doi:10.1006/geno.2002.6770, available online at http://www.idealibrary.com on IDEAL **Article**

PJA1, Encoding a RING-H2 Finger Ubiquitin Ligase, Is a Novel Human X Chromosome Gene Abundantly Expressed in Brain

Ping Yu,[1,*] Yiwang Chen,[2,*] Danilo A. Tagle,[3] and Tao Cai[4,†]

[1]*Structure Biophysics Laboratory, NCI-Frederick, Frederick, Maryland 21702, USA*
[2]*Fujian Medical University, Fuzhou, Fujian, P.R. 350001 China*
[3]*Neurogenetics, Neuroscience Center, NINDS, National Institutes of Health, Bethesda, Maryland 20892, USA*
[4]*Experimental Medical Section, NIDCR, National Institutes of Health, Bethesda, Maryland 20892-4322, USA*

[*]*These authors contributed equally to this work.*

[†]*To whom correspondence and reprint requests should be addressed. Fax: (301) 402-4163. E-mail: tcai@mail.nih.gov.*

RING-finger proteins contain cysteine-rich, zinc-binding domains and are involved in the formation of macromolecular scaffolds important for transcriptional repression and ubiquitination. In this study, we have identified a RING-H2 finger gene, *PJA1* (for praja-1), from a human brain cDNA library and mapped it to human chromosome Xq12 between markers *DXS983* and *DXS1216*, a region implicated in X-linked mental retardation (MRX). Northern blot analysis indicated a 2.7-kb transcript that was abundantly expressed in the brain, including regions of the cerebellum, cerebral cortex, medulla, occipital pole, frontal lobe, temporal lobe, and putamen. Amino acid sequence analysis of the 71-kDa protein PJA1 showed 52.3% identity to human PJA2 (for praja-2, also known as NEURODAP1/KIAA0438) and also a significant identity to its homologs in rat, mouse, and zebrafish. *In vitro* binding and immunoprecipitation assays demonstrated that both PJA1 and PJA2 are able to bind the ubiquitin-conjugating enzyme UbcH5B. Moreover, the ubiquitination assay indicated that PJA1 and PJA2 have an E2-dependent E3 ubiquitin ligase activity. Thus our findings demonstrate that PJA1 can be involved in protein ubiquitination in the brain and is a suitable candidate gene for MRX.

Key Words: RING-H2 finger gene, ubiquitin ligase, chromosome Xq12, X-linked mental retardation, ubiquitination, brain

INTRODUCTION

Protein ubiquitination and subsequent degradation by proteasome are essential for many cellular processes [1]. Ubiquitination of a target protein requires sequential reactions involving three types of enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin-ligase (E3). Protein ubiquitination begins with the formation of a thiol-ester linkage between the carboxy terminus of ubiquitin and the active site cysteine of E1. Ubiquitin is then transferred to an E2 molecule and the interaction of E3 with E2 allows the transfer of ubiquitin to the target protein. One class of E3 ubiquitin-ligase consists of proteins with a RING-finger motif, which does not form a thioester intermediate with ubiquitin [2]. Proteins with RING-finger motifs, including AO7, BRCA1, SIAH-1, TRC8, NF-X1, KF-1, and PJA1 (for praja-1), have been found to interact with the ubiquitin-conjugating enzyme UbcH5B [2]. Other RING proteins have also been found to partner with other E2s: UIP28 interacts with UbcM4 [3], HHARI with UbcH7 [4], c-Cbl with Ubc4 [5], Apc11p with Ubc4 [6], Ubr1p with Ubc2p [7], Mdm2 with UbcH5B [8], RNF2 with Hip2 [9], Rma1 with Ubc4/5 [10], and XIAP with UbcH5B [11]. These RING proteins are involved in diverse cellular functions, such as tumor suppression (Cbl, BRCA1, and Mdm2), apoptosis (c-IAPs and XIAP), cell cycle (Apc11 and Rbx1), DNA repair (Rad5, Rad16, and Rad18), and vesicular transport (Vps8, Pep5, and Vps18).

Several RING finger genes have been found that are mutated in human diseases [12–14]. For instance, the parkin gene was found to be mutated in autosomal recessive familial juvenile Parkinsonism [14]. Later, aSp22 was found to be a substrate for parkin's ubiquitin ligase activity in normal

