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SHORT COMMUNICATION

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Identification of a novel human doublecortin-domain-containing gene (*DCDC1*) expressed mainly in testis

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Abstract Mutations in the X-linked gene *doublecortex* (*DCX*) result in lissencephaly in males or subcortical laminar heterotopia (double cortex) in females. Recently, an evolutionarily conserved doublecortin (DC) domain important for microtubule binding and microtubule polymerization was defined according to detailed sequence analysis of DCX and DCX-like proteins. Subsequently we cloned a novel human cDNA that contained a DC domain during large-scale DNA sequencing of the human fetal brain cDNA library, and termed it *doublecortin-domain-containing 1* (*DCDC1*). According to a search against the human genome database, *DCDC1* was mapped to 11p13. Expression analysis showed that *DCDC1* was mainly expressed in adult testis. Furthermore, the expression level of *DCDC1* in fetal brain was much higher than in adult brain.

Keywords DC domain · Double cortex · Microtubule · Testis · 11p13

Lissencephaly (LIS) is one of the most severe human cerebral cortical malformations (Dobyns and Truwit 1995), which is thought to result from a failure of neuronal migration. Besides the first lissencephaly-associated gene *lissencephaly-1* (*LIS1 or PAFA1B1*) identified on chromosome 17p13, another gene named *doublecortex* (*DCX,* or *XLIS*) was found to be associated with classical type I lissencephaly in humans recently (Portes et al. 1998; Gleeson et al. 1998). Mutations in the X-linked *DCX* cause gross neocortical disorganization (lissencephaly or ''smooth brain'') in hemizygous males, whereas heterozygous females show a mosaic phenotype with a normal cortex, as well as a second band of misplaced (heterotopic) neurons beneath the cortex (''double cortex syndrome''). It was found that DCX co-localized with the microtubules, bound microtubules directly, stabilized them and caused bundling (Horesh et al. 1999).

So far, the closest homologue of *DCX* is a gene named *doublecortin* and *CaM kinase-like 1* (*DCAMKL1*, also known as *KIAA0369*) (Omori et al. 1998). DCX and DCAMKL1 share homology throughout the entire DCX amino acid sequence, but DCAMKL1 is twice larger. The unique C terminus of DCAMKL1 contains a domain similar to Ca^{2+}/calmodulin-dependent (CAM) kinases. Both DCX and DCAMKL1 have microtubule binding and polymerization activities, and are co-expressed in migrating neurons in developing brain (Mizuguchi et al. 1999; Lin et al. 2000). These results suggested that DCX and DCAMKL1 might together form a signaling pathway that regulates microtubules in migrating neurons. Other *DCX*-like genes were also found to be associated with some important inherited diseases. For example, mutations in a retina-specific *DCAMKL1*-like gene named *retinitis pigmentosa 1* (*RP1*) cause autosomal dominant retinitis pigmentosa (Sullivan et al. 1999).

A novel cDNA clone was isolated from a large-scale DNA sequencing of the human fetal brain cDNA library, which was constructed by our laboratory (Xu et al. 2001). Its nucleotide sequence is available from GenBank under accession number AY247970 (Fig. 1a). This 1.7-kb cDNA spans an open reading frame from nucleotide 203 to 1264, encoding a putative 354-amino-acid protein with a predicted molecular mass of 39.8 kDa, and a predicted isoelectric point of 9.40. An in-frame stop codon was found at position 158–160. The presumed initiation codon has a Kozak consensus sequence. And a putative polyadenylation signal, AATAAA, was found near the 3′ end of the sequence. Thus, it was concluded that the coding sequence is complete.

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By using BlastP (NCBI web server, http://www.ncbi.nlm.nih.gov/BLAST), the deduced protein was shown to be homologous to some putative proteins (GenBank accession no. XP_158837, XP_242096, XP_158841, XP_034415, BAA96017, XP_289880, BAC26042, XP_283759, XP_230359, AAF08814, and AF194079) and DCX, with similarities from 46% to 81%. Most of their homologous regions located between residues 170 and 246 in the deduced amino acid sequence of the cDNA we cloned. This region was later identified as a doublecortin domain (Bioinformatics Web server, http://www.isrec.isb-sib.ch/software/PFSCAN_form.html) (Fig. 1b). Therefore, we termed this gene *doublecortin domain containing 1* (*DCDC1*), in agreement with the HUGO Nomenclature Committee. The doublecortin (DC) domain is an evolutionarily conserved domain defined according to detailed sequence analysis of DCX and DCX-like proteins (Sapir et al. 2000). In the large majority of patients, missense mutations in DCX fall within the conserved regions, such as Y125H, A71S, R59H/L, D62N, G253D, R192W, and T203R. The domain commonly appears in the N terminus of proteins and consists of two tandemly repeated 80-amino-acid regions. In vitro and in vivo experiments indicated that each repeat alone bound tubulin, while neither repeat was sufficient for co-assembly with microtubules. However, two tandem repeats were sufficient to mediate microtubule polymerization (Taylor et al. 2000). The DC domain of DCDC1 contains only one repeat. This means that DCDC1 might only have microtubule binding activity, but no ability to mediate microtubule polymerization. The absence of a signal peptide (PSORT II server, http://psort.nibb.ac.jp) and a transmembrane domain suggests that DCDC1 is a hydrophilic, intracellular protein. It might also have some other domains with unknown function.

