Dinucleotide repeat polymorphism in the human X-linked GABA_A receptor α 3-subunit gene

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Source/Description: A clone (λ hGR α 3.1) was isolated from a human genomic library using the bovine GABA_A receptor α 3-subunit cDNA (1). This was shown, by Southern blot hybridization and DNA sequence analysis, to contain an exon of the human GABA_A receptor α 3-subunit gene. An (AC)₁₅ repeat (EMBL database accession number X55438) was similarly identified in the clone, and sequences flanking this were used to design PCR primers.

Primer Sequences: MGD341: 5'-TCCTGAGGGCAGGGTCT-CTGATT-3';

MGD342: 5'-GGGTTTCAGGAGACTGCACAGCAA-3'

Frequency: Four alleles, each observed as an intense band that is flanked by two minor bands, were detected in 72 unrelated European Caucasians. The major band was scored, and its size was determined relative to a DNA sequence ladder.

Allele	Size in nucleotides	Frequency
A1	169	0.80
A2	167	0.01
A3	165	0.18
A4	163	0.01

From these values a PIC of 0.29 was calculated.

Mendelian Inheritance: This polymorphism was shown to be informative in 36% of CEPH parental DNAs tested. Mendelian inheritance was demonstrated in 80 meioses from 10 of the informative families in the CEPH panel.

Chromosomal Localization: Previously, we (2) localized the neuronally-expressed human GABA_A receptor α 3-subunit gene (GABRA3) to the X-chromosome, in Xq28.

Other Comments: PCR amplifications were performed in a total volume of 25 μ l containing: 20–200 ng genomic DNA, 26 pmoles of each primer, 200 μ M of each dNTP, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 0.75 units *Taq* DNA polymerase, 0.01% (w/v) gelatin and 1.5 μ Ci [α -³²P]dCTP (~3000 Ci/mmol; Amersham). Amplification was for 30 cycles with denaturation at 94°C for 30 seconds, annealing at 66°C for 50 seconds, and extension at 72°C for 10 seconds.

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References: 1) Levitan, E.S. *et al.* (1988) *Nature* **335**, 76–79. 2) Buckle, V.J. *et al.* (1989) *Neuron* **3**, 647–654.

PCR detection of the MspI polymorphism in the human IRBP gene (RBP3)

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The gene (Fig. 1) for interphotoreceptor retinoid-binding protein (IRBP) (1) contains a MspI (2) and a StyI polymorphism (3) near each other. Here we show the precise localization of the MspI RFLP in Intron A at position 4591 relative to the Cap site, based on sequence analysis of PCR-amplified human genomic DNAs. A single-base substitution of A to C generates the MspI site:

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ALLELE 1 - CTCTACAAAGTGCTTAGTACAGGGCCAGGTGCTGGTAAAGGTAACAA
ALLELE 2 -      |                      C                      |
                4571                      4591                      4621
  
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Polymorphism: MspI digestion of PCR products identifies two alleles using primers and PCR conditions described below. Allele 1 = 515 bp, Allele 2 = 394 bp and 121 bp. The primers include an EcoRI site for easy subcloning:

Sense oligo:

5'-GACTAGTTACTTAATGAATTCGTGCTTCAGTTTTTCCATGG-3'

Antisense oligo:

5'-TTCAAATACTTCAGGGAATTCGATCTGTAAGAGTTGGAGGT-3'

PCR Conditions: Amplification was performed as recommended by Perkin Elmer-Cetus, with 40 cycles at 94°C for 1 min, 1 min at 62°C, and 1 min at 70°C. The amplified product was digested with MspI for 1 hour at 37°C and electrophoresed through an 8% polyacrylamide gel in 1×TAE buffer.

Frequency/Inheritance: Allele frequencies were calculated from 17 unrelated individuals.

Allele 1 = 0.65,

Allele 2 = 0.35.

Co-dominant segregation is observed in a family (Fig. 2).

Chromosomal Location: The IRBP gene (RBP3 locus) has been assigned to 10q11.2 to 10q21.1 by many groups (for example, 1).

References: 1) Nickerson *et al.* (1988) in Piatigorsky *et al.* Eds.

Molecular Biology of the Eye. Alan R. Liss New York pp 93–105. 2) Simpson, N.E. *et al.* (1987) *Nature* **328**, 528–530.

3) Chin *et al.* (1988) *Nucl. Acids Res.* **16**, 1645.

Fig. 1.

Fig. 2.

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