0888-7543/02 $35.00

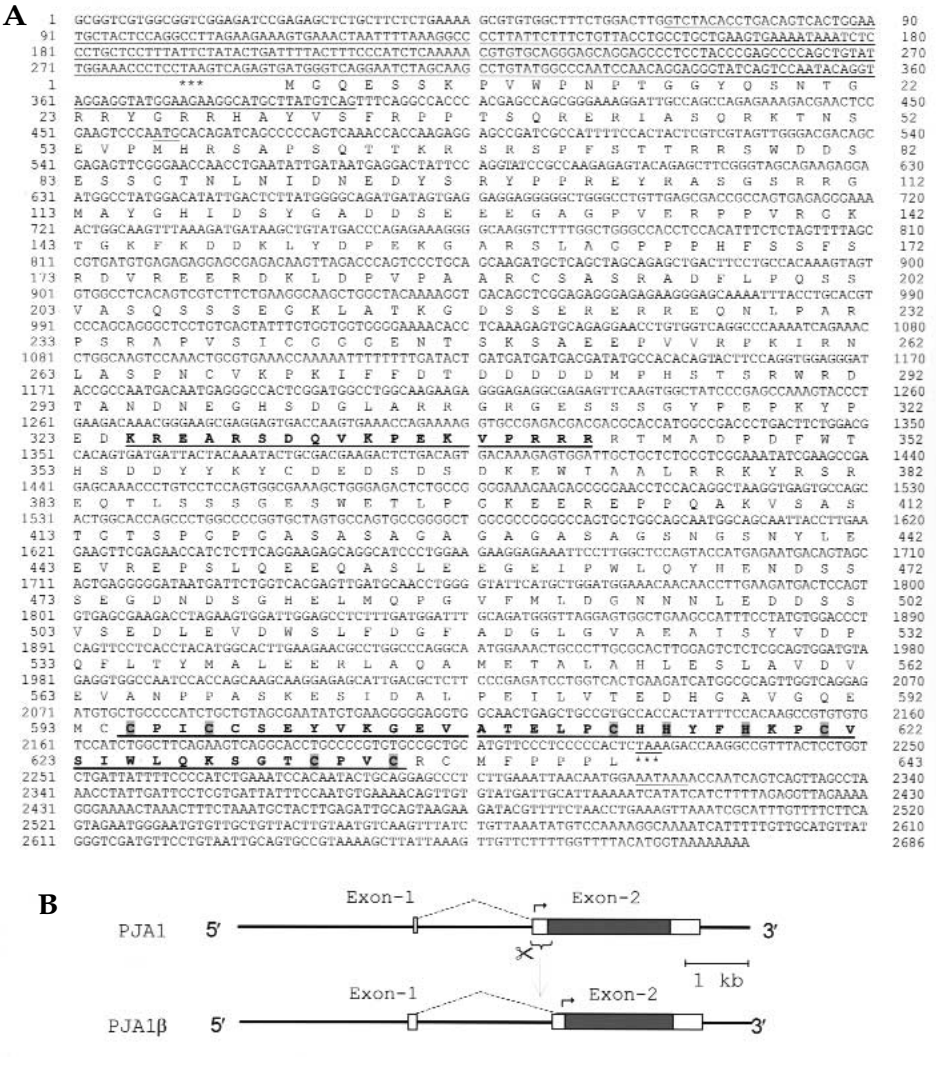

FIG. 1. Sequence and genomic structure of human *PJA1*. (A) Nucleotide and deduced amino acid sequence. The RING-H2 finger motif (amino acids 595–635) is underlined and in boldface. Conserved sequences of the motif are in light gray boxes. The NLS region (amino acids 325–342) is underlined and in boldface. An in-frame stop codon (TAA) is present at nt 283 before the start site and indicated with asterisks. *PJA1β* lacks 326 bp (nt 67–392, underlined) and its start codon is at nt 460 (underlined). The polyadenylation signal (AATAAA) at nt 2313 is underlined. (B) Genomic structure comparison of *PJA1* and *PJA1β*. Exonic sequences are depicted as boxes, with coding regions shown as shaded areas and untranslated region as open boxes. The 326 bp at the beginning of *PJA1* exon 2 were not used in the *PJA1β*, as indicated by the scissors. Start codon is indicated by the arrow.

human brain, and loss of parkin function causes pathological αSp22 accumulation in patient brain [15].

In this study, we have identified a novel human RING-H2 finger gene, *PJA1*, and mapped it to human chromosome Xq12, where several X-linked mental retardation (MRX) disorders have been associated [16–22]. We have also demonstrated that PJA1 can interact with E2 ubiquitin-conjugating enzyme UbcH5B and that PJA1 can function as an E3 ubiquitin-ligase during the ubiquitination process.

RESULTS

Identification and Sequence Analysis of Human *PJA1*

By searching the human EST database with the mouse *Pja1* cDNA sequence, we identified and sequenced two EST clones, which contain the full-length coding sequence of human *PJA1* (GenBank acc. nos. R12654 and R17531). Sequence analysis demonstrates that *PJA1* encodes a 643-amino-acid protein with a calculated molecular weight of 71 kDa (GenBank acc. no. AF262024). The translated product is predicted to be acidic with a p*I* of 5.05. A motif search showed that a RING-H2 ($C_3H_2C_3$) motif [23] is located between amino acids 595 and 635 at the carboxy terminus (Fig. 1A). A motif search using PFSCAN server also showed a potential nuclear localization signal (NLS) between amino acids 325 and 342 (Fig. 1A). Pairwise sequence analysis of human PJA1 and mouse PJA1 [24] demonstrated a significant sequence conservation with 80.5% identity (Table 1) between the two species. We also identified PJA1 homologous sequences in rat and zebrafish (partial sequence), which showed 81.7% and 57.4% identity, respectively (Table 1). *PJA1* also shares high degree of homology with another RING-H2 containing gene, *PJA2* (for praja-2, also known as NEURODAP1 or KIAA0438): 52.3% sequence identity to human *PJA2* [25], 50.3% to rat *Pja2* [26], 50.3% to mouse *Pja2*, and 40.7% to zebrafish *Pja2* (Table 1). Multiple sequence alignment (Fig. 2A) of members of PJA1 and PJA2 across several species shows conservation of the C-terminal RING-H2 motif. Phylogenetic tree reconstruction (Figs. 2B and 2C) illustrates the relatedness of the two subfamilies, *PJA1* and *PJA2*. The placement of *PJA1* and *PJA2* from zebrafish as ancestral sequences likely reflects these as distantly related proteins or possibly the use of partial sequences available for this analysis.

