SHORT COMMUNICATION

DCAMKL1, a Brain-Specific Transmembrane Protein on 13q12.3 That Is Similar to Doublecortin (DCX)

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Mutations in the human doublecortin (DCX), a brain-specific putative signaling protein, cause X-linked lissencephaly and subcortical band heterotopia. A predicted 740-amino-acid protein from human brain has two distinct regions, an N-terminal 345-amino-acid region 78% similar to the DCX protein and a C-terminal 427-amino-acid region that contains two transmembrane domains and is 98% homologous to a rat Ca^{2+}/calmodulin-dependent protein kinase. We have designated this protein DCAMKL1. It maps to chromosome 13q12.3–q13, within a 540-kb YAC clone containing markers D13S805 and D13S1164. Northern analysis detected three major transcript isoforms of the DCAMKL1 gene expressed differentially and predominantly in human fetal and adult brain and during mouse embryogenesis (11–17 dpc). These results and its homology with the DCX and Ca^{2+}/calmodulin dependent kinase proteins suggest a likely role for DCAMKL1 transmembrane protein in developing and adult brain, possibly in a pathway of cortical development.

Several malformations of cortical development in human and mouse involve incomplete or defective migration of cortical neurons (2, 5). Mutations in the 45-kDa α-subunit of the brain isoform of platelet activating factor (PAF) acetylhydrolase cause chromosome 17-linked lissencephaly (LIS1; Ref. 7). A novel human gene, doublecortin (DCX), has recently been implicated in X-linked lissencephaly (LIS) and subcortical band heterotopia (SBH) (Refs. 4, 6, 11). A highly homologous mouse Dcx gene has also been cloned, and a likely role of DCX protein in adult brain in addition to its role in developing brain has also been suggested (11).

A predicted protein, KIAA0369, from human brain (8) showed similarities to the DCX protein in its N-terminal half and to a rat Ca^{2+}/calmodulin-dependent protein kinase (cpg16) in its C-terminal half (4, 6, 11). Given the involvement of DCX in X-linked LIS/SBH and thus a possible role for the KIAA0369 in a pathway of cortical development, we analyzed the KIAA0369 protein and investigated its location and expression profile.

A 5703-bp human KIAA0369 transcript (GenBank Accession No. AB002367) contains an open reading frame (ORF) of 2190 bp. Conceptual translation of the KIAA0369 transcript predicts an 81-kDa protein of 729 amino acids. We isolated several cDNA clones from a human fetal brain cDNA library (Clontech) screened by hybridization with probes derived from the 5′coding region (nt 435–626) and 3′noncoding region (nt 5041–5350) of the KIAA0369 transcript. Partial characterization of seven cDNA clones by PCR assays and sequencing confirmed the identity of the clones and provided an additional 964-bp 3′noncoding region. Sequence comparison in the GenBank database allowed the identification of several ESTs. We compiled a consensus sequence (7.4 kb) and identified a transcript isoform lacking 74 bp (nt 2271–2344) of the KIAA0369 coding region (Fig. 1a). It contains an ORF of 2223 bp and encodes a 740-amino-acid KIAA0369 protein isoform with the C-terminal end different than that of the 729-amino-acid protein isoform (Fig. 1b). We designated the protein isoforms as doublecortin and CaM kinase-like 1 protein (DCAMKL1 alias KIAA0369).[2] Two distinct regions are found in DCAMKL1 protein, an N-terminal 345-amino-acid region 78% homologous to the DCX protein and a C-terminal 427-amino-acid region 98% homologous to a rat CaMK protein cpg16 (GenBank Accession No. U78857) and 40–50% homologous to other protein kinases including CaM kinase family, MAPK-activated kinase, and myosin light chain kinase (data not shown). In fact, a 34-amino-acid segment at the C-terminal domain of the DCX protein (Fig. 1b; aa 315–348) is 78% homologous to the N-terminal segment of the rat Ca^{2+}/calmodulin-dependent kinase, and an identical region was present in DCAMKL1. Protein sequence analysis using the BCM

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[2] The HUGO Nomenclature Committee approved gene symbol.

