Identification and characterization of presenilin I-467, I-463 and I-374

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Abstract We cloned a novel isoform of presenilin I (presenilin I-374) besides previously published presenilin I-467 and I-463 in human lymphocytes. Presenilin I-463 was identical to presenilin I-467 except a 12 bp nucleotides deletion in its amino terminal region. Another isoform, presenilin I-374 was produced by an alternative splicing with an additional exon consisting of 92 bp nucleotides (exon 11), which resulted in the frame shift with a stop codon to generate a truncated presenilin consisting of 374 amino acids. The transcripts for *presenilin I-467/463* was ubiquitously detected while that for *presenilin I-374* was selectively detected in liver, spleen, kidney. Abnormal behavior of presenilin I on gel electrophoresis was found with affinity-purified antibodies against presenilin I.

Key words: Alzheimer's disease; Chromosome 14; Presenilin; Splicing; Exon 11

1. Introduction

Alzheimer's disease (AD) is a progressive intellectual failure with elderly people. The causal genes were identified as *presenilin I* (*S182*) on chromosome 14q24.3 [1], *presenilin II* (*STM2* or *E5-1*) on chromosome 1q31-42 [2,3] and *APP* on chromosome 21 [4–7] while *apolipoprotein E* allele ε4 is established to be a risk factor located on chromosome 19 [8]. The biological function as well as protein-chemical property of presenilin I and II remain to be studied as urgent targets to understand AD pathogenesis. Here we prepared specific antibodies against presenilin I and examined the protein of presenilin I in in vitro translation product using cDNA of *presenilin I*. We found a new isoform of presenilin I and examined the biochemical nature of presenilin I on gel electrophoresis using three antibodies. The loop structure, the only hydrophilic domain in presenilin I, was disturbed in the new presenilin I and discussed its regulatory function.

2. Materials and methods

2.1. cDNA isolation

Total RNAs were prepared from peripheral white blood cells or frozen brain tissues by acid guanidine phenol-chloroform (AGPC) method [9]. Ten μg of total RNAs were reverse-transcribed by Superscript II (Life Technologies) with *Xba*I-anchored specific primer, AD3#2 (5′-GCTCTAGACTAGATATAAAATTGATGGAA-3′). Full length presenilins were amplified from first strand templates using LA PCR Kit (Takara Shuzo, Kyoto) with *Eco*RI-anchored primer, AD3#1 (5′-GCTCTAGAGAATTCGCTCCAATGACAGAGTTACCTG-3′) and AD3#2 primer. PCR condition was 30 cycles of 20 s 98°C, 30 s 54°C, and 8 min 68°C. *Eco*RI/*Xba*I restricted PCR products were then recloned to Bluescript II and the nucleotide sequences were determined by dideoxy chain termination method with Sequenase (Amersham, UK).

2.2. Genome isolation

The genomic fragment containing exon 10, 11, and 12 was amplified from 1 μg of genomic DNA prepared from white blood cells of a Japanese healthy subject using LA PCR Kit with *Eco*RI-anchored primers: AD3#4 (5′-CGGAATTCGTGTGGTTGGTGAATATGGCA-3′) and AD3#5 (5′-CGGAATTCGAAAGTTCCTGGACAGCAGCT-3′). PCR condition was 30 cycles of 20 s 98°C, 1 min 55°C, and 10 min 72°C. A partially digested 6 kb *Eco*RI genomic fragment was recloned to Bluescript II. The genomic clone was then mapped by restriction enzyme digestions and the nucleotide sequences flanking exon 11 were determined using exon 11-specific primers: AD3#6 (5′-AGCCTGTCTGCCTCCTG-3′) and AD3#7 (5′-CCGTGGGCCTGCAGGCA-3′).

2.3. RT-PCR analysis

Total RNAs and first strand cDNAs were prepared from brain, heart, muscle, kidney, liver, and spleen of an autopsy case as described above. Presenilin I-467, I-463, and I-374 were amplified from first strand templates using Taq polymerase (Toyobo, Tokyo) with AD3#1 and AD3#3 (5′-CCCTGGGGTCGTCCATTA-3′) or AD3#4 and AD3#5 primers. The PCR condition was 35 cycles of 1 min 95°C, 1 min 55°C, and 1 min 72°C. PCR products were digested by *Pst*I, *Nco*I, or *Sty*I and resolved on 5% polyacrylamide gel.