TABLE 1: Chromosome location and degree of relatedness to human *PJA1*

Subfamily	Species	Chromosome	Amino acid	I (%)	S (%)	GenBank number	Reference
PJA1	human	Xq12	643	–	–	AF262024	this study
	rat	N.D.	606	81.7	84.7	AW919351	this study
	mouse	Xq12	605	80.5	84.4	NM_008853 AF335250	[24] [31]
	zebrafish	N.D.	195*	57.4	64.5	BG302763	this study
PJA2	human	5p13-q31	708	52.3	58.7	NM_014819	[25]
	rat	N.D.	707	50.3	57.0	BAA06979	[26]
	mouse	N.D.	707	50.3	56.7	AF493070	this study
	zebrafish	N.D.	121*	40.7	49.2	BI892055	this study

Genomic Organization and Chromosomal Localization

Comparison of *PJA1* cDNA with an 119-kb human genomic DNA sequence (GenBank acc. no. NT 019696) revealed that the *PJA1* transcript is encoded by two exons that are separated by a 1.9-kb intron sequence (Fig. 1B). Moreover, we found that the first 326-bp sequence of exon 2 (nt 67–392 of *PJA1*) is missing in several ESTs (GenBank acc. no. BG818926, AL538620, AL534391, and AL554289), which suggests that an alternative start codon at nt 460 is differentially used. We designated this alternative transcript as *PJA1β* (Figs. 1A and 1B), whose predicted translated product is 55 amino acids shorter than the full protein. However, sequence and northern blot analyses indicated that the transcript sizes for both *PJA1* and *PJA1β* (GenBank acc. no. AF264620) are approximately 2.7 kb due to the longer 5′-UTR of *PJA1β* compared with *PJA1* (Fig. 1B).

We have mapped *PJA1* to human chromosome Xq12 by fluorescent *in situ* hybridization (FISH) using the genomic clone F491 BAC DNA (data not shown). Moreover, we performed a fine chromosomal mapping using the G4 chromosomal mapping panel. *PJA1* tightly links to the marker *WI-14304* on chromosome Xq12. The order of flanking markers was defined as *DXS1194–DXS983–PJA1–DXS1216–SGC35157*. Based on genomic sequence analysis, we found that the marker *DXS1216* is located only 15 kb from *PJA1* (GenBank acc. no. AL157699).

Distribution of *PJA1* in Human Brain and Spinal Cord

Northern blot hybridization with an 837-bp cDNA probe from *PJA1* indicated a 2.7-kb transcript that was present in various brain regions (Fig. 3). The cerebral cortex has the highest level of *PJA1* transcript. As the probe contained sequences in common for both *PJA1* and *PJA1β*, we were unable to distinguish the two by northern blot. In addition to the 2.7-kb message, a 5.2-kb transcript was also detected in the brain regions examined for *PJA1*. This hybridization signal is likely due to a cross-reaction of the cDNA probe used with the homologous sequence *PJA2*. The *PJA2* cDNA is approximately 5 kb and shares overall a 58.0% sequence identity with *PJA1*. Moreover, the 837-bp *PJA1* cDNA probe used for the northern analysis shares 68.9% overall identity with *PJA2*, and the last 389 bp of the probe shares 82.9% sequence identity with *PJA2*.

