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SHORT COMMUNICATION

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A novel splice variant of the cell adhesion molecule contactin 4 (*CNTN4*) is mainly expressed in human brain

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Abstract Axon-associated cell adhesion molecules (AxCAMs) of the immunoglobulin superfamily play important roles in the formation, maintenance, and plasticity of functional neuronal networks. Contactin4 (*CNTN4*, *BIG-2*) is a member of the TAG-1/F3 subgroup of AxCAMs. We have cloned a novel splice variant of *CNTN4*, and term it *CNTN4A*. The complete nucleotide sequence of *CNTN4* is also obtained by combining the insert sequences of two clones, which were isolated when screening the human fetal brain cDNA library with *CNTN4A* as a probe. CNTN4A protein has an N-terminal cleavable signal peptide, two FNIII-like domains, and a glycosyl phosphatidylinositol-anchoring domain. According to the search of the human genome database, *CNTN4* was mapped to 3p25–26, a region very close to the breakpoints of the 3p syndrome. Expression analysis of *CNTN4A* shows that *CNTN4A* is mainly expressed in brain.

Key words AxCAM · CNTN4 · FNIII-like domain · Brain · Splice variant · 3p25–26

The precise formation of the neural network is essential for the proper function of the nervous system. In the past decade, evidences have been accumulated that axon-associated cell adhesion molecules (AxCAMs) of the immunoglobulin superfamily play crucial roles in the formation, maintenance, and plasticity of functional neuronal networks. AxCAMs include the TAG-1/F3 subgroup, the Li subgroup, and so forth. In rat, six molecules of the TAG-1/F3 subgroup (Contactin, TAG-1, BIG-1, BIG-2, NB-2, and NB-3) have been identified, and most of them have neurite outgrowth-promoting activity (Yoshihara et al. 1995; Ogawa et al. 2001). Contactin (*CNTN1*)-deficient mice exhibited severe ataxia and died on postnatal day 18 (Berglund et al. 1999). The expression of these genes is tightly regulated in developmental stage-specific and neuronal type-specific manners (Yoshihara et al. 1995; Kamei et al. 2000).

cDNA clone ZL-103 was isolated during large-scale DNA sequencing of the human fetal brain cDNA library, which was constructed by our laboratory (Xu et al. 2001). The 3.1-kb cDNA spans an open reading frame from nucleotide 483 to 1331, encoding a putative 282-amino acid protein with a predicted molecular mass of 31.1 kDa. An in-frame stop codon (position 381–383), and four putative polyadenylation signal AATAAAs are also found in this sequence. Using Blastn, we found that most of the insert sequence of clone ZL-103 shows high similarity to rat *BIG-2* (GenBank accession No.U35371, 91% at DNA level), except for the first 412 nucleotides at the 5′ end.

CNTN4 (*BIG-2*) is a member of the TAG-1/F3 subgroup of AxCAMs. Recombinant rat BIG-2 protein has been reported to show neurite outgrowth-promoting activity *in vitro* (Yoshihara et al. 1995). The expression pattern of rat *BIG-2* during the development and maturation of olfactory sensory neurons suggests that the role of BIG-2 may be concerned with synaptogenesis (Saito et al. 1998). The expression pattern of human *CNTN4* has been investigated, but the complete sequence of this gene has not yet been obtained (Kamei et al. 2000).

To confirm that the cDNA we cloned is a splice variant of *CNTN4* (*BIG-2*), the insert of ZL-103 was labeled with ^{32}P and was used as a probe for the screening of the same cDNA library. The screening yielded four positive clones: clone 1 (2.6 kb), clone 2 (3.2 kb), clone 3 (3.1 kb), and clone 4 (2.7 kb). Clone 2 and clone 4 had an overlapping sequence, which was about 0.9 kb in length. By combining the insert sequence of clone 2 and clone 4, we obtained a putative cDNA sequence (5.0 kb) that spanned an open reading frame from nucleotide 222 to 3302 (position of in-frame stop codon: 204–206). Its deduced 1026 amino acid protein

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is very similar to rat BIG-2 (AAC52262, 1026 amino acids, 94%) and homologous to other members of F3/TAG-1 subgroups, representing overall amino acid identities ranging from 44% to 66% (Fig. 1). This suggests that we isolated a novel splice variant of *CNTN4*, which we termed *CNTN4A*. We also obtained the putative nucleotide sequence of *CNTN4* with a complete open reading frame. The nucleotide sequences of *CNTN4A* and *CNTN4* have been submitted to GenBank under accession numbers AF464063 and AY090737, respectively.

