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Short Communication

Structure of the Gene Coding for Calcineurin B (*PPP3R1*) and Mapping to D2S358-D2S1778 (Chromosomal Region 2p15)

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Calcineurin is a protein phosphatase with an important role in signal transduction; its calcium-binding regulatory subunit, calcineurin B, is widely present in the brain and is coded by the PPP3R1 gene which was mapped recently to human chromosome 2. Calcineurin has long been considered a candidate for psychiatric and/or monogenic brain disorders. The present study reports the intron-exon structure of the PPP3R1 gene with the proximal intronic sequences, its genetic mapping to D25358-D251778 on chromosome 2p15, and its exclusion in a genetic disorder mapped proximal to this locus.

Keywords: Calcineurin B, PPP3R1 gene, chromosome 2p15, radiation hybrid mapping, intron-exon structure

Calcineurin is a heterodimeric protein phosphatase which plays an important role in signal transduction and is widely conserved in evolution (Goto *et al.*, 1992). It binds to calcium and is regulated by calmodulin (Klee *et al.*, 1987). Calcineurin B (PPP3R1) is the calcium-binding regulatory subunit of the enzymatic complex ; it is coded by one gene in all tissues except testes (Klee *et al.*, 1987; Guerini *et al.*, 1989; Mukai *et al.*, 1991). The PPP3R1 gene was mapped in a panel of human/rodent somatic cell hybrids to chromosome 2 to the corresponding cytogenetic bands 2p15-p16 (Wang *et al.*, 1996).

Carney complex, a familial multiple endocrine neoplasia and lentiginosis syndrome, was mapped by linkage analysis to the D2S123 locus (Stratakis *et al.*, 1996), a sequence-tagged site (STS) which is located on chromosome 2p16 (Spurr *et al.*, 1997). A recent analysis of recombination events in kindreds with the complex allowed for a more precise localization of the *CNC*locus between the D2S391 and D2S378 loci (Kirschner *et al.*, 1997), an area that corresponds to the 2p15–16 cytogenetic bands (Spurr *et al.*,1996). The phenotype of patients with Carney

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complex and a molecular genetic analysis of their tumors (Stratakis *et al.*, 1996b) have suggested that the responsible genetic defect(s) lead(s) to activation of a signaling pathway mediating hormonal effects in the endocrine glands that are affected in this disease. Thus, PPP3R1 seemed to be a likely candidate gene for Carney complex.

In the present study, we report the precise genomic organization and sequences of the intron-exon boundaries of the PPP3R1 gene, its exclusion in Carney complex and refined localization of the gene on the short arm of chromosome 2 by radiation hybrid mapping.

The cDNA sequence (2558bp), deduced amino acid sequence, and restriction map of human calcineurin B (PPP3R1) have been reported (Guerini *et al.*, 1989). Genomic clones containing the gene, which have been reported before (Wang *et al.*, 1996), were sequenced following polymerase chain reaction (PCR) amplification of plasmid DNA, using primers designed from the cDNA sequence in random combinations; the products of these reactions were run on ethidium bromide-stained 1% agarose gels and sequenced directly, as previously described (Hurley *et al.*, 1991) or subcloned into the PCR^{TM}-II vector, using the TA-cloning kit (Invitrogen Corporation, San Diego, CA), and sequenced manually by a commercially available plasmid-sequencing kit (United States Biochemical Corp., Cleveland, OH) with M13 forward and reverse primers for the pBluescript cloning vectors, or, with the use of the ABI PRISM dye terminator reaction kit (Perkin-Elmer, Norwalk, CT), in an ABI PRISM 377 automatic DNA sequencer (Perkin-Elmer, Norwalk, CT). In all cases, the dideoxy chain termination sequencing method (Sanger *et al.*, 1977) was used. Synthetic oligonucleotide primers for amplification and sequencing reactions were prepared using a Gene Assembler Plus (Pharmacia LKB).

Subsequently, intron-based primers were designed as illustrated infigure 1. PCR amplifications of human genomic DNA were performed. For each amplicon, six patients with Carney complex from unrelated kindreds (Stratakis *et al.*, 1996a) were screened for single strand conformation polymorphisms (SSCP) (Stratakis *et al.*, 1996c); DNA from normal subjects was used as a control in these reactions.

The 10,000 rad SHGC G3 radiation hydrid (RH) panel was utilized to determine the location of the PPP3R1 gene with regards to STSs mapped to the chromosome 2p15–2p16 area (Cox *et al.*, 1990). PCR was performed with primers from two amplicons of the PPP3R1 gene: one containing the 5′-untranslated sequence and exon 1 of the gene, and another, containing exon 2 and flanking intronic sequences (figure 1). The products of the reactions were run in agarose gels and scored. The RH mapping data were analyzed as previously described (Cox *et al.*, 1990).