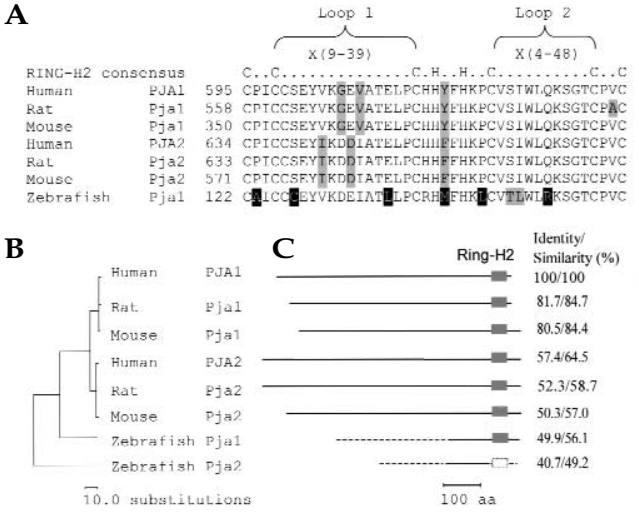

FIG. 2. Human PJA1 and its orthologous and homologous proteins in different species. (A) Multiple sequence alignment of RING-H2 regions from PJA1 and PJA2 sequences across various species. Loops and predicted coordination sites are indicated above. Similar amino acids are in gray and different amino acids are in black boxes. The RING-H2 consensus sequence is $C\times_2C\times(9–39)C\times(1–3)H\times(2–3)H\times_2C\times(4–48)C\times_2C$. (B) A phylogenetic tree of PJA1 and homologous sequences generated from sequence alignments. The scale bar beneath the tree represents 10 substitutions per 100 residues. (C) Representation of PJA1 and PJA2 proteins with RING-H2 motifs. Dashed lines represent incomplete sequences. A dashed open box represents an incomplete sequence of RING-H2 motif. The percent identity/similarity for each of these eight proteins compared with the human PJA1 is shown on the right side. The names of each of these proteins are respectively listed in (B).

doi:10.1006/geno.2002.6770, available online at http://www.idealibrary.com on IDEAL

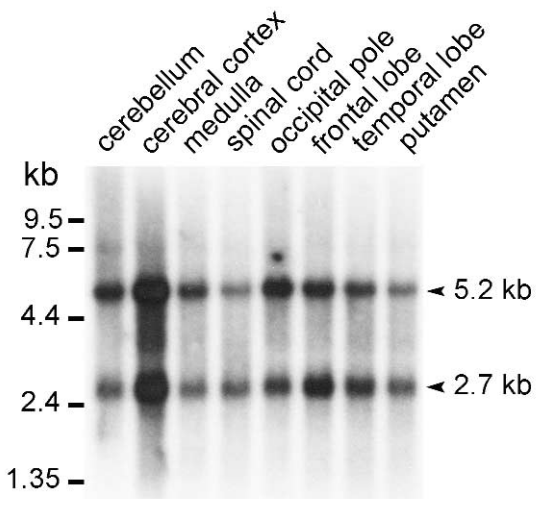

FIG. 3. Expression of *PJA1* by northern blot analysis. The mRNAs of human brain tissues are indicated on the top of each lane. The RNA ladder size marker is shown on the left. The *PJA1* transcript is shown as the 2.7-kb message with the *PJA2* cross-hybridizing message shown as the 5.2-kb signal.

Interaction of PJA1 with UbcH5B *in Vitro* and *in Vivo*

To examine the protein function of PJA1, we carried out the *in vitro* binding assays with UbcH5B and compared the interaction between PJA2 and UbcH5B. We produced the [^{35}S]-labeled PJA1-C (C terminus of PJA1) and PJA2-C proteins using a TnT labeling kit and assessed their capability to form a stable complex with the GST–UbcH5B protein. Both PJA1 and PJA2 bind to GST–UbcH5B protein efficiently, but not GST alone (Fig. 4A).

Furthermore, we have examined the interaction of PJA1 and PJA2 with UbcH5B in mammalian cells by using an *in vivo* binding assay. PJA1-C or PJA2-C was cotransfected with pCMV-Flag-UbcH5B separately into COS-7 cells. After harvesting the cells at 48 hours, we mixed the protein extracts with anti-c-myc monoclonal antibody that was coupled to Sepharose beads.

We used anti-c-myc polyclonal antibody to detect PJA1 and PJA2 from the proteins that bound to the beads, and used anti-FLAG monoclonal antibody to detect the coprecipitated protein UbcH5B. Our results indicated that PJA1 and PJA2 were bound to the Flag–UbcH5B fusion proteins (Fig. 4B).

PJA1 Plays a Role as an E2-Dependent Ubiquitin Ligase

Recent studies have shown that several proteins containing RING-H2 finger domains can function as E3 ubiquitin ligases [27–30]. To determine whether PJA1 has an E2-dependent E3 activity, we carried out *in vitro* ubiquitination assays. We found a ubiquitination pattern in the presence of E1, E2, and PJA1 or PJA2 (lanes 3 and 5 in Fig. 5), whereas no ubiquitination was detected in the absence of PJA1-C (lane 2) or PJA2-C (lane 4). This finding suggests that both PJA1 and PJA2 can function as an E3 ubiquitin-ligase. The orthologous mouse protein PJA1 was similarly observed to generate high molecular weight-smeared bands that indicated a polyubiquitination (lane 6) when combined with E1 and UbcH5B.

