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Monogenic determinants of familial Alzheimer's disease: presenilin-2 mutations

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Abstract. Presenilin-2 (*PS2*) is one of three genes [amyloid precursor protein (APP), presenilin-1 (*PS1*) and *PS2*] shown to cause familial Alzheimer's disease (FAD), and is highly homologous to *PS1*. Currently demonstrated functions of *PS2* include interactions with *APP* and Aβ, and participation in apoptotic pathways. *PS2* FAD mutations influence *APP* processing in a manner predicted to promote amyloid formation and also enhance the proapoptotic effect of wild-type *PS2*. Other possible functions of *PS2* are related to its homology to Notch pathway genes in *Caenorhabditis elegans*, suggesting it may have a developmental role. *PS2*-associated AD is the most reminiscent of the sporadic form of the disease in terms of older age of onset and longer disease duration. Since *PS2* mutations are incompletely penetrant and age of onset in carriers is highly variable (40–88 years), elucidation of *PS2* mechanisms may reveal factors which modify AD and are therapeutically relevant to sporadic AD.

Key words. Alzheimer's disease; presenilin-2; presenilin-1; *APP*; Aβ; sel-12; apoptosis.

Introduction

Presenilin-2 (*PS2*) is the third gene [1, 2], following *APP* [3] and presenilin-1 (*PS1*) [4], in which mutations were shown to cause autosomal dominant, familial Alzheimer's disease (FAD). It was identified by linkage analysis and positional cloning in a group of eight kindreds with FAD, known as the Volga Germans (VG) [5]. The VG are ethnic Germans who migrated to Russia during the reign of Catherine the Great in the 1760s and remained distinct from the surrounding population. Many of their descendants ultimately emigrated to the United States, where FAD was identified in a subset of VG kindreds [6, 7]. Because of their initial genetic isolation, AD in the VG is the result of a founder effect, that is inheritance of a single mutation from a common founder. FAD in the VG was localized to chromosome 1 through a genome-wide scan and linkage analysis [5]. This analysis was complicated by genetic heterogeneity (VG families with FAD but no evidence for linkage to chromosome 1) and the presence of phenocopies (individuals with AD – the phenotype studied – who did not inherit the founder mutation and are essentially sporadic cases within the family). The chromosome 1 FAD gene, *PS2*, was cloned by virtue of its homology to *PS1*. A database search revealed the existence of an expressed gene homologous to *PS1*. This gene was found to be located within the VG FAD region, and subsequently a single mutation within the gene (N141I) was shown to segregate with AD in the VG [1, 2].

Except for younger age of onset, FAD is clinically and neuropathologically indistinguishable from sporadic AD, which accounts for the vast majority of affected individuals [7]. Therefore, although the monogenic forms of AD are rare, the genes which cause them are thought to be relevant to the common, sporadic form of the disease. In this context, the identification of *PS2* offers further opportunities for elucidating what are hoped to be general mechanisms of AD pathogenesis. The following review discusses current knowledge on manifestations of *PS2*-associated AD, and recent insights into the normal and pathogenic roles of *PS2*.

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PS2 mutations

PS2 mutations are a rare cause of FAD, and to date only three *PS2* mutations have been described: N141I in the VG, M239V in an Italian kindred and R62H in a sporadic AD case in the Netherlands [1, 2, 8]. Although N141I and M239V are missense mutations, they are clearly pathogenic. Both segregate with AD in large families, and the amino acid change is nonconservative and occurs in amino acids (aas) conserved in both presenilins and sel-12 (the *Caenorhabditis elegans* presenilin homologue), suggesting they are structurally and/or functionally important. Both mutations occur within putative transmembrane domains (TMDs) of the PS2 protein (PS2p): N141I in TMDII and M239V in TMDV [1, 2]. Notably, the homologous TMDII region of *PS1* is the site of one of two mutation clusters in the *PS1* gene. The R62H mutation was found in a sporadic AD case with onset at 62 years, and familial segregation could not be explored [8]. This mutation represents a conservative aa change in an aa that is not conserved in *PS1* or *sel-12*, and is located in the hydrophilic N-terminal domain that differs between presenilins. It is therefore possible that Arg62His is a rare polymorphism, with or without pathogenic implications [8].