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a

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KIAA0369  AAGCCGAATAGCACAGCAGCTGGAGTTTCTGTCATAGCACTGGACCACGGGTTTACCATCAAGAGATCAGGTCTT  2305
DCAMKL1   ........................................**************************************  2265
cpg16     .......GC........................A......--------------------------------------  1268

KIAA0369  TGGACTACTACCAGCAACCAGGAATGTATTGGATAAGACCACCGCTCTTGATAAGGAGAGGCAGGTTTTCCGACGA  2382
DCAMKL1   **************************************........................................  2308
cpg16     --------------------------------------........................................  1311

KIAA0369  AGACGCAACCAGGATGTGAGGAGCCGGTACAAGGCGCAGCCAGCTCCTCCCGAACTCAACTCGGAATCGGAAGACT  2460
DCAMKL1   ..............................................................................  2386
cpg16     ......................G.............A...........A..A..................A.......  1389

KIAA0369  ACTCCCCAAGCTCCTCCGAGACTGTTCGCTCCCCTAACTCGCCCTTTTAATAAGACCCTTTTACTCAAAGTCCT  2533
DCAMKL1   ................................................TAA........................  2459
cpg16     .......C........T........C........C..T.........TAA........C..C....G.......  1462
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b

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DCAMKL1   MSFGRDMEL--EHFDERDKAQRYSRGSRVNGLPSPTHSAHCSFYRTRTLQTLSSEKKAKK  58
DCX       ------...DFG......TS.NM...M....................A..N......  54

DCAMKL1   VRFYRNGDRYFKGIVYAISPDRFRSFEALLADLTRTLSDNVNLPQGVRTIYTIDGLKKIS  118
DCX       ................V.S.....D.......S...I.......Y.....SR..G  114

DCAMKL1   SLDQLVEGESYVCGSIEPFKKLEYTKNVNPNWSVNVKTTSASRAVSSLATAKGSPSEVRE  178
DCX       .M.E.E.......S.DNF...V...............SANMK.PQ...SSNSA--QA..  172

DCAMKL1   NKDFIRPKLVTIIRSGVKPRKAVRILLNKKTAHSFEQVLTDITDAIKLDSGVVKRLYTLD  238
DCX       ....V.................V.................E....ET....K.....  232

DCAMKL1   GKQVMCLQDFFGDDDIFIACGPEKFRY-QDDFLLDESECRVVKS----TSYTKIASSSRR  293
DCX       ....T..H.......V..........A....S...N....M.GNPSA.AGP.-..PTPQ  291

DCAMKL1   STT-KSPGPSRRSKSPASTSSVNGTPGSQLSTPRSGKSPSPSPTSPGSLRKQRSSQHGGS  352
DCX       K.SA.....M.......D--.A...SS......K.KQ..IST.........HKDLYLPL.  349
cpg16                    MLELIE..............................I......  45

DCAMKL1   STSLASTKVCSSMDENDGPGEEVSEEGFQIPATITERYKVGRTIGDGNFAVVKECVERST  412
DCX       LDDSD.LGD--..*                                               360
cpg16     ....S................E.D.............................I....  105
HCAMKI                        MLGA.EGPRWKQAED.RDI.DFRDVL.T.A.SE.ILAEDKR.  42

DCAMKL1   AREYALKIIKKSKCRGKEHMIQNEVSILRRVKHPNIVLLIEEMDVPTELYLVMELVKGGD  472
cpg16     ............................................................  165
HCAMKI    QKLV.I.C.A.EALE...GSME..IAV.HKI......A.DDIYESGGH...I.Q..S..E  102

DCAMKL1   LFDAITSTNKYTERDASGMLYNLASAIKYLHSLNIVHRDIKPENLLVYEHQDGSKSLKLG  532
cpg16     .......S....................................................  225
HCAMKI    ...R.VEKGF......RLIFQVLD.V....D.G.....L......Y.SLDED..-IMIS  161

DCAMKL1   DFGLATIVD--GPLYTVCGTPTYVAPEIIAETGYGLKVDIWAAGVITYILLCGFPPFRGS  590
cpg16     .........--.................................................  283
HCAMKI    ....SKME.PGSV.S.A....G.....VL.QKP.SKA..C.SI...A......Y...YDE  221

DCAMKL1   GDDQEVLFDQILMGQVDFPSPYWDNVSDSAKELITMMLLVDVDQRFSAVQVLEHPWV-ND  649
cpg16     ..............................N.....N................-..  342
HCAMKI    N.AK--..E...KAEYE.D.....DI.....DF.RHLMEK.PEK..TCE.A.Q...IAG.  279

