Nucleotide sequence of presenilin-1 cDNA fragment of Arabian camel, *Camelus dromedarius*

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ABSTRACT

The cDNA encoding camel presenilin-1 (PS1) fragment was cloned by reverse transcription-polymerase chain reaction (RT-PCR) using primers homologous to conserved sequences of PS1 of human, rat, lemur and mouse. The cDNA fragment, 402 bp in size was well conserved and found to be 79, 89, 91, 91 and 93% homologous to that of chicken, mouse, rat, lemur and human, respectively. The cDNA fragment encodes 130 amino acid protein fragment. The deduced amino acid sequence is also well conserved in various species, exhibiting 98% similarities with those of rat, lemur and human homologues. This cDNA fragment is quite significant as it is the most conserved portion of the PS1 in various animals and encodes four transmembrane regions (TM2, 3, 4, 5) of PS1. Moreover, more than 50% of the amino acid substitutions to which familiar Alzheimer's disease (FAD) have been linked are located in this region.

Key words: *Alzheimer's disease, camel, presenilin-1 cDNA*

GenBank Accession NO. AY134852

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in elderly. The pathogenesis of AD is complex and the common clinical and neuropathological features can arise from several different genetic and non-genetic causes. Nevertheless, approximately 10% of all AD cases are estimated to be early-onset familial AD (FAD) and show autosomal dominant inheritance. Mutations in presenilin-1 (PS1) located on chromosome 14 have been linked with FAD (Schellenberg, 1995). More than 70 mutations have been reported in PS1 gene, their products cause dysfunction/death of vulnerable populations of nerve cells, resulting into clinical syndrome of progressive dementia (Price and Sisodia, 1998; Fraser *et al.*, 2000). In- spite of the significant number of recent studies focusing on AD and its causative factors, the underlying mechanism by which PS1 mutations lead to development of AD remains elusive (Fraser *et al.*, 2000; Saunders, 2001; Esler and Wolfe, 2001; Amtul *et al.*, 2002; Ponting *et al.*, 2002 and Zhou *et al.*, 2002). Although, various hypotheses have been put forward for the physiological function(s) of presenilin, yet it remains undefined and further studies are certainly

required. Investigation of the structure and function of the PS1 is likely to provide powerful clues to resolve pathways culminating to AD linked-neurodegeneration. It has also been suggested that analysis of presenilin and its relationship to Notch signaling pathway may provide valuable insight into AD. 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 various animal models. In the present study, a cDNA encoding camel PS1 fragment was amplified and sequenced, which is the most conserved part of the PS1 in various animals.

MATERIALS AND METHODS

Fresh brain tissues were obtained from the camel (*Camelus dromedarius*) slaughtered at the local abattoir, brought to the laboratory in dry ice and kept at -20°C. Total RNA was extracted from brain tissues by acid guanidium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). The polyadenylated RNA was isolated from the pool of total RNA using mRNA Isolation Kit (Roche, Germany). Primers were designed from the published nucleotide sequences data for highly conserved regions of the PS1 in human (*Homo sapiens*), rat (*Rattus norvigecus*), lemur (*Microcebus murinus*), Mouse (*Mus musculus*) (Sherrington *et al.*, 1995; Calenda *et al.*, 1996; Taniguchi *et al.*, 1997) and chicken (*Gallus gallus*) (accession: AY043492). The following two primers were selected:

Forward primer: 5'-GGA TGA GCA GCT AAT CTA TAC C-3'
Backward primer: 5'-TCC ATT CAG GGA GGT ACT TGA TA-3'

For amplification and sequencing of PS1, 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 the 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 minutes at 50° C. Following the first-strand synthesis, amplification was carried out by 40 cycles of PCR (denaturation at 94°C for 30 seconds., annealing at 50°C for 30 seconds and elongation at 68°C for 1 minute).

The RT-PCR product obtained was separated by electrophoresed on 1.5% agarose gel in TAE buffer. Fragment with the expected size was cut from the gel, purified using Wizard PCR Preps DNA Purification System (Promega Corporation, Madison, USA).