By searching against the human EST database and the human genome database, we found the *DCDC1* gene to be represented by five ESTs and two genomic clones (accession nos. NT_030801.7, AL162614.25 and AL137804.7) from chromosome 11p13. Comparison of the cDNA sequence of *DCDC1* to the genomic sequence revealed that the *DCDC1* gene spanned about 107 kb of genomic DNA and consisted of nine exons. We could thus determine the complete exon-intron structure of the *DCDC1* gene (Table 1).

To investigate the expression pattern of *DCDC1* in different tissues, we used two human multiple tissue cDNA (MTC; Clontech, Palo Alto, Calif., USA) panels as PCR templates according to the manufacturer's protocol. The *DCDC1*-specific primer pairs (DCDC1F: 5′-tgggtgatttctgcatttccaactaag-3′; DCDC1R: 5′-ataaacatcggcttcatggggtatatc-3′) were designed to amplify a 0.7-kb fragment. A *G3PDH* control primer pair included in the panels was used to verify the normalization of the MTC panels. A total of 36 cycles of amplification were performed using rTaq DNA polymerase (TaKaRa, Tokyo, Japan) in a total volume of 50 μl. All the reactions were paused after a total of 27

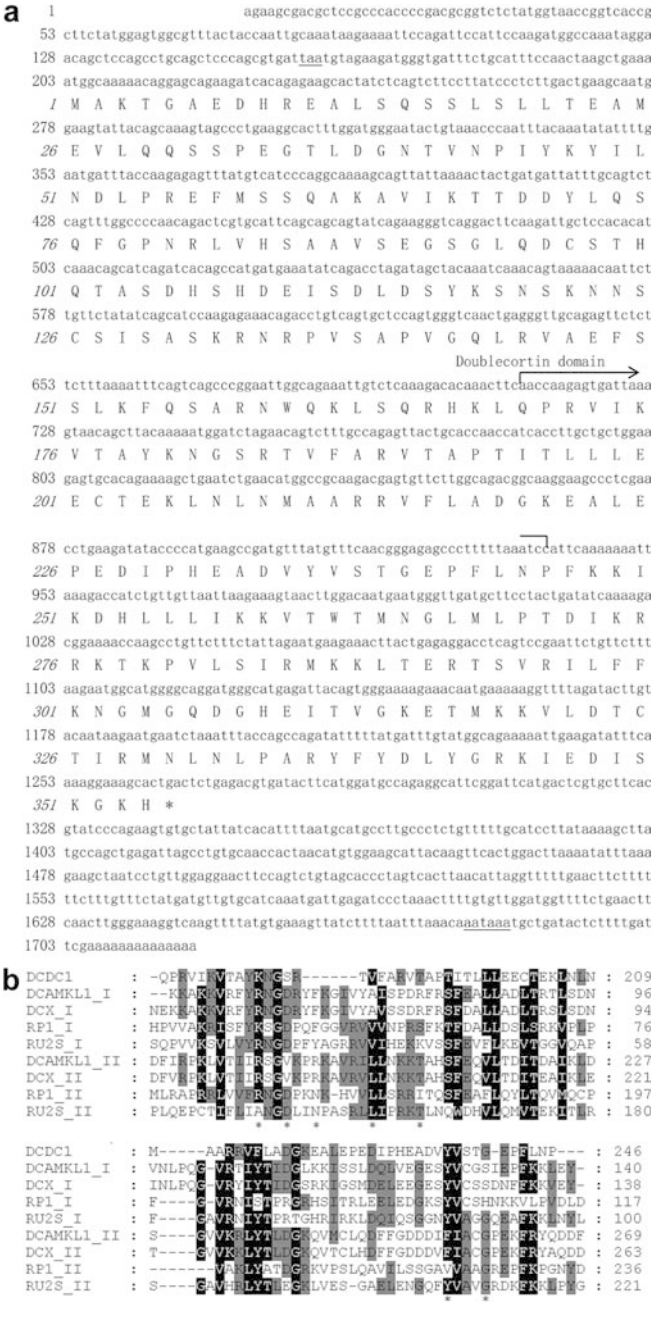

Fig. 1a, b The sequences and domain analysis of *DCDC1*. **a** Sequence of the *DCDC1* gene. The in-frame codons and polyadenylation signals are *underlined*. Amino acids are represented *below* the DNA sequences. The *asterisk* represents the stop codon. The *arrow* indicates the doublecortin (DC) domain. **b** Alignment of the DC domains in DCDC1 and other human DC-domain-containing proteins, including DCX (GenBank accession no. NP_000546; domain I: 48–138, domain II: 175–263), DCAMKL1 (accession no. NP_004725; domain I: 52–142, domain II: 181–269), RP1 (accession no. XP_129368; domain I: 30–117, domain II: 152–236), and RU2S (accession no. AAF23612; domain I: 12–100, domain II 134–221). Alignment was performed by Clustal W algorithms, and amino acids were shaded according to the degree of conservation using GeneDoc (http://www.cris.com/~Ketchup/genedoc.shtml): *black* (80–100% similarity), and *grey* (60–80% similarity). The positions of missence mutations in DCX that cause lissencephaly are shown by *asterisks*

