Genomics **56,** 179–183 (1999)
Article ID geno.1998.5673, available online at http://www.idealibrary.com on IDEAL®

Genomic Structure, Chromosomal Mapping, and Expression Pattern of Human *DCAMKL1* (*KIAA0369*), a Homologue of *DCX* (*XLIS*)

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Received July 27, 1998; accepted October 30, 1998

Human *DCAMKL1*, also known as *KIAA0369*, is a homologue of *DCX* (Xq22.3), a gene associated with X-linked lissencephaly and subcortical band heterotopia. This suggests that *DCAMKL1* may play a role in neuronal migration. The gene also shows similarity to Ca^{2+}/calmodulin-dependent protein kinases. We have determined its genomic structure, regional mapping, and expression pattern in human tissues. *DCAMKL1* consists of at least 18 exons ranging from 58 to 3359 bp in length. We have characterized the exon/intron borders, and primers were designed to amplify each individual exon for mutation analysis. *DCAMKL1* was mapped to chromosome 13q13 by fluorescence *in situ* hybridization. Northern blot analysis showed *DCAMKL1* to be predominantly expressed in human fetal brain as a major transcript of about 5.8 kb.

INTRODUCTION

Classical lissencephaly (LIS) is a neuronal migration disorder characterized by gyral abnormalities ranging from agyria (absent gyri) to pachygyria (abnormal broad gyri). Its spectrum also includes subcortical band heterotopia (bands of gray matter located in the white matter) (Dobyns and Truwit, 1995). Genetic causes of LIS include deletions or intragenic mutations of *LIS1* located on chromosome 17p13.3 (Reiner *et al.,* 1993; Chong *et al.,* 1997; Lo Nigro *et al.,* 1997) and mutations of *DCX* (*Doublecortin, X-linked,* aka *XLIS*) on Xq22.3 (des Portes *et al.,* 1998; Gleeson *et al.,* 1998). We have found that deletions and intragenic mutations of *LIS1* or *DCX* account for 76% of sporadic LIS (Pilz *et al.,* 1998). However, in 24% of these patients, the cause of their lissencephaly remains unexplained, and additional genes involved in neuronal migration are implicated.

DCAMKL1 (doublecortin and CaM kinase-like 1) has been noted to have similarity to *DCX* (des Portes *et al.,* 1998; Gleeson *et al.,* 1998). *DCX* has a 1080-bp open reading frame encoding a 10-kb brain-specific message. At the nucleotide level, *DCAMKL1* shows 73% identity to *DCX* over 825 bp; however the open reading frame of *DCAMKL1* extends an additional 1104 bp in the 3′ direction. The *DCX* protein, which appears to have potential MAP kinase family phosphorylation sites and to function as part of an Abl-dependent signal transduction pathway, is 75% identical at the amino acid level to the predicted protein of *DCAMKL1* (GenBank Accession No. AB002367). However the C-terminus of *DCAMKL1* is considered to be similar to the family of calcium–calmodulin-dependent protein kinases (CaM kinases) and shows high similarity to the rat CaM kinase CPG16 (GenBank U78857). It is thought to be involved in cell signaling/communication (Gleeson *et al.,* 1998).

Ohara *et al.* (1997) found that more than 80% of large proteins (>1000 amino acid residues) in public databases have biologically important functions. They isolated the human *DCAMKL1* gene, originally known as *KIAA0369*, in a project sequencing entire cDNA clones derived from relatively long transcripts. The *DCAMKL1* cDNA was derived from a set of size-fractionated cDNA libraries from human brain, was selected by analysis of the protein-coding potential in an *in vitro* system, and was entirely sequenced. It was mapped to chromosome 13 using a radiation hybrid panel and found to be expressed in human brain by RT-PCR analysis (Nagase *et al.,* 1997).

