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Original Research

Identification, characterization and expression profiles of PSEN2 in the Chinese tree shrew (*Tupaia belangeri chinensis*)

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The gene *PSEN2* encodes presenilin-2, a subunit of γ-secretase. Mutations in *PSEN2* are not only related to Alzheimer's disease but are also involved in other diseases. The Chinese tree shrew (*Tupaia belangeri chinensis*) is a potential animal model for Alzheimer's disease, although little is known about its cDNA sequence, protein structure, and *PSEN2* expression. To better understand *PSEN2* in the tree shrew, we cloned this gene by rapid amplification of cDNA ends technology. Hence, we analyzed the sequence and molecular characteristics of PSEN2 mRNA, predicted its spatial structure, and analyzed its expression profiles. We found that tree shrew *PSEN2* is 1539 base pairs in length and encodes 330 amino acids. It is homologous and genetically similar to humans (97.64% identity). The protein structure of tree shrew PSEN2 indicated similarities to human PSEN2, both being comprised of numerous transmembrane helices. However, tree shrew PSEN2 possesses seven α-helices, and thus lacks three compared with human PSEN2. Tree shrew PSEN2 mRNAs were ubiquitously detected in all tissues, with a tissue- and temporal-specific pattern. These results pave the way towards the function of tree shrew PSEN2, which will give insights into the mechanisms leading to neurodegenerative and other diseases in humans.

Keywords

Alzheimer's disease; tree shrew; presenilin 2; cloning; structural prediction; phylogenetic analysis; neurodegenerative diseases

1. Introduction

Presenilin 2 (PSEN2) is one of the significant components of γ-secretase (as well as Presenilin 1, Nicastrin, and Aph-1) (Wakabayashi and De Strooper, 2008). It is encoded by the *PSEN2* gene, localized to chromosome lq31-42 in humans (Levy-Lahad et al., 1995; Sherrington et al., 1995). Previous research has shown that missense mutations in *PSEN2* are a rare cause of early-onset Alzheimer's disease (AD) (www.alzforum.org/mutations). Other studies have also found *PSEN2* mutations to be involved in frontotemporal dementia, dementia with Lewy bodies, dilated cardiomyopathy, Parkinson's disease with dementia, and breast cancer (Table S1) (Cai et al., 2015).

Human PSEN1 and PSEN2 share 67% of their amino acid identity, and their mutations are associated with AD (Thinakaran et al., 1996). PSEN2 is a core part of γ-secretase. Amyloid precursor protein (APP) is proteolyzed by γ-secretase and β-secretase into β-amyloid (Aβ) Aβ40 and Aβ42 peptides, which have been centrally implicated in AD pathogenesis (Delabio et al., 2014; Selkoe, 1994; Uemura et al., 2009). It has previously been demonstrated that mutation of the *PSEN2* does not damage the enzymatic function of γ-secretase but changes its digestion site leading to the production of aggregative Aβ (Uemura et al., 2009).

It is known that Aβ plaque aggregation is a key pathological feature of AD (Jin, 2014; Karran and De Strooper, 2016; Querfurth and LaFerla, 2010). So far, 13 mutations in *PSEN2* - all of which missense - have been shown to cause early-onset Alzheimer's disease (EOAD) (Bertram and Tanzi, 2004; Rademakers et al., 2005; St George-Hyslop, 2000). The two most common *PSEN2* mutations are the Asn141Ile or N141I (where asparagine is replaced with isoleucine at position 141) and the Met239Val or M239V (where methionine is changed to valine at position 239) (Rocchi et al., 2003; Selkoe, 2001).

The Chinese tree shrew (*Tupaia belangeri chinensis*), a squirrel-like mammal, has a wide distribution in Southeast Asia, South, and Southwest China and has many unique characteristics that make it suitable for use as an experimental animal. It has a

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low cost of maintenance and a short reproductive cycle. It is used for studying human diseases such as social stress (Fuchs, 2005), depression (van Kampen et al., 2002), aging (Keuker et al., 2004, 2005; Yamashita et al., 2012), AD, and Parkinson's disease (Ma et al., 2013).

Fan et al. (2018) analyzed 131 AD-related genes expressed in the tree shrew brain, comparing them with those of human, rhesus monkey, and mouse, finding AD-related genes to be more homologous between human and tree shrew than between human and mouse. Moreover, the expression pattern of accumulated Aβ plaques and neurofibrillary tangles in tree shrew brain tissues were found to resemble that of human brain tissues, with a similar age-dependent effect. These research findings suggest that tree shrews may be a potential animal model for AD.

To date, the presenilin of human, swine, and mouse have been cloned (Lee et al., 1996; Madsen et al., 2007), but the structure and function of tree shrew PSEN2 (tsPSEN2) have not been well studied. In the present study, we identified the ts*PSEN2* sequence by Rapid-amplification of cDNA ends (RACE) technology. We then analyzed the sequence and molecular characteristics of PSEN2, predicted its spatial structure, and analyzed the expression profiles of ts*PSEN2* mRNA.

2. Materials and Methods

2.1 Experimental animals and tissues collection

Chinese tree shrews were obtained from the Center of Tree Shrew Germplasm Resources, Institute of Medical Biology, Chinese Academy of Medical Science and Peking Union Medical College, Kunming, PR China. Nine tree shrews were divided into three groups: group 1, juvenile (2-3 months old, n = 3); group 2, adult (1.5-2 years old, n = 3); and group 3, elderly (over 6 years old, n = 3). The research project was approved by the institutional Ethics Committee, and all the procedures were performed according to ethical standards and practices.

