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Molecular cloning and sequencing of rabbit presenilin-1 cDNA fragment✪✸

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Molecular cloning and sequencing of a cDNA encoding rabbit presenilin-1 (Ps1) fragment was performed by reverse transcription polymerase chain reaction (RT-PCR) using primers: 5′-GGA TGA GCA GCT AAT CTA TAC C-3′ and 5′-TCC ATT CAG GGA GGT ACT TGA TA-3′. The cDNA fragment revealed 402 nucleotides. The sequence was well conserved and found to be 91, 90, 88, 87 and 78% homologous to that of human, lemur, rat, mouse and chicken, respectively. The cDNA translated into a 130 amino-acid protein fragment. The deduced amino-acid sequence was also well conserved in various species and exhibited 98% similarities with those of rat, lemur and human homologues. However, differences were noticed at residues 145, 168 and 212. This cDNA fragment is quite significant because it is the most conserved portion of Ps1 in various animals and encodes four transmembrane regions (TM2, 3, 4, 5) as defined in human Ps1. Moreover, it includes more than 50% of the sites at which substitutions have been reported in familial Alzheimer's disease (FAD). Therefore, it is suggested that the rabbit can be used as an experimental model for future studies on Ps1 and its physiological functions to work out possible pathways leading to FAD linked neurodegeneration.

Presenilin-1 (Ps1) is a multispanning transmembrane (TM) protein encoded by a gene on chromosome 14. Mutations in Ps1 have been linked to familial Alzheimer's disease (FAD) which constitutes approximately 10% of Alzheimer's disease (AD) cases (Schel-

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Abbreviations: aa, amino acid(s); AD, Alzheimer's disease; FAD, familial Alzheimer's disease; Ps1, presenilin-1; TM, transmembrane.

lenberg, 1995). It is a progressive neurodegeneration disorder, characterized by irreversible cognitive and physical deterioration in the elderly (Sherrington *et al.*, 1995). Alzheimer's disease is both genetically and phenotypically a heterologous disorder. Therefore, genetic and/or biochemical markers that support clinical diagnosis and can distinguish AD from cognitive symptoms attributed to aging and from other dementia will be of great value. Thus, much emphasis has been given to the identification of genetic and biochemical markers (Mulder *et al.*, 2000). Further identification of such markers for early diagnosis of AD is mandatory for the development of efficient pharmacological treatment.

Although various studies have been focused on FAD and its causative factors, the underlying mechanism by which Ps1 mutations lead to development of FAD remains speculative (see references in: Fraser *et al.*, 2000; Saunders, 2001; Esler & Wolfe, 2001; Amtul *et al.*, 2002; Ponting *et al.*, 2002; Zhou *et al.*, 2002). Moreover, physiological function(s) of Ps1 is not fully defined and further studies are certainly required. Investigation of the structure and function of Ps1 is likely to provide powerful clues to resolve pathways culminating in FAD linked-neurodegeneration. However, in order to understand the function(s) of Ps1 and its role in the abnormal targeting of proteins into intracellular systems of protein degradation, it is important to investigate Ps1 sequence and structure in a suitable animal model. In the present study we have cloned and sequenced a cDNA encoding rabbit Ps1 fragment.

MATERIALS AND METHODS

Adult rabbits (*Oryctolagus cuniculus*) were slaughtered in the laboratory and fresh brain tissues were obtained and kept at –20°C. Total RNA was extracted from brain tissues by the acid guanidinum thiocynate/phenol/chloroform method (Chomczynski & Sacchi, 1987). mRNA was isolated from total RNA using mRNA Isolation Kit (Roche, Germany). Forward and backward primers were designed from the published nucleotide sequences for highly conserved regions of Ps1 in human (*Homo sapiens*), rat (*Rattus norvegicus*), lemur (*Microcebus murinus*), mouse (*Mus musculus*) (Sherrington *et al.*, 1995; Calenda *et al.*, 1996; Taniguchi *et al.*, 1997) and chicken (*Gallus gallus*) (accession No.: AY043492). The following two primers were selected: 5′-GGA TGA GCA GCT AAT CTA TAC C-3′ and 5′-TCC ATT CAG GGA GGT ACT TGA TA-3′.

