CGG dimorphism in exon 6 of the human p53 gene

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Source/Description: Mutations in the human p53 gene have been demonstrated in a large variety of tumors. The majority of these mutations are missense mutations conferring upon the mutated protein transforming abilities (1). In analysis for mutations in the p53 gene in Burkitt's lymphomas, Gaidano *et al.* (2) described silent mutations in codon 213 occurring in 1.78% of the tumors. To determine if this mutation is a polymorphism we have analyzed normal DNA from native South American, African and North American individuals.

Single Strand Conformation Polymorphism analysis and subsequent sequencing of exon 6 of the human p53 gene revealed a dimorphism at the third position in codon 213. This sequence variation can be screened by digestion of the PCR amplified product of exon 6 with TaqI.

PCR Primers:
5′ ACAGGGCTGGTTGCCCAGGGT 3′
5′ AGTTGCAAACCAGACCTCAG 3′

Method: 1 μg of genomic DNA was amplified using 25 pmol of each primer in a reaction volume of 100 μl containing 1 unit of Taq Polymerase, 200 μM dNTPs, 10 mM Tris–HCl pH 8, 50 mM KCl and 2 mM $MgCl_2$. Reactions were cycled for 30 times at 94°C, 62°C and 72°C for 1 min each. Amplified products were digested with TaqI and analyzed by electrophoresis through a 4% agarose gel.

Polymorphism: After TaqI digestion of the amplified product, two alleles can be identified: A1: 140 bp + 40 bp, A2: 180 bp.

Frequency:
Allelic frequency A1: 90%; A2: 10%
From 50 unrelated individuals from South America.
A1: 97%; A2: 3%
From 30 unrelated individuals from North America.
A1: 100%; A2: –
From 23 unrelated individuals from Africa.

References: 1) Hollstein,M. *et al.* (1991) *Science* **253**, 49–53. 2) Gaidano,G. *et al.* (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5413–5417.

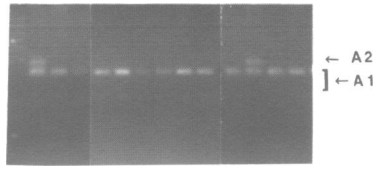

Figure 1. TaqI restricted PCR-amplified DNA product of exon 6 of human p53. DNA samples were obtained from South American individuals. A1 and A2 alleles are highlighted.