RsaI polymorphism of the human growth hormone gene (GH1)

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We report a method to analyze a RsaI polymorphism within the human growth hormone promoter gene using the polymerase chain reaction.

PCR Primers: The primer sequences corresponded to both normal human growth hormone gene (GH1) and the variable human growth hormone gene (GH2), including their promoter regions.
sense oligo 5′ AGAGAGGCAAAGTTGGGTGGTA 3′
antisense oligo 5′ GGTCACAGGGATGCCACC 3′

Polymorphism: The digestion with RsaI (GTAC) produces various fragments proceeding from the two amplified genes. Two of the fragments are very similar in the number of base pairs, therefore a second digestion with BglII (AGATCT) is necessary to be able to differentiate them. BglII is not polymorphic in this region.

The polymorphic fragments obtained are from GH1 and are G1 = 1080 bp + 178 bp and G2 = 1258 bp. Constant fragments: (GH1) — 283 bp, 191 bp, 75 bp and (GH2) — 898 bp, 728 bp, 191 bp. Fragments <728 bp are not shown.

Frequency: Allele frequencies were calculated from 57 unrelated Caucasians.
G1 = 0.87
G2 = 0.13

Chromosomal Localization: The human growth hormone locus has been assigned to chromosome 17q22−24 (1).

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

PCR Conditions: PCR were carried out in a total volume of 100 µl containing: 500 ng of genomic DNA, 0.25 µM of each primer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 50 mM KCl, 10 mM Tris-HCl pH 8.0, 10 µM TMAC (2) and 2.5 U Taq polymerase. The amplification is performed for 30 cycles with an annealing temperature of 65°C. The amplified DNA is digested overnight with a ten-fold excess of RsaI and later with BglII. DNA fragments are resolved by electrophoresis through a 1.5% agarose gel.

Other Comments: In all cases studied a fragment of 1611 bp appears. This fragment was not observed in a patient with deletion of the GH1 gene (data not shown). The digestion with different concentrations of RsaI, with different time-spans and volumes of incubation has not produced any variation.

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PCR-based detection of two MspI polymorphic sites at D18S8

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The polymorphic locus D18S8 has been localised to 18q21.3 (1) and lies within the DCC gene which is deleted in over 70% of human colorectal carcinomas (2). There are two polymorphic MspI sites within the region detected by OLVIIE10 (1) and 15−65 (2), providing four possible haplotypes (2). PCR primers have been designed around the polymorphic MspI sites.

Primer Sequences:
D18S8-M2
D18S8-M2a 5′TTGCACCATGCTGAAGATTGT 3′
D18S8-M2b 5′ACCCTCCCCCTGATGACTTA 3′
The amplified product is 367 bp which on digestion with MspI gives fragments of 367 bp (allele A2 or A3 of OLVIIE10) and 227 bp and 140 bp (allele A1 of OLVIIE10).
D18S8-M3
D18S8-M3a 5′CGACTCGATCCTACAAAATC 3′
D18S8-M3b 5′TCTACCCAGGTCTCAGAG 3′
The amplification conditions used were 35 cycles of 94°C, 30 sec; 58°C, 1 min; 72°C, 1 min.
The amplified product is 240 bp which on digestion with MspI gives fragments of 240 bp (allele A1 or A2 of OLVIIE10) and 137 bp and 103 bp (allele A3 of OLVIIE10).
In each case 200−500 ng of DNA, in a final volume, of 50 µl, was amplified in the presence of 1 µM of each primer, 200 mM dNTP's, 50 mM KCl, 20 mM Tris (pH 8.4), 2.5 mM $MgCl_2$ was used for D18S8-M2 and 1.5 mM $MgCl_2$ for D18S8-M3, and 2 units Amplitaq (Cetus). 10 µl of the amplified product was mixed with 5 units of MspI and 2.4 µl of restriction endonuclease buffer. The DNA fragments were resolved by electrophoresis on a 1.5% agarose gel.

Allele Frequencies: The frequency of the MspI sites was calculated in 20 unrelated Caucasians. The values are in rough agreement with those detected by Southern blotting (1, 2).
D18S8-M2 allele A2 or A3 = 0.43 allele A1 = 0.57
D18S8-M3 allele A1 or A2 = 0.70 Allele A3 = 0.30

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