DNA RESEARCH 5, 393–395 (1998) Short Communication

Genomic Organization and Localization of the Human CRMP-1 Gene

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(Received 12 November 1998)

Abstract

The Collapsin Response Mediator Protein-1 (CRMP-1) is a brain specific protein considered to be involved in the collapsin-induced growth cone collapse during neural development. CRMP-1 belongs to the Unc-33 gene family. Here we report the genomic structure and the localization of the human CRMP-1 gene to chromosome 4p16.1. Sequence analysis revealed that the human CRMP-1 gene consists of 14 exons. We have also established sequencing assays for all its coding exons. This should permit the rapid screening for mutations to assess CRMP-1 role in genetic disorders mapped in the 4p16.1 region.

Key words: Human CRMP-1 gene; gene mapping; Exon-intron structure

The cytoplasmic collapsin response mediator protein-1 (CRMP-1), also known as dihydropyrimidinase related protein-1 (DRP-1) is a brain specific protein that belongs to the Unc-33 related protein family.[1,2,3] CRMP-1 predicted amino acid identity is 84% homologous to the chick crmp-62 gene, which is thought to be involved in the signaling cascade initiated by collapsin-1.[1] Collapsin-1, is a secreted glycoprotein that has been shown to induce the collapse and paralysis of neuronal growth cones *in vitro*[4] and to act as a repulsive cue to dorsal root ganglia axons in culture cells.[5,6] The human CRMP-1 gene has previously been mapped to chromosome 4p15-16.1.[3] The positioning of CRMP-1 to this region of chromosome 4p was immediately relevant for the study of the loci for the Wolfram Syndrome (DIDMOAD),[7] which has also been located near this region by linkage analysis.[8,9] To gain further insight into the involvement of CRMP-1 in human disease, we have precisely mapped it and determined its genomic organization.

A P1 clone library (Genome Systems, St. Louis, MO, USA) was screened using markers D4S562 and D4S543 located in the 4p16.1 region. Four P1 clones were isolated (345H12, 658D8, 805A9, and 99B12). To verify the presence of the CRMP-1 gene in these P1 clones, two sets of STSs from the 3′ end of the CRMP-1 gene were used. The first set, including primers Pr3α 5′-aagacggaaacatcaacgtc-3′ and Pr3β 5′-tgattttgacgcgctggtac-3′ produced a 100 bp amplicon named STS-3. The second set, including primers Pr4α 5′-gtttccaggggcatgtatga-3′ and Pr4β 5′-gagcgggagttgcatatttg-3′ produced a 70 bp amplicon, STS-4. In addition, we designed a primer set from the 5′ end of the CRMP-1 gene; Pr1α 5′-caacgggcggatggttattc-3′ and Pr1β 5′-ttggaagaagtcatcagccg-3′ which produced a 95bp amplicon, STS-1. P1 clones 345H12, 658D8, 805A9 and 99B12 were positive for STSs 3 and 4. 99B12 was the only P1 clone positive for STS-1. Based on STS content mapping, the CRMP-1 gene was located between markers D4S562 and D4S1059 with a 3′–5′ orientation (Fig. 1).

To further characterize the gene, we performed chromosome walking on vectorette libraries constructed from the P1 clone 99B12.[10] PCRs were performed using gene-specific primers designed every 100bp from the 5′ end of the CRMP-1 cDNA, and universal vectorrette primers.[10] The PCR products were directly sequenced as previously described.[11] Exons not identified by the vectorette method were amplified using the Boehringer Mannheim Expand^{TM} Long Template PCR System (Boehringer Mannheim,Indianapolis, IN.USA).

