Codon 367 polymorphism of the human type I β -hydroxysteroid dehydrogenase/isomerase gene (HS5DB3)

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Description, Source and Method: The enzyme β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 isomerase (β -HSD) plays a key role in the biosynthesis of all classes of hormonal steroids. We have recently reported the sequence of the human type I β -HSD gene (1) which possesses a substitution in exon 4 at Asn³⁶⁷, codon AAC being present instead of ACC encoding Thr³⁶⁷ in the previously described human type I cDNA (2) as well as in the human type II β -HSD cDNA (3). PCR was performed using human type I β -HSD specific primers flanking exon 4: 5'-GTGGTTGGCACCTCTTAGGGATATA-3' and 5'-GTGCCCTTGTCACCTTCTGTATGAG-3', thus giving a 971 bp fragment. PCR was carried out in a 100 μ l reaction volume containing 0.25 μ g genomic DNA, 25 pmoles of each primer and reagents from the Gene Amp kit (Perkin-Elmer, Cetus). Amplifications were performed for 30 cycles (95°C, 1 min; 60°C, 1 min; 72°C, 1 min). After removal of the primers (4), 1/30 of the PCR products was subjected to asymmetric amplification for 40 cycles with 1/50 of the reverse primer using, otherwise, the same PCR conditions. One third of the PCR products was then used for direct sequencing with the reverse primer: 5'-TATGAGGCCAGGAGGGTGGAGCTTGA-3'.

Frequency: Frequency of the two alleles in 39 unrelated Caucasians

AAC (Asn³⁶⁷): 0.68 (C1)ACC (Thr³⁶⁷): 0.32 (C2)

Chromosomal Localization: 1p13 (5).

Mendelian Inheritance: Co-dominant segregation was observed in two informative families whose DNA samples were kindly provided by Dr M.C. Thibault from the DNA bank of the Réseau de Médecine Génétique du Québec.

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Two dinucleotide repeat polymorphisms at the D6S202 locus

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Source and Description of Clone: HZ-30 is a lambda clone that was isolated from a human chromosome 6p specific library. Two *Eco*RI subclones of HZ-30, pHZ-30/2.6 and pHZ-30/870, containing a (CA)₂₀ dinucleotide repeat, were isolated. Sequences flanking the dinucleotide repeats were used to design PCR primers. The predicted length of the amplified sequence was 142 bp in the pHZ-30/2.6 subclone (EMBL database accession number X60990) and 150 bp in the pHZ-30/870 subclone (EMBL database accession number X60989).

Primer Sequences:

pHZ-30/2.6: 5'-TCATACTGGAAGCTTAAACCA-3' (AC strand) and

5'-ACTATCTCAGGGTTCTCAAT-3' (GT strand)

pHZ-30/870: 5'-CTCTGCTAGGCACTGTACTA-3' (AC strand) and

5'-TCTGGAAAATAAGAATGCT-3' (GT strand)

Frequency: Estimated from unrelated CEPH individuals using 56 chromosomes for pHZ-30/2.6 (A1–A12) and 36 chromosomes for pHZ-30/870 (B1–B6). The observed heterozygosity is 0.68 for pHZ-30/2.6 and 0.50 for pHZ-30/870.

Allele (bp)	Frequency	Allele (bp)	Frequency
A1 154	0.04	A7 142	0.06
A2 152	0.08	A8 140	0.02
A3 150	0.14	A9 138	0.02
A4 148	0.26	A10 136	0.02
A5 146	0.14	A11 134	0.02
A6 144	0.18	A12 130	0.02

Allele (bp)	Frequency	Allele (bp)	Frequency
B1 155	0.02	B4 147	0.67
B2 153	0.11	B5 145	0.14
B3 149	0.03	B6 143	0.03

Chromosomal Localization and Mendelian Inheritance: Assigned to chromosome 6p using DNAs isolated from a panel of somatic cell hybrids. Codominant segregation was observed in a large seven generation kindred.

PCR Conditions: The PCR reaction was performed using 40 ng of genomic DNA and 100 pmoles of each primer in a volume of 100 μ l. The samples were processed through 30 cycles consisting of 2 min at 94°C, 1 min at 45°C and 1 min 72°C. Allele sizes were determined by comparison to M13 DNA sequencing ladder.

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