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MINI REVIEW

Sequence analyses of presenilin mutations linked to familial Alzheimer's disease

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Abstract Familial Alzheimer's disease (FAD)-linked presenilin (PS) mutations show gain-of-toxic-function characteristics. These FAD PS mutations are scattered throughout the PS molecule, reminiscent of the distribution of cystic fibrosis transmembrane conductance regulator and p53 mutations. Because of the scattered distribution of PS mutations, it is difficult to infer mechanistic insights about how these mutations cause the disease similarly. Recent careful reexamination of γ-secretase activity indicates that some PS mutations decrease the proteolytic activity of γ-secretase, suggesting a loss-of-function nature of PS mutations. To extend this observation to all known PS mutations, a large number of PS mutations were evaluated using bioinformatic tools. The analyses reveal that as many as one third of PS1 residues are highly conserved, that about 75% of FAD mutations are located to the highly conserved residues, and that most PS mutations likely damage the activity of PS. These results are consistent with the idea that the majority of PS mutations lower the activity of PS/γ-secretase.

Keywords Alzheimer's disease · Presenilin mutations · Protein misfolding · Sequence analyses

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease affecting millions of people usually at a later stage of life. Affected individuals suffer from difficulties in memory, judgment, abstraction, and language (see Walsh and Selkoe 2004 for review). These behavioral manifestations are closely linked to lesions in the medial temporal lobe and cortical networks which play a crucial role in long-term, declarative memory (see Buckner 2004 for review). These regions of the brain are invariably studded with characteristic aggregates such as extracellular neuritic plaques and intracellular neurofibrilary tangles in AD patients (Walsh and Selkoe 2004). A major constituent of the amyloid plaque is small Aβ peptide fragments derived from the β-amyloid precursor protein (APP). APP is cleaved along the secretory pathway by several proteases, two of which, β- and γ-secretases, generate the Aβ peptide. Another pathological hallmark of AD is the neurofibrillary tangles that are found in cell bodies and apical dendrites. The major proteinaceous component is abnormally phosphorylated tau proteins (Walsh and Selkoe 2004). A currently accepted model is that soluble forms of extracellular amyloid peptides signal cells to form intraneuronal tangles, which doom neurons to a destructive fate (Haass and Selkoe 2007).

A small fraction of AD is inherited in an autosomal dominant fashion (Price and Sisodia 1998) and is genetically heterogeneous. Most familial AD (FAD) cases are due to mutations in APP and presenilin 1 and 2 (PS1 and PS2). PS is a catalytic subunit of γ-secretase that processes APP (Selkoe and Wolfe 2007). γ-Secretase generates amyloid peptides such as $A\beta_{40}$ and $A\beta_{42}$ in concert with β-secretase from APP. FAD-linked APP mutations act either by increasing overall production of $A\beta_{40}$ and $A\beta_{42}$ or by enhancing propensity of amyloid fragments to aggregate, or by specifically overproducing $A\beta_{42}$ (Walsh and Selkoe 2004). Most FAD-linked PS1 mutations tested so far increase the ratio of $A\beta_{42}/A\beta_{40}$, consistent with the idea that increases of the relative or absolute level of toxic $A\beta_{42}$

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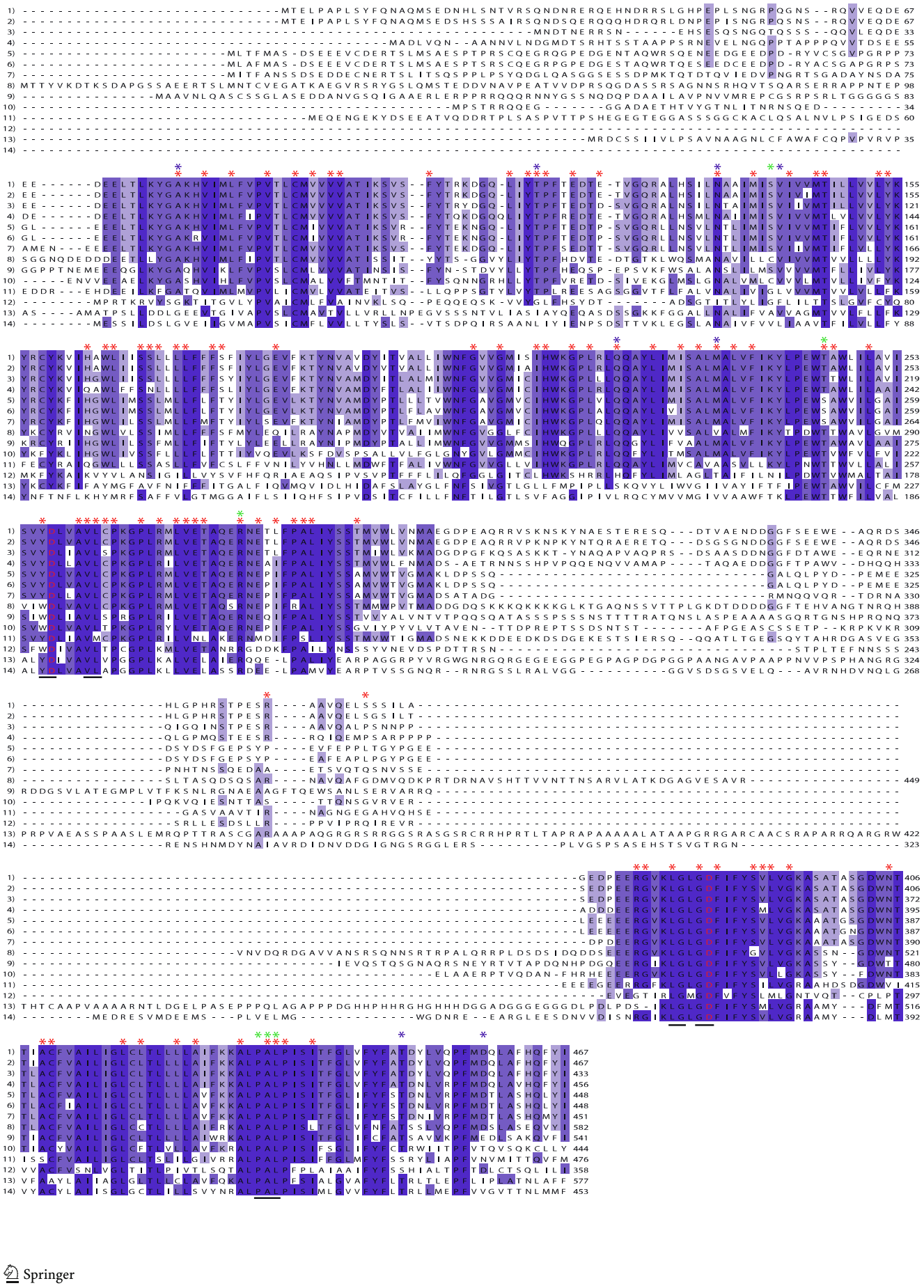