After euthanasia by excessive pentobarbital sodium, tree shrews were quickly dissected, and thirteen different tissues were collected from the heart, liver, spleen, lung, kidney, pancreas, muscle, parietal lobe, frontal lobe, temporal lobe, occipital lobe, hippocampus, and cerebellum. The tissues were immediately frozen in liquid nitrogen and stored at -80 °C.

2.2 Cloning the full length of tsPSEN2

According to the conserved sequences registered in GenBank, including Human *PSEN2* (NM 012486.2), Mouse *PSEN2* (NM 011183.3), and Sumatran orangutan *PSEN2* (NM 001131771.1), four groups of primer were designed to cover the entire coding region of ts*PSEN2* mRNA (Table 1).

Total RNA was extracted from the brain of adult tree shrews using the RNA Extraction Kit (9767, Takara, Japan). The purity and integrity of the RNA were determined by spectrophotometer and electrophoresis. First-strand cDNA was synthesized by reverse transcription (RT) using the PrimeScript™ II 1st Strand cDNA Synthesis Kit (6210A, Takara, Japan). Ts*PSEN2* gene was extracted by general PCR, using an intermediate sequence amplification primer (Table 1) and the Premix Taq™ (LA Taq™ Version 2.0) Kit (RR900A, Takara, Japan).

To secure relatively intact and more accurate mRNA sequences of ts*PSEN2*, the 5' UTR and 3' UTR were amplified by RACE using 5'/3' RACE specific primers (Table 1) and the SMARTer

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ATG CTC ACA TTC ATG GCC TCT GAC AGC GAG GAA GAA GTG TGT GAC GAG CGG ACC TCC CTG
  M  L  T  F  M  A  S  D  S  E  E  E  V  C  D  E  R  T  S  L
ATG TCA GCT GAG AGT CCC TCA CCA CGT ACC TGC CAG GAG GGC AGG CTG GGC CCG GAG GAT
  M  S  A  E  S  P  S  P  R  T  C  Q  E  G  R  L  G  P  E  D
GGT GAG AAT ACC ACC CAG TGG AGA AGC CAG GAT GGC GAG GAA GAT GGC GAG GAG GAC CCT
  G  E  N  T  T  Q  W  R  S  Q  D  G  E  E  D  G  E  E  D  P
GAC CGC TAT GTC TGC AGC GGA GTC CCT GGG CGG CCA CCA GGT CTG GAG GAA GAA CTG ACC
  D  R  Y  V  C  S  G  V  P  G  R  P  P  G  L  E  E  E  L  T
CTC AAA TAT GGG GCA AAG CAC GTG ATC ATG CTG TTT GTG CCT GTC ACA CTG TGC ATG ATC
  L  K  Y  G  A  K  H  V  I  M  L  F  V  P  V  T  L  C  M  I
GTG GTG GTG GCC ACC ATC AAG TCT GTG CGT TTC TAC ACG GAG AAG AAT GGA CAG CTC ATC
  V  V  V  A  T  I  K  S  V  R  F  Y  T  E  K  N  G  Q  L  I
TAC ACG CCG TTC ACT GAG GAC ACG CCC TCA GTG GGC CAG CGC CTC CTC AAC TCT GTA CTC
  Y  T  P  F  T  E  D  T  P  S  V  G  Q  R  L  L  N  S  V  L
AAC ACC CTC ATC ATG ATC AGT GTC ATC GTG GTC ATG ACC ATC TTC CTG GTC GTG CTA TAC
  N  T  L  I  M  I  S  V  I  V  V  M  T  I  F  L  V  V  L  Y
AAG TAC CGC TGC TAC AAG TTC ATC CAT GGC TGG CTG ATT ATG TCC TCC CTG ATG TTG CTG
  K  Y  R  C  Y  K  F  I  H  G  W  L  I  M  S  S  L  M  L  L
TTC CTC TTC ACC TAT ATC TAT CTC GGG GAA GTG CTC AAG ACC TAC AAC GTG GCC ATG GAC
  F  L  F  T  Y  I  Y  L  G  E  V  L  K  T  Y  N  V  A  M  D
TAC CCC ACC CTC TTG CTG ACC GTC TGG AAC TTT GGG GCG GTG GGC ATG GTG TGC ATC CAC
  Y  P  T  L  L  L  T  V  W  N  F  G  A  V  G  M  V  C  I  H
TGG AAG GGC CCC CTG GTG CTG CAG CAG GCC TAC CTC ATC ATG ATC AGC GCG CTC ATG GCC
  W  K  G  P  L  V  L  Q  Q  A  Y  L  I  M  I  S  A  L  M  A
TTG GTC TTC ATC AAG TAC CTC CCA GAG TGG TCC GCC TGG GTC ATC CTG GGT GCC ATC TCT
  L  V  F  I  K  Y  L  P  E  W  S  A  W  V  I  L  G  A  I  S
GTG TAT GAT CTC GTG GCT GTG CTG TGT CCC AAA GGG CCA CTG AGA ATG CTG GTG GAA ACT
  V  Y  D  L  V  A  V  L  C  P  K  G  P  L  R  M  L  V  E  T
GCC CAG GAG AGG AAC GAG CCC ATA TTT CCT GCC TTG ATA TAC TCA TCT TCT ACT TCT CCA
  A  Q  E  R  N  E  P  I  F  P  A  L  I  Y  S  S  S  T  S  P
CAG ACA ACC TGG TGC GCC CCT TTA TGG ACA CCC TGG CCG CCC ATC AGC TCT ATA TCT GAG
  Q  T  T  W  C  A  P  L  W  T  P  W  P  P  I  S  S  I  S  E
GGG CAT GTG GGT GCC GTG GCC TGG AAG CCA TAG
  G  H  V  G  A  V  A  W  K  P  *
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Figure 1. Nucleotide sequences and corresponding amino acids of the tsPSEN2.

