Human Molecular Genetics, 1996, Vol. 5 Review 1449–1455

The presenilin genes: a new gene family involved in Alzheimer disease pathology

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Received June 18, 1996

A positional cloning approach has led to the identification of two closely related genes, the presenilins (PS), for autosomal dominant presenile Alzheimer disease (AD): *PS-1* at 14q24.3 and *PS-2* at 1q31–q42. The *PS-1* gene was identified by direct cDNA selection of yeast artificial chromosomes containing the candidate chromosomal region. Subsequently, the *PS-2* gene was identified due to its high sequence homology with *PS-1* and its location within the candidate region defined by linkage studies. To date, 30 different missense mutations and one in-frame splice site mutation were described in *PS-1*, while only two missense mutations were detected in *PS-2*, suggesting that *PS-1* mutations are more frequently involved in familial presenile AD. The PS transcripts encode novel proteins that resemble integral transmembrane proteins of roughly 450 amino acids and at least seven transmembrane domains. The genomic organization of the PS genes is very similar showing that full length *PS-1* and *PS-2* are encoded by 10 exons. However, different alternative splicing patterns have been observed for *PS-1* and *PS-2* indicating that the corresponding proteins (ps-1 and ps-2) may have similar but not identical biological functions.

INTRODUCTION

Alzheimer disease (AD) is a neurodegenerative disorder of the central nervous system characterized by progressive impairment of memory and intellectual functioning, leading to dementia and ultimately to death. AD brain pathology consistently shows two major lesions known as senile plaques and neurofibrillary tangles. Both lesions have been extensively studied and their major components were identified; however, the exact biochemical pathways leading to AD pathology are still largely unknown. Although AD is basically a disease of the elderly, in approximately 25% of the cases the first symptoms of the disease become apparent before the age of 65 years (presenile AD). Among these presenile AD cases, many were identified that belong to families in which the disease is inherited as a fully penetrant autosomal dominant trait. Several large pedigrees with presenile AD were sampled and a positional cloning approach was employed to identify gene mutations causing presenile AD. To date, three genes have been identified located on chromosomes 1, 14 and 21.

The first presenile AD gene was identified in 1991, maps on chromosome 21 and codes for the amyloid precursor protein (*app*). A total of six different missense mutations were identified in the *app* gene (APP), near or within the βA4 amyloid sequence, a 4 kDa proteolysis product of *app* and the major constituent of the senile plaques (reviewed in 1). Together, APP mutations account for about 5% of all presenile AD families, the latter group representing 10% of all AD cases. *In vitro* studies have shown that each of these mutations in APP alter *app* metabolism in such a way that either more and/or longer βA4 amyloid is formed suggesting a direct link to βA4 amyloid deposition in AD brains (reviewed in 2). Also, transgenic mice bearing the *app* mutation Val717Phe showed progressive βA4 amyloid deposition in senile plaques (3). These observations together with the observation that in humans βA4 amyloid deposition precedes neurofibrillary tangle formation, have led to the amyloid cascade hypothesis, identifying βA4 amyloid deposition as the causative agent of AD pathology (4). However, not all researchers in the field are convinced that βA4 amyloid deposition is the key element in AD pathology (5). In 1995, the chromosome 1 and 14 genes were identified and were shown to be members of a novel gene family: the presenilins.

CLONING OF THE PRESENILIN GENES

A genome-wide search in presenile AD families without APP mutations, led to the identification of a second locus for presenile AD on chromosome 14 at 14q24.3 in an interval of 22.7 cM between the markers *D14S52* and *D14S53* (6). As three other teams independently identified the 14q24.3 locus in their families (7–9), the 14q24.3 gene was recognized as a major locus for familial presenile AD and its contribution was estimated at 70%. Further linkage studies allowed to reduce the candidate area to 6.4 cM between *D14S289* and *D14S61* (10,11). A contiguous physical map of the candidate area was built using yeast artificial chromosomes (YACs) (11,12) showing that several known genes that were potential candidate genes are located within this region,

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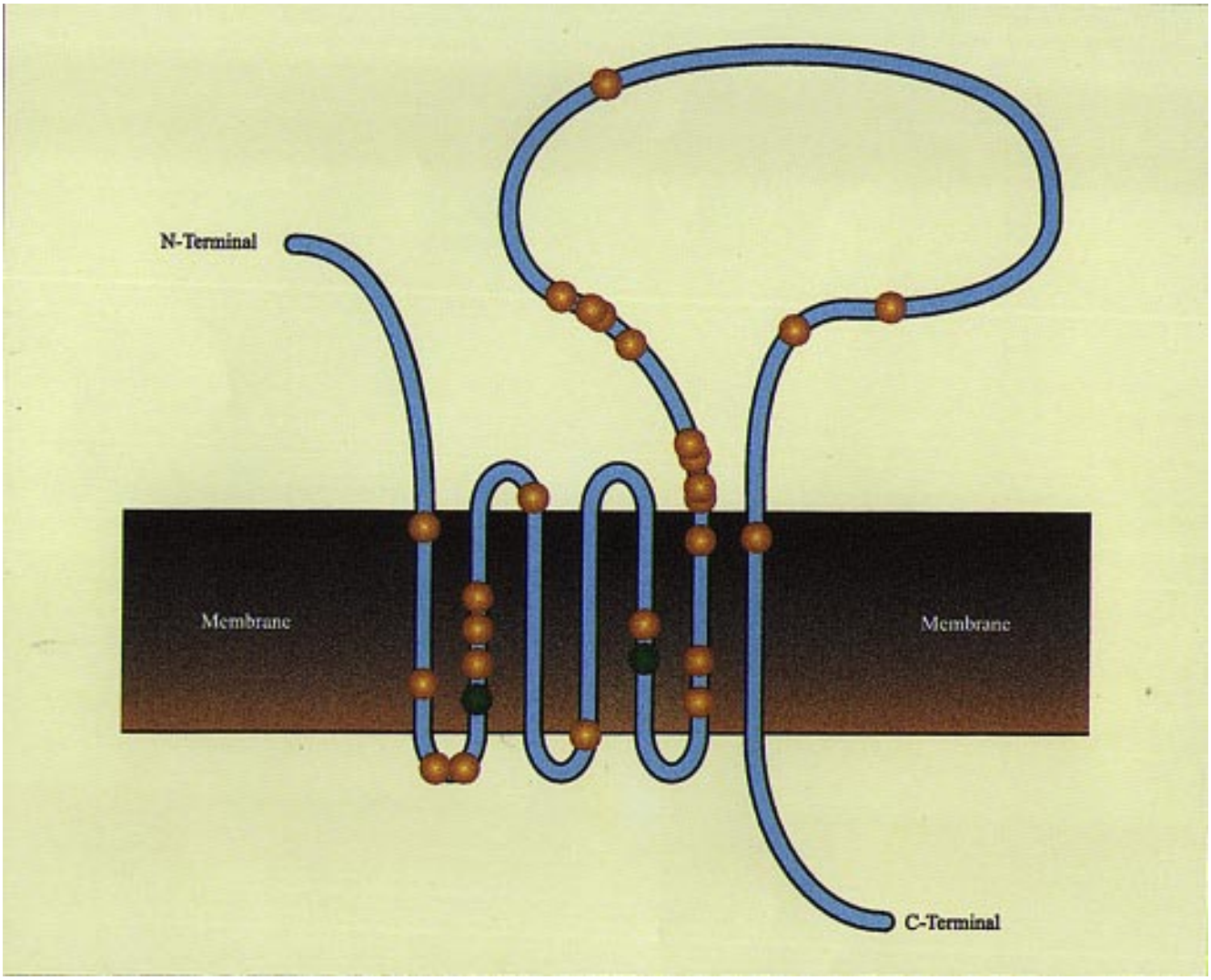