The PPP3R1 gene has five exons; the sequences of the intron-exon boundaries and the approximate size of the introns are shown in figure 1; the sequences of the primers used for SSCP analysis of this gene are indicated. Six unrelated kindreds with Carney complex were screened; no abnormal pattern of migration was observed in any amplicons, with the exception of that containing exon 1 in one patient. Subsequent genomic DNA sequencing revealed that the patient was heterozygous for the C to T polymorphism at codon 32 of the gene (Lin *et al.*, 1994).

RH mapping of the PPP3R1 amplicons placed them centromeric to the region implicated in Carney complex on chromosome 2p16. The placement of the two amplicons was consistent with the presence of a large intronic sequence between exons 1 and 2 of the gene, as predicted by the other data; the amplicon containing exon 1 was most closely linked to framework marker D2S358 (LOD=1,000; $CR_{10,000}$=0), whereas that containing exon 2 was linked to D2S1778 with a LOD of 5.47 and a distance of 31.7 $CR_{10,000}$ (1000 Kb for chromosome 2). D2S1778 is just centromeric to D2S358 in 1:1000 linkage bin 22,

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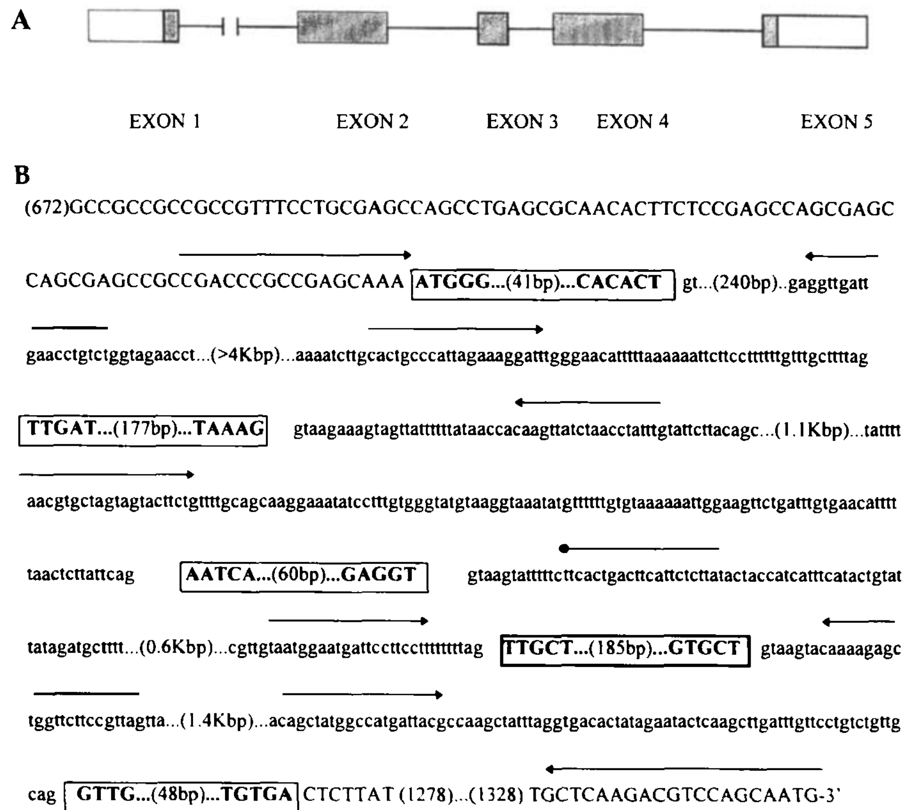

FIGURE 1 (A) Genomic organization of the PPP3R1 gene; the exact size of introns 1 and 4 is not known: ■ coding, and □, untranslated sequences. (B) Partial nucleotide sequence of the intron-exon boundaries of the PPP3R1 gene; the arrows indicate the sequences of the primers used for SSCP and sequence analysis of the gene; the direction of the arrows indicates a forward (->) or reverse (<-) primer. The numbers refer to PPP3R1 cDNA locations as reported by Guerini *et al.* and the sequences in the boxes represent the exons (see also Guerini et al. DNA 8: 675-682, 1989)

while the region implicated in Carney complex is in bin 14.

In conclusion, the present study reports the genomic organization and partial intronic sequences of the PPP3R1 gene, its RH mapping to a linkage bin correponding to cytogenetic bands 2p15–2p16, and its exclusion as a candidate gene in Carney complex.

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