Purified cDNA was sequenced using both forward and backward primers. Subsequently, the results were combined to yield full cDNA sequence of 402 bp. The cDNA sequence was compared with 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 the rat, lemur, human and chicken.

RESULTS AND DISCUSSION

Camel PS1 cDNA fragment was obtained from mRNA by RT-PCR. The cDNA electrophoresed on 1.5 % agarose gel, conformed to 402 bp in size. Comparison of camel cDNA sequence with its counterparts from rat (Taniguchi *et al.*, 1997), lemur

(Calenda *et al.*, 1996), human and mouse (Sherrington *et al.*, 1995) and chicken (accession: AY043492) indicated a high sequence homology. The cDNA sequence is well conserved and was found to be 79, 89, 91, 91 and 93% homologous to that of chicken, mice, rat, lemur and human, respectively. Figure (1) shows cDNA homology with that of rat and Figure (2) depicts some representative nucleotide differences in the electropherogram outputs of rat and camel PS1.

The camel cDNA nucleotide encodes a 130 amino acids protein fragment. The deduced the amino acid sequence was well conserved in various species studied so far. It exhibits 98% similarities with those of rat, lemur and human homologues, whereas 94.6% with that of chicken (Fig. 3). However, a few significant variations were noticed at residue 212 of amino acid sequence, it was found to be alanine, a nonpolar (hydrophobic) amino acid in camel and rat PS1, while it was a serine, a polar (hydrophilic) amino acid, in human and lemur PS1. Another change was found at residue 193, which was methionine in camel PS1 as contrast to valine in rat, lemur and human, though both are nonpolar (hydrophobic) amino acids.

Presenilin-1 is a multispanning transmembrane (TM) protein (Sherrington *et al.*, 1995). It has 10 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 trans-membrane regions. This PS1 cDNA obtained from camel is quite significant as four transmembrane regions (TM 2, 3, 4 and 5) lie in this part of the protein. Moreover, out of over seventy amino acids substitutions which have been reported in early-onset form of FAD, more than 50% are located in the region between 112 and 242 residues. These amino acids were conserved in camel 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 predominantly located in highly conserved TM domains, at/near the putative membrane interfaces or in large hydrophilic loops. However, all the mutations occur at sites that are highly conserved and found in both PS1 and PS2 with the exception of a Glu-to- Lys substitution at the codon 123 in PS1 (Yasuda *et al.*, 1999; Fraser *et al.*, 2000).

A possible role of PS1 in gene expression has also been suggested on the basis of the presence of two TPXX DNA binding motif in the human PS1 protein (Positions 116 and 354) (Li *et al.*, 1995). Both of these are found in mouse also, however, only one (Position 116) is conserved in the lemur (Calenda *et al.*, 1996). Comparison of amino acids sequences of camel PS1 with those of other species (Fig.3) indicates that at least one (Position 116) is conserved in all the five species.

In this study, a cDNA of camel PS1 fragment was characterized which is the most conserved part of nucleotides in various animals studied for PS1 and more than 50% of the mutations that occur in human PS1 with FAD are found in this region. This finding is an addition to our knowledge of PS1 structure and will assist in a comparative study to work out the functional aspect of the PS1 in various animals.

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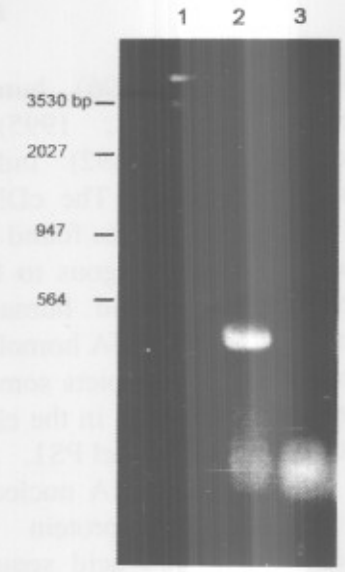

Fig. (1): Amplification of camel PS1 cDNA from mRNA extract.
Lane (1) Lambda DNA digested with Hind III and EcoRI as DNA size marker.
Lane (2) the amplified cDNA fragment of 402 bp in size.
Lane (3) negative control.