Figure 1. Putative seven transmembrane domain structure of ps proteins. The ps-1 mutations are represented by yellow bullets, the ps-2 mutations by green bullets. In the latter case the mutated codons 141 and 239 (Table 1), were converted to their corresponding conserved amino acids 135 and 233 in ps-1.

including the cellular oncogene *c-FOS*. Mutation analyses of *c-FOS*, however, failed to identify AD-related mutations (13–15). Exclusion of *c-FOS* was subsequently confirmed as additional informative recombinants mapped it outside the candidate region (16). The minimal candidate region, flanked by *D14S284* and *D14S277*, measured about 2 Mb on the YAC map and contained the dihydrolipoyl succinyltransferase gene (*DLST*) (11). Again mutation analyses of *DLST* did not reveal AD specific mutations (17). Starting from the YACs, new genes were isolated using exon trapping and direct cDNA selection techniques (16,18,19). Five different missense mutations: Met146Leu, His163Arg, Ala246Glu, Leu286Val and Cys410Tyr (Table 1), were identified in a transcript, S182, corresponding to a novel gene, named presenilin-1 (*PS-1*), whose product resembled an integral transmembrane protein with at least seven transmembrane domains (18) (Fig. 1).

Although many presenile AD families linked to 14q24.3, the initial linkage reports provided evidence that in other families including the Volga-German AD families, the responsible gene had to be located elsewhere in the human genome (6,7). The Volga-German families are a group of related presenile AD families that descend from one German family that first emigrated to Russia, and later to the USA. Although, in these families the onset ages of presenile AD are highly variable, it is believed that the disease in these families is due to the same autosomal dominant gene defect inherited from a common ancestor (20). A genome-wide search in the Volga-German AD families identified a locus on chromosome 1 at 1q31–q42 (21). Subsequently to the identification of the *PS-1* gene, an expressed sequence tagged (EST) sequence, T03796, was identified in the EST database showing high amino acid sequence homology to S182 and mapped within the chromosome 1 candidate region (22,23). The corresponding full-length cDNA, STM-2 or E5–1, was cloned and a missense mutation, Asn141Ile, was identified in seven of the Volga-German families (Table 1). Mutation analysis of the chromosome 1 gene, named presenilin-2 (*PS-2*), identified one other missense mutation, Met239Val, in an Italian family (23).

Table 1. PS mutations. Detailed information on the different mutations can be found in refs 30–35. Exon numbering of the PS genes is as in Figure 2, codon numbering and domain structure are as published (18,22). Location of the mutated codons is illustrated in Figure 1

Gene	Exon	Domain	Mutation	# Families
PS-1	4	TM-I	Val82Leu	1
			Val96Phe	1
	5	HL-I	Tyr115His	1
			Glu120Lys	1
		TM-II	Met139Ile	1
			Met139Thr	1
			Met139Val	4
			Ile143Phe	1
			Ile143Thr	1
			Met146Leu	5
			Met146Val	3
	6	HL-II	His163Arg	6
			His163Tyr	1
	7	TM-IV	Ile213Thr	1
		TM-V	Ala231Thr	1
		TM-VI	Ala246Glu	1
			Leu250Ser	1
	8		Ala260Val	1
		HL-VI	Cys263Arg	1
			Pro264Leu	2
			Pro267Ser	1
			Arg269His	1
			Glu280Ala	4
			Glu280Gly	2
			Ala285Val	1
			Leu286Val	1
			Ser289Cys	1
	9		Glu318Gly	1
	11		Gly384Ala	1
			Leu392Val	2
		TM-VII	Cys410Tyr	3
PS-2	5	TM-II	Asn141Ile	7
PS-2	7	TM-V	Met239Val	1

TM, transmembrane domain; HL, hydrophilic loop.

GENOMIC ORGANIZATION OF PS GENES

The genomic organization of the PS genes has been determined indicating that the open reading frame is encoded by 10 exons (numbered 3–12) (19,24–26). Analysis of 5′ upstream sequences suggests that the untranslated region is contained in at least two exons (18,24). P1 phage and P1 phage-derived artificial chromosome (PAC) clones were isolated that contain the complete PS genes allowing maximal size estimates of respectively 75 kb for *PS-1* (19) and 90 kb for *PS-2* (24,25). Genomic sequencing of *PS-2* clones mapped the 10 coding exons to a genomic region of about 24 kb (24). The intron–exon boundaries of the two PS genes are very similar and in conserved regions of the genes the boundaries are identical (Fig. 2). The intronic sequences immediately flanking the exons are not similar indicating that the PS genes are not the result of a recent gene duplication (24).

