J Hum Genet (2003) 48:217–221
DOI 10.1007/s10038-003-0012-8

ORIGINAL ARTICLE

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Molecular cloning and characterization of a novel human J-domain protein gene (HDJ3) from the fetal brain

Received: 26 December 2002 / Accepted: 17 February 2003 / Published online: 25 April 2003
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Abstract The J-domain is believed to be part of a chaperone involved in protein folding. From a fetal brain cDNA library, we isolated a cDNA of 3249 bp encoding a novel human J-domain protein, which was named as HDJ3. The expression pattern of HDJ3 was examined by reverse transcription/polymerase chain reaction, which suggested that the transcripts were highly expressed in human pancreas and selectively expressed in human brain, lung, liver, skeletal muscle and kidney. The results also showed that a probable splice variant of HDJ3 gene might exist. The HDJ3 gene was located on human chromosome 12q13.1–12q13.2 and consisted of seven exons spanning 8593 bp of the human genome. PSORT analysis indicated that the HDJ3 gene contained a transmembrane domain. The putative protein of the HDJ3 gene was highly homologous to rat dopamine-receptor-interacting protein, suggesting that it was a novel member of the molecular chaperone family and functionally related to dopamine signal transduction.

Keywords HDJ3 gene · Expression pattern · Chaperone · Chromosome 12q13.1–12q13.2

J. Chen and Y. Huang contributed equally to this work.
Electronic database information: the accession number and URL for the data in this article are as follows: GenBank, http://www.ncbi.nlm.nih.gov/Genbank (accession no. AY188447)

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Introduction

Newly synthesized or denatured proteins generally have no function because they do not have the correct topology. Molecular chaperones, which are themselves a series of proteins, interact with these proteins and help them to become properly folded and to reach their final active conformation. Moreover, the molecular chaperones carry these proteins to their correct destination (Ellis and Van 1991). This property may enable molecular chaperones to play several essential roles, such as repairing damaged proteins and assisting proteins in membrane translocation, in addition to helping newly synthesized proteins to fold correctly. Hsp70 proteins are highly versatile chaperones but the family members are highly conserved and ubiquitous. They not only assist a large variety of folding processes, but are also involved in protein transport across membranes and the reactivation of heat-damaged proteins (Lund 1995).

The J-domain protein family is a highly heterogeneous family of chaperones encompassing the J-domain as their only common characteristic. The J-domain stretches over about 70 amino acid residues, which is a protein–protein interaction domain (Kelley et al. 1998). *Escherichia coli* protein DnaJ (eukaryotic homolog, Hsp40), a J-domain protein, has been shown to interact with DnaK, a chaperone of the Hsp70 family. The J-domain mediates the binding of DnaJ to DnaK and transfers the partially folded peptide to the cellular folding machinery (Wall et al. 1994). *E. coli* DnaJ and human Hdj1 J-domains share only 54% sequence identity but the two structures are remarkably similar, as shown by nuclear magnetic resonance (Cheetham et al. 1998), which indicates that the function of the J-domain is highly conserved from prokaryotes to eukaryotes.

Positive-strand RNA virus expression occurs via the synthesis of a polyprotein, which is further processed by cellular and viral proteases. NS2-3 protease, a non-structural protein of the polyprotein, is an extensively characterized viral enzyme. All cellular insertions identified so far in pestivirus genomes have been found within the NS2-3 coding region (Rinck et al. 2001).

We reported here the cloning of a novel human J-domain protein gene (HDJ3), located on human chromosome 12q13.1–12q13.2, together with its sequence characterization and tissue distribution.

Materials and methods

A cDNA library was constructed in a modified pBluescript II SK (+) vector (Stratagene, La Jolla, CA, USA) with human fetal brain mRNA (Clontech). The modified vector was constructed by introducing two *Sfi*I recognition sites, i.e. *Sfi*I A (5'-GGCCAT-TATGGCC-3') and *Sfi*I B (5'-GGCCGCCTCGGCC-3'), between the *Eco*RI and *Not*I sites of pBluescript II SK (+). Double-stranded cDNAs were synthesized by using the SMART cDNA Library Construction Kit (Clontech, Palo Alto, Calif., USA) following the manufacturer's instructions. The cDNA inserts were sequenced on an ABI PRISM 377 DNA sequencer (Perkin–Elmer, San Francisco, Calif., USA) by using the BigDye Terminator Cycle Sequencing Kit and BigDye Primer Cycle Sequencing Kit (Perkin–Elmer) with the −21M13 primer and M13Rev primer. Synthetic internal walking primers were designed according to the obtained cDNA sequence fragments. Each part of the insert was sequenced at least three times bi-directionally. Subsequent editing and assembly of all the sequences from one clone were performed by using Acembly (Sanger Center).