DCAMKL1   DGLPENEHQLSVAGKIKKHFNTGPKPNSTAAGVSVIATTALDKERQVFRRRRNQDVRSRY  709
KIAA0369  ...................................LDHGFTIKRSGSLDYY.QPGMYW  709
cpg16     ........................S...............................G..  402
HCAMKI    TA.DK.I..-..SEQ...N.AKSKWKQAFN.TAV.RHMRK.QLGTSQEGQGQTASHGELL  338

DCAMKL1   KAQPAPPELNSESEDYSPSSSETVRSPNSPF*                              740
KIAA0369  IROOLLIRRGRF.DEDATRM*                                         729
cpg16     ..............................*                               433
HCAMKI    TPVAGGPAAGCCCRDCCVEPGTELS-.TL.HQL*                            370
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FIG. 1. (**a**) Nucleotide comparison of the human DCAMKL1 transcript isoforms with a rat cpg16 transcript sequence. A region deleted in the DCAMKL1 isoform is shown by asterisks. Identities are shown by dots. The termination codons are boxed. (**b**) Homology of the human DCAMKL1 protein with doublecortin (DCX) and calcium/calmodulin-dependent protein kinases (CaMK). Identities are shown as dots. The common protein sequence is boxed. The predicted transmembrane domains are underlined. A potential ATP-binding site (boldface italic type) and a serine/theronine protein kinase active site signature (boldface type) are shown. Other predicted phosphorylation sites are not indicated. DCX, human doublecortin (GenBank Accession No. AF034634); cpg16, rat brain isoform of CaMK (GenBank Accession No. U78857);