RACE 5'/3' Kit (634858, Clontech, USA).

All PCR products were electrophoresed on 1% agarose gel, then purified using the MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0 (9762, Takara, Japan). Subsequently, the purified PCR products were cloned and sequenced in Sangon Biotech (Shanghai) Co., Ltd. Sequences of ts*PSEN2* (Accession Number: MK183108) were obtained from GenBank.

2.3 Sequence and phylogenetic analysis

The homology of *PSEN2* in tree shrew and some other common experimental animals were analyzed by the Basic Local Alignment Search Tool (BLAST) program of the National Center for Biotechnology Information (NCBI). We inferred the phylogenetic position of *PSEN2* from 11 species. The phylogenetic tree for *PSEN2* was constructed using neighbor-joining (NJ) by MAGA 7.0. Xenopus tropicalis was used as the outgroup to root the gene tree. Each tree was measured for robustness by performing 1000 bootstrap iterations to determine the accuracy of the tree branch position.

2.4 Structure prediction (bioinformatics analysis)

The amino acid, molecular weight, and isoelectric point of tsPSEN2 were analyzed using the ExPASy ProtParam tool. The pro-hydrophobicity and the N-terminal signal peptide of tsPSEN2 were predicted using the ExPASy Protscale online analysis software (https://web.expasy.org/protscale/) and SignlP program, respectively. The coiled-coil structure and the N-glycosylation site of tsPSEN2 were detected using the COILS Server and NetNGlyc 1.0 online analysis (http://www.cbs.dtu.dk/services/NetNGlyc/), respectively. The possible distribution of sub-cells and the secon-