Here we report the genomic organization of *DCAMKL1*. The exon/intron borders of *DCAMKL1* were identified, and sizes of the exons and introns were determined. Primers were developed for PCR amplification of the individual exons, which will enable analysis of this gene as a candidate gene for human neuronal migration disorders. We regionally mapped *DCAMKL1* to 13q13 using fluorescence *in situ* hybridization (FISH). Its expression pattern in human tissues demonstrated that *DCAMKL1* is predominantly expressed in human fetal brain. While this paper was under review, Omori *et al.* (1998) reported similar data on the expression pattern and chromosomal localization of this gene.

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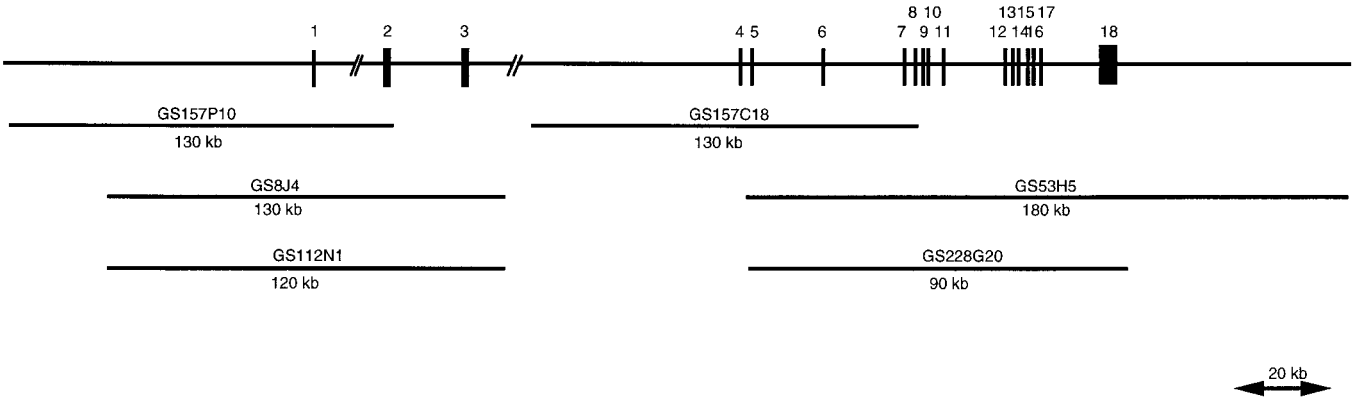

FIG. 1. Genomic structure of the human *DCAMKL1* gene with localization of the exons and size of the introns. The position of the overlapping genomic clones is indicated at the bottom; there is a gap between GS8J4 and GS157C18.

MATERIALS AND METHODS

Isolation of DCAMKL1 genomic clones. A human BAC library (Genome Systems, St. Louis, MO) was screened by PCR according to the manufacturer's protocol using the following primers designed from the *DCAMKL1* cDNA sequence (Accession No. AB002367): SCR-A1(5′-AGACATGGAGCTGGAGCACT-3′, nucleotides 15–34)/SCR-A2(5′-CTTTCTTGGCCTTCTTCTCG-3′, 156–175), SCR-B1(5′-TGATGTGCCTTCAGGACTTTT-3′, 725–745)/SCR-B2(5′-TCCTGGTAACGGAACTTCTCC-3′, 780–800), SCR-C1(5′-TCAATTTAATCATCACCACCG-3′, 5050–5070)/SCR-C2(5′-AAAAGAAAGACGGTCCCCAT-3′, 5130–5149). PCR was performed for 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s.

Pulsed-field gel electrophoresis (PFGE) analysis. The Chef Mapper Pulsed-Field Electrophoresis System (Bio-Rad, Richmond, CA) was used for PFGE analysis. BAC DNA was extracted using an automatic nucleic acid isolation system (AutoGen 740, Integrated Separation Systems, Natick, MA). One microgram of DNA was digested with *Not*I for 3 h and electrophoresed in a 1.0% SeaKem GTG agarose gel (FMC Bioproducts, Rockland, ME) with an autoalgorithm mode ranging from 5 to 300 kb at 14°C. The gel was stained with ethidium bromide.