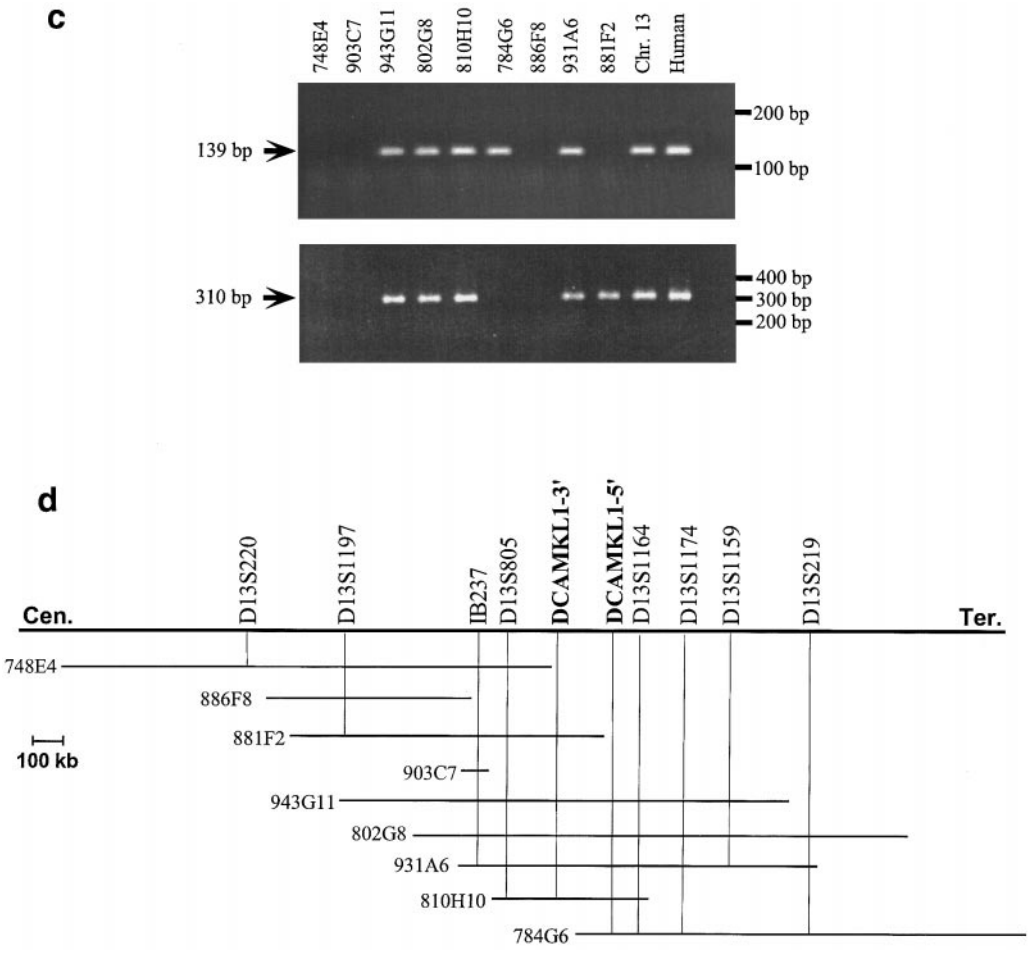


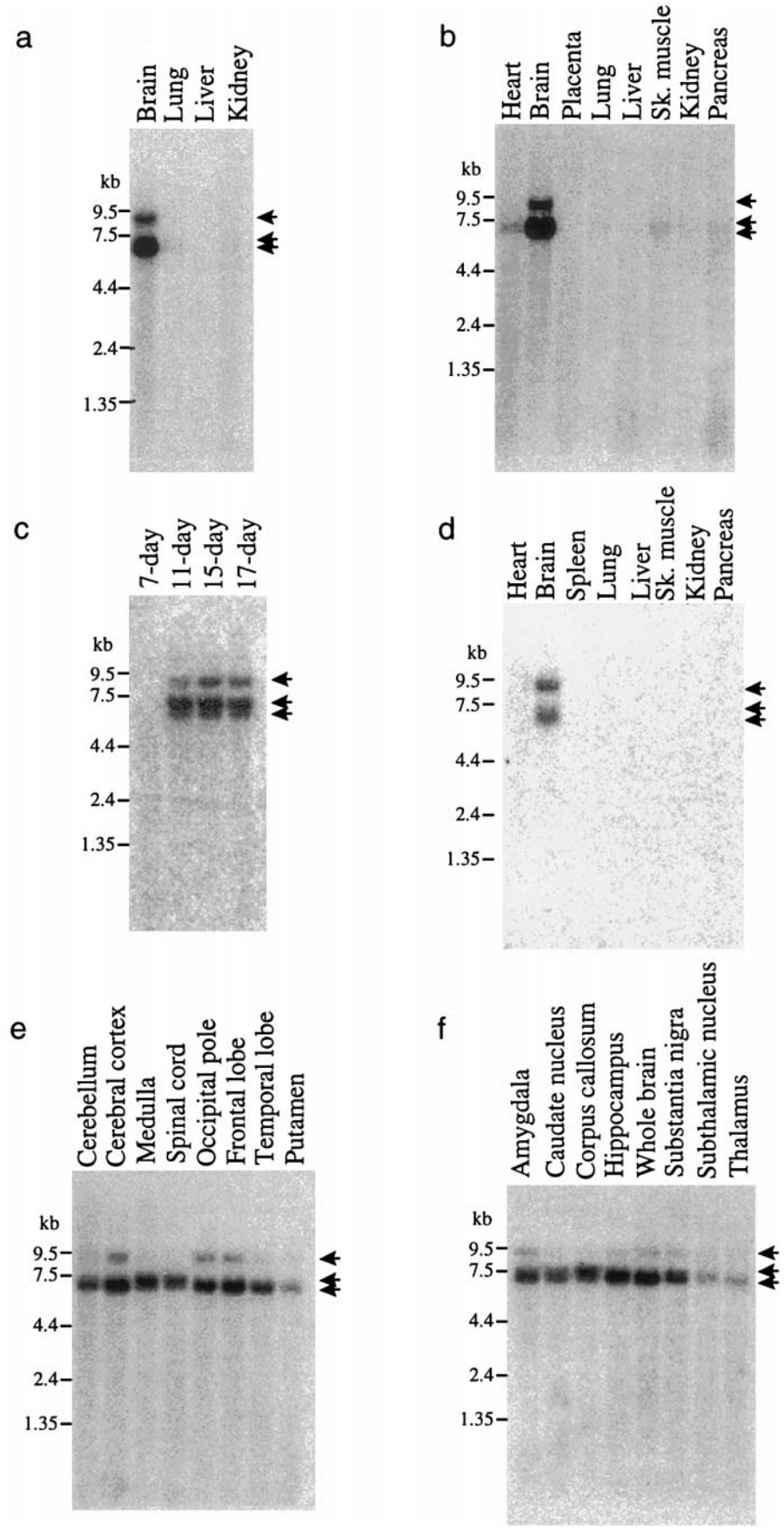
FIG. 1—*Continued*

search launcher (10) identified two transmembrane helices in its C-terminal half (Fig. 1b; aa 534–559 and 568–585). However, PSORT analysis (http://psort.nibb.ac.jp) suggested only one transmembrane domain (aa 568–584). One potential ATP-binding site, one serine/threonine protein kinase active-site signature, and several other potential phosophorylation sites were identified (Fig. 1a). These are consistent with DCAMKL1 in a putative signaling pathway.

The KIAA0369 cDNA was tentatively mapped to chromosome 13 (Ref. 8). We designed several overlapping STSs from the 5′ and 3′ regions of the DCAMKL1 transcript and used them in a PCR test with human genomic DNA. One STS from the 5′ coding region (5′-GCCATCTCCCCAGACCGGTTCCGA-3′ and 5′-CAGGCTGGAAATCTTCTTGAGCCC-3′) gave a specific 139-bp PCR product from the human genomic DNA (Fig. 1c, top). One STS from the 3′ noncoding region (5′-GACAAGCACAAGGCATGCTGACTAC-3′ and 5-GGTCCCCATCACCAGGCCACCACC-3′) gave a specific 310-bp PCR product from the human genomic DNA (Fig. 1c, bottom) and was used to screen the GeneBridge 4 radiation hybrid panel (Research Genetics, Inc., Huntsville, AL). PCR was performed with *Taq* polymerase (Boehringer Mannheim) in a 20-μl reaction volume containing 20–25 ng of genomic DNA according to the manufacturer's instructions. The conditions for PCR were initial denaturation at 95°C for 180 s followed by 35 cycles of 95°C for 20 s, 65°C for 30 s, and 72°C for 60 s. Analysis of the data (http://www-genome.wi.mit.edu) placed the DCAMKL1, 2.4 cR telomeric of WI-5283 (LOD $>$ 3.00) on chromosome 13q12.3–q13 (data not shown).

The map location was further confirmed by clone-based placement in a Whitehead YAC contig, WC-1352, containing marker WI-5283 (D13S1197) in chromosomal region 13q12.3 (http://www-genome.wi.mit.edu; and Ref. 3). Several other markers from the region