◀ **Fig. 1** Multiple protein sequence alignment of PS from various species was performed using ClustalW (Thompson et al. 1994, http://www.ebi.ac.uk/Tools/clustalw/). Conserved residues were marked with *blue shades*. Amino acid sequences are from (*1*) PS1 *Homo sapiens* (NP_000012), (*2*) PS1 *Mus musculus* (NP_032969), (*3*) PS1 *Xenopus tropicalis* (NP_001027027), (*4*) PS1 *Danio rerio* (NP_571099), (*5*) PS2 *Homo sapiens* (P49810), (*6*) PS2 *Mus musculus* (NP_035313), (*7*) PS2 *Xenopus tropicalis* (NP_001017181), (*8*) PS *Helix lucorum* (AAG28518), (*9*) PS *Drosophila melanogaster* (O02194), (*10*) Sel-12 *Caenorhabditis elegans* (P52166), (*11*) PS *Ephydatia fluviatilis* (BAE19681), (*12*) Hop-1 *Caenorhabditis elegans* (O02100), (*13*) PS *Chlamydomonas reinhardtii* (EDP06639), (*14*) *Arabidopsis thaliana* (NP_172346). FAD-linked PS1 residues are marked with *red asterisks*, FAD PS2 residues with *blue asterisks*, and PS1 residues identified by random mutagenesis (Nakaya et al. 2005) with *green asterisks*. Only missense mutations were considered. Two putative active site resides are shown in *red*. Putative catalytic core regions are *underlined* (Weihofen et al. 2002)

may trigger AD (Wolfe 2007), while some FAD-linked PS1 mutations fail to increase neurotoxic $A\beta_{42}$ or the $A\beta_{42}/A\beta_{40}$ ratio (Shioi et al. 2007).

Because many APP or PS mutants change the level of Aβ production, one can expect that any gene or reagent that regulates Aβ metabolism may also affect AD pathology. Many cell-based assays monitoring Aβ metabolism have contributed to the identification of genes or chemicals that modulate Aβ synthesis (Kukar et al. 2005; Zhou et al. 2005; Chen et al. 2006; Weggen et al. 2007).

At the molecular level, it is challenging to figure out how such mutations affect Aβ generation. Most APP mutations are found near cleavage sites of α-, β-, and γ-secretases implying that these mutations compromise the cleavage action by each secretase. On the other hand, more than 100 FAD-linked PS1 mutations are spread throughout the molecule, defeating simple explanations. It is interesting to note that this scattered mutation pattern has been observed in other proteins such as cystic fibrosis transmembrane conductance regulator (CFTR) and p53 (Gelman and Kopito 2002). Remarkably, more than 1,500 mutations in the *CFTR* gene (1,480 amino acids) have been linked to cystic fibrosis (http://www.genet.sickkids.on.ca/cftr). The mutations in CFTR and p53 cause a varying degree of loss of function biochemically and genetically. On the other hand, PS mutations are considered a gain of toxic function genetically. For example, the ablation of one copy of PS1 is asymptomatic in mice (although not confirmed in human), and missense mutations dominantly predispose carriers to AD (De Strooper 2007). It has been recently shown that a few selected PS mutations cause a loss of function biochemically (for example, reduced proteolytic activity; Bentahir et al. 2006) and that the reduction in proteolytic activity of PS may be mechanistically linked to an increased ratio of $A\beta_{42}/A\beta_{40}$ (Wolfe 2007). Thus, it will be important to examine whether the majority of all known PS mutations reduces proteolytic activity of γ-secretase.