In most tissues including brain, northern blot analysis identified major transcripts for *PS-1* and *PS-2* of 2.7 and 2.4 kb respectively (18,22,23), that correspond to the unspliced products of the PS genes. Alternative splice forms were also identified for both *PS-1* and *PS-2*. One splice variant common between *PS-1* and *PS-2* does not contain exon 8, coding for part of the transmembrane domain (TM-VI) and the beginning of the large hydrophilic loop (HL-VI) between TM-VI and TM-VII (18,23,25). Although the transcripts lacking exon 8 are present in brain, the shorter *PS-1* variant is prominent only in leukocytes (23). In *PS-1*, alternative splicing of exon 3 using an alternative splice donor site results in the deletion of four amino acids, VRSQ, a sequence which, when present, constitutes part of two potential phosphorylation sites for protein kinase C and casein kinase II, respectively (16,19). In brain, fibroblasts and lymphoblasts, the isoform lacking the VRSQ motif is 1.5 times more frequent than the longer transcript. The VRSQ motif together with the absence of a signal peptide suggests that the N-terminal domain is intracellular (19). In *PS-2* alternative splice forms have also been described that result from the alternative use of in-frame splice acceptor sites in introns 9 and 10 resulting in the deletion of a Glu at codon 324 or the replacement of Arg at codon 358 by SerGlnGly (24–26). The latter two splice events may not be of functional significance as they occur within a region of HL-VI that is not conserved between *ps*-1 and *ps*-2 (Fig. 2). Alternatively, the splice products may have functions that are specific only for *ps*-2. More intriguing is the observation of a splice variant of *PS-2* lacking exons 3 and 4 and thus TM-I (25). Truncated transcripts of *PS-1* have also been identified resulting from the use of an out-frame splice site in intron 9 or splicing at sites different from the previous defined exon/intron boundaries (27,28). The significance of the spliced and truncated PS transcripts, however, remains unclear as long as the biological function of the *ps* proteins is unknown.

PS MUTATIONS

Since the initial *PS-1* mutation report, several more missense mutations were identified either by RT–PCR sequencing of *PS-1* cDNA or genomic sequencing of *PS-1* exons. Preliminary mutation analyses indicate that *PS-1* mutations account for 30–50% of presenile AD families (29,31, Van Broeckhoven *et al*, unpublished results). To date, 30 different missense mutations have been reported in 52 unrelated presenile AD families of different ethnic background (Table 1) (30–35). All mutations involve amino acids that are conserved between *ps*-1 and *ps*-2. Also, a mutation was detected destroying the splice acceptor site of exon 9 resulting in an in-frame deletion of exon 9 and an amino acid substitution (Ser289Cys) at the splice junction between exons 8 and 10 (36). The mutations are scattered over the *ps-1* protein with most mutations located within or just outside one of the seven TM domains (Fig. 1). However, clustering of mutations is observed with the majority of the mutations occurring in TM-II (30%) and HL-VI (34%).The TM-II domain is encoded by exon 5 of *PS-1*, the HL-VI loop by exons 8–11 (Fig. 2). So far, mutations have been found in seven of the 10 coding exons (4–9 and 11) with exons 5, 8, 9 and 11 accounting for 75% of the mutations. The relevance of this clustering of mutations to the function or dysfunction of *ps-1* is not clear yet. However, as in TM-II the mutations are spaced every three to four residues, they may possibly interfere with an alpha-helical structure of this TM domain (19). Also, it is noteworthy to point out that exon 8 is