HCAMKI, human calcium/calmodulin-dependent protein kinase I (GenBank Accession No. L41816). Only the C-terminal amino acid (aa 650–729) sequence of the KIAA0369 encoded protein is shown. (**c**) PCR based mapping of the DCAMKL1 transcript ends within a YAC (810H10). (**Top**) 5′ coding region STS; (**bottom**) 3′ noncoding region STS. YAC names are indicated. Chr. 13 denotes somatic cell hybrid containing human chromosome 13 (GM10898). Hamster control lane is not shown. (**d**) Physical mapping of the DCAMKL1 gene on chromosome 13q12.3. YAC identification numbers and sizes are indicated. The presence of markers/STSs is indicated with vertical lines crossing the YACs.


a
Brain
Lung
Liver
Kidney
kb
9.5
7.5
4.4
2.4
1.35
b
Heart
Brain
Placenta
Lung
Liver
Sk. muscle
Kidney
Pancreas
kb
9.5
7.5
4.4
2.4
1.35
c
7-day
11-day
15-day
17-day
kb
9.5
7.5
4.4
2.4
1.35
d
Heart
Brain
Spleen
Lung
Liver
Sk. muscle
Kidney
Pancreas
kb
9.5
7.5
4.4
2.4
1.35
e
Cerebellum
Cerebral cortex
Medulla
Spinal cord
Occipital pole
Frontal lobe
Temporal lobe
Putamen
kb
9.5
7.5
4.4
2.4
1.35
f
Amygdala
Caudate nucleus
Corpus callosum
Hippocampus
Whole brain
Substantia nigra
Subthalamic nucleus
Thalamus
kb
9.5
7.5
4.4
2.4
1.35

were tested by PCR analysis using YAC clones from the region. In a YAC contig (Fig. 1d) spanning the marker D13S1197, the order of markers is determined to be cen–D13S1197–IB237–D13S805–DCAMKL1–D13S1164–D13S1174–tel (Fig. 1d). STS content analysis in the overlapping YACs suggested that the gene is transcribed in a tel–5′–3′–cen direction and is contained within a 540-kb YAC clone (810H10) containing markers D13S805 and D13S1164 (Figs. 1c and 1d). Our results excluded the possibility that the KIAA0369 is chimeric since both ends of the KIAA0369 transcript map to a single locus in a YAC.