2.4. Preparation of three antibodies against presenilin I

Three different antibodies, AD3N, AD3L and AD3C were raised in rabbits by immunizing synthetic peptides as antigens. Synthetic peptides were chosen from an amino terminal region (DNRERQEHNDRRSLGHPEPLSNGRPQ) of presenilin I-467 for AD3N, a loop region (MAEGDPEAQRRVSKNSKYNAESTERESQDTV) of presenilin I-467 for AD3L and a carboxyl terminal region (GLVFYFATDYLVQPFMDQLAFHQFYI) of presenilin I-467 for AD3C. The immunizing protocol was as described previously [10]. Polyclonal antibodies were purified by affinity-column conjugated with synthetic peptides. The specific antibodies were eluted by 0.1 M glycine buffer, pH 2.5 and neutralized quickly with 2 M Trizma. Absorption was performed by incubating an antiserum (2–4 μg) with synthetic peptide (10 μg) in 10% calf serum in PBS at room temperature for 60 min.

2.5. In vitro translation and immunoprecipitation

In vitro products were generated from presenilin I-467–BSK and presenilin I-374–BSK as templates by TNT Reticulocyte lysate system (Promega, Madison, WI) with T7 RNA polymerase (Promega) and [^{35}S]methionine (>37 TBq/mmol, Amersham, UK). The products were mixed with either of antibodies overnight at 4°C and immunoprecipitated with Protein A Sepharose 4 Fast Flow (Pharmacia Biotech). The resultant immunoprecipitates were washed with RIPA buffer (0.1% SDS, 1% Triton X-100, 0.5% deoxycholic acid in 50 mM Tris-HCl, pH 7.6, 150 mM NaCl). The final immunoprecipitates were resolved on SDS-polyacrylamide gel. The gel was amplified by Amplify (Amersham), dried, and exposed to Kodak-XAR5 film for 24 h.

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Abbreviations: AD, Alzheimer's disease; PS I-467, presenilin I-467; PS I-463, presenilin I-463; PS I-374, presenilin I-374; SDS, sodium dodecyl sulfate