Alignment of the sequence of these PCR fragments with the CRMP-1 cDNA (GenBank accession number D78012) allowed us to determine the intron-exon splice junctions and the intronic sequences flanking exons 2 to 14 (Table 1A, Fig. 1). The 14 exons defined here contain all of the nucleic acid sequences observed in the human DRP-1 cDNA. The genomic organization of CRMP-1 is given in Fig. 1. Sizes of exons and introns

Communicated by Mituru Takanami

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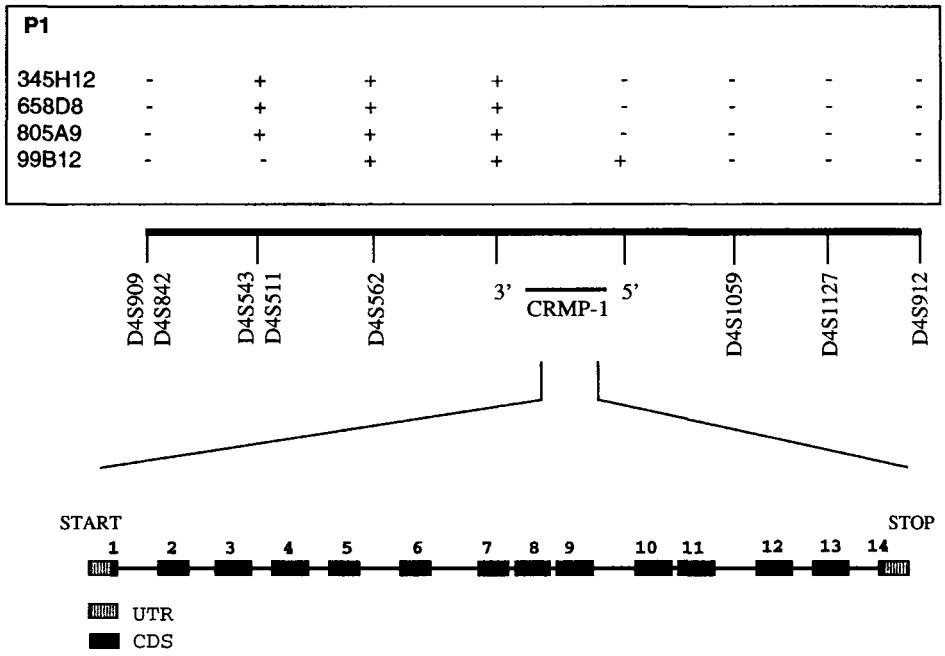

Figure 1. Genomic structure and localization of the human CRMP-1 gene. (Top) STS content mapping of the CRMP-1 gene with P1 clones, 345H12, 658D8, 805A9 and 99B12. (Middle) Localization of the CRMP-1 gene to region 4p16.1 between STSs D4S562 and D4S1059. (Bottom) Genomic structure of the CRMP-1 gene. A schematic representation of the CRMP-1 gene is shown with the exons indicated by solid boxes (black boxes for CDS and gray boxes for UTR). Introns are indicated by an horizontal line.

Table 1. (A) Sizes and location of exons and introns and sequences at exon-intron boundaries.

Exon	Size (bp)	Intron Location in cDNA	Sequence at exon-intron juction: 3'splice acceptor INTRON exon	5' splice donor exon INTRON	Intron size (kb)
2	89	279	TGCTTTGC**AG**agtgaccgac.....gacttatcaa**GT**GGGTTGAT		5.5
3	185	463	TTGCCTCC**AG**acaaatagga.....acgatgatca**GT**GAGTGACA		6.0
4	165	628	TCTGTCCC**AG**ttgaccatgt.....caggacaaag**GT**CAGTTAaA		5.0
5	62	690	CTTGAAAC**AG**gcgtcaattc.....cgacagccag**GT**AGGTCCCT		4.0
6	81	771	CTTTCCAT**AG**ctctatgaag.....gatagctcag**GT**GAGTGGCA		9.0
7	69	840	TGTCTTGT**AG**gaacaaaagc.....acctgaagag**GT**AAGGACTC		1.3
8	121	961	CCGTGTTC**AG**ctggaggccg.....aggaagaaag**GT**ATGCAGCC		1.3
9	157	1118	CCCTCCTAAGggcccctagt.....tactggcctg**GT**GAGACCTG		ND
10	142	1260	TCCTTGCC**AG**tggggacttg.....caaggcggtg**GT**AAGGCTGG		0.8
11	171	1431	TTGCCTGT**AG**gctactggca.....tcacaagtcg**GT**ANGTCNTG		10.0
12	180	1611	GTTGGTGC**AG**gcggtggagt.....caggaataag**GT**GAGTGTGT		3.0
13	168	1779	CCTCTGTT**AG**gtttttggat.....agcttatcag**GT**AGGAAAGA		3.8
14	91		TCTCTTTC**AG**gtgcccagat.....		