DISCUSSION

Here, we have identified a novel human chromosome X gene *PJA1* from human brain. *PJA1* is abundantly expressed in human brain tissues, including the cerebellum, cerebral cortex, medulla, occipital pole, frontal lobe, temporal lobe, and putamen. A similar expression pattern was observed for mouse *Pja1*, which is abundantly expressed in brain and to a lesser degree in liver and kidney [24]. mRNA expression of mouse *Pja1* has been shown in cortical and subcortical brain areas with highest levels in the neurons of the basolateral nucleus of

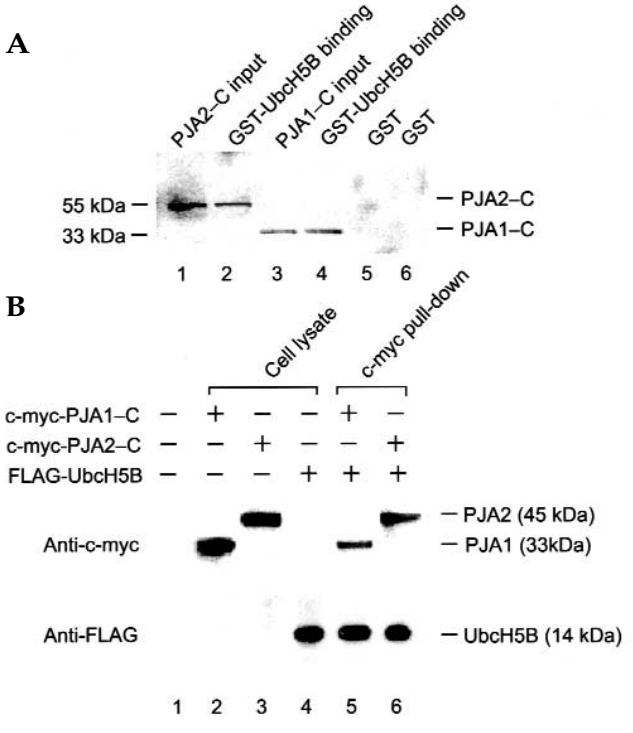

FIG. 4. PJA1-C and PJA2-C interact with UbcH5B. (A) [^{35}S]-Radiolabeled PJA1-C is shown as a 33-kDa band (lane 3) and it forms a complex with UbcH5B (lane 4), but not GST (lane 6). [^{35}S]-Radiolabeled PJA2-C is shown as 55 kDa (lane 1) and bound to UbcH5B (lane 2), but not GST (lane 5). (B) Coprecipitation of UbcH5B with PJA1-C or PJA2-C. c-myc-tagged PJA1-C (lane 2) or c-myc-tagged PJA2-C (lane 3) and Flag-tagged UbcH5B (lane 4) were cotransfected separately into COS-7 cells and detected by anti-c-myc and anti-Flag antibodies. Pellets of cell lysates from cotransfected Flag-tagged UbcH5B with c-myc-tagged PJA1-C or c-myc-tagged PJA2-C were incubated with anti-c-myc monoclonal antibody conjugated with Sepharose beads (lanes 5, 6), and transferred onto nitrocellulose membrane for western blot. PJA1-C and PJA2-C were precipitated as shown by an anti-c-myc polyclonal antibody. UbcH5B was coprecipitated as shown by an anti-FLAG antibody.

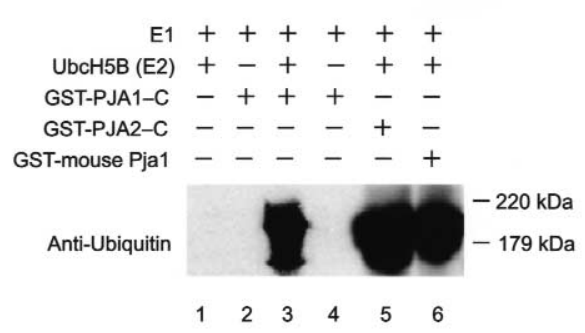

FIG. 5. PJA1 is involved in the E2-dependent ubiquitination *in vitro*. GST-PJA1-C fusion protein was incubated with (lane 3) or without (lane 2) UbcH5B; GST-PJA2-C fusion protein was also incubated with (lane 5) or without (lane 4) UbcH5B. Ubiquitination patterns of both proteins were investigated using a ubiquitin antibody. UbcH5B and mouse PJA1 interaction was used as a positive control (lane 6). No E3 load was used as a negative control (lane 1).

the amygdala, but not in the fiber tracts [31]. Moreover, *Pja1* expression was pronounced and showed learning-specific induction in the basolateral complex of the amygdala during formation of fear memory [31]. These findings support an important role in mice for PJA1 in neuronal plasticity that is the basis for learning and memory. In addition, rat PJA2 (NEURODAP1) is involved in protein sorting at the postsynaptic density region of axosomatic synapses and possibly plays a role in synaptic communication and plasticity [26].

Sequence analysis of the PJA family indicates that it belongs to a class of RING finger proteins with emerging functions as transcriptional repressors and ubiquitination factors. Protein ubiquitination is a highly regulated cellular process with E3 ubiquitin-ligases that play central roles. As a unique category of E3 ubiquitin-ligases, several RING-H2 finger containing proteins have been shown to interact with E2 proteins [2]. We have demonstrated that both PJA1 and PJA2 are ubiquitin protein ligases, and that they can interact and activate UbcH5B. Furthermore, we have showed that PJA1 and PJA2 are able to mediate their own ubiquitination and require no other eukaryotic proteins other than E1 ubiquitin-activating enzyme and an E2 ubiquitin-conjugating enzyme. It is apparent, therefore, that PJA1 and PJA2 manifest an intrinsic capacity to mediate ubiquitination. PJA1 and PJA2 could now be added to the growing category of E3 ligases, each of which has the potential to mediate substrate-specific ubiquitination via the RING finger domain. Moreover, the identification of PJA1 and PJA2 as additional E3 ubiquitin-ligases may help better understand how cells orchestrate the complexities of rapidly regulating heterologous protein levels and activity.