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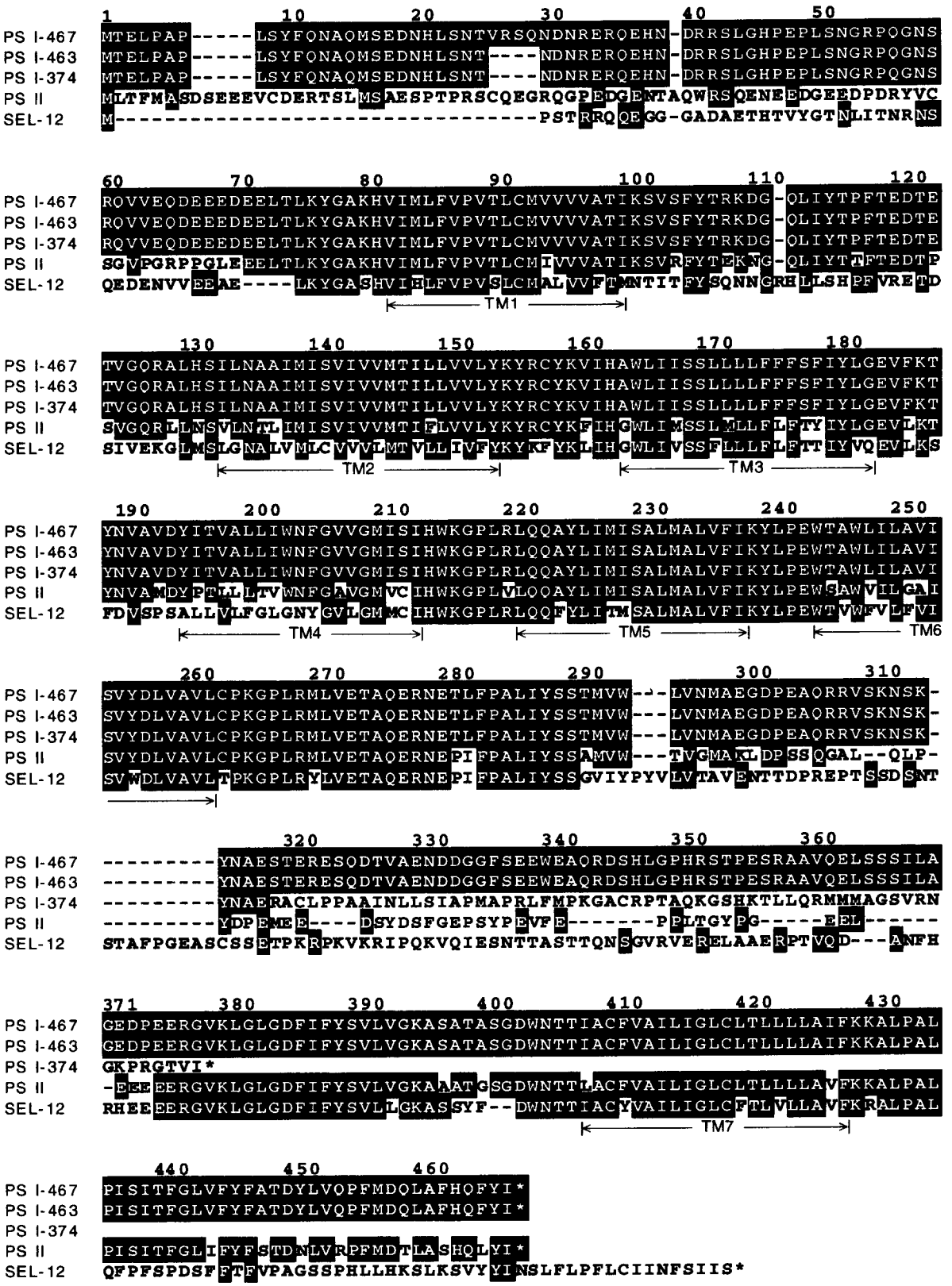

Fig. 1. Amino acid alignment among presenilin I isoforms and other presenilin members. The amino acid sequence (in single-letter code) of presenilin I-467 (S182) has been aligned with those of presenilin I-463, presenilin I-374, presenilin II, and Sel-12 using Clustal method contained in Lasergene software package (DNASTAR); dashes denote gaps that have been introduced to maximize the alignment. The sequences identical to those of presenilin I-467 were shown by black boxes. The numbering of the sequences was based on the amino acid sequence of presenilin I-476. Predicted TM domains are underlined. The nucleotide sequences of presenilin I-463 and presenilin I-374 have been deposited in GenBank under accession numbers of U40379 and U40380, respectively.

3. Results

3.1. Isoforms of presenilin I

Presenilin I cDNA was amplified from human peripheral blood cells and brains by RT-PCR using primers based on the published sequence [1]. We sequenced 9 clones and identified 3 species of transcripts as shown in Fig. 1. A clone identical to published presenilin I was referred to as presenilin I-467 according to the number of amino acid residues [1]. Presenilin I-463 lacked 12 bp nucleotides in the amino terminal region, resulting in the deletion of VRSQ between the 26th and 29th amino acids sequence of presenilin I-467 as also documented recently [11]. Another clone, designated as presenilin I-374 was found to contain the additional insert sequence consisting of 92 bp nucleotides to generate a frame shift in the loop structure followed by a stop codon, which resulted in the shorter isoform consisting of 374 amino acids.

3.2. Exon 11

In order to identify and locate the additional exon in the genome, we cloned a 6 kb genomic locus spanning exon 10 and the following exon. As shown in Fig. 2, we identified exon 11 in that genomic locus and determined the flanking sequence of exon 11, revealing that presenilin I-374 was generated by an alternative splicing. PCR-amplified presenilin I-374 was further verified by restriction enzyme digestions: *Pst*I, *Nco*I, and *Sty*I (Fig. 2c,d).