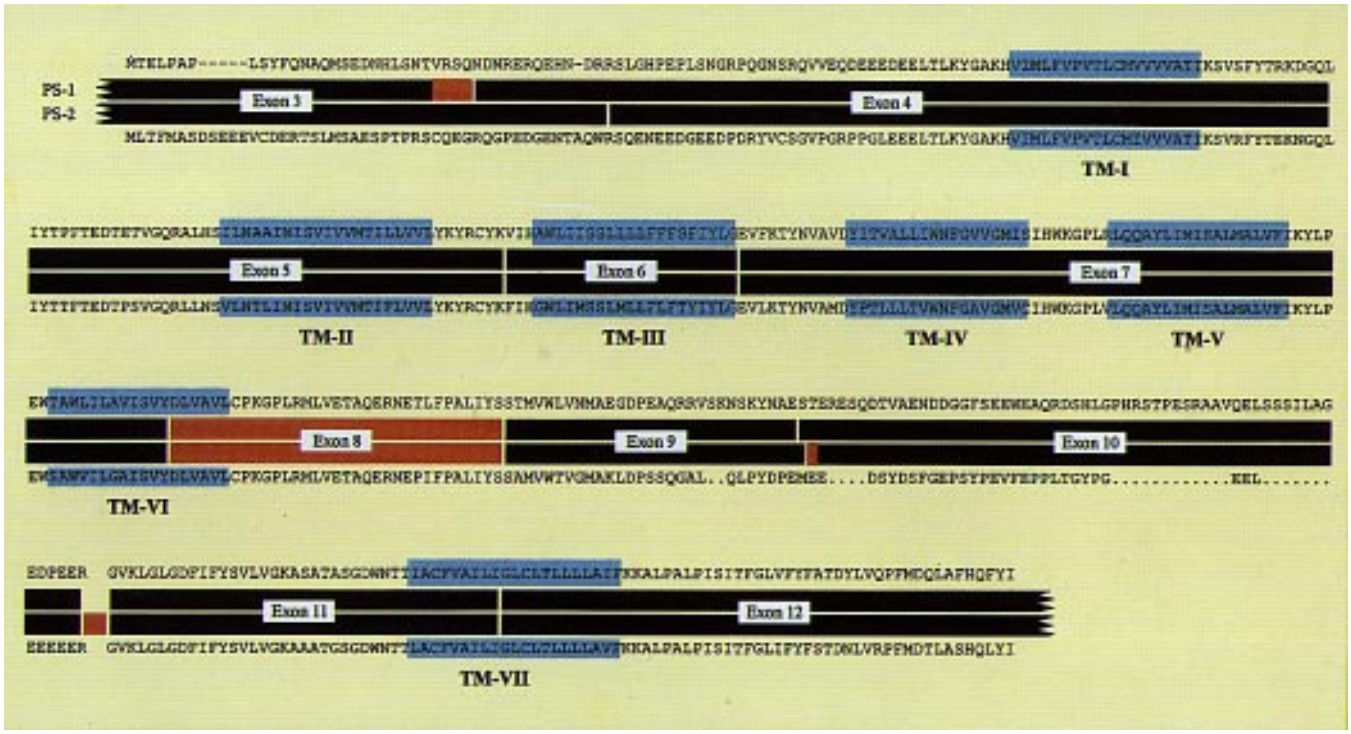

Figure 2. Genomic organization of the PS genes. The amino acid sequences of ps-1 (top) and ps-2 (bottom) are aligned. The exons are indicated by black bars and orange bars represent sites of alternative in-frame splicing. The putative TM domains are depicted in blue.

alternatively spliced with the shorter splice form being most prominent in leukocytes but not in brain (discussed above).

PS-1 mutations cause autosomal dominant presenile AD with mean onset ages between 35 and 55 years (30). In general, *PS-1* mutations are fully penetrant in these families showing a narrow onset age range; however, one example of incomplete penetrance of a *PS-1* mutation has been described (37). Within the *PS-1* gene, the onset age is determined by both the position and the nature of the mutation: families with the same *PS-1* mutation have very similar onset ages. While in some APP families onset age was decreased in the presence of the apolipoprotein E (apoE)ε4 allele and increased in the presence of an apoE ε2 allele, no such a modifying effect was observed in *PS-1* families (38,39). As some mutations result in a later onset age it can be predicted that *PS-1* mutations may also result in late-onset (>65 years) or senile AD. Interesting in this respect is the observation of an association between presenile AD and a polymorphism located in intron 8 of *PS-1* (40). Confirmatory findings were obtained in two of four independent studies of late-onset AD (41–44). Allelic association was also reported previously with *D14S52* in families with mean onset ages between 60 and 70 years (45). We recently identified a *PS-1* mutation at codon 79 in an autosomal dominant AD case with onset age of 64 years (Van Broeckhoven *et al*., unpublished results). Whether *PS-1* also contributes to senile AD either as a genetic risk factor or a causative gene, however, awaits more and larger studies in an epidemiological setting.

Analysis of additional families with presenile AD not linked to 14q24.3, failed to identify more *PS-2* mutations suggesting that *PS-2* mutations are less frequent than *PS-1* mutations (26). However, it cannot be excluded that the higher *PS-1* mutation frequency is due to an ascertainment bias as families with early-onset ages are more likely to be included in a linkage study. In contrast to *PS-1* families, age at onset of disease symptoms is later and highly variable in *PS-2* families with some mutation carriers developing the disease in their seventies or eighties (22,23,26). This variability was not attributable to differences in apolipoprotein E (APOE) genotypes (26,46) suggesting that other genetic and/or environmental factors modify the AD phenotype associated with *PS-2* mutations.

PS PROTEINS AND EXPRESSION

ps-1 and ps-2 with 467 and 448 amino acids, respectively, share an overall homology of 67% with the highest similarity observed in the putative TM domains (Fig. 2) (22,23,30). Less similarity is found in the N-terminal domain and the central region of HL-VI (Fig. 2). Western blot analysis and immunoprecipitation of *PS-1* cDNA transfected cells or *in vitro* translated *PS-1* cDNA detected major bands at about 43–50 kDa. In addition higher and lower molecular weight bands were observed, most likely derived from protein aggregation and proteolysis, respectively (27,47–49). Particularly interesting is the observation that cells transfected with mutant PS-1 cDNA, fail to produce a 28 kDa proteolytic product, as compared to wild-type PS-1 cDNA transfected cells (49). Northern blot analysis showed that both *PS-1* and *PS-2* are expressed in most tissues, e.g. heart, liver, pancreas, spleen, kidney, testis and brain (18,23,50). In rat and human brain, *PS-1* and *PS-2* transcripts are present in the neuronal cell population of hippocampus, cerebral cortex, cerebellum and particularly the choroid plexus (48,50). Immunohistochemical analysis of brain sections of AD cases using a C-terminal ps-1 polyclonal antibody, localized ps-1 to the senile plaques similar to βA4 amyloid (51).

However, using a N-terminal ps-1 polyclonal antibody we detected specific neuronal staining in the absence of senile plaque staining (Hendriks *et al.*, unpublished results). Neuronal staining by ps-1 antisera was also observed in mouse brain (47). Immunocytochemical analysis of cultured cells, localized ps proteins to the perinuclear zone of the neuronal cells, most likely in the endoplasmic reticulum and the Golgi complex in, yet unidentified, intracellular vesicles (48) (Hendriks *et al.*, unpublished results).

PS HOMOLOGIES AND FUNCTION

The high sequence homology between ps-1 and ps-2 suggests that these proteins may have a similar biological function. However, one ps protein cannot compensate for the other, as a mutation in one ps protein is sufficient to cause AD. Also, to date only missense mutations have been observed and no deletions or nonsense mutations that would result in absence or truncation of the ps proteins. The splice site mutation and subsequent exon skipping, also results in an in-frame amino acid substitution. Therefore, it is predicted that ps mutations cause AD because of a gain of (mis)function of the corresponding ps protein rather than a loss of function.