In order to obtained cDNA from mRNA, reverse transcription (RT) and polymerase chain reaction (PCR) was carried out using Titan one tube RT-PCR system (Roche, Germany). The reaction is a one step system. It utilizes *AMV* reverse transcriptase (*AMV* RT) from avian myeloblastosis virus for first strand DNA synthesis, and the expand high fidelity enzyme blend (*Taq* DNA polymerase and a proof reading polymerase) for PCR in a single optimized RT-PCR buffer.

First-strand cDNA synthesis was accomplished in 30 min at 50°C. Following first-strand synthesis, amplification was carried out by 40 cycles of PCR at 94°C for 30 s, 50°C for 30 s and 68°C for 1 min.

The RT-PCR products obtained were separated by electrophoresis on 1.5% agarose gel in Tris/acetate/EDTA (TAE) buffer. Fragments with the expected size were cut out from the gel and purified using Wizard® PCR Preps DNA Purification System (Promega® Corporation, Madison, U.S.A.). Purified cDNA was sequenced using both forward and backward primers (Davis sequencing, CA, U.S.A.). Subsequently, the results were combined to yield full cDNA sequence. The cDNA sequence was compared with the sequences of Ps1 of rat, lemur, human and chicken using blast-2 software. The cDNA sequence was also translated into amino-acids sequence by Translator® software and compared with the amino-acid sequences of Ps1 of rat, lemur, hu-

man and chicken and homology was determined.

RESULTS AND DISCUSSION

A rabbit Ps1 cDNA fragment was obtained by RT-PCR using mRNA. The RT-PCR product separated on 1.5% agarose gel conformed to about 400 bp in size (Fig. 1) and revealed 402

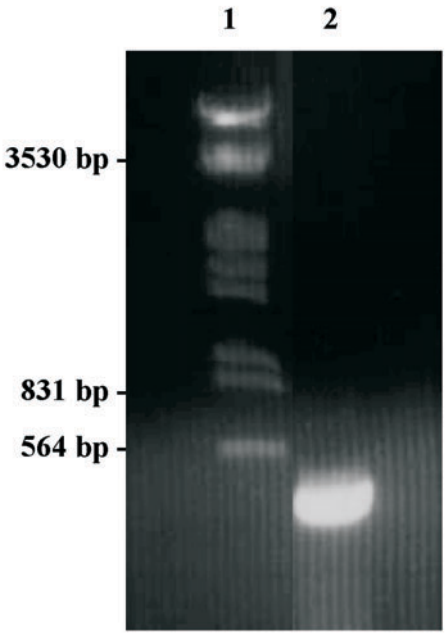

Figure 1. Amplification of rabbit Ps1 cDNA from mRNA extracts.

Lane 1 shows lambda DNA digested with *Hin*dIII and *Eco*RI as DNA size marker. Lane 2 shows the amplified cDNA fragment of 402 bp in size.

nucleotides on sequencing. Comparison of the rabbit cDNA sequence with its counterparts from rat, lemur, human, mouse (Sherrington *et al.*, 1995; Calenda *et al.*, 1996; Taniguchi *et al.*, 1997) and chicken (accession No.: AY043492) indicated a high sequence homology. The cDNA sequence was well conserved and found to be 78, 87, 88, 90 and 91% homologous to those of chicken, mouse, rat, lemur and human, respectively. Figure 2 shows the rabbit cDNA homology with that of human indicating 91% similarity in the sequences. However, some real variations were noticed in the electrophoregram outputs of Ps1 sequences of various species.