Like rat BIG-2, deduced CNTN4 protein also possesses six Ig-like domains, four fibronectin type III (FNIII)-like repeats, one glycosylphosphatidylinositol (GPI)-anchoring domain, and one N-terminal cleavable signal peptide. All of its potential asparagine-linked glycosylation sites, cysteine residues in Ig-like domains, and characteristic tryptophan and tyrosine (or phenylalanine) acid residues in the FNIII-like domains are conserved between human and rat. Deduced CNTN4A protein seems to be a truncated form of CNTN4 protein. It only has two FNIII-like domains and one GPI-anchoring domain. Interestingly, CNTN4A also has an N-terminal cleavable signal peptide at amino acid position 1–13 (Fig. 2a). In CNTN4, these amino acids are in the region of the second FNIII-like repeat (amino acid position 745–757). Possession of this signal peptide suggests that CNTN4A is also a membrane molecule, like CNTN4.

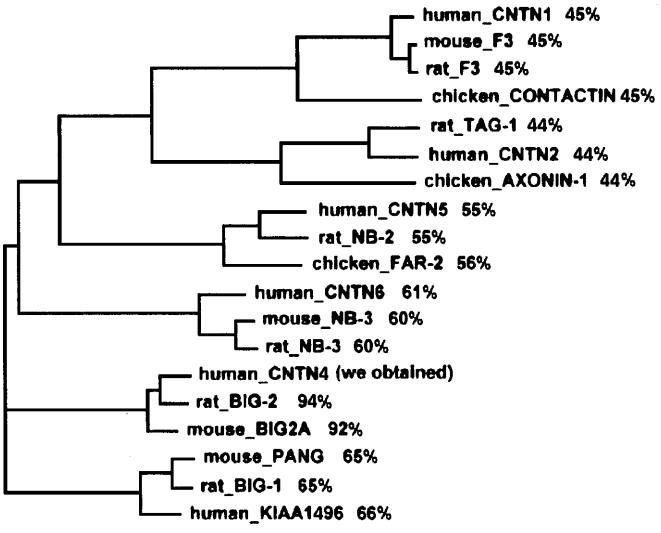

Fig. 1. A gene tree representing the TAG-1/F3 subgroup of axon-associated cell adhesion molecules. The gene tree was generated using the AlignX program of Vector NTI Suite 5.5. Sequence data were derived from GenBank as the following accession numbers: AAA67920, CAA33075, BAA07504, CAA68753, AAA42201, CAA48335, CAA44815, BAA36579, BAA13311, CAC51431, BAA82612, BAA92367, BAA13320, AY090737, AAC52262, CAA67504, A53449, AAA63607, BAA96020 (from *top to bottom*). The amino acid identities between human *CNTN4* and other genes are shown *beside* the tree

It has been reported that most of the genes of the human contactin subgroup, except for *CNTN4*, are located in chromosomal regions responsible for neurological disorders (Kamei et al. 2000). By searching the human expressed sequence tag (EST) database and the human genome database, we found that *CNTN4* was represented by 14 ESTs, and it perfectly matched a genomic clone (GenBank accession No. NT_005927.8) from chromosome 3q25–q26. This provisional chromosomal localization was confirmed by electronic polymerase chain reaction (e-PCR). These results suggest that, like *CNTN3* and *CNTN6*, *CNTN4* might also be responsible for the 3p syndrome, which is characterized by multiple congenital anomalies and mental retardation.