The normal or abnormal biological function of ps in normal and AD brains is unknown. Their putative seven transmembrane domain structure is compatible with a function as a receptor molecule, an ion channel or a membrane structural protein. Homologies of ps with other known proteins allows to formulate hypotheses that can be tested in *in vitro* and *in vivo* systems. Two proteins of *Caenorhabditis elegans*, spe-4 and *sel-12*, displayed significant homologies with ps-1 of 24–37% and 48%, respectively (30). In *C.elegans*, spe-4 participates in intracellular protein trafficking and sorting during spermatogenesis. Similarly, in human ps-1 may also be involved in protein transport within cells. Interesting is that in human testis the ps-1 expression is very high (50). The seven TM domain protein sel-12 has a role in the lin-12 signalling pathway, which during development of *C.elegans*, is responsible for direct signal transmission from the cell surface to the nucleus. The exact role of sel-12 is not known, but it seems to modulate signal transmission through lin-12. In the mutated sel-12 that activates lin-12 signal transmission, the mutation site corresponds to a site of out-frame splicing resulting in a truncated ps-1 (27). Interestingly, 27 of the 33 ps mutations (82%) involve amino acids conserved in sel-12. A strong identity was also detected between the C-terminal part of ps-2 and alg-3, a protein which blocks signal transduction during T-cell-induced apoptotic cell death (52). Possibly, a mutation in ps-2 restores signal transmission resulting in neurodegeneration and cell death as seen in AD brains.

Knowledge about the pathological function of ps can also be gained by comparing ps metabolism in transfected cells or transgenic mice expressing mutant PS genes. However, these experiments are still ongoing and no data have been published yet. A major question is whether ps proteins also fit into the amyloid cascade, in other words, do mutated ps proteins interfere directly or indirectly with normal app metabolism leading to βA4 amyloid deposition and thus AD. In fibroblasts of some PS mutation carriers increased APP transcription (53), and increased secretion of βA4 amyloid were observed (54,55), suggesting that ps proteins have indeed a function in the amyloid cascade. However, more data will be needed to confirm these observations.

ACKNOWLEDGEMENTS

The authors like to thank J. Hardy, P. St George-Hyslop, G. Schellenberg, R. Tanzi, S. Younkin, R. Sandbrink and M. Mercken for sharing in press data and J. Hardy for critical reading of the manuscript. The AD research work in the Laboratory of Neurogenetics is funded by the Flemish Biotechnology Program, the National Fund of Scientific Research, BIOMED (GENE-CT93–0015) and BIOTECH (BIO2-CT94–2056) programs of the European Community and by the Alzheimer's Disease Research Program of the American Health Assistance Foundation (AHAF).

ABBREVIATIONS

AD, Alzheimer's disease; PS, presenilin genes; *PS-1*(-2), presenilin-1 (-2) genes; ps, presenilin proteins; ps-1(-2), presenilin-1(-2) proteins; APP, amyloid precursor protein gene; app, amyloid precursor protein; DLST, dihydrolipoyl succinyltransferase gene; *c-FOS*, c-fos gene; APOE, apolipoproteine E gene; TM, transmembrane domain; HL, hydrophylic loop; YAC, yeast artificial chromosome; EST, expressed sequence tag.

REFERENCES

1. Van Broeckhoven, C. (1995) Molecular genetics of Alzheimer disease: Identification of genes and gene mutations. *Eur. Neurol.* **35,** 8–19.
2. Hendriks, L. and Van Broeckhoven, C. (1996) The βA4 amyloid precursor protein gene and Alzheimer's disease. *Eur. J. Biochem.* **237,** 6–15.
3. Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donalson, T., Gillespi, F., Guido, T., Hagoplan, S., Johnson-Wood, K., Khan, K., Lee, M., Leibowitz, P., Lieberburg, I., Little, S., Masliah, E., McConlogue, L., Montoya-Zavala, M., Mucke, L., Paganini, L., Penniman, E., Power, M., Schenk, D., Seubert, P., Snyder, B., Soriano, F., Tan, H., Vitale, J., Wadsworth, S., Wolozin, B. and Zhao, J. (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F β amyloid- precursor protein. *Nature* **373,** 523–527.
4. Hardy, J.A. and Higgins, G.A. (1992) Alzheimer's disease: The amyloid cascade hypothesis. *Science* **256,** 184–185.
5. Roses, A.D. (1994) Apolipoprotein E affects the rate of Alzheimer disease expression: β amyloid burden is a secondary consequence dependent on APOE genotype and duration of disease. *J. Neuropathol. Exp. Neurol.* **53,** 429–437.
6. Schellenberg, G.D., Bird, T.D., Wijsman, E.M., Orr, H.T., Anderson, L., Nemens, E., White, J.A., Bonnycastle, L., Weber, J.L., Alonso, M.E., Potter, H., Heston, L.L. and Martin, G.M. (1992) Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science* **258,** 668–671.
7. St George-Hyslop, P., Haines, J., Rogaev, E., Mortilla, M., Vaula, G., Pericak-Vance, M., Foncin, J., Montesi, M., Bruni, A., Sorbi, S., Rainero, I., Pinessi, L., Pollen, D., Polinsky, R., Nee, L., Kennedy, J., Macciardi, F., Rogaeva, E., Liang, Y., Alexandrova, N., Lukiw, W., Schlumpf, K., Tanzi, R.E., Tsuda, T., Farrer, L., Cantu, J., Duara, R., Amaducci, L., Bergamini, L., Gusella, J., Roses, A. and Crapper-McLachlan, D. (1992) Genetic evidence for a novel familial Alzheimer's disease locus on chromosome 14. *Nature Genet.* **2,** 330–334.
8. Van Broeckhoven, C., Backhovens, H., Cruts, M., De Winter, G., Bruyland, M., Cras, P. and Martin, J. (1992) Mapping of a gene predisposing to early-onset Alzheimer's disease to chromosome 14q24.3. *Nature Genet.* **2,** 335–339.
9. Mullan, M., Houlden, H., Windelspecht, M., Fidani, L., Lombardi, C., Diaz, P., Rossor, M., Crook, R., Hardy, J., Duff, K. and Crawford, F. (1992) A locus for familial early-onset Alzheimer's disease on the long arm of chromosome 14, proximal to the α1-antichymotrypsin gene. *Nature Genet.* **2,** 340–342.