To test whether the characteristics of FAD PS mutations provide an insight with regard to structure and function of PS/γ-secretase, 14 PS sequences from distantly related species were analyzed using bioinformatic approaches. The results suggest that most FAD PS mutations lead to a loss of structure and function of PS/γ-secretase biochemically.

Table 1 Highly conserved residues of PS are preferred targets for FAD mutation

Degree of conservation[a]	Number of conserved residues	Number of FAD-linked PS1 residues	Mutation rate (%) of PS1 residues[b]	Number of FAD-linked PS2 residues	Mutation rate (%) of PS2 residues[c]	Number of PS1 residues hit by random mutagenesis	Hit rate (%) of PS1 residues[d]
14/14	43	21	48.8			4	9.3
13/14	30	12	40	1	3.3		
12/14	32	13	40.6	1	3.2		
11/14	54	22	40.7	4	21.1	1	1.8
10/14	32	5	15.6				
9/14	34	5	14.7	2	5.9		
8/14	35	6	17.1			1	2.9
7/14	22	4	18.2				
6/14	19	1	5.3				
5/14 to 0/14	166	2	1.2				
Total	467	91	19.5%				

[a] For example, when a residue is conserved in 12 PS sequences among 14 sequences (Fig. 1), it is shown as 12 of 14.
[b] The mutation rate was calculated based on number of FAD-linked PS1 residues from number of conserved residues under a given degree of conservation.
[c] The mutation rate was calculated based on number of FAD-linked PS2 residues from number of conserved residues under a given degree of conservation.
[d] The mutation rate was calculated based on number of PS1 residues hit by random mutagenesis from number of conserved residues under a given degree of conservation.

Conservation of PS and pattern of FAD-linked PS mutations

Because not all the functionally and structurally critical residues of PS are known, it was necessary to find a way to recognize such residues. On the assumption that critical residues would be conserved across different species, 14 PS sequences from various species including *Chlamydomonas* and *Arabidopsis* were compared (Fig. 1). This analysis resulted in 43 conserved residues in all 14 species. In support of the assumption, Aβ production was significantly altered when the PS1 Y288 residue (one of the 43 conserved residues) was artificially mutated (Laudon et al. 2004), although this residue has not been linked to AD so far.

Table 2 Effects of FAD PS1 mutations on PS1/γ-secretase activity

Degree of conservation (14/14)[a]	Mutation	Functional significance (SIFT)[b]	Functional significance (PANTHER)[c]	Functional significance (GV/GD)[d]
V89	L	*0.00*[e]	*−3.62373*[e]	*0.00/30.92*[e]
C92	S	*0.00*	*−3.44456*	*0.00/111.67*
T147	I	*0.00*	−1.87486	*0.00/89.28*
G209	R	*0.00*	*−4.56674*	*0.00/125.13*
G209	E	*0.00*	*−4.41973*	*0.00/97.85*
G209	V	*0.00*	*−3.91328*	*0.00/109.55*
L250	S	*0.00*	*−3.35853*	*0.00/144.08*
A260	V	*0.00*	*−3.97167*	*0.00/65.28*
V261	F	*0.00*	*−4.59946*	*0.00/48.95*
P264	L	*0.00*	*−4.06675*	*0.00/97.78*
P267	S	*0.00*	−2.25417	*0.00/73.35*
P267	L	*0.00*	*−3.84044*	*0.00/97.78*
L271	V	*0.00*	*−3.05205*	*0.00/30.92*
V272	A	*0.00*	*−3.13143*	*0.00/65.28*
R278	K	*0.00*	*−3.05745*	*0.00/26.00*
R278	T	*0.00*	*−3.91372*	*0.00/70.97*
R278	S	*0.00*	*−3.70071*	*0.00/109.21*
L381	V	*0.00*	*−3.99591*	*0.00/30.92*
G384	A	*0.00*	*−4.85237*	*0.00/60.00*
F386	S	*0.00*	*−5.37049*	*0.00/154.81*
G394	V	*0.00*	*−5.85813*	*0.00/109.55*
L418	F	*0.00*	*−4.35042*	*0.00/21.82*
A431	E	*0.00*	*−4.73687*	*0.00/106.71*
A431	V	*0.00*	*−4.08271*	*0.00/65.28*
A434	C	*0.00*	*−5.39513*	*0.00/195.00*
L435	F	*0.00*	*−4.35042*	*0.00/21.82*
P436	S	*0.00*	*−5.25856*	*0.00/73.35*
P436	Q	*0.00*	*−6.16212*	*0.00/75.14*

[a] The degree of conservation was according to Table 1 and accurate for SIFT. For PANTHER PSEC and Align-GVGD, it may change due to different set of homologous sequences.

[b] Normalized probabilities. Positions with normalized probabilities less than 0.05 are predicted to be deleterious; those greater than or equal to 0.05 are predicted to be tolerated. The analysis was performed using PS sequences in Fig. 1 (Ng and Henikoff 2006).