The rabbit cDNA nucleotide sequence was translated into a protein fragment containing 130 amino acids (aa). Comparison of the aa sequence of rabbit Ps1 with those of other species indicated that it was well conserved. It exhibited 98% similarity with those of rat, lemur, and human homologues and 93% with that of chicken (Fig. 3). However, some significant differences were noticed at residues 145, 168 and 212.

The residue 145 of the amino-acid sequence was isoleucine in rabbit and valine in human, rat, lemur and chicken Ps1. Contrary to this, at residue 168 it was valine in rabbit and rat, but isoleucine in lemur, human and chicken Ps1. The residue 212 was found to be serine, a polar amino acid in rabbit, human and lemur but alanine, a nonpolar amino acid, in rat and cysteine, a polar one, in chicken Ps1. Additional differences were also noticed in chicken Ps1 at residues 121, 125, 131, 144, 164, 193 and 201 in comparison with that of rabbit. These variations at the structural level of Ps1 may result in differences at the functional level as well.

Ps1 is a multispanning transmembrane (TM) protein (Sherrington *et al.*, 1995). It has ten hydrophobic domains that represent potential TM helices indicated as TM1–6 and hydrophobic domains 7–10 (Fraser *et al.*, 2000). Alignment of amino-acid sequences of Ps1 of different species showed that they were highly conserved especially within the transmembrane regions. The cDNA of Ps1 fragment obtained from rabbit is quite significant as four transmembrane regions (TM2, 3, 4 and 5) of human Ps1 lie in this part of the protein. Moreover, more than 50% of the over 70 amino-acid substitutions which have been reported in FAD, are located in the region between residues 112 and 242. These residues are conserved in rabbit Ps1 similar to those in other species studied earlier (Fig. 3). The majority of the pathological mutations reported in the Ps1 gene are missense mutations and

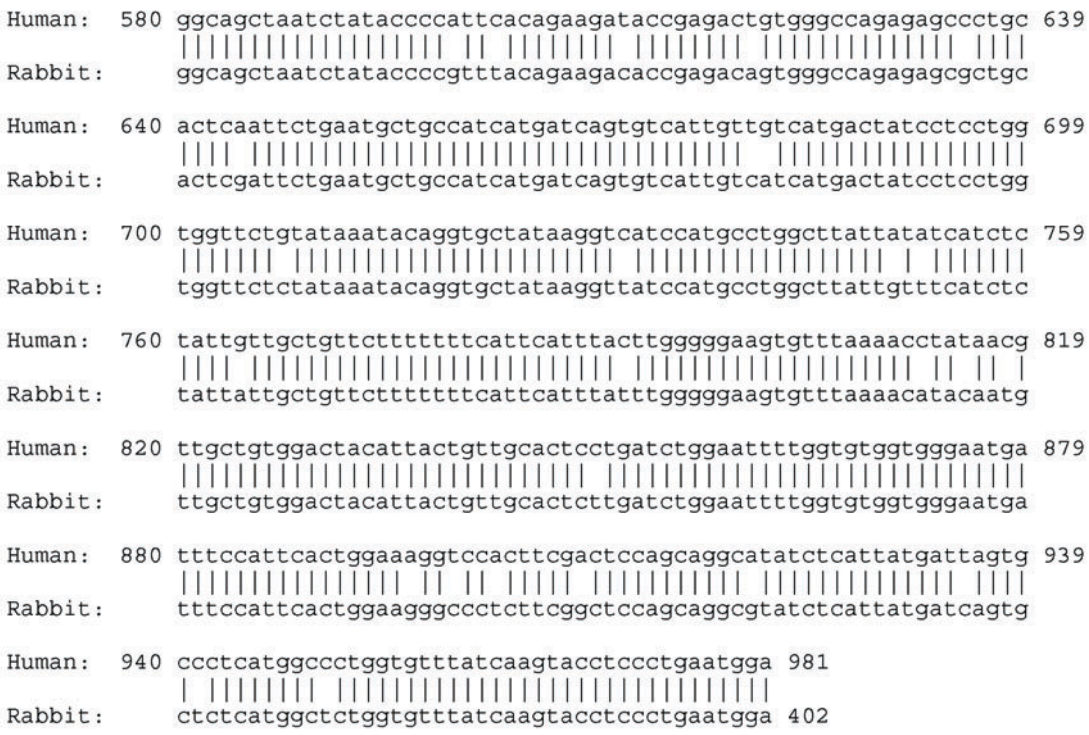

Figure 2. Comparison of cDNA sequences of presenilin-1 of rabbit and human.