Frequency of *PS2* mutations

Linkage of late onset (>60 years) FAD kindreds to chromosome 1 has been excluded [9], and no mutations in the *PS2*-coding region were identified in 71 FAD pedigrees and 56 familial AD cases from various countries [8, 10]. However, because of the later and more variable age of onset in *PS2*-associated AD, families with *PS2* mutations are less likely to be ascertained as FAD pedigrees, and probands would then be considered as sporadic cases. In a population-based study in the Netherlands one of 34 young-onset sporadic cases was found to have a *PS2* mutation (R62H). Together with the FAD and familial cases in which no mutation was identified, the authors estimated that frequency of *PS2* mutations in young-onset AD is 1% [8]. In comparison, *PS1* mutations have been described in scores of families worldwide, and in the same study in the Netherlands they were found to account for 18% of early-onset FAD and 6% of young-onset AD [8]. *PS2* mutation frequency and the possibility of mutations in intronic or regulatory *PS2* sequences can also be addressed by utilizing a number of biallelic polymorphisms found throughout the *PS2* gene [8, 10]. One study of 82 nonautopsy proven AD cases found that homozygosity for a polymorphism in the *PS2* 3′UTR (untranslated region) was associated with AD, with an attributable risk of 21% [11]. Larger studies would be needed to accurately determine the contribution of *PS2* mutations and/or polymorphisms to late-onset and sporadic AD.

Clinical and neuropathological features of *PS2* mutations

Descriptions of the *PS2* mutant phenotype are essentially limited to the VG and the Italian M239V kindred. Except for younger age of onset, in both families the clinical course and neuropathology of AD are generally indistinguishable from those found in the sporadic form of the disease. However, in addition to characteristic AD pathology, some affected VG subjects display prominent cerebral amyloid angiopathy, which has manifested in one person as a lethal intracranial hemorrhage [12, 13]. This suggests that *PS2* mutations may also affect vascular deposition of amyloid, as observed in two *APP* mutations that cause combined dementia and cerebral hemorrhage [14, 15].

In the VG, mean age of onset of AD is 54.9 (±8.4) years, significantly older than in families with *PS1* mutations, in which family mean ages of onset generally range from 30 to 55. Mean disease duration in the VG is 11.3 (±4.6) years, significantly longer than in *PS1* families, in which mean disease durations vary from 5.8 to 7.6 years [16]. In the M239V Italian family, mean age of onset is over 60 years, with disease duration of up to 20 years [2, 10]. An especially striking feature of both *PS2* pedigrees is the intrafamilial variability in ages of onset, which ranges from 40 to 75 years in the VG [16], and from 45 to 88 years in the Italian M239V kindred [10]. In the VG there is also one case of probable nonpenetrance of AD in an 89-year-old person [16]. This variability is in contrast to the complete penetrance and relatively narrow range of intrafamilial age of onset in *PS1* families, and seems to be a feature of *PS2*-associated disease rather than of the specific mutations observed. In a family with a *PS1* mutation at the site corresponding to the VG mutation, ages of onset in five cases ranged only from 34 to 37 years [17]. Therefore, the clinical parameters of age of onset, penetrance and disease duration all point to the less aggressive nature of *PS2*-associated AD compared with *PS1*-associated AD. At the neuropathological level this is reflected by the observation that amyloid load, and more specifically deposition of Aβ42, is significantly higher in *PS1* mutation carriers compared with *PS2* mutation carriers, who are not different from sporadic cases in this respect [13, 18].

Incomplete penetrance and the large variability in age of onset suggest that *PS2*-associated AD can be modulated by other genetic and/or environmental factors, which is akin to the polygenic/multifactorial basis likely to be true for sporadic AD [16]. The apolipo-

protein E (*ApoE* ε4) allele, which modifies age of onset in familial and sporadic AD, is potentially such a factor [19], but has not been found to influence age of onset in either the VG or the Italian M239V kindred [10, 16, 20]. However, better understanding of *PS2* function could lead to identification of disease-modifying factors which may have major therapeutic implications.

Structure and evolution of the *PS2* gene

The *PS2* gene, originally named *STM-2* (for *seven-transmembrane domain*), encodes a 448-aa transmembrane protein that is 67% identical to the PS1p. The regions of greatest identity (84%) correspond to the hydrophobic TMDs, and the regions of greatest divergence are the hydrophilic N-terminus and the extramembranous 'loop' between TMDs 6 and 7, near the C-terminus [1, 2]. The genomic organization of both presenilins is also highly similar, and intron/exon boundaries are identical in conserved regions of the gene [21, 22]. Both presenilins are highly conserved in evolution. In terms of sequence similarities, there is a single presenilin gene in both *C. elegans* and *Drosophila*: *C. elegans sel-12* (50% aa identity) and *DPS* (*Drosophila* presenilin, 53% aa identity, 60% in the TMDs) [23–25]. Rat and mouse have both *PS1* and *PS2* homologues, which together with the highly similar genomic organization of human presenilins suggests that mammalian presenilins arose by duplication after the divergence of arthropods and vertebrates. At the aa level both rat and mouse PS2 proteins are 95% identical to human PS2p [26, 27].