Note. The location of the introns in the CRMP-1 (DRP-1) cDNA are numbered relative to the published DRP-1 cDNA (accession number D78012). ND abbreviation stands for not determined.

and intron-exon junction sequences are summarized in Table 1. The intron-exon junctions conform to the consensus sequences established for intron donor and acceptor splice signals (Table 1A). Exon 1 contains the 5′ UTR of DRP-1 and the translation start codon at nucleotide 151. Exon 14 contains the stop codon at nucleotide 90 and 3′ UTR. GCC at position-3 to -1 matches the kozak consensus sequence.[12]

Based on the intron-exon structure of the CRMP-1 gene, we have developed intronic oligonucleotides and PCR conditions to specifically amplify each of the fourteen coding exons from genomic DNA (Table 1B). The identification of the intron/exon boundaries and the determination of the intronic sequences flanking these boundaries will enable the systematic screen for mutations in the gene.

Table 1. (B) Primers used to amplify the 14 coding exons of DRP-1.

Exon	Position in the DRP-1 cDNA sequence	Accesion number	Name of primer	Primer Sequences (5′ ⟶ 3′)	Size of amplicon (bp)
1	...-191	D78012	CRMP-1F	agcctccgtccgtgtctcta	150
			CRMP-1R	ttcttgccctggtacgacat	
2	191-278	AF096140	CRMP-2F	aggatccttaatactttgcc	143
			CRMP-2R	aaggtccgttttgatcaacc	
3	279-463	AF096141	CRMP-3F	ctacttttcctttaagaacc	259
			CRMP-3R	ctaaggcaggggacagtgtc	
4	464-628	AF096142	CRMP-4F	agtctgaagcatcatgatgc	253
			CRMP-4R	accaaagaacaagggattcc	
5	629-690	AF096143	CRMP-5F	ctctgctctgtggtcattga	190
			CRMP-5R	tgcccagacaagagaggaga	
6	691-771	AF096144	CRMP-6F	ctcacacccacccctgttta	137
			CRMP-6R	ggagtggaaattcttgccac	
7	772-840	AF096145	CRMP-7F	gtcacttctcccagctctga	163
			CRMP-7R	gtgtcagagtcatgcccaag	
8	841-961	AF096146	CRMP-8F	ttccctcctgtgacactgtg	226
			CRMP-8R	aacttgaacctgcaggacac	
9	962-1118	AF096147	CRMP-9F	aaccaggtttatgttttccc	213
			CRMP-9R	aaaccccacgttctcacagg	
10	1119-1260	AF096148	CRMP-10F	gtctgaagtcaggatttgcc	234
			CRMP-10R	tcctgagaagagatccagcc	
11	1261-1431	AF096149	CRMP-11F	cttctcatctgtgggtcttg	229
			CRMP-11R	tctcaacagcacaaataggc	
12	1432-1611	AF096150	CRMP-12F	gaagtaacgctgaggctgtt	266
			CRMP-12R	aagagacgctgtcggctctg	
13	1612-1778	AF096151	CRMP-13F	gtcgatcaatcacactctcc	248
			CRMP-13R	cctgaactgctgcaattgtg	
14	1779-...	AF096152	CRMP-14F	atcccatccagcagcgattg	184
			CRMP-14R	aaagggatggacatgattcc	

Note. Primer CRMP-1F is in the 5′UTR of the gene and primer CRMP-1R is exonic.

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