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EXPRESSION PROFILING OF HUMAN PRESENILINS

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ABSTRACT

Alzheimer's disease (AD), is the most common form of dementia caused by abnormal fragmentation of amyloid precursor protein by presenilins of γ-secretase protease complex. There are 2 types of presenilins called 1 and 2 and each exists in 2 isoforms. The aim of this study was to get the presenilin sequences from the protein data base and to study functional motifs using motif scan tool and to interpret their functions by computational methods. Motif scan tool identified motifs in presenilin 1 viz., Asn_Glycosylation, Camp_Phospho_Site, Ck2_Phospho_Site, Myristyl, Pkc_Phospho_Site,Arteri_GP4, Coq4,NADHDh, Presenilin, c4dic_mal_tran, Oxidored_q5_N. Motifs identified in presenilin 2 were Asn_Glycosylation,Ck2_Phospho_Site,Myristyl,Pkc_Phospho_Site,Arteri_GP4,Pr esenilin. Clustal W results showed both presenilin 1 and 2 have 65% identical sequences.

KEY WORDS

Gamma secretase, amyloid plaque, presenilin, amyloid beta, alzheimer's disease

INTRODUCTION

Alzheimer's disease has been called "the disease of the century" with staggering medical and social dimensions. Epidemiological studies point out that the disease affects 5% of the population over 65[1]. As the life span is being prolonged with the advances in medical science, the incidence of diseases related to aging has dramatically risen. Current demographic projections indicate increased percentages of the elderly in developed countries and similar trends are emerging in the developing nations thanks to the spectrum of interacting social forces and the improvement in quality of life. Alois Alzheimer first published his observations on the typical neuro pathological changes of the disease nearly a century ago.[2]

A wide range of cognitive impairments is manifested in AD[1]. The most common cognitive failure is memory loss for recent events[1,2.] Progressive impairment of cognitive functions in AD parallels the pathological neural degeneration. Not only memory, AD gives rise to a range of symptoms like attention deficit, depression, panic, lack of self-care, sleep disturbances, paranoid and delusion[3,4].

Pathological hallmarks common to the disease include β amyloid plaques, dystrophic neurite associated with plaques and neurofibrillary tangles within nerve cell bodies[5,6].

Senile plaques are extracellular deposits composed primarily of amyloid β-protein (Aβ), which is a 40-42 amino acid long peptide derived by proteolytic cleavages of the amyloid-precursor protein (APP), with surrounding neuritic alterations and reactive glial cells. Aβ has taken a central role in Alzheimer's disease research for the past two decades in large part because of the amyloid cascade hypothesis (Figure 1) which posits that Aβ is the common initiating factor in AD pathogenesis.[5,6]

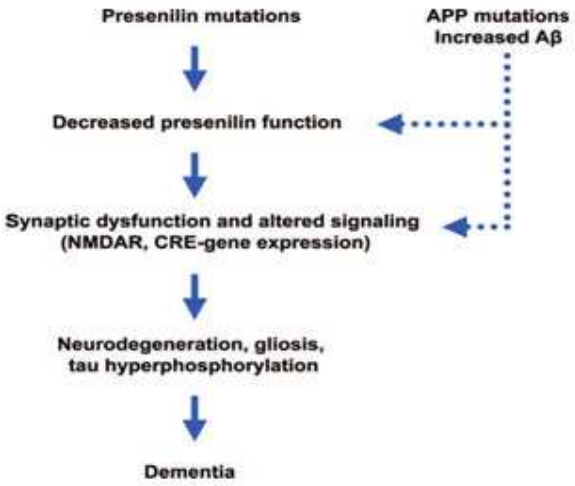

Figure. 1.
The presenilin hypothesis. This diagramdepicts the cascade of events leading to neurodegeneration and dementia in AD, as proposed by the presenilin hypothesis.

The processing of APP and generation of Aβ from APP have become areas of substantial research focus. APP is a member of a family of conserved type I membrane proteins.[7]

APP mutations that cause familial Alzheimer's disease increase the intracellular accumulation of potentially amyloidogenic and neurotoxic carboxyl-terminal fragments of APP in neurons[7] .This indicates that the carboxyl-terminal fragment of APP might be significantly involved in the pathogenesis in AD.[7]

The main determinant of amyloid formation is the conformation adopted by the peptide in the stage before aggregation[8]. Full-length APP is sequentially processed by at least three proteases termed α-, β and γ-secretases.[9]