[c] PANTHER subPSEC values were acquired using its own set of PS1 homologous sequences from its database (http://www.pantherdb.org/; Thomas et al. 2003). A subPSEC score of −3 or less corresponds to a deleterious mutation. This prediction method reveals gain-of-function mutations in some instances (Thomas et al. 2003). The sequences were from PS1_HUMAN (P49768, *Homo sapiens*), Q6RH31_CANFA (Q6RH31, *Canis lupus familiaris*), Q6RH32_CANFA (Q6RH32, *Canis familiaris*), PSN1_BOVIN (Q9XT97, *Bos Taurus*), PSN1_RAT (P97887, *Rattus norvegicus*), PSN1_MOUSE (P49769, *Mus musculus*), Q90X08_CHICK (Q90X08, *Gallus gallus*), PSN1_XENLA (O12976, *Xenopus laevis*), PSN1_BRARE (Q9W6T7, *Danio rerio*), PSN_CAEEL (P52166, *Caenorhabditis elegans*), HOP-1 (O02100, *Caenorhabditis elegans*), SPE-4 (Q01608, *Caenorhabditis elegans*), PSNA_ARATH (O64668, *Arabidopsis thaliana*), and PSNB_ARATH (Q9SIK7, *Arabidopsis thaliana*).

[d] Grantham variation (GV) measures the degree of biochemical variation among amino acids found at a given position in the multiple sequence alignment, and Grantham deviation (GD) scores the biochemical distance of the mutant amino acid from the observed amino acid at a particular position (Grantham 1974; Mathe et al. 2006). The criteria for prediction are described in Mathe et al. (2006). The current Align-GVGD analysis used PS sequences in Fig. 1 except sequences from *Arabidopsis thaliana* (NP_172346) and *Chlamydomonas reinhardtii* (EDP06639) because adding more distantly related sequences decreased the number of mutants predicted as deleterious (Mathe et al. 2006). However, the current set of PS sequences is still more divergent than the set of p53 sequences used to develop the Align-GVGD method (Mathe et al. 2006).

[e] Values predicted to damage the activity of PS1 are shown in italics.

Table 3 Effects of FAD PS1 mutations on PS1/γ-secretase activity

Degree of conservation (13/14)[a]	Mutation	Functional significance (SIFT)[b]	Functional significance (PANTHER)[c]	Functional significance (GV/GD)[d]
V96	F	*0.01*[e]	−2.55121	*0.00/48.95*[e]
V97	L	*0.03*	−2.14694	*0.00/30.92*
N135	D	0.11	*−3.19811*[e]	*0.00/23.01*
N135	S	0.11	*−3.11384*	*0.00/46.24*
L174	M	*0.03*	−2.56868	*0.00/14.30*
L174	R	*0.00*	*−5.26216*	*0.00/101.88*
L262	F	*0.02*	*−4.09855*	*0.00/21.82*
E273	A	*0.02*	*−3.73922*	*0.00/106.71*
P284	S	*0.03*	*−3.16234*	*0.00/73.35*
P284	L	*0.02*	*−3.35216*	*0.00/97.78*
A285	V	*0.01*	*−3.10351*	*0.00/65.28*
S390	I	*0.00*	*−5.42803*	*0.00/141.80*
L392	V	*0.02*	*−3.54057*	*0.00/30.92*
L392	P	*0.00*	*−5.82317*	*0.00/97.78*
A409	T	*0.01*	*−3.66939*	*0.00/58.02*
C410	Y	*0.00*	*−5.50834*	*0.00/193.72*

[a, b, c, d, e] See legend of Table 2.

Conserved residues were classified according to the degree of conservation (Table 1; when a residue is conserved in 12 PS sequences among 14 sequences, it is represented as 12/14). As expected, previously identified motifs such as the active site residues and their surrounding residues were found among the conserved residues (Fig. 1; Wolfe et al. 1999; Weihofen et al. 2002). It is interesting to note that 21 out of 43 absolutely conserved residues (14 of

Table 4 Effects of FAD PS1 mutations on PS1/γ-secretase activity

Degree of conservation (12/14)[a]	Mutation	Functional significance (SIFT)[b]	Functional significance (PANTHER)[c]	Functional significance (GV/GD)[d]
V82	L	*0.04*[e]	−2.10435	*0.00/30.92*[e]
Y115	H	0.12	*−4.10728*[e]	*0.00/83.33*
Y115	D	0.37	*−5.28542*	*0.00/159.94*
Y115	C	*0.04*	*−5.33748*	*0.00/193.72*
M146	V	0.28	−1.63467	*0.00/20.52*
M146	I	0.09	−2.23949	*0.00/10.12*
M146	L	0.09	−2.04936	*0.00/14.30*
L153	V	*0.05*	−2.32119	*0.00/30.92*
S169	L	*0.01*	−2.24316	*0.00/144.08*
S169	P	*0.02*	*−3.71787*	*0.00/73.35*
G206	S	0.22	−2.33284	*0.00/55.27*
G206	D	*0.03*	*−3.95033*	*0.00/93.77*
G206	A	0.09	−2.67628	*0.00/60.00*
G206	V	*0.02*	−2.10266	*0.00/109.55*
H214	Y	0.20	−2.9983	*0.00/83.33*
G217	D	*0.04*	−2.90745	*0.00/93.77*
L226	R	*0.01*	*−3.57015*	*0.00/101.88*
L226	F	0.13	*−3.95858*	*0.00/21.82*
L286	V	*0.03*	−2.53727	*0.00/30.92*
R377	M	*0.02*	*−4.49467*	*0.00/91.64*
G378	V	*0.02*	−2.28787	*0.00/109.55*
G378	E	*0.02*	*−4.13214*	*0.00/97.85*
I439	V	0.06	−1.86884	*0.00/28.68*

[a, b, c, d, e] See legend of Table 2.