DNA and protein sequence comparisons were carried out by using BLAST 2.0 at the National Center for Biothechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/blast). PROFILESCAN was performed at the Swiss Institute of Bioinformatics (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html). Protein alignment was performed by GeneDoc program (http://www.cris.com/~Ketchup/genedoc.shtml). Transmembrane analysis was carried out by using the program in PSORT (http://psort.ims.u-tokyo.ac.jp/).

Adult multiple tissue cDNA (MTC) panels (Clontech) were used as the polymerase chain reaction (PCR) template. The MTC-based reverse transcription/PCR (RT-PCR) was performed according to the manufacturer's recommendations. The sequences for HDJ3-specific primers were 5'-gaaggcctatagacagctggcagtgatg-3' (F, 1501–1529) and 5'-ggagatacctacacgctggcatccagc-3' (R, 1922–1949). Thirty–six cycles of amplification (30 s at 94°C, 30 s at 58°C, 1 min at 72°C) were performed by using ELONGASE DNA polymerase (Gibco Brl, Gaithersburg, Md., USA) and the PCR products were then resolved on 1.5% Metaphor agarose gel (FMC, Philadelphia, Pa., USA). The targeted PCR product was cloned into T-vector and sequenced with both M13 consensus primers.

Results

A novel cDNA clone was isolated from the human fetal brain cDNA library that we had constructed. This cDNA was 3249 bp in length and contained an open reading frame from nucleotides 122–2230 (Fig. 1A). Its deduced protein was composed of 702 amino acid residues, which showed significant homology to the J-domain protein. The putative initiation ATG codon is in the context of gtcATGG, satisfying the Kozak consensus, A/GXXATGG, which apparently controls the translational efficiency of mammalian mRNAs (Kozak 1987). Only the *Homo sapiens* cDNA fragment, FLJ31383 fis (AK055945), in the GenBank was about 100% identical to the 3'-end nucleotide sequence of the cDNA that we had cloned. Since the cDNA was highly homologous to the *Bos taurus* J-domain protein (Jiv) mRNA, it was named the human DnaJ 3 gene (HDJ3). The nucleotide sequence has been submitted to the GenBank Database under accession no. AY188447.

PROFILESCAN indicated that the deduced amino acid sequence of the HDJ3 gene contained a J-domain from amino acid residues 443–507. Multiple sequence alignment with other homologous proteins (NP_446142, AAH11146, AAK28640, AAB19180) showed that the J-domain was highly conserved among them. HDJ3 protein was a 702-amino-acid-residue peptide, which corresponded to full-length proteins in other organisms, such as rat, mouse and cow. Human NP_115740 (412 amino acids in length) was 100% identical to the sequence of amino acid residues 291–702 of the HDJ3 protein, suggesting that it was an N-terminal truncated HDJ3 protein. The alignment of these homologies is shown in Fig. 1B. All the sequences aligned had a domain highly homologous to NS2-3 protein in bovine viral diarrhea virus (BVDV).

The gene of AY188447 was mapped to contig NT–009458.11 on 12q13.1–12q13.2 by using BLAST analysis against human genome databases at NCBI. Comparing AY188447 with the genome suggested that the gene had seven exons. All the sequences at the exon–intron junctions were consistent with the AG-GT rule (Table 1).

The PSORT program was also used to detect the potential transmembrane domain in the HDJ3 protein. The results showed that the HDJ3 protein was a membrane protein with one transmembrane domain from amino acid residues 326 to 342.

The tissue distribution of the HDJ3 gene was determined by RT-PCR. The desired length of the PCR product was 448 bp. The specific transcript band was detected in brain, lung, liver, skeletal muscle, kidney and pancreas (Fig. 2), whereas no obvious PCR band was detected in the heart and placenta. The expression level in the pancreas was extremely high and two bands were detected. This led to the suggestion that another J-domain family member or alternatively spliced variant of HDJ3 gene might exist.