Presenilin 1 (PS1) and PS2 genes are linked to autosomal dominant early onset familial AD[10,11]. PS are multifunctional transmembrane proteins that contain the catalytic core of γ-secretase, an aspartyl protease that mediates the processing of β amyloid precursor protein.[10,11]

PS undergo regulated endoproteolysis to generate N- and C-terminal fragments, which are the predominant PS species that accumulate *in vivo* within the enzymatic complex[12,13]. The functional active γ-secretase complex is composed of PS dimers and other components such as Nicastrin, Pen-2 and Aph1[14,15]. The mechanism of complex assembly involves stabilization of full-length presenilin by Aph-1/nicastrin, followed by binding of Pen-2, which facilitates endoproteolysis of presenilin and confers the γ-secretase activity.[16]

Two presenilin homologs presenilin-1 (PS1) and presenilin-2 (PS2) located on chromosomes 14q24.3 and 1q31-q42, respectively, are present in mammals[17]. Gene expression studies in different species have revealed wide-spread expression of PS in different cell types and tissues. PS1 and PS2 are ubiquitously expressed in most tissues including the brain.[17]

In human brain, PS1 protein is highly expressed in pyramidal neurons of the hippocampus and neocortex, magnocellular neurons of the basal forebrain, brainstem motor neurons and some interneuron populations[18,19]. Although PS aremainly expressed in neurons, PS1 and PS2 mRNAs are detected at low levels in white matter glial cells and cultured astrocytes.[17]However, PS1 expression is upregulated in reactive astrocytes and activated microglia under pathological conditions such as those occuring in AD, brain injury and hypoxia.[20,21,22]

Mutations in PS alter the cleavage of APP resulting in generation of distinct amyloidogenic Aβ peptides.[23] Thus, several mutations in distinct domains of PS1 (EΔ9, A79V, I143T,L166P, A231V, L262F, L282V, G384A) or PS2 (N141I) decrease total Aβ or Aβ40 levels with little or unappreciable changes on the more amyloidogenic Aβ42 species.[23,24] Other studies have demonstrated increased Aβ42(43) peptides, and/or increased Aβ42/Aβ40 ratio in cells or transgenic mice expressing mutant PS1 or PS2 genes.[25]The age of onset of dementia in families with PS mutations correlates with increase of Aβ42/Aβ ratio and decrease Aβ40 levels.[52]The fact that PS pathogenic mutations suppress the γ- and/or β-secretase cleavage of several substrates including APP has lead to the hypothesis that these mutations may act through a loss of function mechanism.[23,26]

Mutations in the presenilin genes accelerate age of onset and cause earlier and severe progression of neurodegeneration than sporadic AD. The presence of some PS mutations results in quantitative differences in brain neuropathology compared to sporadic forms of AD.[26] Thus, despite similar disease duration, familial cases show similar or greater atrophy and neuronal loss, specially in the medial temporal lobes and frontal/temporal cortices, than sporadic AD cases.[27,28,29]

Similarly, an increase of NFTs and higher NFT-associated neuritic pathology have been reported in PS-linked familial AD cases compared to sporadic AD.[28,30,31]Several studies have also shown increased over all amyloid plaques, especially those containing higher deposition of Aβ42 in genetics forms of AD.[27]

By contrast, other reports show similar amyloid plaque deposition in PSEN1 and sporadic AD

cases.[32,33]The distinct effect of PS mutations on disease progression may reflect a differential effect of PS mutations on Aβ processing, tau phosphorylation and other signaling pathways (GSK3β, β-catenin, calsenilin, Notch, CREB,E/N-cadherin, etc…).[32,33]

The aim of this study was to find the molecular expression profile of human presenilins from their sequences.

MATERIALS AND METHODS

Tools:

1. Motif scan[34]
2. Clustal w[35]

   The sequences of Presenilin 1(Isoforms 463,467) and Presenilin 2 (Isoforms 448,447) were retrieved from NCBI database and the sequences were submitted to Motif scan tool for motifs identification, and clustal W for multiple sequence alignment.