DNA sequencing. A primer-walking strategy was adopted with primers designed using *DCAMKL1* cDNA sequence and isolated BACs as templates. Purified BAC DNA was isolated using Qiagen Midi-Prep columns (Chatsworth, CA) according to the manufacturer's protocol, with the following modifications: Qiagen-Tip 100 columns were used for a 250-ml overnight BAC culture with 10 ml of P1, 10 ml of P2, and 10 ml of P3 solutions as described elsewhere (Hubert *et al.*, 1997). One microgram of BAC DNA and 40 pmol of primers were used for sequencing with the ABI Prism Dye or dRhodamine Terminator Cycle Sequencing Ready Reaction Kits (PE Applied Biosystems, Inc., Foster, CA) followed by analysis on an ABI 377 automated DNA sequencer. The sequencing reaction was cycled 50 times at 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s. PCR products were also used for direct sequencing after purification using Microcon 100 (Amicon, Inc., Beverly, MA). Thirty nanograms of DNA from PCR products and 4 pmol of primers were used for the sequencing reaction with the above mentioned kits. The sequencing reaction was performed for 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min.

Analysis of intron lengths (intron analysis). Standard PCR or XL-PCR (Perkin–Elmer/Roche, Branchburg, NJ) was performed in a volume of 15 μl containing 100 ng of BAC DNA as a template, 1 μM of each primer, 200 μM of each dNTP, 0.033 U/μl *Taq* Gold (standard PCR) or 0.025 U/μl r*Tth* (XL-PCR) polymerase, and 1× standard PCR buffer (1.5 mM $MgCl_2$) or 1× XL-PCR buffer [1.25 mM $Mg(OAc)_2$]. Initial denaturation at 95°C for 10 min was followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 55°C for 30 s, and extension at 72°C for 2.5 min (standard PCR) or at 72°C for 10 min (XL-PCR). Furthermore, for XL-PCR, 35 cycles of denaturation at 95°C for 15 s and annealing and extension at 60–62°C for 10 min were used. Products were analyzed in a 0.8 or 0.4% agarose gel.

Fluorescence in situ hybridization. Chromosome preparations were made from normal lymphoblastoid cell lines by conventional methods. Slides were incubated in 2× SSC at 37°C for 30 min, serially dehydrated in 70, 80, and 95% ethanol at room temperature, denatured in 70% formamide/2× SSC at 72°C for 2 min, and then serially dehydrated at −20°C in 70, 80, 90, and 100% ethanol. BAC DNAs were labeled with digoxigenin-11–dUTP (Boehringer Mannheim, Indianapolis, IN) by nick- translation, precipitated in ethanol with a 50× excess of human Cot-1 and herring testis DNA (Gibco BRL, Gaithersburg, MD), and resuspended to a final concentration of 20 ng/μl in hybridization solution (50% formamide, 2× SSC, 10% dextran sulfate). Probes were denatured at 76°C for 10 min and preassociated at 37°C for 15 min prior to hybridization. The probe-hybridization mix (10 μl) was applied under a 22 mm × 22 mm coverslip, and slides were incubated in a moist chamber for 16 h at 37°C and then washed as previously described (Chong *et al.*, 1997). Probes were detected in 50 μl of rhodamine anti-digoxigenin (Boehringer Mannheim) at 1 μg/ml. Slides were washed three times in 4× SSC, 0.1% Tween 20 at 45°C and mounted in antifade solution

