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

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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.

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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Y. Huang United Gene Holdings, 200092, Shanghai, P.R. China **Keywords** HDJ3 gene · Expression pattern · Chaperone · Chromosome 12q13.1–12q13.2

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 SfiI recognition sites, i.e. SfiI A (5'-GGCCAT-TATGGCC-3') and SfiI B (5'-GGCCGCCTCGGCC-3'), between the EcoRI and NotI sites of pBluescript II SK (+). Doublestranded 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). PRO-FILESCAN 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 MTCbased 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'-ggagatacctacagctggcatccagc-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-acidresidue 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) membraneassociated 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

Α 61 CTTCGGCATCAGAACACACCATGATGGCTGTGACCTAAGACCCTCAGGAAGCCCCGGGGT 120 121 CATGGCCCAGAAGCACCCCGGAGAAAGAGGGTTGTATGGAGCCCACCACAGTGGTGGTGC 180 M A Q K H P G E R G L Y G A H H S G G A 181 CTCCCTCAGGACTTTAGGACCCTCCGTGGACCCTGAAATACCTTCATTCTCAGGACTCAG 240 S L R T L G P S V D P E I P S F S G L R 241 GGACTCAGCAGGGACTGCTCCTAATGGTACCCGCTGCCTCACAGAGCACTCTGGTCCTAA 300 D S A G T A P N G T R C L T E H S G P K 301 GCACACACAGCACCCAAACCCAGCCCATTGGTTGGACCCAAGCCATGGCCCCCCAGGGGG 360 ТОНР N P A H W L D P S H G P 361 TCCAGGACCACCTAGAGATGCAGAGGACCCTGATCAGAGTGAGACGTCTTCAGAAGAAGA 420 P G P P R D A E D P D Q S E T S S E E E 421 ATCAGGAGTGGACCAGGAACTCTCAAAAGAAAACGAGACTGGGAACCAGAAGGATGGGAA 480 S G V D O E L S K E N E T G N O K D G N 481 CTCTTTTCCATTCCATCTGCTTGCAACTGCCAGGGAACACCTGGAATTCCAGAAGG 540 S F L S I P S A C N C Q G T P G I P E G 541 GCCTTACTCTGAGGGAGGAAATGGTTCTTCTAGCAACTTTTGCCACCACTGTACCTCTCC 600 P Y S E G G N G S S S N F C H H C T S P A L G E D E L E E E Y D D E E S L K F 661 CAGTGATTTTTCACGTGTGTCCAGCGGAAAGAAACCCCCCATCCCGGAGACAGCGGCACCG 720 S D F S R V S S G K K P P S R R Q R H R 721 CTTTCCAACGAAGGAGGATACTCGGGAGGGTGGACGTAGGGATCCCAGGTCCCCTGGTCG 780 F P T K E D T R E G G R R D P R S P G R 781 ACATCGGCTGGGTCGGAAACGAAGTCAGGCAGATAAGCGCAAAGGCCTGGGATTGTGGGG 840 H R L G R K R S Q A D K R K G L G L W G AGCCGAGGAACTATGTCAACTTGGACAGGCAGGCTTTTGGTGGCTGATTGAACTGCTGGT 900 841 A E E L C Q L G Q A G F W W L I E L L V 901 ATTGGTGGGAGAGTACGTAGAAACTTGTGGCCATCTCATCTATGCCTGCAGGCAACTGAA 960 LVGEYVETCGHLIYACROLK 961 AAGCAGTGATTTGAACCTTTTTCGAGTTTGGATGGGAGTGTGGACAGGGCGGTTAGGGGG 1020 S S D L N L F R V W M G V W T G R L 1021 CTGGGCCCAGGTCATGTTTCAGTTTCTAAGCCAGGGGTTTTACTGTGGAGTAGGACTGTT 1080 W A O V M F O F L S O G F Y C G V G L F 1081 TACTCGTTTTCTTAAGCTGCTGGGTGCTTTGCTGCTCCTGGCTCTGGCCCCTCTTTTTGGG 1140 T R F L K L L G A L L L L A L A L F L G 1141 CTTTCTACAGTTGGGATGGCGGTTTCTGGTGGGACTAGGTGACCGGTTAGGCTGGAGGGA 