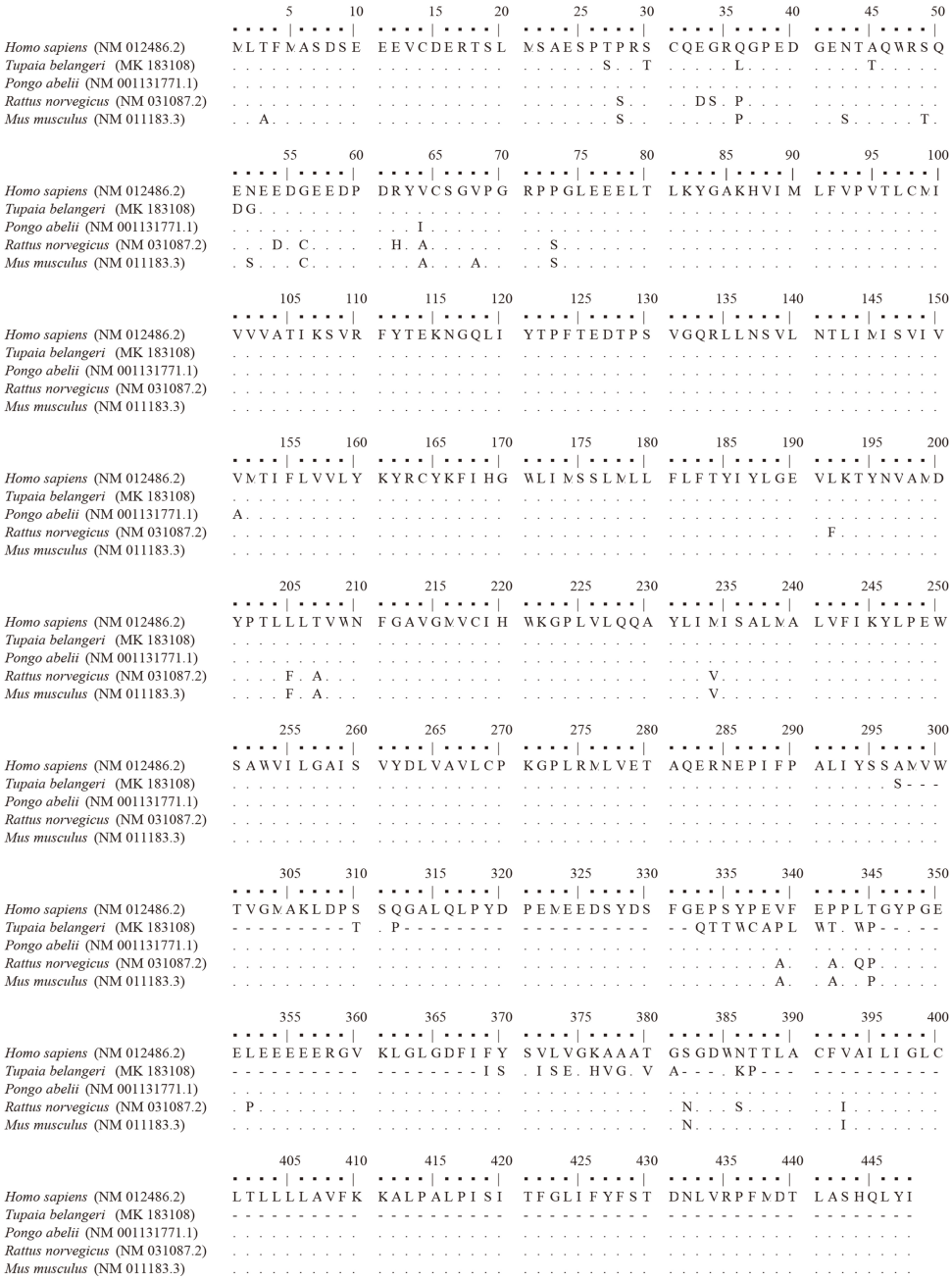

Figure 2. A comparison of the amino acid sequence of PSEN2 in tree shrews and other species using MAGA 7.0 software. Conserved domains are marked with black boxes. The tree shrew and Human protein sequences are 97.64 % identical, higher than Sumatran orangutan (96.97%), Mouse (93.94%), and Rat (93.60%).

dary structure of tsPSEN2 were predicted using the POSTT II's k-NN analysis program and NPS@MLRC online analysis system (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_mlrc.html) respectively. The domain of tsPSEN2 was predicted using the SMART program. The tertiary structure of tsPSEN2 was predicted using the SWISS-MODEL online analysis platform (https://swissmodel.expasy.org/). Transmembrane structure of tsPSEN2 was predicted using TMHMM.

2.5 RNA extraction and expression quantification

Total RNA from thirteen tissues of tree shrews of different ages was extracted using the RNA Extraction Kit (9767, Takara, Japan). After removing genomic DNA contamination, the total RNA quality was measured using a UV VIS spectrophotometer (nanophotometer np80, Implen, Germany). Reverse transcription-quantitative real-time PCR (RT-qPCR) was performed using the Real-Time PCR Detection system (CFX96™, Bio-Rad, America), using the One-Step TB Green® PrimeScript PLUS RT-PCR Kit (Perfect Real Time) (RR096A, Takara, Japan) and RT-qPCR specific primer (Table 2). The ts*GAPDH* was used as an internal control. The relative ts*PSEN2* gene expression was calculated with the double-standard curve method.

Table 1. Primer design of the ts*PSEN2*

Primers	Sequence (5' to 3')	Groups
5' adaptor	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGCCCCCCCCCCCCCCC	Adaptor primer
3' adaptor	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTTTTTTTTTTTTTTTT	
5.3' outer	GCTGTCAACGATACGCTACGTAAC	
5.3' inner	GCTACGTAACGGCATGACAGTG	
PSEN2-F	CGCCTCCTCAACTCTGTACTCA	Intermediate sequence amplification primer
PSEN2-R	CAGTTTCCACCAGCATTCTCAGT	
PSEN2-F1	GGCATGGTGTGCATCCACTGGAAG	3' RACE specific primer
PSEN2-F2	TCATGGCCTTGGTCTTCATCAAGTACCTC	
PSEN2-R1	CAGGGAGGACATAATCAGCCAGCCAT	5' RACE specific primer
PSEN2-R2	GGGTAGTCCATGGCCACGTTGTAGGT	
PSEN2-RT1	TCAAGGCAGGAAATATGGGCT	
PSEN2-RT2	ACCCACATGCCCCTCAGATATA	

Table 2. RT-qPCR primer sequences

Primers	Sequence (5' to 3')	Groups
PSEN2-F	GTGTGTGACGAGCGGACCTC	qRT-PCR specific primer
PSEN2-R	CGCTGCAGACATAGCGGTCA	
GAPDH-F	CTTCAACTCTGGCAAGGT	
GAPDH-R	AAGATGGTGATGGACTTCC	

2.6 Statistical analysis

All tsPSEN2 mRNA expression data were analyzed using IBM SPSS Statistics. Data were presented as mean ± SEM. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1 Identification, cDNA cloning and homologous analyses of tsPSEN2

We identified the full length of ts*PSEN2* cDNA in the brain of three individual specimens of the adult tree shrew group. The ts*PSEN2* is 1539 base pairs (bp) in length, and the open reading frame (ORF) sequence is 993 bp, encoding 330 amino acids (Fig. 1). The amino acid sequence of tsPSEN2 was deduced based on the ts*PSEN2* cDNA sequence.

To further research the relation between the tree shrew and other species, we compared the PSEN2 amino acid sequence of human, Sumatran orangutan, mouse, and rat. The multiple sequence alignment (MSA) results indicated that tsPSEN2 was more homologous to human (97.64%) and Sumatran orangutan (96.97%) PSEN2 than it was to mouse (93.94%) or rat (93.60%) PSEN2 (Fig. 2).

