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RFLP Report

IDENTIFICATION OF A SINGLE BASE POLYMORPHISM IN INTRON 2 OF THE c-*fos* GENE AND ITS DETECTION BY MISMATCHED PCR-RFLP

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A T→C transition in intron 2 of the c-*fos* gene was identified by sequencing analysis (Fig. 1). A simple method to detect this polymorphism was developed by mismatched PCR-RFLP. Allele frequency of this polymorphism was determined in the Japanese and in the Caucasian, separately.

Key Words Alzheimer's disease, c-*fos*, polymorphism, mismatched PCR-RFLP

Primer sequences for mismatched PCR.
FOS3-5: 5′-CAGACACTTTTACTGAATGTCG-3′
FOS3-3K: 5′-TCTTCTTCTGGAGATACCTAG-3′

This primer has a single base mismatch (underlined) which creates a detectable *Sty*I site for allele b (CCTAGG), but not for allele a (TCTAGG) (Straaten *et al.*, 1983). PCR amplification followed by digestion with *Sty*I shows 62 bp for allele a, two bands of 41 bp and 21 bp for allele b (Fig. 2). These products can be analyzed by 20% polyacrylamide gel (29:1).

Allele frequency. Determined by analysis of 51 CEPH parents: a:0.94; b:0.06. Observed heterozygosity was 0.08. Allele frequency was also analyzed in 20 unrelated Japanese: a: 0.92; b: 0.08, observed heterozygosity: 0.15.

Mendelian inheritance. Mendelian inheritance was demonstrated in two three-generation and one two-generation families.

PCR conditions. PCR was performed in 20 μl containing 100 ng DNA, 1.5 mM of each primer, 150 μM dNTP, 1% formamide, 2 μl 10× buffer (1.5 mM $MgCl_2$) and 1 U Taq polymerase (Perkin-Elmer) for 35 cycles as follows: 94°C for 45 sec, 60°C for 30 sec, 72°C for 45 sec with additional 1 sec in each cycle.

Comments. The c-*fos* gene was evaluated in 4 patients of 4 independent families of early-onset Alzheimer's disease. This polymorphism was not co-transmitted

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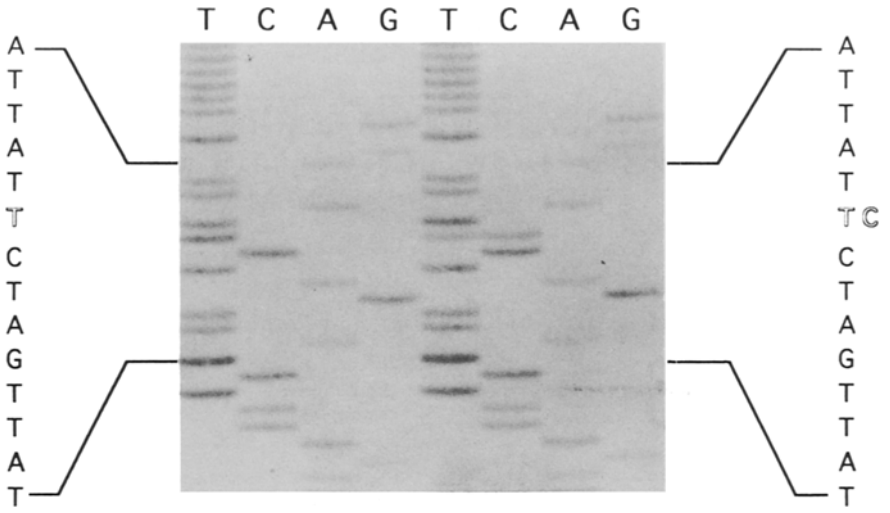

Fig. 1. Direct sequencing of the exon 3-intron 2 boundary of the c-*fos* gene. ATTAT T/C CTAG is the sequence of intron 2 and TTAT is that of exon 3.

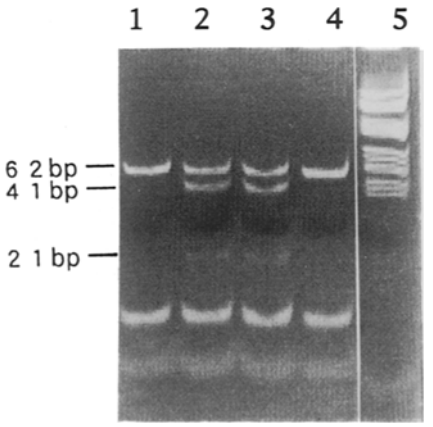

Fig. 2. Detection of a single base polymorphism by mismatched PCR-RFLP. Homozygotes of allele a (line 1 and 4), and heterozygotes of allele a and b (lane 2 and 3) are shown. Lane 5 is MW marker (marker V, Boehringer-Mannheim).

with the onset of the disease. No other base changes were found in the exons and the exon-intron boundaries of the c-*fos* gene.

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References

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