1200 FLQLGWRFLVGLGDRLGWRD 1201 TAAGGCTACCTGGCTCTTCTCTTGGCTGGATTCTCCAGCCTTGCAGCGTTGCTTGACTCT 1260 KATWLFSWLDSPALORCLTL L R D S R P W Q R L V R I V Q W G W 1321 GTTGCCTTGGGTCAAGCAGAATATTAATAGGCAGGGGAATGCACCTGTAGCTAGTGGGCG 1380 L P W V K O N I N R O G N A P V A S G R 1381 CTACTGCCAGCCTGAAGAGGAAGTGGCTCGACTCTTGACCATGGCTGGGGTTCCTGAGGA 1440 Y C Q P E E E V A R L L T M A G V P E D 1441 TGAGCTAAACCCTTTCCATGTACTGGGGGGTTGAGGCCACAGCATCAGATGTTGAACTGAG 1500 E L N P F H V L G V E A T A S D V E L F 1501 GAAGGCCTATAGACAGCTGGCAGTGATGGTTCATCCTGACAAAAATCATCATCCCCGGGC 1560 K A Y R O L A V M V H P D K N H H P R A TGAGGAGGCCTTCAAGGTTTTGCGAGCAGCTTGGGACATTGTCAGCAATGCTGAAAAGCG 1620 A F K V L R A A W D I V S N A E K R 1621 AAAGGAGTATGAGATGAAACGAATGGCAGAGAATGAGCTGAGCCGGTCAGTAAATGAGTT 1680 K E Y E M K R M A E N E L S R S V N E I 1681 TCTGTCCAAGCTGCAAGATGACCTCAAGGAGGCAATGAATACTATGATGTGTAGCCGATG 1740 L S K L O D L K E A M N T M M C S R C 1741 CCAAGGAAAGCATAGGAGGTTTGAAATGGACCGGGAACCTAAGAGTGCTAGATACTGTGC 1800 Q G K H R R F E M D R E P K S A R Y 1801 TGAGTGTAATAGGCTGCATCCTGCTGAGGAAGGAGACTTTTGGGCAGAGTCAAGCATGTT 1860 E C N R L H P A E E G D F W A E S S M L 1861 GGGCCTCAAGATCACCTACTTTGCACTGATGGATGGATAGGTGTATGACATCACAGAGTG 1920 G L K I T Y F A L M D G K V Y D 1921 GGCTGGATGCCAGCGTGTAGGTATCTCCCCAGATACCCACAGAGTCCCCTATCACATCTC 1980 A G C O R V G I S P D T H R V P Y H I S 1981 ATTTGGTTCTCGGATTCCAGGCACCAGAGGGCGGCAGAGAGCCACCCCAGATGCCCCTCC 2040 FGSRIPGTRGRQRATPDAPP 2041 TGCTGATCTTCAGGATTTCTTGAGTCGGATCTTTCAAGTACCCCCAGGGCAGATGCCCAA 2100 A D L Q D F L S R I F Q V P P G Q M P N 2101 TGGGAACTTCTTTGCAGCTCCTCAGCCTGCCCCTGGAGCCGCTGCAGCCTCTAAGCCCAA 2160 G N F F A A P O P A P G A A A A S K P N 2161 CAGCACAGTACCCAAGGGAGAAGCCAAACCTAAGCGGCGGAAGAAAGTGAGGAGGCCCTT 2220 T V P K G E A K P K R R K K V 2221 CCAACGTTGATGCCCCTTCTCTTTCCTCAAATCAATGTCAGGGAGTCAAAAGGGCTGTAG 2280 0 R 2281 CACAGGATGGAGTTTGATTTATCCTTCCTCCCCCAACACCTAGGAACTGAATCTTTTTCT 2340 2341 TTTTATTTTTTGAGATGGAGTCTTGCTCTGTTGCCCAGCTGGAGTGCAGTGGTGTGATCT 2400 2401 CAGCTTACTGCAACCTCTGTCTCCCGGGTTCAAGCAATTCTCCCATCTCAGCCTCCTGAG 2460 2461 TAGCTGGGATTACAGGCACACCACCACCACCTGGCCCAGCTAATTCTTTTTGTATTTT 2520 2521 TAGTAGAGACGGGGTTTCACCATGTTGCCCAGGCTGGTCTCGAACTCCTGAGCTCAGGTG 2580 2581 ATCCACCCGTCTTGGCCTCCCAAAGTGCTGGATTACAGGCATAAGCCACTGTGCCCGGCC 2640 2641 TGAATCTTGTCTTTTGACAATACCAAAGAAATAGGGGGGTAGCTAGAGTAAAGAACCTAGG 2700 2701 GCCTGGACCTGGGCTGGACAGTGTATCCCTTTAGGTGTGGGAACTGGGTATTTCCCTGGG 2760 ${\tt 2761}\ {\tt GTCTGTATGCCTTTGTCTTGTCATTTGCTTTTAGGGCAGATGACACTTTTTCCCACCCTT}\ {\tt 2820}$ 2881 CCTGATATAATATTTAAAAGACAGAACAAGAAAGCATGTAGCCCTAATGATAGGAGATTA 2940 2941 TCGCATAGAGTTCAGAGACTGGAAACTGAATTTTCCCTCGACTTTCACTTTGTGGGTAAA 3000 3001 TCACCCAATTTTAGGCTCTTTTCTGCAAGGATGGCCAAAATTAATCATTTTTAAAAAGTA 3060 3061 GATTCATGCCCACTGCCCTTGGGTGAGGGGGAAGAATACGGGGGTTCCCAGAAGCCCCCA 3120 3181 TTTAAAGCCAGGGGATTATCTTTCTATAAATGTATAACTGGCAACCTGTATCTTCCCTCT 3240 3241 TTGTTGCCC