3.2 Phylogenetic analysis of tsPSEN2

To gain an insight into the evolutionary relationship between the tree shrew and other species, a phylogenetic tree was constructed using the Neighbor-Joining (NJ) method, based on the PSEN2 protein sequences using the genetic database from GenBank. In our diagram of the phylogenetic tree, ts*PSEN2* was genetically close to humans (Homo sapiens) and Sumatran orangutan (Pongo abelii), but more distant from mouse (Mus musculus) and rat (Rattus norvegicus) (Fig. 3). This result, together with the MSA result, indicates that ts*PSEN2* is close to human and non-human primates in evolutionary relationship terms.

3.3 The structure analysis of tsPSEN2 protein

Using the ExPASy ProtParam tool to analyze its amino acid sequence, the molecular weight of tsPSEN2 was calculated to be 37105.40Da, its theoretical isoelectric point 4.90, including 5240 atoms in total, and the molecular formula was $C_{1701}H_{2634}N_{408}O_{475}S_{22}$. It is composed of 20 different amino acids, among which leucine content was highest (11.5%), followed by valine (9.4%), and relatively high proportions of serine (7.3%), glutamine (7.0%), proline (7.0%) and threonine (7.0%). The instability index of tsPSEN2 was found to be 43.03, the fat index 102.12, and the hydrophilic-hydrophobic average (GRAVY) 0.278. The pro-hydrophobicity analysis revealed considerable amino acid distributions in the positive and negative regions (Fig. 4A), with the positive region distribution slightly higher than that of negative regions, which was consistent with the average of the pro-hydrophobicity (0.278) predicted by ExPASy Protscale. TsPSEN2 demonstrated six transmembrane regions (Fig. 4B), which was also consistent with the predicted results of hydrophobicity.

Using the SignlP program and the COILS Server, we found that tsPSEN2 had no N-terminal signal peptide and no coiled-coil structure. There was an N-glycosylation site at the 43rd amino acid (predicted by on-line analysis using NetNGlyc 1.0) (Fig. 4C). Using the POSTT Π's k-NN analysis program to predict the possible distribution of sub-cells, it was found that 56.5% was distributed in the cell membrane, 21.7% in the endoplasmic reticulum, 4.3% in the Golgi, and 4.3% in the nucleus.

Analysis of the secondary structure of tsPSEN2 reveal it to be 47.58% α-helix (h), while the extended chain (e) accounted for 11.21%, and the random curl (c) 41.21% (Fig. 4D). Using the SMART program to predict the domain of tsPSEN2, we found a PSN, which is a domain unique to the presenilin signal peptide kinase family, at position 136-328 of the protein (Fig. 4E).

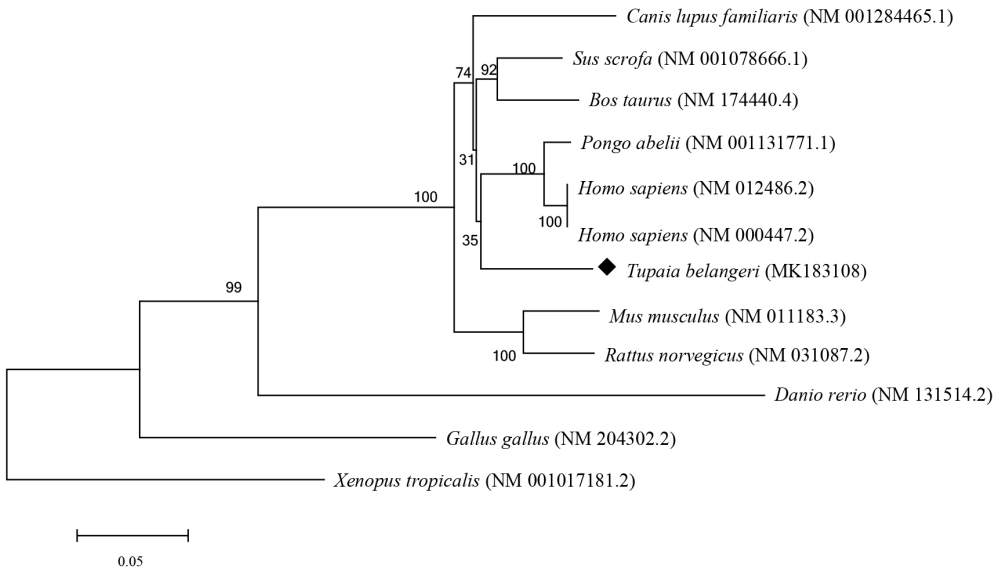

Figure 3. Phylogenetic tree of the ts*PSEN2*. A phylogenetic tree was reconstructed according to the Neighbor-joining (NJ) method, with 1000 bootstrap replications.

To understand the tertiary structure similarity of PSEN2 between human and tree shrew, we formed predictions based on the primary structure of PSEN2 using the SWISS-MODEL online. The tsPSEN2 was found to possess six transmembrane regions (TM1-6) in seven α-helices, while the human PSEN2 had eight transmembrane regions (TM1-8) in nine α-helices (Fig. 5). Similar to human PSEN2, tsPSEN2 demonstrated a conserved conformation that included six curved transmembrane regions connected by seven α-helices. But tsPSEN2 lacked two transmembrane regions and one intramembrane region in three α-helices compared with human PSEN2. As such, the human and tree shrew PSEN2 share similar but not identical structures.