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Rat:   332 ggcagctaatctataccccattcacagaagacaccgagactgtaggccagagagccctgc 391
           ||||||||||||||||||||||||||||||||||||||||||||||| || ||||||||||||
Camel:     ggcagctaatctataccccattcacagaagacaccgagactgtggggcagagagccctgc

Rat:   392 actcgatcttgaatgccgccatcatgatcagtgtcatcgtcgttatgaccatcctcctgg 451
           |||||||| ||||||| |||||||||||||| |||||||||| ||||| || |||||||
Camel:     actcgatcctgaatgctgccatcatgatcagcgtcatcgtcgtcatgactattctcctgg

Rat:   452 tggtcctgtataagtacaggtgctacaaggtcatccatgcctggcttattgtttcatctc 511
           |||| |||||||| |||||||||| |||||||||||||||||||||||| ||||||| |
Camel:     tggttctgtataaatacaggtgctataaggtcatccatgcctggcttattatttcatccc

Rat:   512 tgttgttgctgttctttttttcattcatttacttaggggaagtattcaagacctacaatg 571
           | ||||||||||||||||||||||||||||||||||||||||||| || || ||||| || |
Camel:     tattgttgctgttctttttctcattcatttacttaggggaagtgtttaaaacctataacg

Rat:   572 tcgccgtggactatattacggttgcactcctgatctggaattttggtgtggtcgggatga 631
           | ||| ||||||| ||||||||||||||||||||||||||||||||||||||| || ||||
Camel:     ttgccatggactacattacggttgcactcctgatctggaattttggtgtggtgggaatga

Rat:   632 ttgccattcactggaaaggcccactccgactgcagcaggcgtatctcattatgatcagtg 691
           |||||||||||||||||| ||||| ||||| |||||||| ||||||||||||||||||
Camel:     ttgccattcactggaaaggtccactgcgactccagcaggcttatctcattatgatcagtg

Rat:   692 ccctcatggccctggtatttatcaagtacctccctg 727
           |||||||||||||||||||||||||||||||||||
Camel:     ccctcatggccctggtatttatcaagtacctccctg
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Fig. (2): Comparison of cDNA sequences of presenilin-1 of camel and rat.

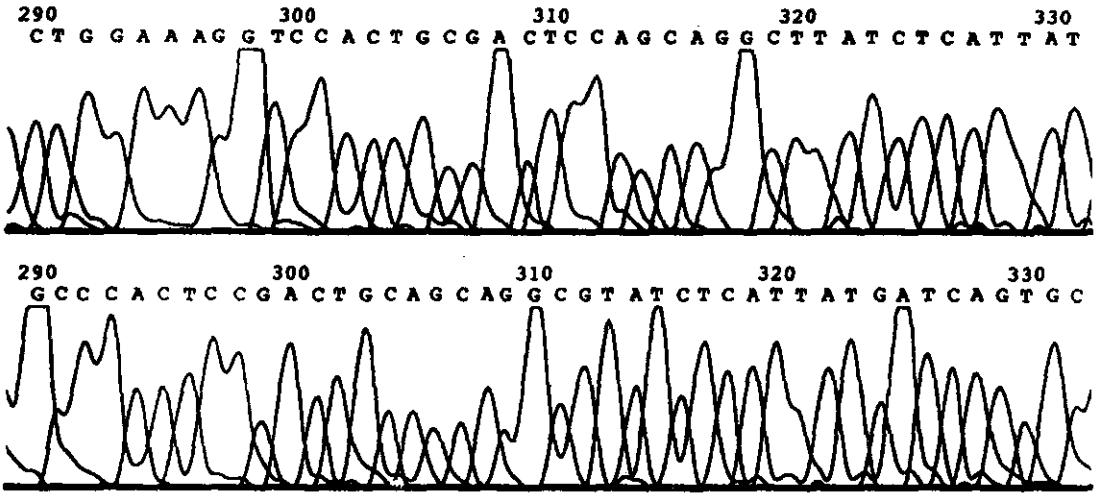

Fig. (3): Some of the variations in cDNA sequence of camel.
Upper: Portion of the electropherogram of camel cDNA sequence.
Lower: Portion of the electropherogram of rat cDNA sequence.