Discussion

The DnaJ domain is believed to be part of a chaperone involved in protein folding. J-domain proteins with highly specialized functions have been described in eukaryotes. There are many of these proteins, such as

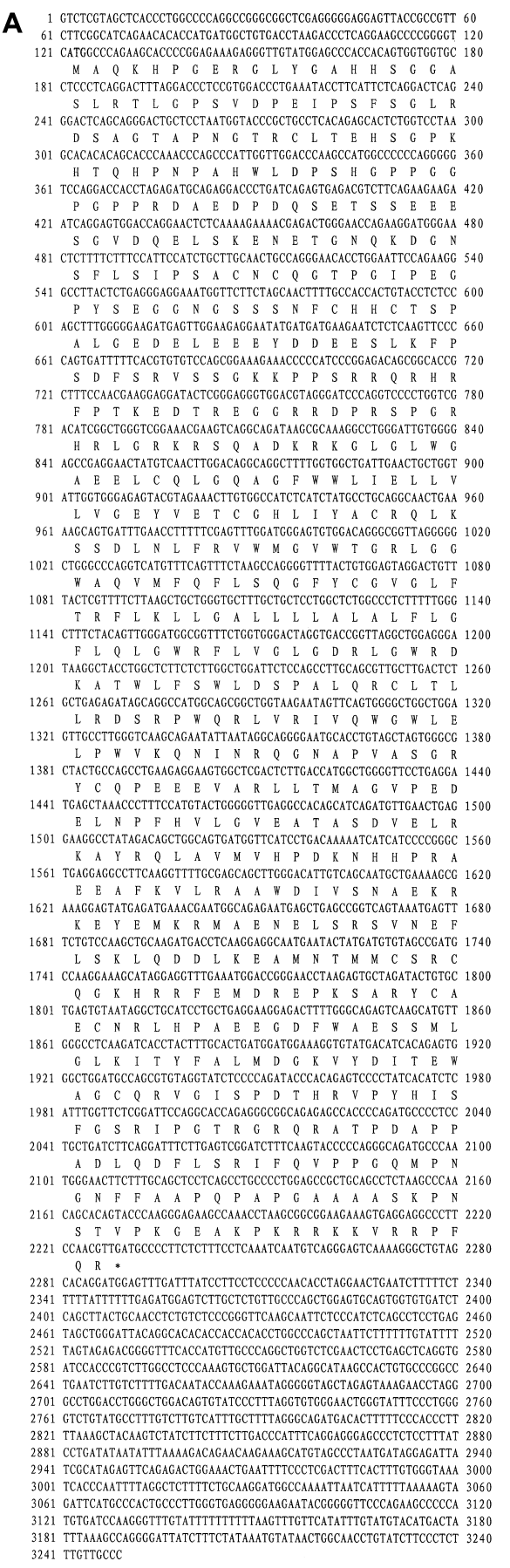

Fig. 1 **A** Nucleotide and deduced amino acid sequences of the HDJ3 gene (GenBank accession no. AY188447). *Top* Nucleotide sequence of the 3249-bp cDNA, *bottom* its predicted amino acid sequence in single-letter code, *numbers right* last nucleotide or last amino acid in each corresponding line. The open reading frame extended from nucleotide 122 to 2230 and encoded a protein of 702 amino acids. *Asterisk* Terminator in the protein sequence. **B** Alignment of HDJ3 with its homologous proteins: *AY188447* (*Homo sapiens* HDJ3), *NP_446142* (*Rattus norvegicus* dopamine receptor interacting protein), *AAH11146* (*Mus musculus* RIKEN cDNA 5730551F12 gene), *AAK28640* (*Bos taurus* J-domain protein Jiv) and *AAB19180* (BVDV2 non-structural protein NS2-3). *Thin line* Conserved J-domain, *bold line* NS2-3 protein homologous for these proteins. Alignment was performed by the GeneDoc program (http://www.cris.com/~Ketchup/genedoc.shtml). *Black* 100% similarity, *grey* 80%–90% similarity, *light grey* 60%–70% similarity

MDJ2P, SEC63, auxilin, virus T-antigens and Csp. They are involved in protein importing and sorting, protein translocation into the endoplasmic reticulum and interaction with hsp70, cell cycle regulation and exocytosis (Kanazawa et al 1997; Nishikawa et al 2001; Misselwitz et al. 1999). For example, auxilin forms part of the clathrin baskets of clathrin-coated vesicles (Ma et al. 2002).