10. Mitsunaga, Y., Takahashi, K., Tabira, T., Tasaki, H. and Watanabe, S. (1994) Assignment of a familial Alzheimer's disease locus between D14S289 and D14S53. *Lancet* **344,** 1154–1155.
11. Cruts, M., Backhovens, H., Theuns, J., Clark, R.F., Le Paslier, D., Weissenbach, J., Goate, A.M., Martin, J. and Van Broeckhoven, C. (1995) Genetic and physical characterisation of the early-onset Alzheimer's disease AD3 locus on chromosome 14q24.3. *Hum. Mol. Genet.* **4,** 1355–1364.
12. Clark, R.F., Cruts, M., Korenblat, K.M., Chengshi, H., Talbot, C., Van Broeckhoven, C. and Goate, A.M. (1995) A yeast artificial chromosome contig from human chromosome 14q24 spanning the Alzheimer's disease locus AD3. *Hum. Mol. Genet.* **4,** 1347–1354.
13. Bonnycastle, L.L.C., Chang-En, Y., Wijsman, E.M., Orr, H.T., Patterson, D., Clancy, K.P., Goddard, K.A.B., Alonso, M.E., Nemens, E., White, J.A., Heston, L.L., Martin, G.M., Bird, T.D. and Schellenberg, G.D. (1993) The c-fos gene and early-onset familial Alzheimer's disease. *Neurosci. Lett.* **160,** 33–36.
14. Rogaev, E.I., Lukiw, W.J., Vaula, G., Haines, J.L., Rogaeva, E.A., Tsuda, T., Alexandrova, N., Liang, Y., Mortilla, M., Amaducci, L., Bergamini, L., Bruni, A.C., Foncin, J., Macciardi, F., Montesi, M.P., Sorbi, S., Rainero, I., Pinessi, L., Polinsky, R.J., Frommelt, P., Duara, R., Lopez, R., Pollen, D., Gusella, J.F., Tanzi, R., Crapper-McLachlan, D., FRCP(C) and St George-Hyslop, P.H. (1993) Analysis of the *c-fos* gene on chromosome 14 and the promoter of the amyloid precursor protein gene in familial Alzheimer's disease. *Neurology* **43,** 2275–2279.
15. Cruts, M., Backhovens, H., Martin, J. and Van Broeckhoven, C. (1994) Genetic analysis of the cellular oncogene fos in patients with chromosome 14 encoded Alzheimer's disease. *Neurosci. Lett.* **174,** 97–100.
16. Cruts, M., Backhovens, H., Wang, S-Y., Van Gassen, G., Theuns, J., De Jonghe, C., Wehnert, A., De Voecht, J., De Winter, G., Cras, P., Bruyland, M., Datson, N., Weissenbach, J., den Dunnen, J.T., Martin, J-J., Hendriks, L. and Van Broeckhoven, C. (1995) Molecular genetic analysis of familial early-onset Alzheimer's disease linked to chromosome 14q24.3. *Hum. Mol. Genet.* **4,** 2363–2372.
17. Cruts, M., Backhovens, H., Van Gassen, G., Theuns, J., Wang, S., Wehnert, A., van Duijn, C.M., Karlsson, T., Hofman, A., Adolfsson, R., Martin, J. and Van Broeckhoven, C. (1995) Mutation analysis of the 14q24.3 dihydrolipoyl succinyltransferase (DLST) gene in patients with early-onset Alzheimer disease. *Neurosci. Lett.* **199,** 73–77.
18. Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levasque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Tsuda, T., Mar, L., Foncin, J.F., Bruni, A.C., Montesi, M.P., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., Chumakov, I., Pollen, D., Brookes, A., Sansosu, P., Polinsky, R.J., Wasco, W., Da Silva, H.A.R., Haines, J.L., Pericak-Vance, M.A., Tanzi, R.E., Roses, A.D., Fraser, P.E., Rommens, J.M. and St George-Hyslop, P. (1995) Cloning of a gene bearing mis-sense mutations in early-onset familial Alzheimer's disease. *Nature* **375,** 754–760.
19. Alzheimer's Disease Collaborative Group:, Clark, R.F., Hutton, M., Fuldner, R.A., Froelich, S., Karran, E., Talbot, C., Crook, R., Lendon, C., Prihar, G., He, C., Korenblat, K., Martinez, A., Wragg, M., Busfield, F., Behrens, M.I., Myers, A., Norton, J., Morris, J., Mehta, N., Pearson, C., Lincoln, C., Baker, M., Duff, K., Zehr, C., Perez-Tur, J., Houlden, H., Ruiz, A., Ossa, J., Lopera, F., Arcos, M., Madrigal, L., Collinge, J., Humphreys, C., Ashworth, A., Sarner, S., Fox, N., Harvey, R., Kennedy, A., Roques, P., Cline, R.T., Philips, C.A., Venter, J.C., Forsell, L., Axelman, K., Lilius, L., Johnston, J., Cowburn, R., Viitanen, M., Windblad, B., Kosik, K., Haltia, M., Poyhonen, M., Dickson, D., Mann, D., Neary, D., Snowdon, J., Lantos, P., Lannfelt, L., Rossor, M., Roberts, G.W., Adams, M.D., Hardy, J. and Goate, A. (1995) The structure of the Presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. *Nature Genet.* **11,** 219–222.
20. Bird, T.D., Lampe, T.H., Nemens, E.J., Miner, G.W., Sumi, S.M. and Schellenberg, G.D. (1988) Familial Alzheimer's disease in American descendants of the Volga Germans: Probable genetic founder effect. *Ann. Neurol.* **23,** 25–31.
21. Levy-Lahad, E., Wijsman, E.M., Nemens, E., Anderson, L., Goddart, K.A.B., Weber, J.L., Bird, T.D. and Schellenberg, G.D. (1995) A familial Alzheimer's disease locus on chromosome 1. *Science* **269,** 970–973.
22. Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D.M., Oshima, J., Pettingell, W.H., Yu, C., Jondro, P.D., Schmidt, S.D., Wang, K., Crowley, A.C., Fu, Y., Guenette, S.Y., Galas, D., Nemens, E., Wijsman, E.M., Bird, T.D., Schellenberg, G.D. and Tanzi, R.E. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269,** 973–977.