RESULTS

A. Presenilin 1 : Isoforms I and II

The motifs identified were

Asn_Glycosylation,cAMP_Phospho_Site, Ck2_Phospho_Site, Myristyl,Pkc_Phospho_Site, Arteri_GP4, Coq4, NADHdh, Presenilin, C4dic_mal_tran, Oxidored_q5_N (Table:1)

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CLUSTAL 2.0.12 multiple sequence alignment


gi|195947397|ref|NP_015557.2|      MTELPAPLSYFQNAQMSEDNHLSN----TNDNRERQEHNDRRSLGHPEPL 46
gi|4506163|ref|NP_000012.1|        MTELPAPLSYFQNAQMSEDNHLSNTVRSQNDNRERQEHNDRRSLGHPEPL 50
                                   ************************     ********************

gi|195947397|ref|NP_015557.2|      SNGRPQGNSRQVVEQDEEEDEELTLKYGAKHVIMLFVPVTLCMVVVVATI 96
gi|4506163|ref|NP_000012.1|        SNGRPQGNSRQVVEQDEEEDEELTLKYGAKHVIMLFVPVTLCMVVVVATI 100
                                   **************************************************

gi|195947397|ref|NP_015557.2|      KSVSFYTRKDGQLIYTPFTEDTETVGQRALHSILNAAIMISVIVVMTILL 146
gi|4506163|ref|NP_000012.1|        KSVSFYTRKDGQLIYTPFTEDTETVGQRALHSILNAAIMISVIVVMTILL 150
                                   **************************************************

gi|195947397|ref|NP_015557.2|      VVLYKYRCYKVIHAWLIISSLLLLFFFSFIYLGEVFKTYNVAVDYITVAL 196
gi|4506163|ref|NP_000012.1|        VVLYKYRCYKVIHAWLIISSLLLLFFFSFIYLGEVFKTYNVAVDYITVAL 200
                                   **************************************************

gi|195947397|ref|NP_015557.2|      LIWNFGVVGMISIHWKGPLRLQQAYLIMISALMALVFIKYLPEWTAWLIL 246
gi|4506163|ref|NP_000012.1|        LIWNFGVVGMISIHWKGPLRLQQAYLIMISALMALVFIKYLPEWTAWLIL 250
                                   **************************************************

gi|195947397|ref|NP_015557.2|      AVISVYDLVAVLCPKGPLRMLVETAQERNETLFPALIYSSTMVWLVNMAE 296
gi|4506163|ref|NP_000012.1|        AVISVYDLVAVLCPKGPLRMLVETAQERNETLFPALIYSSTMVWLVNMAE 300
                                   **************************************************

gi|195947397|ref|NP_015557.2|      GDPEAQRRVSKNSKYNAESTERESQDTVAENDDGGFSEEWEAQRDSHLGP 346
gi|4506163|ref|NP_000012.1|        GDPEAQRRVSKNSKYNAESTERESQDTVAENDDGGFSEEWEAQRDSHLGP 350
                                   **************************************************

gi|195947397|ref|NP_015557.2|      HRSTPESRAAVQELSSSILAGEDPEERGVKLGLGDFIFYSVLVGKASATA 396
gi|4506163|ref|NP_000012.1|        HRSTPESRAAVQELSSSILAGEDPEERGVKLGLGDFIFYSVLVGKASATA 400
                                   **************************************************
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Figure 2:

Clustal w results showed deletion or insertion in one of the isoforms of Presenilin 1. Aminoacids (TVRS) which were present in isoform 467 (I) were absent in isoform 463(II). Apart from this difference, all amino acids were same, except for only one amino acid which was differed in 29th position(Q in isoform 467(I), T in isoform 463(II)).

B. Presenilin 2: Isoforms I and II:

The motifs identified were

Asn_Glycosylation, Ck2_Phospho_Site, Myristyl, Pkc_Phospho_Site, Arteri_GP4,presenilin. Apart from these motifs all other motifs identified in presenilin1 were not identified in presenilin 2. (Table: 1)