220

в		
AY188447 NP_446142 AAH11146 AAK28650 AAB19180	MAQKHPCERCLYCAHHSGCASLETLCPSVDPETDSFSGLEDSACTAPNGTRGLTEHSGPRHTQHPMPAHWLDPSH MAQKHPGERGLCCVHHSGGSSLTTSGSSVDPETLSFSGLEDSKETAPNGTRGLKEHSDPRCTQPSMPAHWSDPSH MAQKHPGERRLCCAHRSGGTSLSTSGSSVDPETLSFSGLEDSAETAPNGTRGLKEHSGPRYTQPPMPAHWSDPSH MAQKHPGECCLCCAHHSGGASLPTLGPSVDPDHLSFSGLEDSAGSAPNGTRGLTEHSSPRYTQPPMPAHWSDPSH	75 75 75 75 -
AY188447 NP_446142 AAH11146 AAK28650 AAB19180	CPPCGPCPPRDAEDPDQSETSSEEBSGVDQELSKENETCN <mark>OKDCNS.FLSIPSACNCQCTPCIPEC</mark> PYSECCNCS CPPRCPCPPREGGYPDESETCSEE.SGVDQELSRENETCTOEDCSPSFLPIPSACNCQCSPGVPECTCSEECDCS CPPRCPCPPRCGGYPDESETCSEE.SGVDQELSRENETCYQEDCSPSFLSIPSACNCQCSPGVPECTYSEECDCS CPPRCPCPPLAEEDPDQSEASSEE.SGVDQELSRENETCYQDDCNSSFLSIPSTCNCQCTPCIPECPYSECRDSS	149 149 149 149 -
A Y188447 NP_446142 AAH11146 AAK28650 AAB19180	SSNFCHHCTSPALCEDE, LEBEYDDEESLKFPSDFSRVSSCKKPPSRQPHRFPTKEDTRECCRRDPRSPGRHRL 33 <mark>37</mark> CHHCTSPALCEDELEBEYDDEEPLKFP3DF3RV3SCKKPPSRQPHRFLTKEDVRDSGRRDPNAPGRHRL SS <mark>SL</mark> CHHCTSPALCEDEELEBEYDDEEPLKFPSDFSRVSSCKKPLSRQKHRFFTKEDVRDSGRREPKAPGRHRL SSNFCHHCTSPALCEDEELEGEYDBEEPLKFPSDVSRVPSEKKPAPRQPHRVPAKEDTRECGRRDPRSPGRHRL	223 224 224 224 -
AY188447 ND_446142 AAH11146 AAK28650 AAB19180	GRERSQADEREGLGLWCABELCQLGQAGFWWLIELLVLVGBYVETCCHLIYACEQLESSDLNLFEVWMGVW <mark>TCRL AEV</mark> DQITDECLCLWOV <mark>EC</mark> LCQLCQACFWWLIELLVLVCEYVETCC <mark>Y</mark> LIYACDCLKCSDLDLFDIWVCVWADDL ARERSQTDERRGLGLWCVEELCQLGQAGFWWLIELLVLVGBYVETCCHLIYACER <mark>LKC</mark> SDLDLFEVWVGVWAREL G <mark>RERSQADEREGLGLWC</mark> ABELCQLGQAGFWWLIELLVLVGBYVETCCHLIYACEQLKCSDLDLF	298 297 299 299 -
A Y188447 NP 446142 AAH11146 AAK28650 AAB19180	CGWAQWHFQFLSQCFYCCWGLFTRFLKLLGALLLLALALFLCFLQLCWRFLWGLCDRLGWRDKATWLFSWLDSPA CGWARWMFQFLSQSFFSVAGLFTRLLRVWGAFLLLALALFLCGLQLGWRFLWGLCDRLGWRCKAAWLFSWLDSPA CGWARMMFQFLSQSFFCVWGLLTRTLRVWGAFLLLALALFLGGLQLGWRFSWGCNRLGWRDKTAWLFSWLGSPA RGWAQVMFQFLSQCFCYGAGLFTRFLRLWGALLLLALALLLGCLQLGWRFLVGLSDRLGWRDKATWIFSWLASP	373 372 374 374 -