3.4 Tissue-specific expression of the tsPSEN2

We measured the mRNA levels of ts*PSEN2* in thirteen tissues from three age groups (juvenile, adult, and elderly). The ts*PSEN2* mRNAs were ubiquitously detected in all tissues, with a tissue- and temporal-specific pattern (Fig. 6). In particular, ts*PSEN2* had a relatively high mRNA level in the pancreas, liver, lung, spleen, and cerebellum, whereas lower levels were present in heart and muscle tissues. Ts*PSEN2* mRNA was highly expressed in almost all juvenile tree shrews. When the ts*PSEN2* mRNA expression levels of the three age groups were compared, the juvenile and adult groups demonstrated a higher basal expression level than the elderly in nearly all tissues. The tissue- and temporal-specific expression patterns of ts*PSEN2* may be significant concerning AD development.

4. Discussion

We identified the ts*PSEN2* sequence, finding it to be 1539 bp in length with an ORF sequence of 993 bp, encoding 330 amino acids (Fig. 1). In contrast, the PSEN2 of humans, swine, and mouse consists of 448 amino acid residues, and nucleotide sequence alignment revealed a 92% sequence identity between the swine and the human PSEN2 (Madsen et al., 2007). Multiple amino acid sequence alignment revealed a 97.64% sequence identity between tsPSEN2 and human PSEN2. By generating a phylogenetic tree, it was found that ts*PSEN2* was genetically close to humans and Sumatran orangutan but distant from mouse and rat (Fig. 2).

Furthermore, we found a PSN at position 136-328 of the protein, which is a domain unique to the presenilin signal peptide kinase family. We also found that tsPSEN2 had a similar structure to human PSEN2. The tsPSEN2 protein has six transmembrane regions - the α-helix accounting for 47.58% - and has a strong hydrophobicity. The hydrophilic and hydrophobic areas have a similar protein distribution, which indicates that the characteristics of its membrane protein are consistent with its transmembrane structure.

According to previous reports of human PSEN2 structure (De Strooper and Annaert, 2010; Escamilla-Ayala et al., 2020), tsPSEN2 has the same structure of the extracellular domain, transmembrane, and intracellular domains, and has highly similar ligand binding sites. In our findings, the spatial structure of tsPSEN2 had seven curved α-helices connected by a short linker, which was identical to that of human PSEN2 (Fig. 5). However, tsPSEN2 lacked three α-helices compared with human PSEN2. These differences may affect the ligand binding of tsPSEN2. Also, tsPSEN2 has one N-glycosylation site. Posttranslational glycosylation may be related to its transport function.

Expression profiling showed a tissue and temporal-specific regulation of ts*PSEN2* transcription patterns in various tissues and different age groups (Fig. 6). Specifically, relatively high mRNA levels were found in the pancreas, liver, lung, spleen, and cerebellum, whereas lower levels were found in the heart and muscle tissues.