Comparison of the cDNA sequence of *CNTN4* to the genomic sequence NT_005927.8 revealed that the *CNTN4* gene spanned about 957 kb of genomic DNA and consisted of 24 exons. *CNTN4A* consists of 6 exons, with only 1 exon different from *CNTN4* (Fig. 2b). All sequences of the exon–intron junctions were consistent with the AG–GT rule. It was reported that another truncated splice variant of *CNTN4* was identified in mouse (*BIG-2A*, GenBank accession No. CAA67504), and its deduced protein contained an N-terminal cleavable signal peptide, six Ig-like domains, and a single FNIII-like repeat (Mimmack et al. 1997). By using Blastn, it was found that the different exon of the *BIG-2A* sequence from BIG-2 also matched well with the genome clone NT_005927.8. This result indicates that the homologue of mouse *BIG-2A* may exist in humans (Fig. 2b). Because the 5′-end of *CNTN4A* is just behind the 3′-end of this putative homologue of *BIG-2A* in the human

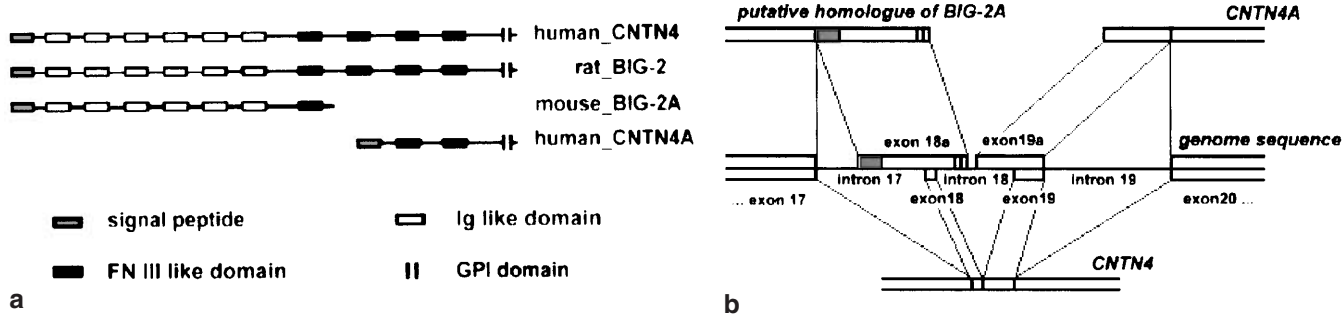

Fig. 2a,b. The structure and alternative splicing patterns of the human *CNTN4* gene. **a** Comparison of the structures of deduced CNTN4, CNTN4A, rat BIG-2, and mouse BIG-2A protein. **b** Alternative splicing pattern of the human *CNTN4* gene. *CNTN4* consists of 24 exons. *CNTN4A* consists of 6 exons, and the first exon (exon 19a) is different from *CNTN4*. The putative homologue of mouse *BIG-2A* consists of 18 exons. The eighteenth exon (exon 18a), which is different from *CNTN4*, may start at a region (*shade* in intron 17) highly similar to mouse BIG-2, and end with three putative polyadenylation signals (in intron 18)