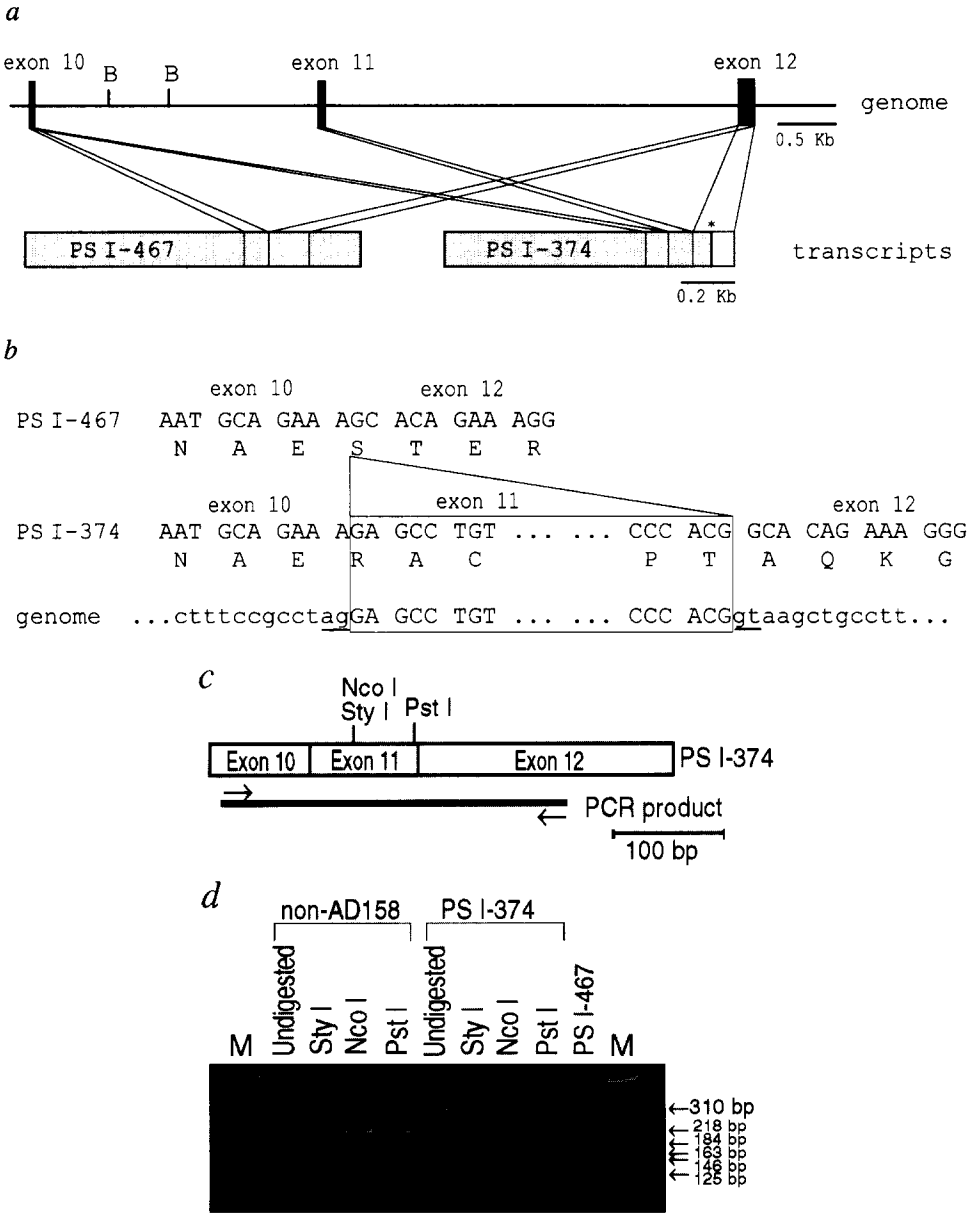

Fig. 2. Genomic organization of exon 10, 11, and 12 of presenilin I gene. a, Presenilin I-467 and presenilin I-374 are generated by an alternative splicing of exon 11. Presenilin I-374 showed the new stop codon (indicated by an asterisk) in exon 12 due to a frame shift generated by the insertion of exon 11. B, *Bam*HI. b, Junctional sequences between exon 10 and exon 12 of presenilin I-467 and between exon 10 and 11 or exon 11 and 12 of presenilin I-374, which shows a frame shift in presenilin I-374. Boundary sequences between exon 11 and intron sequences are shown in the lower panel. Donor and acceptor sites for splice are underlined in the genomic sequence. c, Schematic representation of RT-PCR strategy for the detection of presenilin I-374. d, Presenilin I-374 amplified from non-AD brain was verified by restriction enzyme digestion; *Sty*I, *Nco*I, and *Pst*I.

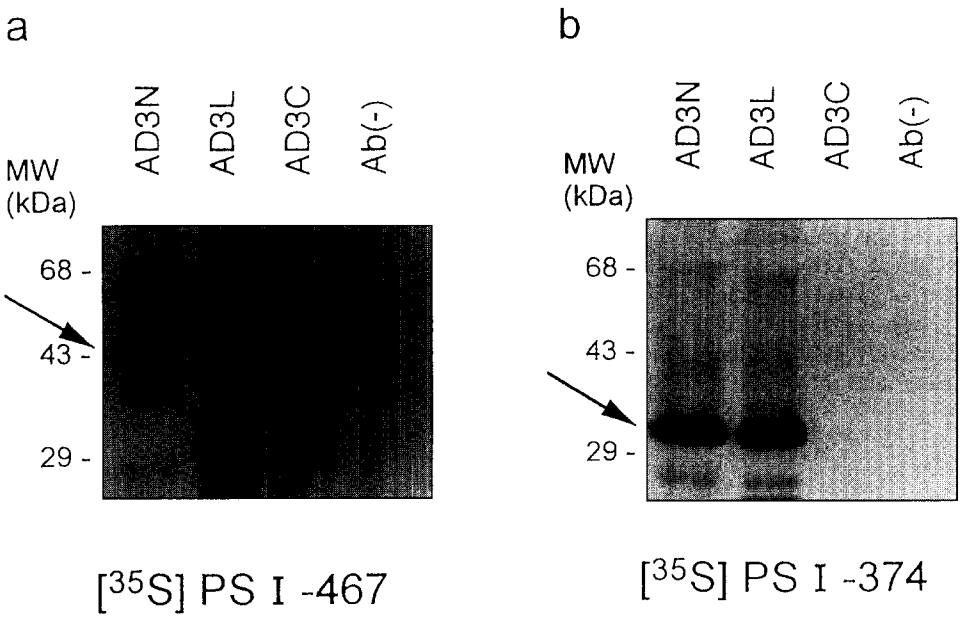

Fig. 3. Characterization of presenilin I-467 and presenilin I-374. Two cDNA clones of presenilin I-467 and -374 were in vitro translated in the presence of [^{35}S]methionine and their products were immunoprecipitated by antibodies against the N-terminal region (AD3N), loop region (AD3L), and C-terminal region (AD3C) of presenilin I-467. a, [^{35}S]presenilin I-467 was immunoprecipitated by all three antibodies. b, [^{35}S]presenilin I-374 was immunoprecipitated by AD3N and AD3L but not by AD3C (right panel).

3.3. The gene products of presenilin I

To characterize the gene products of presenilin isoforms, we analyzed the in vitro translated products of presenilin I-467 and presenilin I-374 by immunoprecipitations with antibodies against N-terminal, C-terminal, and loop sequence of presenilin I-467. As clearly demonstrated in Fig. 3, presenilin I-467 was identified as a single band with M_r 43,000 by all three antibodies while presenilin I-374 was identified as a single band with M_r 31,000 by antibodies against the N-terminal and loop sequences but not by antibody against the C-terminal sequence. Thus, immunoprecipitated presenilins showed their abnormalities in electrophoretic mobility in SDS-polyacrylamide gel because they were found to move faster than the calculated M_r (52,202 and 42,290). Both gene products showed proteolytic fragments beside full-sized forms, suggesting the occurrence of an endoproteolytic cleavage (unpublished). When presenilin I were heat-treated, the molecular masses of presenilin I-467 and presenilin I-374 were observed as 74,000 and 65,000, respectively. Moreover, when both samples were extensively incubated in a boiling bath, these bands were markedly decreased and detected on the gel top (manuscript in preparation). Such abnormal property of presenilin I was similar to those of some membrane proteins [12,13].