are predominantly located in TM domains, at/near putative membrane interfaces or in a large hydrophilic loop. However, all the mutations occur at sites that are highly conserved. Li *et al.* (1995) suggested a possible role of Ps1 in gene expression, on the basis of the pres-

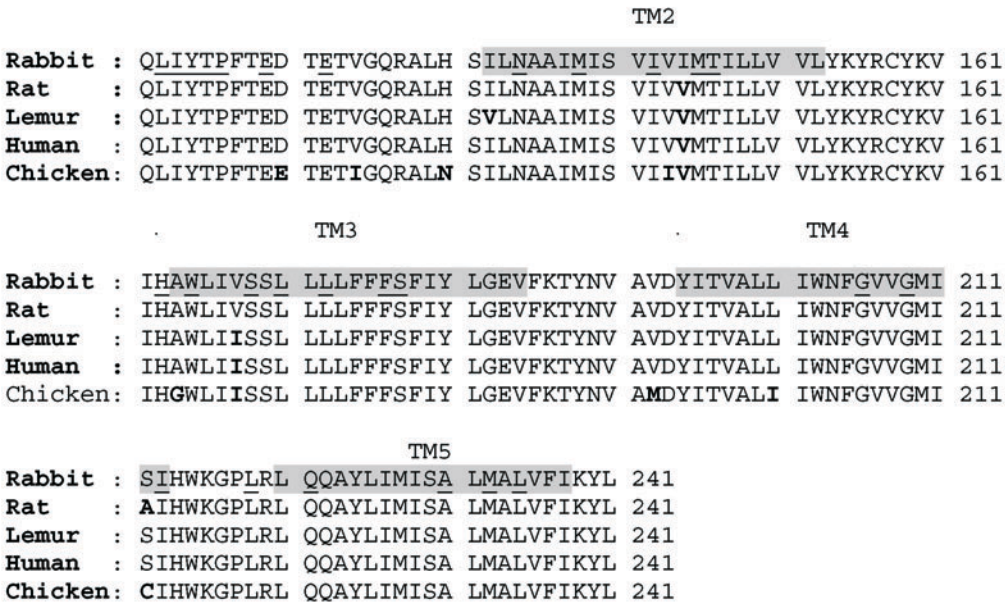

Figure 3. Comparison of the predicted amino-acid sequences of rabbit presenilin-1 with that of rat, lemur, human and chicken.

Underlines indicate positions at which substitutions occur in the early-onset form of FAD in human. TM (shaded) represents the putative transmembrane domains defined on the basis of the human sequence. Amino-acid differences in sequence of various animals are indicated in bold.

ence of two TPXX DNA binding motifs in the human Ps1 protein (positions 116 and 354). They are both present in the mouse sequence but only one (position 116) is conserved in the lemur (Calenda *et al.*, 1996). Comparison of amino-acid sequences of rabbit Ps1 with those of other species (Fig. 3) indicated that at least one (position 116) was conserved in all the five species.

In this study, molecular cloning and sequencing of a cDNA fragment of rabbit Ps1 was performed. This fragment is the most conserved part of the nucleotide sequence in various animals studied for Ps1 and includes the residues at which more than 50% of the mutations linked with FAD occur. This indicates the significance of the present study. Therefore, it is suggested that the rabbit can be a good experimental model for future studies on Ps1 and its association with FAD to work out the physiological function(s) of Ps1 that will help not only in the early diagnosis of FAD but also in developing efficient treatment.

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