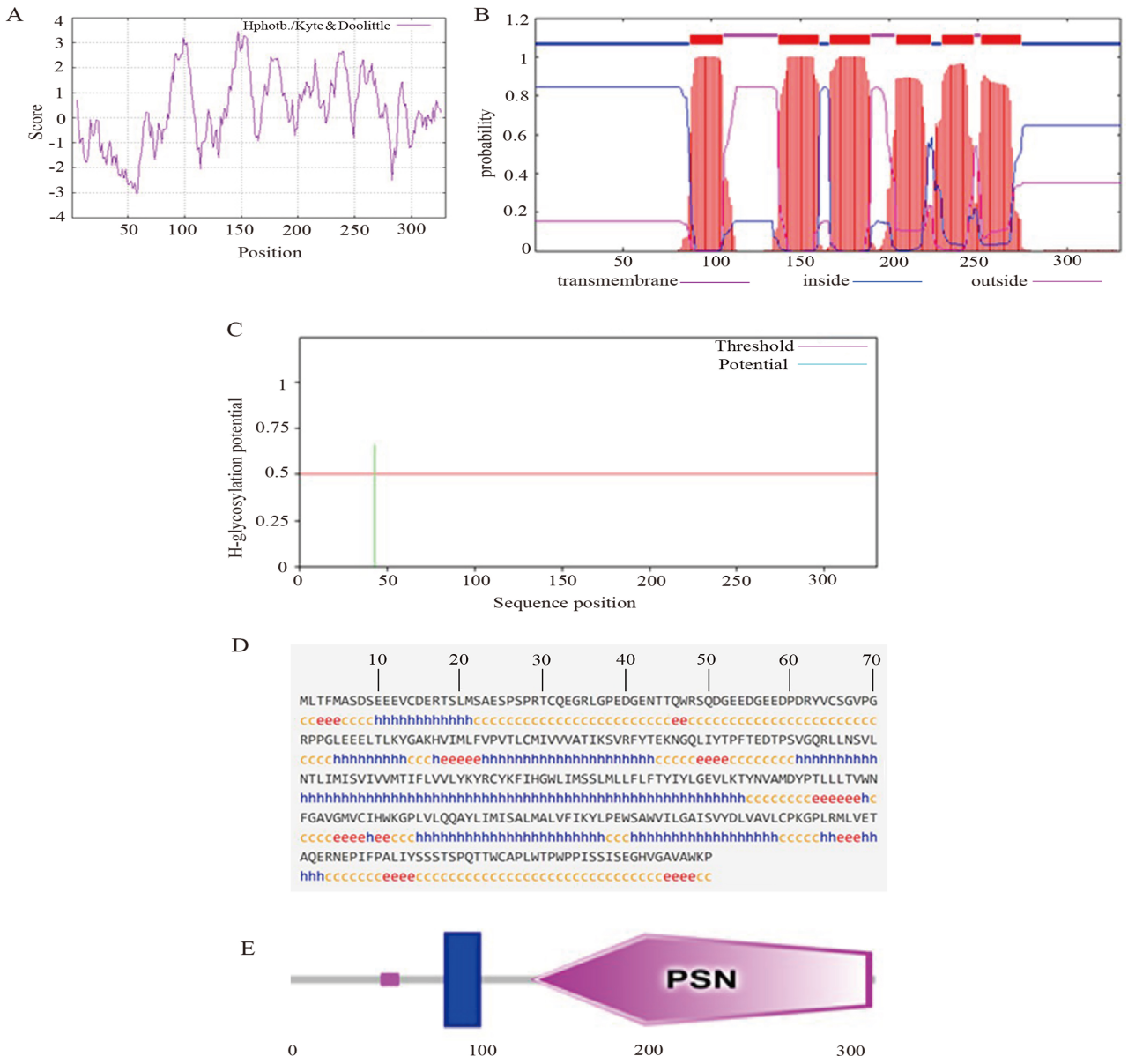

Figure 4. The structure analysis of tsPSEN2 protein. A. Hydrophobicity prediction of tsPSEN2 protein. The pro-hydrophobicity of the tsPSEN2 was predicted using the ExPASy Protscale online analysis software. The positive values indicate hydrophobicity, and negative values indicate hydrophilicity. B. Transmembrane structure prediction of tsPSEN2. The transmembrane structure of the tsPSEN2 was predicted using TMHMM online analysis software. C. N-glycosylation sites analysis of tsPSEN2. The N-glycosylation sites of tsPSEN2 were predicated using NetNGlyc 1.0 online analysis. D. Secondary structure prediction of tsPSEN2. The secondary structure of the tsPSEN2 was predicted by NPS@MLRC online analysis system. E. The protein domain prediction of tsPSEN2. The tsPSEN2 domain was predicted using the SMART program.

In a previous study by Li et al. (2019), ts*PSEN1* expression was shown to be high in the frontal lobe, parietal lobe, and hippocampus, but low in the pancreas, lung, and kidney. The ts*PSEN2* mRNA of the juvenile and adult group had a higher basal expression level relative to the elderly in nearly all tissues. Madsen et al. (2007) found that PSEN1 and PSEN2 were expressed in the frontal cortex, cerebellum, hippocampus, basal ganglia, and brain stem at the different stages of porcine gestation, which indicated that PSEN1 and PSEN2 were multifunctional and of great importance in embryonic development. Delabio et al. (2014) examined differences in PSEN2 expression in postmortem brain tissue acquired from individuals with and without clinical and neuropathological signs of AD, finding that PSEN2 expression was significantly downregulated in individuals with AD. Lee et al. (1996) have found that PSEN1 and PSEN2 transcripts are expressed in many tissues and that *PSEN1* mRNA transcripts are more abundant than those of PSEN2. The unique expression pattern of ts*PSEN2* mRNA suggests that ts*PSEN2* may play different biological roles in tissue- and development-specific processes.

Cai et al. (2015) have indicated that mutations in *PSEN2* are not only associated with both early- and late-onset AD, but that they are also closely involved in other diseases, including dilated

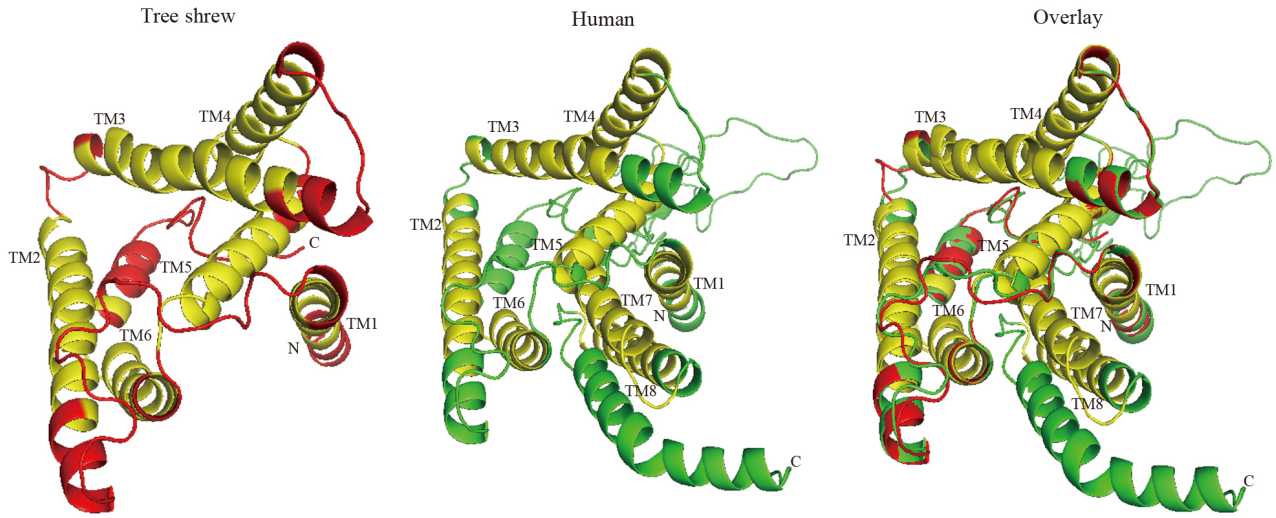

Figure 5. The spatial structure of PSEN2 predicted using the SWISS-MODEL Workspace. Tree shrew (red) and human (green) PSEN2 proteins. TM represent transmembrane domains (yellow). N-terminal (N); C-terminal (C).