14) were linked to FAD (48.8%; Table 1). Considering the possibility that more naturally occurring FAD PS1 mutations would be discovered in the future, it is reasonable to predict that the number of FAD mutations in this category will continue to increase. Sixty-eight among 91 FAD PS1 residues (74.7%) were found in highly conserved residues (degree of conservation from 14 of 14 to 11 of 14). Other FAD PS1 mutations (20 residues out of 91; 22%) were found mostly in moderately conserved residues (degree of conservation from 10 of 14 to 7 of 14). Therefore, most FAD PS1 mutations (88 out of 91; 96.7%) were mapped to highly or moderately conserved residues. Only 3 out of 91 FAD mutations (3%) were found in less conserved residues. These observations indicate that the preferred spots for FAD PS1 mutations are the highly conserved residues.

Table 5 Effects of FAD PS1 mutations on PS1/γ-secretase activity

Degree of conservation (11/14)[a]	Mutation	Functional significance (SIFT)[b]	Functional significance (PANTHER)[c]	Functional significance (GV/GD)[d]
A79	V	*0.02*[e]	*−4.02789*[e]	*0.00/65.28*[e]
L85	P	*0.00*	*−4.42532*	*0.00/97.78*
L113	P	*0.02*	*−4.94282*	*0.00/97.78*
L113	V	0.36	−2.37417	*0.00/30.92*
L113	Q	*0.01*	*−4.64537*	*0.00/112.44*
T116	N	0.19	*−3.90259*	*0.00/64.77*
T116	I	0.82	*−4.37086*	*0.00/89.28*
P117	S	0.14	−2.65492	*0.00/73.35*
P117	L	1.00	−1.78495	*0.00/97.78*
P117	R	0.07	*−3.11267*	*0.00/102.71*
E120	K	0.06	−2.11044	*0.00/56.87*
E120	D	0.19	−2.5923	*0.00/44.60*
Y154	C	*0.00*	*−5.3361*	*0.00/193.72*
Y154	N	*0.00*	*−4.65004*	*0.00/142.23*
W165	G	*0.00*	*−3.80029*	*0.00/183.79*
W165	C	*0.00*	*−4.1291*	*0.00/214.36*
L166	H	*0.00*	*−4.86668*	*0.00/98.69*
L166	P	*0.01*	−2.81463	*0.00/97.78*
L166	R	*0.00*	*−4.43624*	*0.00/101.88*
S170	F	*0.02*	*−4.10627*	*46.24/135.88*
L173	W	*0.00*	*−4.97603*	*0.00/60.98*
F177	L	0.10	−1.80095	*0.00/21.82*
F177	S	*0.02*	*−3.13367*	*0.00/154.81*
I213	L	0.36	−2.09768	*0.00/4.86*
I213	F	*0.01*	*−3.58038*	*0.00/21.28*
I213	T	*0.01*	*−3.32339*	*0.00/89.28*
L219	F	0.17	−3.17101	*0.00/21.82*
L219	P	0.16	−3.96853	*0.00/97.78*
Q222	R	0.29	−2.66321	*0.00/42.81*
Q222	H	0.28	−2.51753	*0.00/24.08*
A231	T	*0.02*[e]	−2.73211	*0.00/58.02*
A231	V	*0.02*	−2.43329	*0.00/65.28*
T245	P	*0.01*	*−4.08141*	*57.75/24.03*
R269	G	*0.01*	*−3.65651*	*0.00/125.13*
R269	H	*0.01*	*−3.72602*	*0.00/28.82*
T274	R	0.08	*−3.9915*	*0.00/70.97*
E280	A	0.06	*−3.19891*	*0.00/106.71*
E280	G	*0.02*	*−3.52354*	*0.00/97.85*
L424	H	*0.00*	*−5.84841*	*0.00/98.69*
L424	R	*0.01*	*−5.33629*	*0.00/101.88*
A426	P	*0.03*	*−4.17643*	*0.00/26.87*

[a, b, c, d, e] See legend of Table 2.

Table 6 Effects of FAD PS1 mutations on PS1/γ-secretase activity

Degree of conservation (10/14)[a]	Mutation	Functional significance (SIFT)[b]	Functional significance (PANTHER)[c]	Functional significance (GV/GD)[d]
L235	V	0.44	−2.35073	*0.00/30.92*[e]
L235	P	0.07	*−4.05115*[e]	*0.00/97.78*
F237	L	0.53	*−3.04552*	*0.00/21.82*
F237	I	0.47	−2.5801	*0.00/21.28*
Y256	S	*0.01*[e]	−2.17096	*0.00/143.11*
V391	F	*0.02*	*−3.88813*	*20.52/28.53*
L420	R	*0.01*	*−4.15521*	*0.00/101.88*

[a, b, c, d, e] See legend of Table 2.

The multiple sequence alignment also showed that many PS1 residues are highly conserved (159 out of 467; 34%; Fig. 1 and Table 1). The percentage of conservation was increased to 60.3% when moderately conserved residues were also counted (123 out of 467; 26.3%). This high conservation is consistent with the fact that PS has multiple domains/functions for proteolytic cleavage, Ca^{2+} ion channeling, PI3K signaling, and cytoskeletal regulation (Selkoe and Wolfe 2007; Baki et al. 2004; Fraering et al. 2005; Tu et al. 2006; Khandelwal et al. 2007; Zhao et al. 2008 and references therein) and suggests that these domains/functions are important across many different species. Because highly conserved residues span over the PS molecule and a majority of FAD mutations fall onto these residues, it is natural to observe a scattered distribution of FAD PS mutations.