Table 1 Exon-intron boundary sequence of *DCDC1*. Intron and exon nucleotide sequences are shown in *lowercase* and *uppercase* letters, respectively. *Bold italic* lettering indicates donor and acceptor splice sites. All sequences of the exon-intron junctions are consistent with the AG-GT rule

Exon		cDNA position		Splicing acceptor	Splicing donor	Intron	
Number	Length (bp)	Position at NT_030801.7	*DCDC1*			Number	Length (bp)
1	78	10796482–10796405	1–78		TTTACTACCA***gt***aagttccc		
2	118	10762272–10762155	79–196	tattttcc***ag***ATTGCAAATA	TCCAACTAAG***gt***acctggtt	1	34,132
3	170	10754994–10754825	197–366	tcatttttt***ag***CTGAAAATGG	ATTTACCAAG***gt***aagaacat	2	7,160
4	270	10734616–10734347	367–636	tttcttcc***ag***AGAGTTTATG	GTCAACTGAG***gt***aatcaaag	3	20,208
5	157	10733096–10732940	637–793	ttttcttc***ag***GGTTGCAGAG	CATCACCTTG***gt***aactagtg	4	1,250
6	163	10732485–10732323	794–956	tttcttct***ag***CTGCTGGAGG	AAAATTAAAG***gt***aaaaaaga	5	454
7	206	10717560–10717355	957–1162	tatcttct***ag***ACCATCTGTT	AATGAAAAAG***gt***aattttta	6	14,762
8	94	10692308–10692215	1163–1256	tcctgcac***ag***GTTTTAGATA	ATTTCAAAAG***gt***aagtggca	7	25,046
9	450	10689781–10689332	1257–1706	atcaaaac***ag***GAAAGCACTG		8	2,433

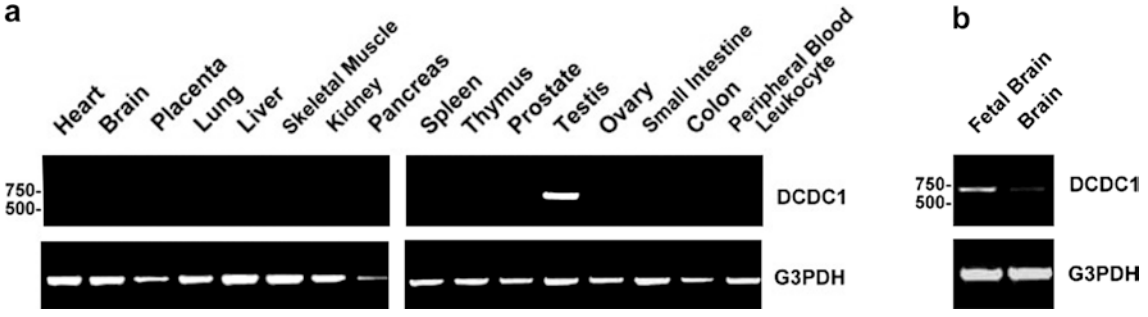

Fig. 2a, b Expression pattern of *DCDC1*. **a** Reverse transcription-PCR analysis of human adult tissues cDNA for *DCDC1* and *G3PDH* (positive control). Results of 36 cycles (for *DCDC1*) and 27 cycles (for *G3PDH*) of amplification are shown. **b** Reverse transcription-PCR analysis of human fetal brain and brain tissues cDNA for *DCDC1* and *G3PDH* (positive control). Results of 38 cycles (for *DCDC1*) and 27 cycles (for *G3PDH*) are shown

cycles, 30 cycles, 33 cycles and 36 cycles. Each time, 5-μl samples of each reaction mixture were removed to run on a gel, and the rest put back in the thermal cycler. The cycling conditions were as follows: 2 min at 94 °C, followed by cycles of 30 s at 94 °C, 90 s at 68 °C, with a 5 min 68 °C step to finish. When 27 cycles of amplification was performed, *G3PDH* (positive control) RT-PCR products were detected in all tissues tested. After 36 cycles the signal was detected in testis, lung, kidney and pancreas. However, the band in testis was much brighter than in either of the other tissues (Fig. 2a). Thus, we concluded that *DCDC1* was expressed mostly in testis.

We noticed that *DCDC1* cDNA could be isolated from fetal brain but could not be detected in adult brain tissue. We further used the cDNAs of these two tissues from MTC as PCR templates to compare the expression levels in fetal brain and adult brain. Both the primers and the cycling conditions were the same as above. After 38 cycles, it was found that the expression level of *DCDC1* in fetal brain was much higher than in adult brain (Fig. 2b). Further study should be made to corroborate DCDC1 as a microtubule-associated protein and to clarify the precise role of *DCDC1* gene in testis.

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