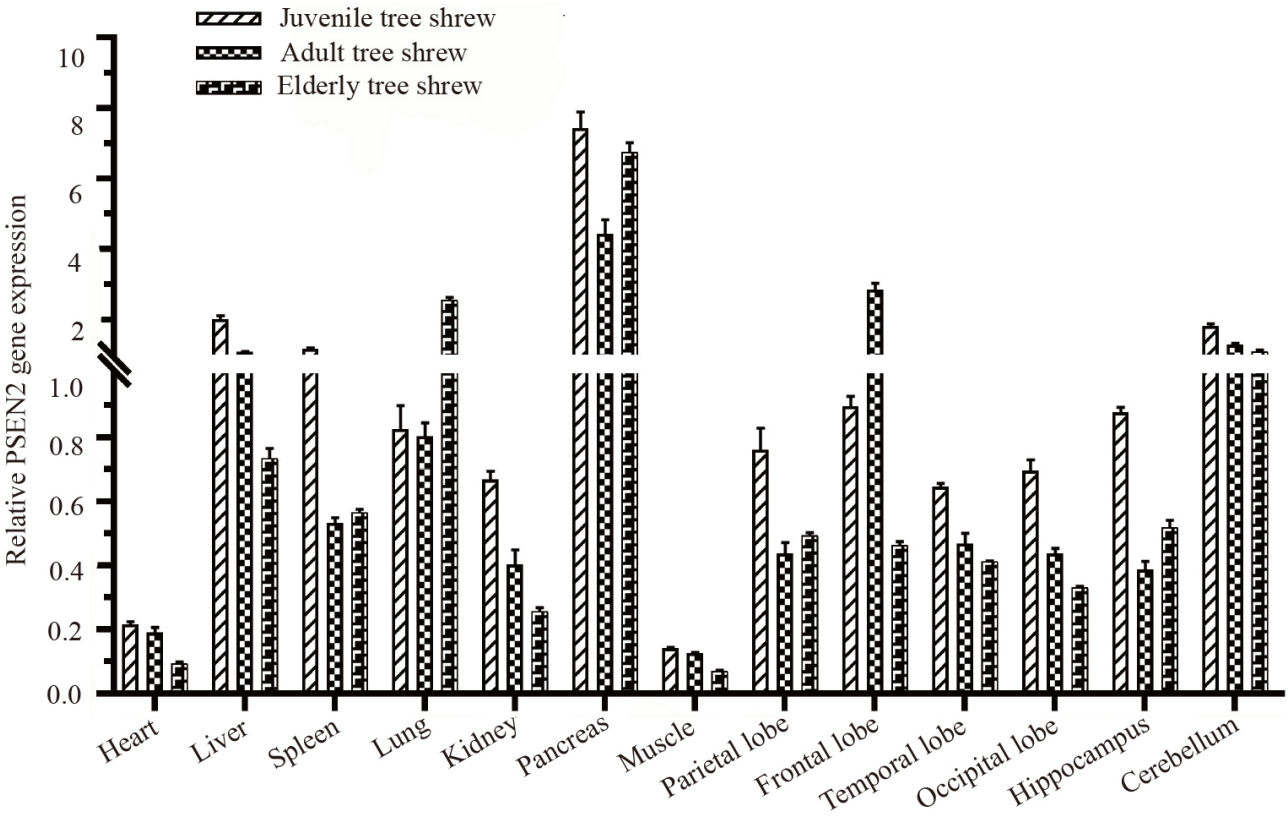

Figure 6. The mRNA expression pattern of ts*PSEN2* in different tissues of the tree shrews. The ts*PSEN2* mRNA expression was measured by RT-qPCR with gene-specific primers (Table 2) in thirteen tissues of the tree shrews. The ts*GAPDH* gene was used for normalization.

cardiomyopathy, breast cancer, dementia with Lewy bodies, frontotemporal dementia, and Parkinson's disease with dementia (Table S1). The mechanisms by which mutations in human *PSEN2* are associated with multiple diseases is unclear. It is also unknown whether mutations in ts*PSEN2* contribute to the development of multiple diseases in the same way that human *PSEN2* does. In the current study, we cloned the ts*PSEN2* gene, analyzed the protein's molecular characteristics, and detected its expression profiles.

In conclusion, our results show that the sequence and structure of PSEN2 are evolutionarily conserved from tree shrew to humans. The gene is ubiquitously expressed in different age groups and tissues, suggesting that similar functions have been phylogenetically

preserved. Here we presented a characterization of the tsPSENs, which is the first step in relating the tree shrew as a suitable animal model for understanding human diseases.

Ethics approval and consent to participate

The research project was approved by the institutional Ethics Committee of the Institute of Medical Biology, Chinese Academy of Medical Sciences, and all the procedures were performed according to ethical standards and practices.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://jin.imrpress.com/EN/10.31083/j.jin.2020.02.28.

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