HDJ3 protein is highly homologous to BVDV2 NS2-3 protein, which is a metalloprotease in the viral genome. Processing of the NS2-3 protein in cytopathic BVDV1 occurs by several different strategies depending on the viral strain and the insertion of the sequences into the viral genome (Rinck et al. 2001) but autoproteolysis at the NS2-NS3 junction has been found in the hepatitis C virus polyprotein (Wu et al. 1998). Recently, the cow cellular protein Jiv, which is highly homologous to HDJ3 protein, has been identified. Jiv contains the J-domain and forms a stable complex with pestiviral non-structural protein NS2. Jiv had the potential to induce the specific processing step of the cleavage of NS2-3 (Rinck et al. 2001). This has raised an interesting question regarding why and how the J-domain and NS2-3-like domain owner, Jiv protein, triggers the cleavage of the viral NS2-3 protein.

Another highly homologous protein of HDJ3 is the rat dopamine-receptor-interacting protein DRiP78. DRiP78 shares 76% sequence identity and is 80% sequence-positive to HDJ3. Dopamine modulates synaptic transmission in neural circuits acting through D1 receptors. D1–dopamine receptors stimulate the formation of adenosine 3',5'-monophosphate (cAMP) by coupling to Gs heterotrimeric GTP-binding (G) proteins (Huang et al. 1995). DRiP78 is the newly identified endoplasmic-reticulum (ER) membrane-associated protein that has been shown to bind to the carboxy-terminal hydrophobic motif, FxxxFxxxF (with x representing any amino acid), which is highly conserved among GPCRs, suggesting that DRiP78 might regulate the transport of a GPCR by binding to a specific ER-export signal (Bermak et al. 2001). PSROT analysis has revealed that the HDJ3 protein is a membrane-associated protein with similar

Fig. 1 (Continued)