Previous studies established an important role for E3 ubiquitin-ligases in hereditary neurological disorders. Parkin contains an amino-terminal ubiquitin-like domain and its C-terminal half includes two atypical RING finger sequences. Parkin has been shown to associate with UbcH7 in a RING-dependent manner and mutations in parkin have been associated with juvenile familial Parkinson's disease [14]. Angelman syndrome, a neurobehavioral disorder with severe mental retardation, can be caused by mutations in the E3 ubiquitin protein ligase gene (*UBE3A*) [32,33]. Mice with maternal deficiency for *Ube3a* show motor dysfunction, inducible seizures, and a context-dependent learning deficit [34]. These mice also show severe impairment in long-term potentiation (LTP), indicating that ubiquitination may have a role in mammalian LTP.

Using FISH and radiation hybrid mapping, we were able to position PJA1 to Xq12 between markers *DXS983* and *DXS1216*. Several primary or nonspecific MRX disorders have also been linked to Xq12, for example MRXS6 [16], MRXS7 [17], MRX9 [18], MRX14 [19], MRX20 [20], MRX58 [21], and MRXSSD [22]. The causative genes in these MRX patients have not been identified [35] and our findings in this study and other converging evidence would suggest that it would be reasonable to search for mutations in *PJA1* in this cohort of MRX patients.

MATERIALS AND METHODS

Identification of PJA1 cDNA and genomic DNA clones. The human EST database was searched using the mouse *Pja1* cDNA as the query sequence. Two homologous EST clones derived from a human infant brain cDNA library were obtained from ResGen (Huntsville, AL) and sequenced. Both of them contain the full-length sequence of *PJA1*. The BAC clone F491 was isolated using the *PJA1* full-length cDNA as a probe to screen the High-Density Human Genomic Filters (Incyte Genomics, Palo Alto, CA). Rat *Pja1*, mouse *Pja2*, and zebrafish *Pja1* and *Pja2* were identified by sequencing EST clones (Table 1). A stretch of 123 bp or the corresponding C-terminal 41 amino acids of RING-H2 domains was aligned and a phylogenetic analysis was performed using PILEUP, DISTANCES (that is, unweighted pair group method), and GROWTREE programs of the Genetics Computer Group package (GCG, Wisconsin Package Version 10.2, Madison, WI). Motif sequences were identified using MOTIFS (GCG) and PFSCAN server (http://hits.isb-sib.ch/cgi-bin/PFSCAN).

Determination of PJA1 expression in human brain and spinal cord. Distribution of *PJA1* mRNA in human CNS was examined in northern blot, including eight neural tissues: cerebellum, cerebral cortex, medulla, spinal cord, occipital pole, frontal lobe, temporal lobe, and putamen (Clontech, Palo Alto, CA). An 837-bp probe was PCR-amplified from nt 1170–2006 of *PJA1* (for less cross reaction, RING-H2 motif was excluded) with primers (sense 5′-TACCGCCAATGACAATGAGG-3′; antisense 5′-TTGCTTGCTGGTGGATTGG-3′). The blot was probed with ^{32}P-labeled PCR product in ExpressHyb hybridization solution according to the manufacturer's instructions (Clontech). The PCR and northern blot conditions were described [36].

Chromosomal mapping. Digoxigenin dUTP-labeled DNA from BAC clone F491 was used as probe against normal metaphase chromosomes derived from PHA-stimulated peripheral blood lymphocytes. Specific hybridization signals were detected by incubating the hybridized slides with fluoresceinated anti-digoxigenin antibodies and followed with DAPI counterstaining. Fine chromosomal mapping was carried out using a G4 radiation hybrid panel (ResGen). A 473-bp fragment at 3′-UTR of *PJA1* was PCR-amplified as described [36] (primers: 5′-TGTCCATCTGGCTTCAGAAGTC-3′, 5′-GCAATTACAGGAACATCGACC-3′). The PCR result was used to query the RHserver (http://www.genome.wi.mit.edu/cgi-bin/mouse_rh/rhmap-auto/rhmapper.cgi) to determine chromosome position.