Based on the pattern of FAD PS1 mutations, we asked whether or not FAD PS2 mutations follow this trend. Indeed, despite a small pool of FAD-linked mutations, a similar pattern was observed with 75% (six out of eight residues) of FAD mutations being located to highly conserved residues (Table 1). Strikingly, Nakaya et al. (2005) identified residues affecting proteolytic activity of PS1 by randomly mutagenizing the PS1 gene. When these mutations were mapped (Fig. 1), five out of six residues were actually highly conserved residues according to the current analysis (Table 1). The percentage is comparable to that from naturally occurring mutations of PS1 and PS2. These results show that FAD PS mutations involve highly conserved residues.

The core region (CR) of p53 is highly enriched with missense mutations (Soussi and Wiman 2007). All residues of the CR have been found to be mutated in human cancers. This is because the CR of p53 is highly flexible and cycles between folded and unfolded states, rendering fragility to the protein (Joerger and Fersht 2007) and making the CR of p53 a binding site for Hsp90 (Rüdiger et al. 2002). The mutations on the CR lead to a loss of function ranging from partial to complete loss of activity (Kato et al. 2003). In fact, it has been shown that the probability of a random mutation to cause a genetic

Table 7 Effects of FAD PS1 mutations on PS1/γ-secretase activity

Degree of conservation (9/14)[a]	Mutation	Functional significance (SIFT)[b]	Functional significance (PANTHER)[c]	Functional significance (GV/GD)[d]
F105	I	0.39	−2.66139	*0.00/21.28*[e]
F105	L	0.68	−2.39242	*0.00/21.82*
I143	F	*0.01*[e]	*−4.18757*[e]	*0.00/21.28*
I143	N	*0.00*	*−5.41713*	*0.00/148.91*
I143	T	*0.01*	*−4.00722*	*0.00/89.28*
I143	M	*0.01*	*−3.93209*	*0.00/10.12*
E184	D	0.17	−2.92255	*0.00/44.60*
M233	L	0.25	−2.4775	*0.00/14.30*
M233	I	0.54	−2.68173	*0.00/10.12*
M233	V	1.00	−2.13273	*0.00/20.52*
M233	T	0.40	−2.39566	*0.00/81.04*
N405	S	0.72	−2.23365	*0.00/46.24*

[a, b, c, d, e] See legend of Table 2.

Table 8 Effects of FAD PS1 mutations on PS1/γ-secretase activity

Degree of conservation (8/14)[a]	Mutation	Functional significance (SIFT)[b]	Functional significance (PANTHER)[c]	Functional significance (GV/GD)[d]
M139	K	*0.01*[e]	*−3.3509*[e]	*0.00/94.49*[e]
M139	T	*0.02*	−2.68458	*0.00/81.04*
M139	I	0.20	−2.23428	*0.00/10.12*
H163	Y	0.44	*−3.82384*	*24.08/83.33*
H163	R	0.57	−2.45471	*24.08/28.37*
A246	E	*0.04*	−2.78519	*58.02/65.50*
C263	R	*0.03*	−1.63645	111.67/90.15
C263	F	*0.02*	−2.90381	111.67/124.98
L282	R	*0.03*	−2.65841	*4.86/97.59*
L282	V	0.35	−1.11025	*4.86/28.68*
R358	Q	0.45	−2.11737	111.84/16.48

[a, b, c, d, e] See legend of Table 2.

disease increases as the degree of conservation goes up (Vitkup et al. 2003). Similarly, many FAD PS mutations found in the highly conserved regions of PS also likely cause a varying degree of loss of activity of PS because conserved residues likely play an important role for proper structure and function of the protein (Ng and Henikoff 2006).

Prediction for activity of PS variants based on bioinformatic approaches

To predict the extent to which PS1 missense mutations affect the activity of γ-secretase, three independent prediction methods were employed (Tables 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12). These methods score whether an amino acid substitution at a certain position is tolerated based on sequence homology and physicochemical properties of amino acids (Sorting Intolerant From Tolerant [SIFT], http://blocks.fhcrc.org/sift/SIFT.html; Protein Analysis Through Evolutionary Relationships [PANTHER] PSEC, http://www.pantherdb.org/; Align-Grantham variation–Grantham deviation [GVGD], http://agvgd.iarc.fr/). These approaches have been used to evaluate how missense mutations abrogate or increase/decrease protein function (Vitkup et al. 2003; Brunham et al. 2005; Mathe et al. 2006). When these analyses were applied to PS1 missense mutations, SIFT, PANTHER, and A-GVGD predicted 94, 90, and 139 mutations (out of 145 mutations) to be deleterious (Tables 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12). As a control, nonpathogenic mutations (R35Q, F175S, and E318G) were also analyzed. The PANTHER prediction turned out to be the most stringent of the three methods. This is consistent with the report that PANTHER yields a high false-negative rate (59%; the percentage of substitutions predicted to be functionally neutral on a set of amino acid substitutions that are known to affect protein function) compared to SIFT (31%; Ng and Henikoff 2006). The accuracy of the Align-GVGD on p53 for deleterious and neutral mutants was 88.1% and 71.2%, respectively (Mathe et al. 2006). Similarly, the Aligh-GVGD prediction for deleterious and neutral mutants of PS1 was 95.9% (139 of 145) and 67% (two of three), respectively. These results suggest that most FAD-linked PS1 mutations reduce the activity of PS1.

