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RFLP Report

DINUCLEOTIDE REPEAT POLYMORPHISM IN 65k-GLUTAMATE DECARBOXYLASE GENE*

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65k-Glutamic acid decarboxylase (GAD65), γ-amino butyric acid synthesizing enzyme, plays a role not only in regulation of inhibitory neurotransmission, but also in pathogenesis of insulin-dependent diabetes mellitus and Stiffman's syndrome (Baekkeskov *et al.*, 1990). The GAD65 gene is located in chromosome 10p11.23 (Bu *et al.*, 1992). To facilitate the genetic studies of the GAD65 related disorders, we identified a polymorphic CA repeat marker in the GAD65 gene. A human genomic EMBL3 clone encoding the GAD65 gene was isolated and a CA repeat region was identified in the intron 15 (Kure *et al.*, 1994). The repeat number of the CA was highly polymorphic, suggesting the usefulness of this genetic marker. A $(CT)_7(CA)_n$ compound dinucleotide repeat was previously reported in the other region of the GAD65 gene (Wapelhorst *et al.*, 1995). Those two polymorphic markers may provide the useful tools for studying the GAD65 related disorders.

PCR primers

HGAD62 (CA primer): 5'GTATCATGTCCAGGAACCAG3'

HGAD53 (GT primer): 5'FAM-ACTGGACAGCATAGATTATTTCACC3'

Polymorphism and frequency

Thirteen alleles were detected in 508 chromosomes of unrelated Japanese individuals and the observed heterozygosity frequency was 0.71.

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Allele	Size (bp)	Frequency
A1	128	0.03
A2	130	0.53
A3	132	0.04
A4	134	0.02
A5	136	0.01
A6	138	0.01
A7	140	0.06
A8	142	0.17
A9	144	0.04
A10	146	0.05
A11	148	0.01
A12	150	0.02
A13	152	0.01

Chromosomal localization. The GAD65 gene has been assigned to chromosome 10p11.23 (Bu *et al.*, 1992).

Mendelian inheritance. Codominant inheritance was observed in two families.

Other comments. We screened the EMBL3 human genomic library with a GAD65 cDNA fragment and identified a CA repeat region by probing with $(CA)_{15}$ oligonucleotide. Sequencing analysis disclosed the franking sequences of the repeat region and a search of Genebank revealed no matching sequences. To elucidate the polymorphic profile of the CA repeat region, the regions of many individuals were amplified by PCR and the length of each PCR product was determined. The PCR reaction was carried out in a volume of 15 μl containing 10 ng genomic DNA, 2.5 pmol of PCR primers, 200 μM dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, 0.001% gelatin, 10 mM Tris-HCl (pH 8.4), and 1 U *Taq* polymerase (Amersham, Little Chalfon, England) in a 96-well type thermal cycler (PCR9600, Perkin-Elmer Corp. Norwalk, Conn., USA). Each thermocycle consisted of 94°C for 8 sec, 55°C for 1 sec, and 72°C for 10 sec, and was repeated for 32 cycles. The forward primer was labelled with a fluorescence dye, FAM (Perkin-Elmer Corp.) to measure the length of the PCR products in the automated fluorescence DNA sequencer (A.L.F. Sequencer, Pharmacia, Uppsala, Sweden).

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