Pull-down, coimmunoprecipitation, and ubiquitination assays. PJA1(C was cloned into pCMV-c-myc (Stratagene, La Jolla, CA) and pGEX-4T (Amersham Pharmacia Biotech, Piscataway, NJ) vectors with PCR-amplified *PJA1* cDNA (amino acids 345–643; primer, 5′-TGGATCCATGGCCGACCCTGACTTCT-3′; 5′-AGAATTCTTAGAGTGGGGGAGGGAAC-3′). PJA2-C was linked into pCMV-c-myc and pGEX-4T with *PJA2* cDNA (amino acids 306–708; 5′-AGAATTCCATGGAAGTTCTCCTGAA-3′; 5′-ACTCGAGTTAGGGTGCTTCTGCAATA-3′) amplified from an EST (GenBank acc. no. BE786046). *UbcH5B* (amino acids 1–147, GenBank no. U39317; 5′-AGGATCCATGGCTCTGAAGA-

GAATC-3′; 5′-AGAATTCTTACATCGCATACTTCTGA-3′) [37] was amplified from a human brain cDNA library and linked with pCMV-FLAG vector (Stratagene). Mouse *Pja1* was cloned into pGEX-4T (amino acids 1–424; 5′-AGAATTCATGAGCCACCAGGAAAG-3′; 5′-ACTCGAGTTAGAACAAG GGCTCCTGTA-3′). The underlined parts of primer sequences indicate the restriction endonuclease sites used for cloning. ^{35}S-labeled PJA1-C and PJA2-C were generated using a TNT *in vitro* translation system (Promega, Madison, WI).

For coprecipitation experiments, pCMV-FLAG-tagged UbcH5B was cotransfected into COS-7 cells with c-myc-tagged PJA1–C or c-myc-tagged PJA2–C. A pull-down assay was performed as described [36]. Anti-Flag M2 and anti-c-myc antibodies were purchased from Stratagene.

GST-PJA1–C or GST-PJA2–C immobilized to the glutathione-Sepharose beads was combined with 20 ng E1, 20 ng UbcH5B, and 10 mg ubiquitin (Affiniti Research Products Ltd, Exeter, UK) in a ubiquitination buffer. The buffer consists of 50 mM Tris·HCl (pH 7.4), 2 mM ATP, 5 mM $MgCl_2$, 2 mM DTT, 1 mM creatine phosphate, 15 units of creatine phosphokinase, and BL-21 cell lysates. The reaction mixture was incubated for 1.5 hours at 30°C and terminated by adding a protein sample buffer (Invitrogen, Carlsbad, CA) before running on a SDS-PAGE gel. Western blot experiments were carried out using anti-ubiquitin antibody (Sigma-Aldrich, Inc., St Louis, MO). The experiment was carried out according to a previously published report with modifications [2,9].

ACKNOWLEDGMENT

We thank Charles Wohlenberg for helpful discussion.

RECEIVED FOR PUBLICATION OCTOBER 9, 2001;
ACCEPTED MARCH 11, 2002.