PS2 expression

Although AD pathology is confined to the central nervous system, the 2.3-kb *PS2* transcript is expressed in almost all tissues tested. General expression levels in the brain are actually lower than in other tissues, especially pancreas, heart and skeletal muscle, in which *PS2* is most highly expressed [1, 2, 21]. Within the brain, in situ messenger RNA (mRNA) hybridization reveals that *PS2* expression is widespread but occurs exclusively in neurons [28, 29]. In the temporal lobe, expression is high in the hippocampal formation, parahippocampal gyrus and entorhinal cortex, regions in which characteristic AD pathology occurs early and is most pronounced [28, 30]. It is unclear whether *PS2* mRNA levels are affected in AD brains beyond the effect of neuronal loss. In one study of six subjects with AD and four controls, there were no significant differences in *PS2* mRNA levels, but elevated *PS2* mRNA was observed in one AD case [30]. Another study of seven AD cases and three controls found AD was associated with decreased *PS2* mRNA per neuron in the hippocampus, but increased *PS2* mRNA in hippocampal astrocytes [31]. Antibodies directed at a shared PS1p/PS2p epitope were shown to stain a subset of intraneuronal neurofibrillary tangles (NFTs), with a similar pattern observed in both sporadic AD cases and affected subjects with the N141I *PS2* mutation [32]. These antibodies did not stain senile plaques, which reportedly stain with PS1p antibodies directed at other epitopes [33]. Thus, although the cellular and regional pattern of *PS2* expression is not limited to areas most vulnerable to AD neuropathology, it does occur in these regions, and PS2p immunoreactivity has been observed in NFTs. However, these parameters do not clearly differ between AD cases, including *PS2* mutation carriers, and controls.

PS2 protein (PS2p): subcellular localization, structure and metabolism

Since the presenilins are expressed in almost all tissues, including the brain, their functions are almost certainly not limited to neuronal pathways. Determination of the subcellular localization and membrane topology of the presenilin proteins can provide important clues as to their functions in general and their role in AD in particular.

Subcellular localization

The majority of cellular immunoflourescent studies have identified PS1p and PS2p on the membranes of the endoplasmic reticulum (ER) and Golgi apparatus, based on a pattern of intracellular staining comparable to ER/Golgi proteins (e.g. K58 and β-amyloid precursor protein (APPp) [28]) or double-labelling with ER-specific proteins (e.g. grp78 [34] and calreticulin [35]). These results were obtained in a variety of cell lines (COS, CHO, H4 human neuroglioma PC12 and HeLa), either endogenously producing or overexpressing transfected *PS2* [28, 34–37]. Recently, the 166 N-terminal aas of PS2p were shown to be sufficient to direct the specific localization of PS2p to the ER [34, 35]. The VG N141I mutation does not appear to grossly alter subcellular localization of PS2p [28]. Localization to the ER, along with the homology to the *C. elegans* gene *spe-4*, which is involved in cytoplasmic protein partitioning, fostered the idea that the presenilins are involved in protein trafficking [4]. In particular it has been proposed that they influence APPp processing in the ER and Golgi compartments to favour pathological amyloid production [28].

The presence of high levels of presenilins in the ER does not preclude the possibility that presenilins have important functions at other cellular locations, albeit at lower

levels. In particular, a membrane protein could be found largely in the ER and Golgi, depending on the balance between its production and transport through the ER/Golgi to the target membrane. Indeed, Dewji and Singer have demonstrated expression of both endogenous and transfected *PS1* and *PS2* on the cell surface of live DAMI (human megakaryocyte) and neuronally differentiated NT2N cells [38]. This location is compatible with the their hypothesis that cell surface APPp and presenilins may bind transcellularly in an interaction that would be crucial to the ultimate formation of Aβ [39]. In addition, a perinuclear/nuclear localization of PS1p and PS2p was observed in primary cultures of rat hippocampal neurons [40], in *PS2*-transfected HeLa cells [35] and in untransfected, dividing human fibroblasts, where PS1p and PS2p were seen mainly in the nuclear membrane, in centrosomes and with interphase kinetochores [41]. Li et al. speculate that the physiological roles of the PS1p and PS2p may be related to chromosome organization and segregation, in keeping with evidence they present for trisomy 21 mosaicism as a possible unifying mechanism for AD in genetic, sporadic and Down's syndrome cases [41].