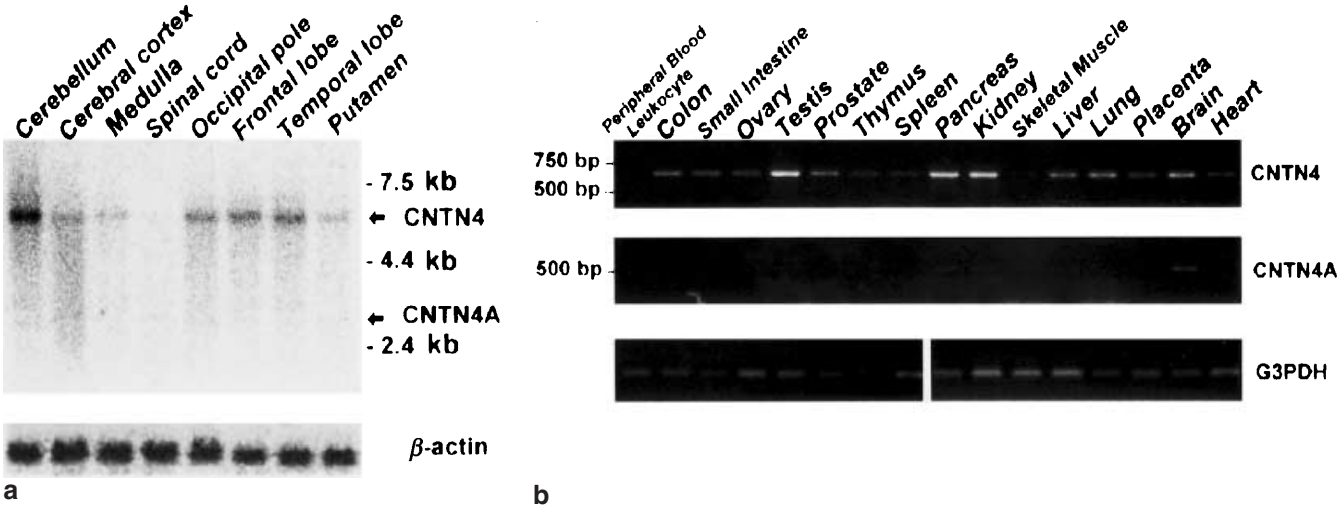

Fig. 3a,b. Expression patterns of *CNTN4* and *CNTN4A*. **a** Northern blot analysis of *CNTN4* and *CNTN4A* in eight regions of the adult human nervous system (*upper panel*). Numbers on the *right* indicate the molecular weight markers. The blot was stripped and reprobed with β-actin as an indicator of RNA loading (*lower panel*). **b** Reverse transcriptase-polymerase chain reaction analysis of human adult tissue cDNA for *CNTN4*(*A*) and *G3PDH* (positive control). Results of 33 cycles (for *CNTN4*), 35 cycles (for *CNTN4A*), and 24 cycles (for *G3PDH*) of amplification are illustrated

genome sequence, its expression may be tightly associated with the expression of *CNTN4A*.

Expression of the *CNTN4* and *CNTN4A* in human adult brain was then investigated by Northern blot hybridization. A common DNA fragment of two splice variants, which is about 1 kb in length (the region corresponding to *CNTN4A* cDNA, position 1182–2195), was hybridized to a human brain MTN blot (Clontech, Palo Alto, CA, USA). As a result, *CNTN4* mRNA appears as a main band, and was expressed at high levels in cerebellum and at low levels in spinal cord (Fig. 3a). This result is coincident with previously published data (Kamei et al. 2000). Only a weak signal of *CNTN4A* mRNA was observed in cerebral cortex, and it was less well resolved on the autoradiograph. To confirm the existence of this transcript, and to investigate the expression patterns of two splice variants in different tissues, we used two human multiple tissue cDNA panels (MTC, Clontech) as PCR templates according to the manufacturer's protocol. The sequences of *CNTN4*-specific and *CNTN4A*-specific sense primer were 5′-CCCCAGAGG CTGTGACAATAG ACGA-3′ and 5′-GCTGGGTCTG CCCCATCAAAGAG CA-3′, respectively. The sequence of *CNTN4*(*A*) common antisense primer was 5′-TTCTGGCAAAGATACTGGCT GGT GGT-3′. The cycling conditions were as follows: 5 min at 94°C, followed by cycles of 30 s at 94°C and 90 s at 68°C, with a 5-min 68°C step to finish. When 33 cycles of reverse transcriptase (RT)-PCR were performed, *CNTN4* RT-PCR products were detected in all human adult tissues tested except skeletal muscle. The expression levels in testis, pancreas, and kidney are much higher than in the other tissues. The difference between this result and previously published data (Kamei et al. 2000) may be due to the existence of other splice variants of *CNTN4* that are not yet identified. When 35 cycles of RT-PCR were performed, *CNTN4A* RT-PCR product was detected in human adult brain, suggesting that *CNTN4A* mRNA is mainly expressed in brain (Fig. 3b). Further experiments are necessary to clarify the precise role of *CNTN4* and *CNTN4A*.

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