23. Rogaev, E.I., Sherrington, R., Rogeava, E.A., Levesque, G., Ikeda, M., Liang, Y., Chi, H., Lin, C., Holman, K., Tsuda, T., Mar, L., Sorbi, S., Nacmias, B., Piancentini, S., Amaducci, L., Chumakov, I., Cohen, D., Lannfelt, L., Fraser, P.E., Rommens, J.M. and St George-Hyslop, P.H. (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* **376,** 775–778.
24. Levy-Lahad, E., Poorkaj, P., Wang, K., Hui Fu, Y., Oshima, J., Mulligan, J. and Schellenberg, G.D. (1996) Genomic structure and expression of STM2, the chromosome 1 familial Alzheimer's disease gene. *Genomics* **34**, 198–204.
25. Prihar, G., Fuldner, R.A., Perez-Tur, J., Lincoln, S., Duff, K., Crook, R., Hardy, J., Phillips, C.A., Venter, C., Talbot, C., Clark, R.F., Goate, A., Li, J., Potter, H., Karran, E., Roberts, G.W., Hutton, M. and Adams, M.D. (1996) Structure and alternative splicing of the presenilin-2 gene. *Neuroreport* (in press).
26. Sherrington, R., Froelich, S., Sorbi, S., Campion, D., Chi, H., Rogaeva, E.A., Levesque, G., Rogaev, E.I., Lin, C., Liang, Y., Ikeda, M., Mar, L., Brice, A., Agid, Y., Percy, M.E., Clerget-Darpoux, F., Piacentini, S., Marcon, G., Nacmias, B., Amaducci, L., Frebourg, T., Lannfelt, L., Rommens, J.M. and St George-Hyslop, P.H. (1996) Alzheimer's disease associated with mutations in presenilin 2 is rare and variably penetrant. *Hum. Mol. Genet.* **5**, 985–988.
27. Sahara, N., Yahagi, Y., Takagi, H., Kondo, T., Okochi, M., Usami, M., Shirasawa, T. and Mori, H. (1996) Identification and characterization of presenilin I-467, I-463 and I-374 *FEBS Lett.* **381,** 7–11.
28. Anwar, R., Moynihan, T.P., Ardley, H., Brindle, N., Coletta, P.L., Cairns, N., Markham, A.F. and Robinson, P.A. (1996) Molecular analysis of the presenilin 1 (S182) gene in 'sporadic' cases of Alzheimer's disease: Identification and characterisation of unusual splice variants. *J. Neurochem.* **66,** 1774–1777.
29. Campion, D., Flaman, J-M., Brice, A., Hannequin, D., Dubois, B., Martin, C., Moreau, V., Charbonnier, F., Didierjean, O., Tardieu, S., Penet, C., Puel, M., Pasquier, F., Bellis, G., Calenda, A., Heilig, R., Martinez, M., Mallet, J., Bellis, M., Clerget-Darpoux, F., Agid, Y. and Frebourg, T.(1995) Mutations of the *presenilin-1* gene in families with early-onset Alzheimer's disease. *Hum. Mol. Genet.* **4**, 2372
30. Van Broeckhoven, C. (1995) Presenilins and Alzheimer disease. *Nature Genet.* **11,** 230–232.
31. Hutton, M., Busfield, F., Wragg, M., Crook, R., Perez-Tur, J., Clark, R.F., Prihar, G., Talbot, C., Phillips, H., Wright, K., Baker, M., Lendon, C., Duff, K., Martinez, A., Houlden, H., Nichols, A., Karran, E., Roberts, G., Venter, J.C., Adams, M.D., Cline, R.T., Phillips, C.A., Fuldner, R.A., Hardy, J. and Goate, A. (1996) Complete analysis of the presenilin 1 gene in families with early onset Alzheimer's disease. *Neuroreport*, **7,** 801–805.
32. Sandbrink, R., Zhang, D., Schaeffer, S., Masters, C.L., Bauer, J., Förstl, H. and Beyreuther, K. (1996) Missense mutations of the *S182/PS1* gene in German early-onset Alzheimer's disease patients. *Ann. Neurol*. (in press).
33. Wasco, W. and Tanzi, R.E. (1996) Etiological clues from gene defects causing early-onset familial Alzheimer's disease. In: *Molecular mechanisms of dementia* (eds Wasco,W. and Tanzi, R.E.). Humana Press (in press).
34. Boteva, K., Vitek, M., Mitsuda, H., de Silva, H., Xu, P.T., Small, G. and Gilbert, J.R. (1996) Mutation analysis of presenilin 1 gene in Alzheimer's disease. *Lancet* **347,** 130–131.
35. Kamino, K., Sato, S., Sakaki, Y., Yoshiiwa, A., Nishiwaki, Y., Takeda, H., Tanabe, H., Nishimura, T., Ii, K., St George-Hyslop, P., Miki, T. and Ogihara, T. (1996) Three different mutations of presenilin 1 gene in early-onset Alzheimer's disease families. *Neurosci. Lett.* **208,** 195–198.
36. Perez-Tur, J., Froelich, S., Prihar, G., Crook, R., Baker, M., Duff, K., Wragg, M., Busfield, F., Lendon, C., Clark, R.F., Roques, P., Fuldner, R.A., Johnston, J., Cowburn, R., Forsell, C., Axelman, K., Lilius, L., Houlden, H., Karran, E., Roberts, G.W., Rossor, M., Adams, M.D., Hardy, J., Goate, A., Lannfelt, L. and Hutton, M. (1996) A mutation in Alzheimer's disease destroying a splice acceptor site in the presenilin-1 gene. *Neuroreport* **7,** 297–301.
37. Rossor, M.N., Fox, N.C., Beck, J., Campbell, T.C. and Collinge, J. (1996) Incomplete penetrance of familial Alzheimer's disease in a pedigree with a novel presenilin-1 gene mutation. *Lancet* **347,** 1560
38. Van Broeckhoven, C., Backhovens, H., Cruts, M., Martin, J., Crook, R., Houlden, H. and Hardy, J. (1994) APOE genotype does not modulate age of onset in families with chromosome 14 encoded Alzheimer's disease. *Neurosci. Lett.* **169,** 179–180.