Differences in the localizations observed may reflect not only levels of expression, but also tissue-specific and/or developmental events. For example, in primary cultures of rat hippocampal neurons, PS1p and PS2p were found mainly in the somatodendritic compartment, rather than in the ER. During differentiation their distribution changed from uniform intracellular, to the growth cones, then to the somatodendritic compartment and ultimately to the nuclear envelope [40]. Further analysis of PSp distribution in different tissues and developmental stages may offer additional clues as to their normal and deleterious functions.

PS2 protein structure

Initial hydrophobicity plots of the deduced PS1p and PS2p amino acid sequences indicated that presenilins were most likely transmembrane proteins with 7 TMDs, a structure reminiscent of G protein-coupled receptors [1, 4]. Later analyses suggested that up to 9 of the 10 hydrophobic domains observed in presenilins could potentially span a membrane [42]. The N-terminus and the loop between TMDs 6 and 7 are the two major hydrophilic domains of the presenilins, and are also the most divergent between *PS1* and *PS2*. Thus, they are candidate regions for gene-specific interactions with other proteins, and determining their orientation would direct the search towards cytoplasmic or ER proteins. Although most published experiments have all been performed on PS1p and sel-12p proteins, the high degree of homology in the TMDs suggests it is reasonable to assume they also pertain to PS2p.

Experiments based on different chimeric peptides with 'topologically neutral reporter proteins' fused to some or all of the putative TMDs have localized the PS1 and sel-12p N-terminus, large hydrophilic loop and C-terminus to the cytosolic side of the ER membrane, implying an even number of TMDs [36, 43–45]. Li and Greenwald concluded that sel-12p has 8 TMDs [44], while Lehman et al. found PS1p hydrophobic domains 7–10 to be cytoplasmic and suggest a 6-TMD model [45]. Doan et al. tested only TMDs 1 and 2 and showed that the N-terminus is cytoplasmic and the loop between TMDs 1 and 2 is intraluminal, results compatible with either a 6- or 8-TMD model [43]. These differences could be the result of limitations in the methods used by either group, or, less likely, of differing structures of sel-12p and PS1p in the C-terminal region. An interesting aspect of the 6-TMD structure is that the proteolytic cleavage observed in presenilins at a site following TMD6 (see below), would generate a potentially soluble C-terminal fragment which could act as a signal transducer.

In addition to chimeric protein experiments, Doan et al. used domain-specific anti-PSp antibodies to confirm the cytoplasmic orientation of the N-terminus, hydrophilic loop and C-terminus [43]. By selectively permeabilizing the plasma membrane while leaving the ER/Golgi intact, they observed staining with antibodies to cytoplasmic domains, but not with antibodies to intraluminal epitopes. No staining was observed on the cellular plasma membrane.

On the other hand, using a similar approach with specific PS1p/PS2p antibodies, Dewji and Singer localized presenilins primarily to the cell surface [38]. They found that live cells, impermeable to antibodies, were labelled by antibodies directed to the N-terminus and the large hydrophilic loop, indicating that they face the external cell surface. Antibodies directed to the C-terminus and to the small hydrophilic loop between TMDs 1 and 2 labelled the cell surface (and ER/Golgi) only after the cells were fixed and permeabilized, showing that these regions are cytoplasmic. Such a staining pattern is compatible with a 7-TMD structure, but not with an even number of TMDs [38]. Although it is quite possible that fusion-protein experiments of single TMDs are not representative of the topology of the full-length protein, this would not explain conflicting antibody results, and these authors suggest that localization of the N-terminus to the cytoplasmic side of the ER by Doan et al. may reflect an intermediate step in the integration of presenilins into the membrane [38].

To summarize, in all the proposed models (6, 7 and 8 TMDs), the C-terminus has been shown to be cytoplasmic. However, while the N-terminus and the large hydrophilic loop are agreed to share a common orientation, there is disagreement as to whether they are

cytoplasmic or luminal/extracellular. This controversy parallels respectively that of ER vs. plasma membrane localization of the presenilins.