Table 9 Effects of FAD PS1 mutations on PS1/γ-secretase activity

Degree of conservation (7/14)[a]	Mutation	Functional significance (SIFT)[b]	Functional significance (PANTHER)[c]	Functional significance (GV/GD)[d]
V94	M	0.08	−2.61745	28.68/0.00
L171	P	*0.03*[e]	*−4.41596*[e]	*0.00/97.78*[e]
I229	F	0.08	*−3.62133*	*0.00/21.28*
T291	P	0.27	−2.94048	*58.02/1.62*

[a, b, c, d, e] See legend of Table 2.

Table 10 Effects of FAD PS1 mutations on PS1/γ-secretase activity

Degree of conservation (6/14)[a]	Mutation	Functional significance (SIFT)[b]	Functional significance (PANTHER)[c]	Functional significance (GV/GD)[d]
S178	P	0.13	*−4.04264*[e]	*57.75/24.03*[e]

[a, b, c, d, e] See legend of Table 2.

Experimental observations consistent with loss of activities of clinically isolated PS1 variants

The reduction/elimination of the activity of *sel-12* (a PS homolog) causes an egg-laying defective phenotype due to defective signaling of the Lin-12/Notch pathway in *Caenorhabditis elegans* (Sundaram and Greenwald 1993). Normal human PS can substitute for the *C. elegans* SEL-12 protein in vivo, whereas FAD-linked mutant human PSs have reduced ability to rescue the phenotype caused by *sel-12* disruption (Levitan et al. 1996). Proteolytic release of the Notch-1 intracellular domain (NICD) is critical for Notch signaling, and this proteolysis is impaired in PS1 null cells and restored by PS1 expression (Song et al. 1999). However, some FAD-linked PS1 mutations display reduced ability to generate the NICD, consistent with the genetic data obtained from *C. elegans* (Levitan et al. 1996). FAD-linked PS1 mutations also appear to have a reduced activity toward other PS substrates (Marambaud et al. 2003; Bentahir et al. 2006).

The absence of PS1 causes the accumulation of cytosolic β-catenin, resulting in accelerated entry into the S phase of the cell cycle (Soriano et al. 2001). This hyperproliferative response is rescued by PS1 expression but not by two different FAD-linked PS1 mutants (Soriano et al. 2001). Wild-type PS1 may also activate the PI3K/Akt signaling pathway. In contrast, PS1 FAD mutations inhibit PS1-dependent PI3K/Akt activation, thus promoting glycogen synthase kinase 3 (GSK-3) activity and tau overphosphorylation (Baki et al. 2004).

PS is believed to regulate capacitative calcium entry (CCE) – a mechanism for refilling intracellular Ca^{2+} through plasma membrane channels. FAD-linked PS variants attenuate CCE (Yoo et al. 2000). PS also appears to be responsible for approximately 80% of passive Ca^{2+} leak from the endoplasmic reticulum (ER; Tu et al. 2006). Clinical PS mutations are defective in Ca^{2+} leak from the ER (Tu et al. 2006).

The anterograde fast axonal transport of APP and Trk receptors is impaired in the sciatic nerves of transgenic mice expressing two independent FAD-linked PS1 variants compared to the transport in the comparable nerves of transgenic mice expressing the wild-type human PS1 (Lazarov et al. 2007). The expression of FAD-linked PS1 mutants likely leads to increased GSK-3 activity and the reduction in kinesin-1-based transport of a subset of membrane cargo proteins (Pigino et al. 2003).

However, despite the various defects described in this section, it remains to be discovered how these defective activities of mutant PS contribute to AD.

Defective protein folding as an underlying cause of impaired PS functions

A PS1 mutation seems to cause multiple defects in PS functions. Pleiotropic defects associated with a mutation may be caused by protein misfolding. For example, CFTR ΔF508 displays loss-of-function phenotypes as indicated from defective export from the ER and unstable response to agonists. This is due to the disruption in protein folding (see Gelman and Kopito 2002 for review). The incubation of cells expressing CFTR ΔF508 in a medium containing a high concentration of chemical chaperones such as glycerol and trimethylamine *N*-oxide (TMAO) increased the steady-state level of mature CFTR at the cell surface and restored a functional Cl^- ion channel activity by assisting protein folding (Welch and Brown 1996; Brown et al. 1996; see

Table 11 Effects of FAD PS1 mutations on PS1/γ-secretase activity

Degree of conservation (Rest)[a]	Mutation	Functional significance (SIFT)[b]	Functional significance (PANTHER)[c]	Functional significance (GV/GD)[d]
E123	K	0.08	−1.69647	117.42/36.71
S365	Y	0.15	−2.12378	155.34/25.33

[a, b, c, d] See legend of Table 2.