3.4. The tissue-specific expression of presenilin isoforms

We next investigated the expression of 3 isoforms of presenilin I in various tissues by RT-PCR, showing that presenilin I-463 and presenilin I-467 were both ubiquitously transcribed in brain tissue and other tissues (Fig. 4a). Presenilin I-374, however, was selectively detected in liver, kidney, and spleen by RT-PCR analysis (Fig. 4b).

4. Discussion

These variations of gene expression among presenilin I isoforms may reflect the temporal and tissue-specific expression of presenilin I-467, presenilin I-463 and presenilin I-374. Although the biological significance of these isoforms remains to be studied, it is tempting to speculate that the truncated presenilin I-374 may regulate the function of presenilin I-467/463 by forming a mosaic complex since these isoforms only differ in loop and C-terminal TM domain sequence (Figs. 1 and 2). It is also likely that presenilin protein is vulnerable to protease digestion. Hence full-sized presenilin may be the min-

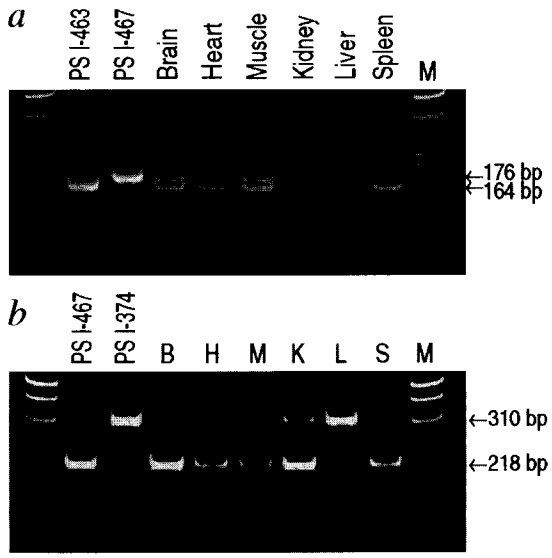

Fig. 4. Expression of presenilin I-467, I-463, and I-374 in AD brains and tissues. a, Transcripts for presenilin I-463 (164 bp) and presenilin I-467 (176 bp) were ubiquitously detected in tissues. b, The transcript for presenilin I-467/463 (218 bp) was ubiquitously detected in tissues while the transcript for presenilin I-374 (310 bp) with exon 11 (92 bp) was selectively detected in kidney, liver, and spleen.

or species in brain tissues. Thus, abnormal gene expression of presenilin I such as presenilin I-374 or another unknown dysfunctioning mechanism on presenilin I may play a pathological role in sporadic AD. Levitan and Greenwald recently reported that Sel-12, a suppressor and/or enhancer with 467 amino acids of lin-12/Notch, was identified to be a *C. elegans* homolog for human presenilin I [14]. Sequence alignment between presenilin-467/463 and Sel-12 showed the well-conserved structure of seven transmembranous domains and some part of loop domain (Fig. 1). This strongly suggests the evolutional conservation of presenilin/Sel-12 family beyond species of organisms. Moreover, a mutated allele (ar133:W381 → stop) of Sel-12 was a recessive supressor of lin-12 and might correspond to presenilin I-374 on the viewpoint of their truncation in size and homology. Thus, taken together with the evolutional conservation between presenilin I and Sel-12, the present finding provides the supporting evidence for the recent hypothesis that the presenilin family may function as a suppressor and/or enhancer of the lin-12/Notch family, receptors for intercellular signals to specify cell fates [15].

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