TABLE 1

Exon/Intron Organization of the Human *DCAMKL1* Gene

Exon	Size (bp)	5′ splice donor	Intron size (kb)	3′ splice acceptor
1	(ND)	ND	(ND)	cgt**ag**GTCCTA
2	(395)	TGGAAG**gt**gac	(18)	ctt**ag**GAGAGT
3	(347)	AAACAG**gt**aag	(ND)	tgt**ag**GTGATG
4	(100)	AAAGTG**gt**aag	(3.8)	ccc**ag**AATGTC
5	(117)	GCTCAG**gt**aaa	(15)	tct**ag**TTAATG
6	(95)	CAGAGG**gt**aag	(20)	tac**ag**ATCTCT
7	(85)	GAGAAG**gt**gat	(2.9)	ttc**ag**AAGTGT
8	(109)	AGAAAG**gt**gag	(1.8)	cgc**ag**ATCGAC
9	(58)	GGCAAA**gt**atg	(0.8)	gac**ag**GAGCAC
10	(120)	GTAAAG**gt**gtg	(4.0)	ctc**ag**GGGGGA
11	(147)	CTGCTG**gt**aag	(15)	ttt**ag**GTGTAT
12	(172)	GACTGG**gt**aaa	(2.0)	tcc**ag**ATACGG
13	(78)	CCGTGG**gt**atg	(0.8)	aac**ag**AAGTGG
14	(97)	GCAAAG**gt**agg	(2.4)	cac**ag**GAGCTC
15	(81)	GTTAAT**gt**aag	(0.7)	tcc**ag**GATGAT
16	(114)	ATAGCA**gt**aag	(1.6)	ctc**ag**CTGGAC
17	(74)	GATAAG**gt**atg	(18)	gct**ag**ACCACC
18	(>3359)			

Note. Intron sequences are in lowercase letters, and exons are in uppercase letters. ND, not determined.

TABLE 2

Intron Primers for Amplification of *DCAMKL1* Exons

Exon	Primer sequences	Product size (bp)	Annealing temperature (°C)
2	5′-AAC CTA CAT CAA TAT GTG TGT CTG C-3′ 5′-TGG AAC CTGTAG GGT CAC G-3′	484	55
3	5′-AGT AGG AAA AAA GAA ATG GGC A-3′ 5′-TTG CCT TAA AGT CAA AGT CAC A-3′	449	55
4	5′-CGT GGG GTT GCA TTG TTG T-3′ 5′-TTT CGA AAG CTG CCT CAA AT-3′	219	50
5	5′-CCC CAT AAT GAT GTG TGG TTT-3′ 5′-GGA ACA AGG CAA TTT CTT CAA-3′	194	55
6	5′-CAT GAA AAT TAT TTA ACG TGC CA-3′ 5′-GGC TGA GAC AGG TCT GAA ACA-3′	194	55
7	5′-AAA CTC AGG CAT TCT GAC CC-3′ 5′-CAT CCT CTG CCT CCT CAG AT-3′	180	55
8	5′-TGT GTT CCC TTT TCA ACC AA-3′ 5′-GGA AAA CAT AAC GCC AAA TG-3′	213	55
9	5′-TGA TCC CTG ACA AAA TAA TTC AA-3′ 5′-AAC ACT CTT ATC TCA TAA GAA C-3′	168	55
10	5′-TAT GGA AGC TGA TGA AGA TAA C-3′ 5′-CGG TGC CAT CAA TAA AGA CT-3′	200	55
11	5′-TGT GCT GCT TCA GCT TCT GT-3′ 5′-GGA ACT CAG GAT GCC CTG TA-3′	215	55
12	5′-TGG TTT TCA GAC AAT TGT GAT TTT-3′ 5′-TTT GCA AGC ATA GCA CTT AAA CA-3′	211	55
13	5′-CGA GCC TGA CCC TCC TAA CT-3′ 5′-TAG AGA AAT GCT TTG TCG GC-3′	166	55
14	5′-AGT GCT GTT TTT CCT TGT TGT C-3′ 5′-CGT TAA GAC ACA CAG AAT ATA GGG A-3′	181	55
15	5′-CAG CTT TTC ACC TTA AAT TCA GA-3′ 5′-AAA AGG AAT GTT AGG AGA GAA CAA A-3′	180	55
16	5′-CAT TTT TCA TTT ATC TCA TTT TCT TTC-3′ 5′-AAA AGT TAT AGG TGG ATA TTT CAG ATG-3′	205	50
17	5′-GCT TGG TCT GGA GTC TTA ATT CTT T-3′ 5′-GGT TAA TGG AGA CTG CCA GC-3′	209	50
18	5′-CCT CGC CCT AAT TGT CCT CT-3′ 5′-GGG TCT TAT TAA AAG GGC GA-3′	216	50

(Vector) containing DAPI. Analysis of more than 50 cells was performed using a Zeiss Axiophot microscope equipped with filters to detect DAPI and rhodamine separately, as well as a triple-bandpass filter (Chroma Technology Corp.) to detect signals simultaneously. Images were collected and merged using a cooled CCD camera (KAF 1400, Photometrics) and IP Lab Spectrum software (Signal Analytics Corp.) or Quips mFISH Imaging software (Vysis, Inc.).