Table 12 Effects of nonpathogenic mutations on PS1/γ-secretase activity

	Mutation	Functional significance (SIFT)[a]	Functional significance (PANTHER)[b]	Functional significance (GV/GD)[c]
R35	Q	0.59	−0.8905	147.42/0.00
F175	S	*0.04*[d]	−2.84132	*0.00/154.81*[d]
E318	G	0.55	−2.6434	353.86/0.00

[a] Normalized probabilities. Positions with normalized probabilities less than 0.05 are predicted to be deleterious; those greater than or equal to 0.05 are predicted to be tolerated. The analysis was performed using PS sequences in Fig. 1 (Ng and Henikoff 2006).
[b] PANTHER subPSEC values were acquired using its own set of PS1 homologous sequences from its database (http://www.pantherdb.org/; Thomas et al. 2003). A subPSEC score of −3 or less corresponds to a deleterious mutation. This prediction method reveals gain-of-function mutations in some instances (Thomas et al. 2003). The sequences were from PS1_HUMAN (P49768, *Homo sapiens*), Q6RH31_CANFA (Q6RH31, *Canis lupus familiaris*), Q6RH32_CANFA (Q6RH32, *Canis familiaris*), PSN1_BOVIN (Q9XT97, *Bos Taurus*), PSN1_RAT (P97887, *Rattus norvegicus*), PSN1_MOUSE (P49769, *Mus musculus*), Q90X08_CHICK (Q90X08, *Gallus gallus*), PSN1_XENLA (O12976, *Xenopus laevis*), PSN1_BRARE (Q9W6T7, *Danio rerio*), PSN_CAEEL (P52166, *Caenorhabditis elegans*), HOP-1 (O02100, *Caenorhabditis elegans*), SPE-4 (Q01608, *Caenorhabditis elegans*), PSNA_ARATH (O64668, *Arabidopsis thaliana*), and PSNB_ARATH (Q9SIK7, *Arabidopsis thaliana*).
[c] Grantham variation (GV) measures the degree of biochemical variation among amino acids found at a given position in the multiple sequence alignment, and Grantham deviation (GD) scores the biochemical distance of the mutant amino acid from the observed amino acid at a particular position (Grantham 1974; Mathe et al. 2006). The criteria for prediction are described in Mathe et al. (2006). The current Align-GVGD analysis used PS sequences in Fig. 1 except sequences from *Arabidopsis thaliana* (NP_172346) and *Chlamydomonas reinhardtii* (EDP06639) because adding more distantly related sequences decreased the number of mutants predicted as deleterious (Mathe et al. 2006). However, the current set of PS sequences is still more divergent than the set of p53 sequences used to develop the Align-GVGD method (Mathe et al. 2006).
[d] Values predicted to damage the activity of PS1 are shown in italics.

Brown et al. 1997 for a review). It has been suggested that some FAD PS1 mutations change the conformation of PS1 (Berezovska et al. 2005). It is interesting to note that two FAD PS1 variants, ΔE9 and M146L, displayed a defect in export from the ER (Kim et al. 2007). However, the ER export defect of PS1 ΔE9 was milder than that of CFTR ΔF508 as indicated in the mild disruption of the steady-state distribution of PS1 ΔE9 (Kim et al. 2000). In addition, TMAO partially rescued the ER export defect of PS1 ΔE9 (Kim et al. 2007). This result suggests that indeed some FAD PS mutations alter PS folding, contributing to pleiotropic impairments of PS functions.

Do human genetic data support the loss-of-function theory?

Although our sequence analyses point to a loss-of-function property of PS mutations in a biochemical sense, genetically, FAD PS mutations clearly demonstrate gain-of-toxic-function characteristics as illustrated by De Strooper (2007). Nevertheless, there are a few occasional reports supporting a genetic loss-of-function theory such as PS1 promoter mutations (Theuns et al. 2000, 2003; Lambert et al. 2001) and a PS1 truncation mutation (Tysoe et al. 1998). However, the frequency of these mutations is far less than that of PS missense mutations, and these observations await independent confirmation. Thus, more of such mutations should surface to support the loss-of-function theory of FAD PS mutations from a genetic point of view.

Loss of function and aberrant Aβ production

An important question is how an apparently reduced activity of γ-secretase can result in a gain of toxic function. It has been suggested that the reduced activity of γ-secretase can be linked to an increased ratio of $A\beta_{42}/A\beta_{40}$ due to the processive nature of this enzyme (Sato et al 2003; Qi-Takahara et al. 2005; Wolfe 2007). For example, γ-secretase consecutively trims $A\beta_{49}$ or $A\beta_{48}$ (precursors of Aβ or new cleavage products of APP by γ-secretase) to $A\beta_{46}$, $A\beta_{43}$, $A\beta_{40}$, or to $A\beta_{45}$, $A\beta_{42}$, and $A\beta_{39}$, respectively. If γ-secretase becomes inefficient due to a PS mutation or an APP mutation, γ-secretase fails to process the substrate to smaller fragments and prematurely releases longer forms of amyloid peptide. This will result in an increased ratio of $A\beta_{42}/A\beta_{40}$. This also explains why deleting one copy of PS1 does not affect the ratio of $A\beta_{42}/A\beta_{40}$.

Conclusion

In summary, this bioinformatic analysis extends the notion that most FAD-linked PS mutations dampen the activity of γ-secretase. More mutations may be found in highly conserved regions of PS in the future. The scattered distribution of PS mutations appears to reflect the distribution of conserved residues of PS which are structurally and functionally critical to the enzymatic activity of γ-secretase.

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