TABLE: 1
Functional Motifs identified in the presenilins

S.No	Motif-id	Description	presenilin-1 isoform I-463	presenilin-1 isoform I-467	Presenilin-2 isoform 1 (448 aa)	presenilin-2 isoform 2 (447 aa)	*Investigator*
1	Asn_Glycosylation	*N-glycosylation site.*	275 278 401 404	279 282 405 408	386 389	385 388	Volkmargiesel mann*, Andreas polten, Joachim kreysing, and Kurt von figura
2	Camp_Phospho_Site	*cAMP- and cGMP-dependent protein kinase phosphorylation site.*	303 306	307 310	--- ----	--- ----	Kalderon D , Rubin GM
3	Ck2_Phospho_Site	*Casein kinase II phosphorylation site.*	103 106 250 253 270 273 316 319 323 326 349 352	28 31 107 110 254 257 274 277 320 323 327 330 353 356	7 10 30 33 260 263 280 283 330 333 335 338	7 10 30 33 260 263 280 283 329 332 334 337	Ashim K. Gupta, Tapas Das and Amiya K. Banerjee
4	Myristyl	*N-myristoylation site.*	374 379 413 418	378 383 417 422	359 364 398 403	358 363 397 402	Aniko v. Paul*t, alanschultzt, steven e. Pincus*§, s. Oroszlant, and eckardwimmer
5	Pkc_Phospho_Site	*Protein kinase C phosphorylation site.*	70 72 95 97 103 105 316 318	25 27 74 76 99 101 107 109 320 322	27 29 80 82 105 107 108 110 113 115	27 29 80 82 105 107 108 110 113 115	Mark H. Rider[a], Josef Van Damme[b], Didier Vertommen[a], Alain Michel[c], JoëlVandekerc khove[b] and Louis Hue
6	Arteri_GP4	*Arterivirus glycoprotein*	400 414	404 418	385 399	384 398	New Motif
7	Coq4	*Coenzyme Q (ubiquinone) biosynthesis protein Coq4*	216 232	220 236	--- ----	--- ----	New Motif
8	NADHDH	*NADH dehydrogenase*	82 98	86 102	--- ----	--- ----	New Motif
9	Presenilin	*Presenilin*	66 454	70 458	76 439	76 438	Sherrington-presenilin1 Rudolph Tanzi and Jerry Schellenberg in 1995-presenilin2
		C4-dicarboxylate			--- ----	---- ----	

10	C4dic_Mal_Tran	*transporter/malic acid transport protein*	85	446	89	450				New Motif
11	Oxidored_Q5_N	*NADH-ubiquinone oxidoreductase chain 4, amino terminus*	135	235	139	239	---	----	---- ----	New Motif

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CLUSTAL 2.0.12 multiple sequence alignment


gi|156105679|ref|NP_000438.2|      MLTFMASDSEEEVCDERTSLMSAESPTPRSCQEGRQGPEDGENTAQWRSQ 50
gi|156105681|ref|NP_036618.2|      MLTFMASDSEEEVCDERTSLMSAESPTPRSCQEGRQGPEDGENTAQWRSQ 50
                                   **************************************************

gi|156105679|ref|NP_000438.2|      ENEEDGEEDPDRYVCSGVPGRPPGLEEELTLKYGAKHVIMLFVPVTLCMI 100
gi|156105681|ref|NP_036618.2|      ENEEDGEEDPDRYVCSGVPGRPPGLEEELTLKYGAKHVIMLFVPVTLCMI 100
                                   **************************************************

gi|156105679|ref|NP_000438.2|      VVVATIKSVRFYTEKNGQLIYTPFTEDTPSVGQRLLNSVLNTLIMISVIV 150
gi|156105681|ref|NP_036618.2|      VVVATIKSVRFYTEKNGQLIYTPFTEDTPSVGQRLLNSVLNTLIMISVIV 150
                                   **************************************************

gi|156105679|ref|NP_000438.2|      VMTIFLVVLYKYRCYKFIHGWLIMSSLMLLFLFTYIYLGEVLKTYNVAMD 200
gi|156105681|ref|NP_036618.2|      VMTIFLVVLYKYRCYKFIHGWLIMSSLMLLFLFTYIYLGEVLKTYNVAMD 200

                                   **************************************************

gi|156105679|ref|NP_000438.2|      YPTLLLTVWNFGAVGMVCIHWKGPLVLQQAYLIMISALMALVFIKYLPEW 250
gi|156105681|ref|NP_036618.2|      YPTLLLTVWNFGAVGMVCIHWKGPLVLQQAYLIMISALMALVFIKYLPEW 250
                                   **************************************************

gi|156105679|ref|NP_000438.2|      SAWVILGAISVYDLVAVLCPKGPLRMLVETAQERNEPIFPALIYSSAMVW 300
gi|156105681|ref|NP_036618.2|      SAWVILGAISVYDLVAVLCPKGPLRMLVETAQERNEPIFPALIYSSAMVW 300
                                   **************************************************

gi|156105679|ref|NP_000438.2|      TVGMAKLDPSSQGALQLPYDPEMEEDSYDSFGEPSYPEVFEPPLTGYPGE 350
gi|156105681|ref|NP_036618.2|      TVGMAKLDPSSQGALQLPYDPEM-EDSYDSFGEPSYPEVFEPPLTGYPGE 349
                                   ********************** ************************

gi|156105679|ref|NP_000438.2|      ELEEEEERGVKLGLGDFIFYSVLVGKAAATGSGDWNTTLACFVAILIGLC 400
gi|156105681|ref|NP_036618.2|      ELEEEEERGVKLGLGDFIFYSVLVGKAAATGSGDWNTTLACFVAILIGLC 399
                                   **************************************************

gi|156105679|ref|NP_000438.2|      LTLLLLAVFKKALPALPISITFGLIFYFSTDNLVRPFMDTLASHQLYI 448
gi|156105681|ref|NP_036618.2|      LTLLLLAVFKKALPALPISITFGLIFYFSTDNLVRPFMDTLASHQLYI 447
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Figure 3:

Clustal W results showed only one amino acid difference (E in 324th position in isoform I) between two isoforms of presenilin2.