Northern analysis. Expression of *DCAMKL1* mRNA was examined by Northern hybridization using a 330-bp PCR product of the coding region of *DCAMKL1* as a probe corresponding to nucleotides 15–344. Human multiple tissue blots (2 μg of poly(A)$^+$ RNA loaded per lane) were obtained from commercial sources (Clontech Laboratories, Inc., Palo Alto, CA). The blots were hybridized with the ^{32}P-labeled probe in ExpressHyb Hybridization Solution (Clontech), washed in 0.1× SSC/0.1× SDS at 50°C, and analyzed on a Storm 860 imaging system and with ImageQuant software from the manufacturer (Molecular Dynamics, Inc., Sunnyvale, CA).

RESULTS AND DISCUSSION

Genomic Structure of DCAMKL1

We isolated the BAC clones GS8J4, GS157P10, and GS112N1 with primer SCR-A1/SCR-A2, GS157C18 with SCR-B1/SCR-B2, and GS53H5 and GS228G20 with SCR-C1/SCR-C2 (Fig. 1). PFGE analysis showed that the sizes of the BAC clone inserts ranged from 90 to 180 kb (data not shown). Of these clones, GS8J4 (130 kb), GS157C18 (130 kb), and GS53H5 (180 kb) were used for further analysis because of their large inserts. Sequence analysis with primers designed from the cDNA sequence (Nagase *et al.*, 1997) and BACs as templates revealed that the human *DCAMKL1* gene consists of at least 18 exons and 17 introns. The organization of the gene is depicted in Fig.1. The size of each exon and intron and the sequence of the exon/intron junctions are shown in Table 1. The size of these exons range from 58 bp (exon 9) to more than 3359 bp (exon 18). The exon/intron junctions conform to the 5′-donor and 3′-acceptor consensus (GT. . .AG) (Table 1). Exon 1 and part of exon 2 encode the 5′-untranslated region of the *DCAMKL1* cDNA. The *DCAMKL1* coding region starts at base 21 of exon 2, spans exons 3 to 17, and extends to the first 58 bp of exon 18. Exon 18 is the largest among the exons, and most of it constitutes the 3′- untranslated region of the *DCAMKL1* cDNA. The length of 15 of the 16 introns from introns 2 to 17 in the *DCAMKL1* gene was determined by PCR; the smallest is intron 15 (0.7 kb). The size of intron 3 could not be determined due to a gap in the BAC contigs. The primers designed to amplify the individual

exons and the appropriate PCR conditions are listed in Table 2.

Regional Chromosomal Mapping and Expression Pattern of DCAMKL1

The regional chromosomal localization of *DCAMKL1* was determined by FISH using BACs GS8J4 (covering exons 2 and 3) and GS53H5 (covering the 3′-UTR). Two signals were observed on chromosome 13, band q13, with both BACs (Fig. 2), and no signal was seen on any other chromosomes.

Northern blot analysis showed that *DCAMKL1* is expressed as a major transcript of about 5.8 kb. Weak hybridization signals of about 6.0 and 9.0 kb were also observed (Fig. 3). These signals could be due to hybridization of the *DCAMKL1* probe to unprocessed *DCAMKL1* transcripts or minor splicing variants. Omori *et al.* (1998) showed four splicing variants of *DCAMKL1*. Two variants contained both the *DCX*-like domain and the CAM kinase-like domain of the gene, and the other two contained only the CAM kinase-like domain. The former was expressed mainly in fetal brain, and the latter showed similar expression in fetal and adult brain. We also found expression of *DCAMKL1* mRNA at high levels only in the fetal brain using a probe in the *DCX*-like domain. Low levels of expression were observed in adult brain, ovary, and prostate.