39. Brice, A., Tardieu, S., Didierjean, O., LeGuern, E., Michon, A., Pillon, B., Hahn, V., Darpoux, F., Bellis, M., Calanda, A., Heilig, R., Weissenbach, J., Mallet, J., Frebourg, T., Hannequin, D., Puel, M., Ledoze, F. and Pasquier, F. (1996) Apolipoprotein E genotype does not effect age at onset in patients with chromosome 14 encoded Alzheimer's disease. *J. Med. Genet.* **33,** 174–175.
40. Wragg, M., Hutton, M., Talbot, C., Busfield, F., Han, S.W., Lendon, C., Clark, R.F., Morris, J.C., Edwards, D., Goate, A., Pfeiffer, E., Crook, R., Prihar, G., Philips, H., Baker, M., Hardy, J., Rossor, M., Houlden, H., Karran, E., Roberts, G. and Craddock, N. (1996) Genetic association between intronic polymorphism in presenilin-1 gene and late-onset Alzheimer's disease. *Lancet* **347,** 509–512.
41. Kehoe, P., Williams, J., Lovestone, S., Wilcock, G. and Owen, M.J. (1996) Presenilin-1 polymorphism and Alzheimer's disease. *Lancet* **347,** 1185.
42. Higuchi, S., Muramatsu, T., Matsushita, S., Arai, H. and Sasaki, H. (1996) Presenilin-1 polymorphism and Alzheimer's disease. *Lancet* **347,** 1186.
43. Scott, W.K., Growdon, J.H., Roses, A.D., Haines, J.L. and Pericak-Vance, M.A. (1996) Presenilin-1 polymorphism and Alzheimer's disease. *Lancet* **347,** 1186–1187.
44. Perez-Tur, J., Wavrant-De Vrieze, F., Lambert, J.C., Chartier-Harlin, M. and the Alzheimer's Study Group (1996) Presenilin-1 polymorphism and Alzheimer's disease. *Lancet* **347,** 1560–1561.
45. Schellenberg, G.D., Payami, H., Wijsman, E.M., Orr, H.T., Goddard, K.A.B., Anderson, L., Nemens, E., White, J.A., Alonso, M.E., Ball, M.J., Kaye, J., Morris, J.C., Chui, H., Sadovnick, A.D., Heston, L.L., Martin, G.M. and Bird, T.D. (1993) Chromosome 14 and late-onset familial Alzheimer disease (FAD). *Am. J. Hum. Genet.* **53,** 619–628.
46. Levy-Lahad, E., Lahad, A., Wijsman, E.M., Bird, T.D. and Schellenberg, G.D. (1995) Apolipoprotein E genotypes and age of onset in early-onset familial Alzheimer's disease. *Ann. Neurol.* **38,** 678–680.
47. Moussaoui, S., Czech, C., Pradier, L., Blanchard, V., Bonici, B., Gohin, M., Imperato, A. and Revah, F. (1996) Immunohistochemical analysis of presenilin-1 expression in the mouse brain *FEBS Lett.* **383,** 219–222.
48. Kovacs, D.M., Fausett, H.J., Page, K.J., Kim, T., Moir, R.D., Merriam, D.E., Hollister, R.D., Hallmark, O.G., Mancini, R., Felsenstein, K.M., Hyman, B.T., Tanzi, R.E. and Wasco, W. (1996) Alzheimer-associated presenilins 1 and 2: Neuronal expression in brain and localization to intracelllular membranes in mammalian cells. *Nature Med.* **2,** 224–229.
49. Mercken, M., Takahashi, H., Honda, T., Sato, K., Murayama, M., Nakazato, Y., Noguchi, K., Imahori, K., Takashima, A. (1996) Characterization of human presenilin 1 using N-terminal specific monoclonal antibodies: evidence that Alzheimer mutations affect proteolytic processing. *FEBS Letts* (in press).
50. Suzuki, T., Nishiyama, K., Murayama, S., Yamamoto, A., Sato, S., Kanazawa, I. and Sakaki, Y. (1996) Regional and cellular presenilin-1 gene expression in human and rat tissues. *Biochem. Biophys. Res. Commun.* **219,** 708–713.
51. Wisniewski, T., Palha, J.A., Ghiso, J. and Frangione, B. (1995) S182 protein in Alzheimer's disease neuritic plaques. *Lancet* **346,** 1366.
52. Vito, P., Lacana, E. and d'Adamio, L. (1996) Interfering with apoptosis: Ca-binding proteine ALG-2 and Alzheimer's disease gene ALG-3. *Science* **271,** 521–524.
53. Querfurth, H.W., Wijsman, E.M., St George-Hyslop, P.H. and Selkoe, D.J. (1995) βAPP mRNA transcription is increased in cultured fibroblasts from the familial Alzheimer's disease-1 family. *Mol. Brain Res.* **28,** 319–337.
54. Martins, R.H., Turner, B.A., Carroll, R.T., Sweeney, D., Kim, K.S., Wisniewski, H.M., Blass, J.P., Gibson, G.E. and Gandy, S. (1995) High levels of amyloid-β protein from S182 (Glu^{246}) familial Alzheimer's cells. *Neuroreport* **7,** 217–220.
55. Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J., Hutton, M., Kuruli, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D. and Younkin, S. (1996) The amyloid β protein deposited in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and *APP* mutations linked to familial Alzheimer's disease. *Nature Med.* (in press).