Figure 4:
Multiple sequence alignment with Clustal W for presenilin 1 and 2 showed 65% sequence homology

DISCUSSION

Glycosylation is an enzymatic process that links saccharides to produce glycans, attached to proteins, lipids, or other organic molecules. Glycans serve a variety of structural and functional roles in membrane and secreted proteins.[36]

Cyclic AMP (cAMP) is a key intracellular regulator of cell function in both prokaryotes and eukaryotes. One of the ways in which it regulates enzymes is by binding to and causing activation of cAMP-dependent protein kinases, which in turn activate or deactivate other enzymes by phosphorylating them.[37]

Casein kinase II is a serine/threonine protein kinase that phosphorylates acidic proteins.[38]

Myristoylation is an irreversible, co-translational (during translation) protein modification found in animals, plants, fungi and viruses. In this protein modification, a myristoyl group (derived from myristic acid) is covalently attached via an amide bond to the alpha-amino group of an N-terminal amino acid of a nascent polypeptide.[39]

Protein kinase C also known as PKC is a family of enzymes that are involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins.[40]

The membrane glycoprotein GP5 is found in the porcine reproductive and respiratory

syndrome virus (PRRSV) [1].This is a family of structural glycoproteins from Arteriviridae that corresponds to open reading frame 4 (ORF4) of the virus.[41]

Coenzyme Q biosynthesis Coq4p (also known as ubiquinone biosynthesis protein COQ4) was shown to peripherally associate with the matrix face of the mitochondrial inner membrane. The putative mitochondrial-targeting sequence present at the N terminus of the polypeptide efficiently imports it to mitochondria.[42]

NADHdh (also referred to as "NADH:quinone reductase" or "Complex I") is an enzyme located in the inner mitochondrial membrane that catalyzes the transfer of electrons from NADH to coenzyme Q (CoQ). It is the "entry enzyme" of oxidative phosphorylation in the mitochondria.[43]

Presenilins play a key role in the modulation of intracellular Ca^{2+} involved in presynaptic neurotransmitter release and long-term potentiation induction.[44]

Dominant mutations in the genes that encode Presenilin1 proteins are the most common cause of familial early-onset Alzheimer's disease. Presenilin 2 mutations in this gene cause type 4 FAD.[45]

Two members of the Tellurite-Resistance/Dicarboxylate Transporter (TDT) family have been functionally characterised. One is the TehA protein of Escherichia coli which has been implicated in resistance to tellurite; the other is the Mae1 protein of Schizosaccharomyces pombe which functions in the uptake of malate and other dicarboxylates by a proton symport mechanism. These proteins exhibit 10 putative transmembrane α-helical spanners (TMSs).[46]

NADH: ubiquinone oxidoreductase (complex I) (EC:1.6.5.3) is a respiratory-chain enzyme that catalyses the transfer of two electrons from NADH to ubiquinone in a reaction that is associated with proton translocation across the membrane (NADH $^{+}$ ubiquinone = NAD^{+} $^{+}$ubiquinol).[47]

Presenilin1:

The result shows that the presenilin I isoforms 463 and 467 have one cAMP phosphorylation site, so it gets activated by cAMPin membrane signaling .A new motif called Arteri_GP4 may get involved in AD.Other motifs like Coq4, NADHdh, C4dic_mal_tran, Oxidored_q5_N may be involved in mitochondrial targeting of presenilin which ultimately leads to mitochondrial damage.

Presenilin 2:

Absence of cAMP phosphorylation site confirmed that this protein does not get activated by cAMP. Only one new motif is identified in presenilin 2 isoforms I and II, called as Arteri_GP4 which may get involved in AD by inducing abnormal processing of APP.

CONCLUSION

This computational study identified new motifs like Arteri_GP4, Coq4, NADHdh, C4dic_mal_tran, Oxidored_q5_N, which confirmed the role of presenilin 1 and presenilin 2 in Alzheimer's disease and role of presenilin 1 in mitochondrial damage involved in alzheimers' disease. So drugs need to be designed separately targeting each presenilin for the treatment of Alzheimer's disease .

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