A Y188447 NP_446142 AAH11146 AAK28650 AAB19180	LQRCLTLLRDSRPWQRLVRIVQWGWLELPWVKO <mark>NINROGNAPWAS</mark> GRYCQPEEEWARLLTHAGVPEDELNPFHVL LHHFLTLLKDSRPWQQLVRVIQWGWLELPWVKORTOROGTANWPSGRYCQPEEEWARLLTHAGVPEDELNPFHVL LHHCLTLLKDSRPWQQLVRLIQWGWLELPWVKORTKKUGNAPWASGRYCQPEEEWARLLTHAGVPEDELNPFHVL WQRCLTLLRESRPWQQLVRIVQWGWLELPWVKORTNROANAPWAGGRYCQPEEEWARLLTHAGVPEDELNPFHVL P.KLV	448 447 449 449 4
AY188447 NP_446142 AAH11146 AAK28650 AAB19180	GVEATASDVELRKAYRQLAVMVHPD. KNHH. PRAEEAFKVLRAAWDIVSN <mark>A</mark> EKRKEYEMKRMAENELSRSVNEFL GVEATASDIELKKAYRQLAVMVHPD. KNHH. PRAEEAFKVLRAAWDIVSNPERRKEYEMKRMAENELSRSVNEFL GVEATASD <mark>I</mark> ELNKATRQLAVMVHPD. KNHH. PRAEEAFKILRAAWDIVSNPERRKETEMKRMAENELSRSVNEFL GVEATASDVELKKAYRQLAVMVHPD. KNHH. PRAEEAFKVLRAAWDIVSNPERRKEYEMKRMAENELSRSVNEFL S <mark>V.VKAAT</mark> L <mark>SRSBHCILCTVCESRDWKGETCPKCGRFGPSLSCCMTLSDFEERWKKIFI</mark> RBAENELSRSVNEF.	521 520 522 522 77
AY188447 NP_446142 AAH11146 AAK28650 AAB19180	SKL QDDLR BANNTMMCSRCQCKHRRF END REPKSARYCAECNRLHPAEEGD FWAESSMLGLKI TYFALMDGKVYD SKL QDDLR BANNTMMCSRCQCKHRRF END REPKSARYCAECNRLHPAEEGD FWAESSMLGLKI TYFALMDGKVYD SKL QDDLUBANNTMMCSRCQCKHRRF END REPKSARYCAECNRLHPAEEGD FWAESSMLGLKI TYFALMDGKVYD SKL QBANNTMMCSRCQCKHRRF END REPKSARYCAECNRLHPAEEGD FWAESSMLGLKI TYFALMDGKVYD LSK. LQEANNTMMCSRCQCKHRRF END REPKSARYCAECNRLHPAEEGD FWAESSMLGLKI TYFALMDGKVYD	596 595 597 593 149
A Y188447 NP_446142 AAH11145 AAK28650 AAB19180	ITEWAGCQRVGISPDTHRVPYHISFGSRIPGT <mark>R</mark> GRQRATPDAPPADLQDFLSRIFQVPPCQMPNGNFFAAPQPAP ITEWAGCQRVGISPDTHRVPYHISFGSRVPGTSGRQRATPESPPADLQDFLSRIFQVPPCPMSNCNFFAAPHPGP ITEWAGCQRVGISPDTHRVPYHISFGSRVPGISGRQRATPESPPADLQDFLSRIFQVPPCOMSNGNFFAAPHPGP ITEWAGCQRVGISPDTHRVPYHISFGSRMPGTSGRQRATPDAPPADLQDFLSRIFQVPPCOMSNGNFFAAPAQPCP ITEWAGCQRVGISPDTHRVPYHISFGSRMPGTSGRQRATPDAPPADQSD	671 670 672 668 206
A Y188447 NP_446142 AAH11146 AAK28650 AAB19180	CAAAASKPNSTVPRGEARPKRRKKVRRPFQR CTTSTSRPNSSVPRGEARPKRRKVRRPFQR CTTSTSRPNSSVPRGEARPKRRKVRRPFQR CATAASKPNSTVPRGEARPKRRKVRRPFQR CYLQYKARCQLFL <mark>R</mark> NL. <mark>P</mark> ILAT <mark>KVR</mark> LLLVGNLGSEVGDLEHLGWILRGPAVCKKITDHERCHVSIMDKLTTFFG	702 701 700 695 275