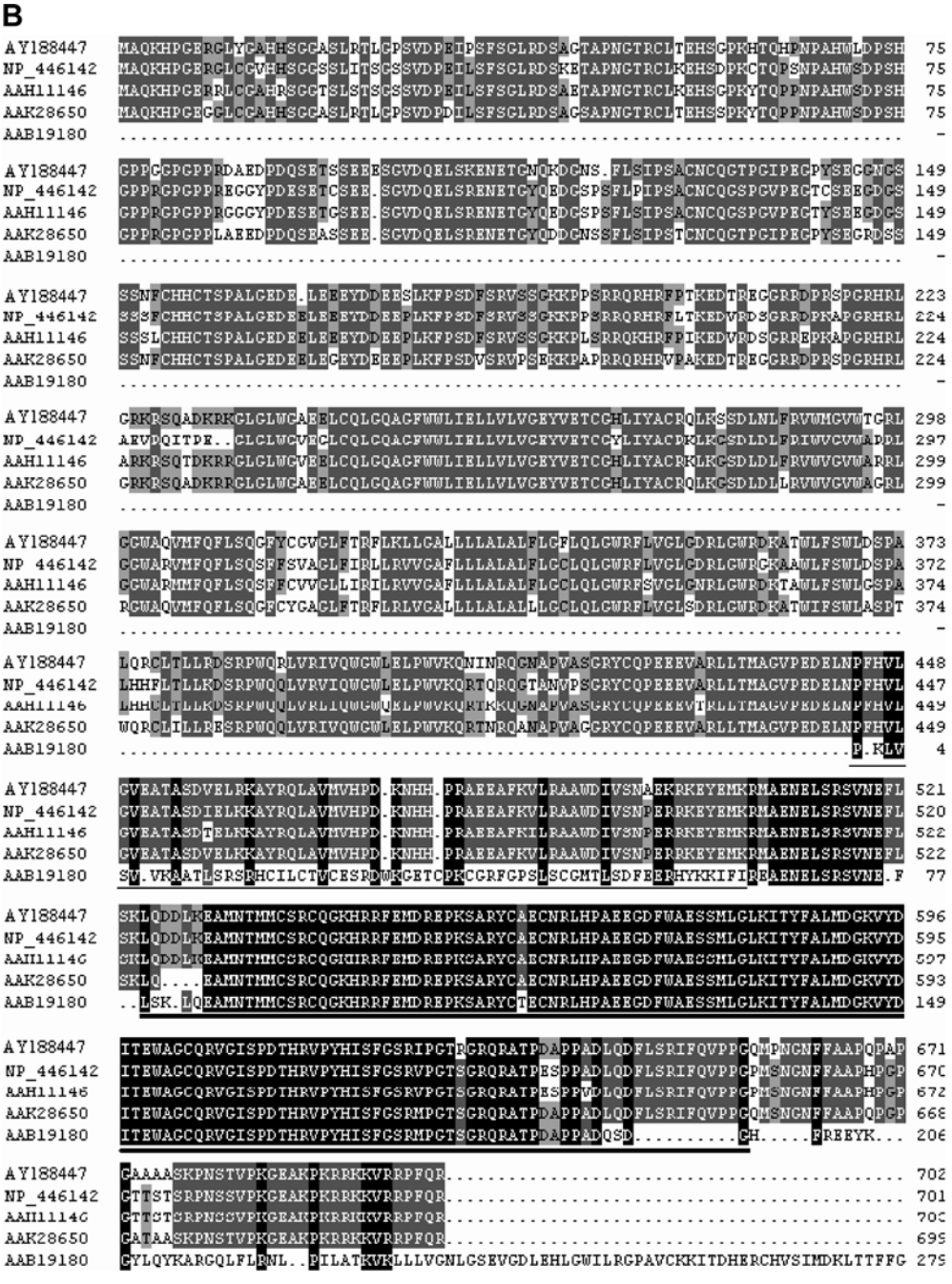

Table 1 Nucleotide sequence of exon–intron junctions of the HDJ3 gene. The intron sequence is shown in *lowercase* and the exon sequence is shown in *uppercase*

3' Splice acceptor	Exon	Size (bp)	5' Splice donor	Exon	Intron Size (bp)
cDNA end TCTCGTAGCTCA	1	64	CGCCGTTCTTCG**gt**acgtccgtct	1	772
tatctgttac**ag**GCATCAGAACAC	2	1463	CTGGCAGTGATG**gt**gagtaccctt	2	3743
ctgtcttggt**ag**GTTCATCCTGAC	3	107	GGAGTATGAGAT**gt**aagttggaga	3	212
ttcttctatt**ag**GAAACGAATGGC	4	120	AGGAAAGCATAG**gt**atgaaagaga	4	337
tgttggttgc**ag**GAGGTTTGAAAT	5	161	ATGACATCACAG**gt**actctctgtc	5	96
tcatgtttat**ag**GCTGGGCTGGAT	6	103	AGGGCGGCAGAG**gt**aggtggtatt	6	185
gttttacctc**ag**AGCCACCCCAGA	7	1230	TCTTTGTTGCCC–	7	–

characteristics to DRiP78 protein. Taken together with its expression in the brain, HDJ3 protein might also act as a protein translocation machinery at the ER, like the J-domain yeast protein, Sec63p (Misselwitz et al. 1999), which mediates the signal transduction of dopamine.

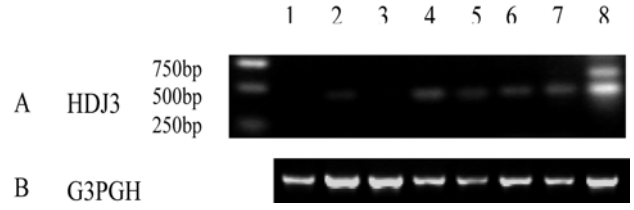

Fig. 2A, B Expression of HDJ3 gene in eight adult human tissues. **A** Normal tissue distribution of HDJ3. **B** Glucose-3-phosphate dehydrogenase (*G3PDH*) expression as a positive control. *Lanes 1–8* Human tissue heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas, respectively

Acknowledgements This research was funded by a grant from the Natural Science Foundation of China (30100192).

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