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                                                    TM2
CAMEL   : QLIYTPFTED TETVGQRALH SILNAAIMIS VIVVMTILLV VLYKYRCYKV 161
Rat     : QLIYTPFTED TETVGQRALH SILNAAIMIS VIVVMTILLV VLYKYRCYKV 161
Lemur   : QLIYTPFTED TETVGQRALH SVLNAAIMIS VIVVMTILLV VLYKYRCYKV 161
Human   : QLIYTPFTED TETVGQRALH SILNAAIMIS VIVVMTILLV VLYKYRCYKV 161
Chicken: QLIYTPFTEE TETIGQRALN SILNAAIMIS VIIVMTILLV VLYKYRCYKV 161

                TM3                                 TM4
CAMEL   : IHAWLIISSL LLLFFFSFIY LGEVFKTYNV AMDYITVALL IWNFGVVGMI 211
Rat     : IHAWLIVSSL LLLFFFSFIY LGEVFKTYNV AVDYITVALL IWNFGVVGMI 211
Lemur   : IHAWLIISSL LLLFFFSFIY LGEVFKTYNV AVDYITVALL IWNFGVVGMI 211
Human   : IHAWLIISSL LLLFFFSFIY LGEVFKTYNV AVDYITVALL IWNFGVVGMI 211
Chicken: IHGWLIISSL LLLFFFSFIY LGEVFKTYNV AMDYITVALI IWNFGVVGMI 211

                TM5
CAMEL   : AIHWKGPLRL QQAYLIMISA LMALVFIKYL 241
Rat     : AIHWKGPLRL QQAYLIMISA LMALVFIKYL 241
Lemur   : SIHWKGPLRL QQAYLIMISA LMALVFIKYL 241
Human   : SIHWKGPLRL QQAYLIMISA LMALVFIKYL 241
Chicken: CIHWKGPLRL QQAYLIMISA LMALVFIKYL 241
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Fig. (4): Comparison of the predicted amino acids sequence of camel presenilin-1with that of rat, lemur, human and chicken. Underlines indicate positions at which substitutions occur in the early-onset form of FAD. TM represents the putative transmembrane domains defined on the basis of the human PS1sequence. Amino acid differences of various species are indicated in bold.

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الملخص العربي

تتابع نيوكليوتيدات جزء دنا التكاملي مشفر لبروتين البريسينلين-1 من الجمل العربي

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تـم تنسـيل الدنـا التكاملي المشفر لجزء من بروتين البريسنلين 1 الخاص بالجمل باستخدام تفاعل السلسلة المبلمرة الـرجعى وبادئات مطابقة لتتابعات نيو كليوتيدية أصيلة لمورث البريسينلين-1 من الإنسان ،الجرذ، قرود الليمور والفار. وقد كـان حجـم الدنا التكاملى 402 نيوكليوتيدة مزدوجة وأظهرت تشابها بواقع 79%،89% ،91%، 91%و 93% مع المكافئ الجزيـئى فى الدجـاج ،الفار ،الجرذ، قرود الليمور والانسان على التوالى .ويحمل الدنا التكاملى المتحصل عليه شفرات 130 حمـض أمنى من بروتين البريسينلين_1. ويظهر تتابع الأحماض الأمينية تشابها مع المثيل فى كائنات حية عديدة بواقع 98% فى كـل مـن الجرذ، قرود الليمور والانسان .ويعد هذا الجزء من الدنا التكاملى مهما نظرا لكونه الجزء الاكثر اصالة ويحمل الشفرات الوراثية للأجزاء البروتينية المتداخلة مع الغشاء البلازمى وهى اللأجزاء 2،3،4،5 من البريسينلين-1. كما يظهر أن أكثر من 50% من طفرات التبديل المرتبطة بمرض الزهايمر العائلى محصورة بهذا المكافئ المعزول من الجمل .