REFERENCES

1. Hershko, A., and Ciechanover, A. (1998). The ubiquitin system. *Annu. Rev. Biochem.* **67:** 425–479.
2. Lorick, K. L., *et al.* (1999). RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc. Natl. Acad. Sci. USA* **96:** 11364–11369.
3. Martinez-Noel, G., Niedenthal, R., Tamura, T., and Harbers, K. (1999). A family of structurally related RING finger proteins interacts specifically with the ubiquitin-conjugating enzyme UbcM4. *FEBS Lett.* **454:** 257–261.
4. Moynihan, T. P., *et al.* (1999). The ubiquitin-conjugating enzymes UbcH7 and UbcH8 interact with RING finger/IBR motif-containing domains of HHARI and H7-AP1. *J. Biol. Chem.* **274:** 30963–30968.
5. Joazeiro, C. A., *et al.* (1999). The tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase. *Science* **286:** 309–312.
6. Leverson, J. D., *et al.* (2000). The APC11 RING-H2 finger mediates E2-dependent ubiquitination. *Mol. Biol. Cell* **11:** 2315–2325.
7. Xie, Y., and Varshavsky, A. (1999). The E2-E3 interaction in the N-end rule pathway: the RING-H2 finger of E3 is required for the synthesis of multiubiquitin chain. *EMBO J.* **18:** 6832–6844.
8. Fang, S., Jensen, J. P., Ludwig, R. L., Vousden, K. H., and Weissman, A. M. (2000). Mdm2 is a RING finger-dependent ubiquitin protein ligase for itself and p53. *J. Biol. Chem.* **275:** 8945–8951.
9. Lee, S., *et al.* (2001). E3 ligase activity of RING finger proteins that interact with Hip-2, a human ubiquitin-conjugating enzyme. *FEBS Lett.* **503:** 61–64.
10. Matsuda, N., Suzuki, T., Tanaka, K., and Nakano, A. (2001). Rma1, a novel type of RING finger protein conserved from Arabidopsis to human, is a membrane-bound ubiquitin ligase. *J. Cell Sci.* **114:** 1949–1957.
11. Suzuki, Y., Nakabayashi, Y., and Takahashi, R. (2001). Ubiquitin-protein ligase activity of X-linked inhibitor of apoptosis protein promotes proteasomal degradation of caspase-3 and enhances its anti-apoptotic effect in Fas-induced cell death. *Proc. Natl. Acad. Sci. USA* **98:** 8662–8667.
12. Mattsson, K., Pokrovskaja, K., Kiss, C., Klein, G., and Szekely, L. (2001). Proteins associated with the promyelocytic leukemia gene product (PML)-containing nuclear body move to the nucleolus upon inhibition of proteasome-dependent protein degradation. *Proc. Natl. Acad. Sci. USA* **98:** 1012–1017.
13. Ruffner, H., Joazeiro, C. A., Hemmati, D., Hunter, T., and Verma, I. M. (2001). Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proc. Natl. Acad. Sci. USA* **98:** 5134–5139.
14. Kitada, T., *et al.* (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392:** 605–608.
15. Shimura, H., *et al.* (2001). Ubiquitination of a new form of α-synuclein by parkin from human brain: implications for Parkinson's disease. *Science* **293:** 263–269.
16. Wilson, M., Mulley, J., Gedeon, A., Robinson, H., and Turner, G. (1991). New X-linked syndrome of mental retardation, gynecomastia, and obesity is linked to DXS255. *Am. J. Med. Genet.* **40:** 406–413.
17. Ahmad, W., *et al.* (1999). Linkage mapping of a new syndromic form of X-linked mental retardation, MRXS7, associated with obesity. *Eur. J. Hum. Genet.* **7:** 828–832.
18. Willems, P., *et al.* (1993). Localization of a gene responsible for nonspecific mental retardation (MRX9) to the pericentromeric region of the X chromosome. *Genomics* **18:** 290–294.
19. Gendrot, C., *et al.* (1994). X-linked mental retardation exhibiting linkage to DXS255 and PGKP1: a new MRX family (MRX14) with localization in the pericentromeric region. *Clin. Genet.* **45:** 145–153.
20. Lazzarini, A., *et al.* (1995). Short tandem repeat polymorphism linkage studies in a new family with X-linked mental retardation (MRX20). *Am. J. Med. Genet.* **57:** 552–557.
21. Holinski-Feder, E., *et al.* (1999). Nonsyndromic X-linked mental retardation: mapping of MRX58 to the pericentromeric region. *Am. J. Med. Genet.* **86:** 102–106.
22. Siderius, L. E., *et al.* (1999). X-linked mental retardation associated with cleft lip/palate maps to Xp11.3-q21.3. *Am. J. Med. Genet.* **85:** 216–220.
23. Saurin, A. J., Borden, K. L., Boddy, M. N., and Freemont, P. S. (1996). Does this have a familiar RING? *Trends Biochem. Sci.* **21:** 208–214.
24. Mishra, L., *et al.* (1997). Praja1, a novel gene encoding a RING-H2 motif in mouse development. *Oncogene* **15:** 2361–2368.
25. Ishikawa, K., *et al.* (1997). Prediction of the coding sequences of unidentified human genes. VIII. 78 new cDNA clones from brain which code for large proteins in vitro. *DNA Res.* **4:** 307–313.
26. Nakayama, M., Miyake, T., Gahara, Y., Ohara, O., and Kitamura, T. (1995). A novel RING-H2 motif protein downregulated by axotomy: its characteristic localization at the postsynaptic density of axosomatic synapse. *J. Neurosci.* **15:** 5238–5248.
27. Freemont, P. S. (2000). RING for destruction? *Curr. Biol.* **10:** R84–87.
28. Tyers, M., and Jorgensen, P. (2000). Proteolysis and the cell cycle: with this RING I do thee destroy. *Curr. Opin. Genet. Dev.* **10:** 54–64.
29. Jackson, P. K., *et al.* (2000). The lore of the RINGs: substrate recognition and catalysis by ubiquitin ligases. *Trends. Cell Biol.* **10:** 429–439.
30. Joazeiro, C. A., and Hunter, T. (2000). Biochemistry. Ubiquitination—more than two to tango. *Science* **289:** 2061–2062.
31. Stork, O., Stork, S., Pape, H. C., and Obata, K. (2001). Identification of genes expressed in the amygdala during the formation of fear memory. *Learn. Mem.* **8:** 209–219.
32. Kishino, T., Lalande, M., and Wagstaff, J. (1997). UBE3A/E6-AP mutations cause Angelman syndrome. *Nat. Genet.* **15:** 70–73.
33. Matsuura, T., *et al.* (1997). De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. *Nat. Genet.* **15:** 74–77.
34. Jiang, Y. H., *et al.* (1998). Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. *Neuron* **21:** 799–811.
35. Lubs, H. A., *et al.* (1996). XLMR genes: update 1996. *Am. J. Med. Genet.* **64:** 147–157.
36. Chen, Y. W., Yu, P., Lu, D., Tagle, D. A., and Cai, T. (2001). A novel isoform of β-spectrin II localizes to cerebellar Purkinje cell bodies and interacts with neurofibromatosis type 2 gene product schwannomin. *J. Mol. Neurosci.* **17:** 59–70.
37. Jensen, J. P., Bates, P. W., Yang, M., Vierstra, R. D., and Weissman, A. M. (1995). Identification of a family of closely related human ubiquitin conjugating enzymes. *J. Biol. Chem.* **270:** 30408–30414.

Sequence data from this article have been deposited in the DDBJ/EMBL/GenBank Data Libraries under accession numbers AF262024 (human PRAJA1) and AF264620 (human PRAJA1β).