Table 1Nucleotide sequenceof exon-intron junctions of theHDJ3 gene. The intronsequence is shown in *lowercase*and the exon sequence is shownin uppercase

3' Splice acceptor	Exon Size (bp) 5' Splice donor			Intron Size (bp)	
cDNA end TCTCGTAGCTCA tatctgttacagGCATCAGAACAC ctgtcttggtagGTTCATCCTGAC ttcttctattagGAAACGAATGGC tgttggttgcagGAGGTTTGAAAT tcatgtttatagGCTGGGCTGGAT gttttacctcagAGCCACCCCAGA	2 14 3 4 5 6	463 107 120 161 103	CGCCGTTCTTCG gt acgtccgtct CTGGCAGTGATG gt gagtaccctt GGAGTATGAGAT g taagttggaga AGGAAAGCATAG gt atgaaagaga ATGACATCACAG gt actctctgtc AGGGCGGCAGAG gt aggtggtatt TCTTTGTTGCCC-	4 5	772 3743 212 337 96 185

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.

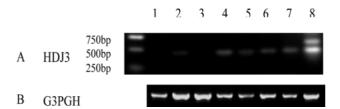


Fig. 2A, B Expression of HDJ3 gene in eight adult human tissues. **A** Normal tissue distribution of HDJ3. **B** Glucose-3-phosphate dehydrogenase (G3PDH) expression as a positive control. Lanes 1-8 Human tissue heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas, respectively

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