Two neurological disorders, nocturnal enuresis 1 (ENUR1) (OMIM600631) and Moebius syndrome (OMIM157900), have been previously mapped to this region. ENUR1 has been linked to the markers D13S291 and D13S263 at 13q13–q14.3 (Eiberg *et al.*, 1995). Some Moebius syndrome patients have shown structural chromosomal abnormalities including 13q12.2–q13 (Slee *et al.*, 1991; Ziter *et al.*, 1977). Although abnormal neuronal migration phenotypes have so far not been mapped to 13q13, sequence similarity of *DCAMKL1* to *DCX*, their coexpression in developing brain, and simultaneous temporal expression pattern in the mouse embryo after day E-11 (Omori *et al.*, 1998) place *DCAMKL1* as a potential candidate gene for neuronal migration disorders.

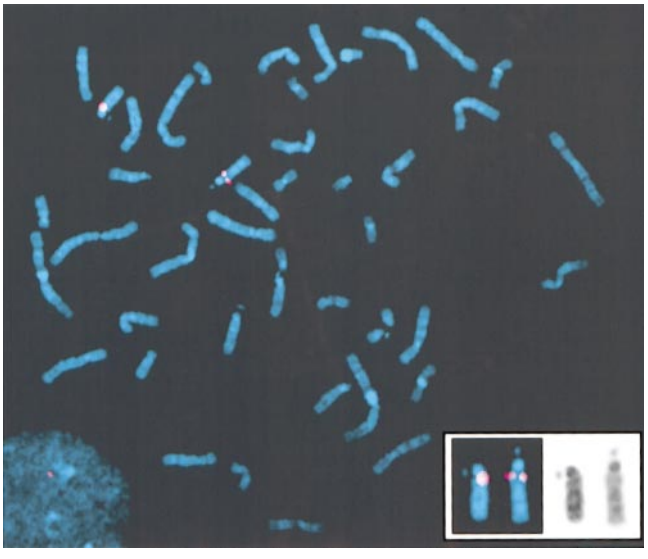

FIG. 2. Regional mapping of the *DCAMKL1* gene by fluorescence *in situ* hybridization. The *DCAMKL1* gene is localized to human chromosome 13q13 using a digoxigenin–dUTP-labeled GS53H5 clone containing the *DCAMKL1* gene as a probe. Reverse DAPI banding pattern of both chromosomes 13 is shown in bottom right corner.

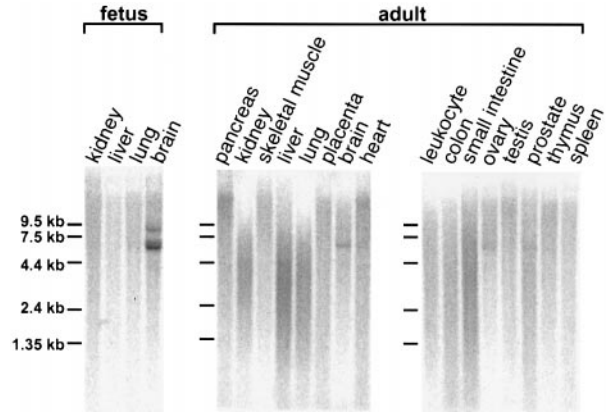

FIG. 3. Northern analysis of human *DCAMKL1* showing predominant expression in the fetal brain as a major transcript of 5.8 kb and minor transcripts of 6.0 and 9.0 kb. The image was obtained after overnight exposure (12 h) to a storage phosphor screen.

ACKNOWLEDGMENTS

We are grateful to Dr. Naohiko Seki at the Kazusa DNA Institute, Japan, and Dr. Elizabeth M. Ross at the Laboratory of Molecular Neurobiology and Development, University of Minnesota for their helpful comments, and we thank Dr. Judy A. Fantes and Ms. Stephanie Mewborn for expert technical assistance with the FISH experiments. N.M. was supported in part by a research fellowship of the Uehara Memorial Foundation.

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