The expression pattern of the DCAMKL1 was determined using a specific probe derived from the 5′coding region of the DCAMKL1 transcript. The 192-bp probe from the human fetal cDNA library was generated using primers 5′-GCCATCTCCCCAGACCGGTTCCGA-3′ and 5′-CTTGAAGGGCTCTATGGAGCCACA-3′ and hybridized to multiple tissue Northern blots (Clontech) containing poly(A) selected RNA from normal human adult and fetal tissues. Three major transcripts ranging in size from 9.0 to 6.1 kb were expressed differentially and predominantly in human fetal and adult brain (Figs. 2a and 2b). Comparatively very-low-level expression of the 6.1-kb transcript was noted in fetal lung (Fig. 2a) and several other adult tissues (heart, lung, skeletal muscle, pancreas, and kidney) only after longer exposure of X-ray film (Fig. 2b).

We have also analyzed the expression of the homologous gene in mouse, using a Northern blot containing poly(A)-enriched mRNAs, isolated from mouse embryos of different gestational stages and from mouse adult tissues with human cDNA probe (Figs. 2c and 2d). The transcripts of 9.0 and 6.1 kb were present exclusively in mouse adult brain and during embryogenesis (11–17 dpc). An approximately 7.0-kb transcript that was highly expressed during mouse embryogenesis was not expressed in the adult brain or present at a very low level of detectability (Figs. 2c and 2d). The three major transcripts were expressed differently in various regions of the adult brain (Figs. 2e and 2f). The 9.0-kb transcript was expressed at a low level in the adult brain (Figs. 2b and 2f). This transcript was present specifically in cerebral cortex, occipital lobe, frontal lobe, amygdala, hippocampus and substantia nigra (Figs. 2e and 2f). In addition, the greater intensity of bands in cerebral cortex, occipital lobe, frontal lobe, and amygdala (Figs. 2e and 2f) relative to whole brain (Fig. 2f) indicates a relatively higher concentration of this transcript in specific brain regions. A similar expression pattern was obtained with a 310-bp probe from the 3′ noncoding region (data not shown). This probe also contains regions homologous to the 3′ noncoding region of the rat calmodulin-dependent kinase (cpg16) and thus detected an additional 5.2-kb transcript expressed exclusively in fetal and adult brain; it is likely to represent a human homologue of the rat CaMK (cpg16) transcript. However, expression of this transcript was comparatively higher in the adult brain (data not shown). Expression of the 7.0-kb DCAMKL1 transcript isoform specifically in fetal brain further suggests a developmental regulation of this gene.

We can draw several possibilities for the likely function of the DCAMKL1 gene based on its similarity with DCX and CaMK and its expression in the developing brain. The DCAMKL1 protein homology with the DCX protein strongly suggests a likely role for this CNS gene in neuronal migration in the developing brain and perhaps in the adult brain. While two genes in human have now been isolated in which mutations result in LIS or the milder SBH (4, 6, 7, 11), several clinical observations suggest that additional LIS and SBH genes exist (1, 9). DCAMKL1 provides another candidate locus that may be involved in a pathway of cortical development and may facilitate the analysis of the patients with lissencephaly and other neuronal migration disorders.

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FIG. 2. Northern blot analysis of the DCAMKL1 gene in human and mice. Northern blot containing RNA from human fetal tissues (**a**), human adult tissues (**b**), staged mouse embryos (**c**), mouse adult tissues (**d**), and from different human brain sections (**e, f**) hybridized with a 192-bp probe from the 5′ coding region of the DCAMKL1. Filters were washed at high stringency according to the manufacturer's (Clontech) recommendations. Three major transcripts are indicated by arrows.

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