PS2p metabolism: endoproteolytic cleavage, proteasomal degradation and phosphorylation

Endoproteolytic processing of presenilins within the hydrophilic loop was originally shown to occur in cell lines and transgenic mice expressing *PS1*, in which relatively little full-length PS1 protein was observed. Most of the protein was found in its cleaved form, as N-terminal and C-terminal fragments (NTF and CTF, respectively) [46]. Full-length PS2p (50–55 kDa) was subsequently found to undergo similar proteolysis in a number of cell lines (H4 neuroglioma, COS-1, N2a). In addition to a small amount of full-length protein, an ~35-kDa NTF and multiple CTFs of either ~23–25 kDa or 18–20 kDa were observed [47–50]. The smaller CTFs are the result of cleavage by caspases, enzymes in the apoptotic pathway, and are discussed below. The larger, 23–25 kDa CTFs are thought to be products of an unidentified 'presenilinase'. In stably transfected SH-SY5Y neuroblastoma cells they have been shown to be the result of proteolysis at Lys^{306}/Leu^{307} [49] in the proximal portion of the hydrophilic loop, analogous to 'presenilinase' processing sites reported for PS1p [51]. Overexpression of *PS2* results in accumulation of the full-length protein and little change in CTF levels, suggesting that this cleavage reaction is saturable [46]. Interestingly, the endoproteolytic pathways involving PS1p and PS2p were found to be interdependent [50]. In cultured mouse N2a cells and in brains of transgenic mice overexpressing human *PS1*, saturable accumulation of human PS1p endoproteolytic fragments is accompanied by a compensatory decrease in accumulated murine PS1p and PS2p derivatives, and concomitantly, human *PS2* overexpression in mouse cells decreased murine PS1p and PS2p derivatives. This suggests that PS1p and PS2p are processed by common but limiting cellular factors, and indeed, while full-length PS2p is rapidly turned over ($t_{1/2}$ of ~1 h), PS2p derivatives are longer lived ($t_{1/2}$ of ~24 h) [47, 50].

Although many investigators have observed proteolysis of PS2p, this finding is by no means universal and may be dependent on length of labelling in pulse-chase experiments, cell type or extraction method. In HeLa [35], COS-7 [34, 52] and DAMI [53] cells, PS2p was found primarily in its full-length form, and little or no proteolytic fragments were observed. Even when proteolysis is observed, its biological significance is unclear. Dewji and Singer found that using either more or less aggressive cell extraction methods could respectively induce or inhibit appearance of proteolytic fragments in DAMI, CHO, NT2 and other cell types [53]. In addition, neither the N141I (VG) nor the M239V mutations alter the proteolytic pattern in HeLa, SH-SY5Y, COS-1 or N2a cells [35, 48, 49], raising doubts as to the significance of endoproteolysis in the disease process.

Full-length PS2p is also processed through a proteasomal pathway, in which PS2p is polyubiquinylated and appears in Western blots as a diffuse high molecular mass (HMW) species of PS2p [47]. Proteasome inhibitors elevate HMW-PS2p levels, suggesting it is an intermediary product in the degradation of full-length PS-2p, but they do not influence NTF/CTF levels, suggesting that this proteolysis does not occur in the proteasome. Since endogenous amounts of PS2 protein are low, proteasomal degradation may be important in regulating PS2p levels in a pathway unrelated to endoproteolysis. It remains to be determined whether perturbation of this process is related to AD.

The only other posttranslational modification observed in presenilins is phosphorylation. They are not glycosylated, acylated or sulphated, in keeping with their localization to the ER [34, 36]. Both presenilins are phosphorylated on serine residues, but PS2p phosphorylation is more pronounced than PS1p phosphorylation [34, 36]. The site(s) of PS2p phosphorylation are in the N-terminal domain, which is divergent from PS1p. In vitro, PS2p is phosphorylated by both CK-1 and CK-2, but not by protein kinase C (PKC). No differences were detected between phosphorylation of the N141I mutant and of wild-type PS2p [34], and no functional role has yet been identified for this modification.

PS2p and AD pathogenesis

An obvious difficulty in elucidating mechanisms of a degenerative disease is discerning cause from effect. Indeed, delineation of the basis of AD by studying the endpoint of a lifetime of accumulated neuropathology has been likened to 'molecular archaeology' [54]. The presenilins were not identified using a biochemical approach based on their role in the pathology of AD, but by positional cloning in families with the disease. This gives them the advantage of being 'genetically proven' to be a cause of at least some forms of AD, but does not provide any clues as to their function(s) in this regard. The presenilins probably have multiple roles. Their evolutionary conservation in species which do not have an AD correlate suggests that some fundamental presenilin functions may not be related to AD in humans. After divergence into two presenilins, certain roles could have been kept or lost in either *PS1* or *PS2*,

and new roles could have evolved for one or both of these genes. However, those functional aspects which are sufficient to cause AD should be affected by the known mutations. As mentioned above, distribution and levels of *PS2* mRNA and subcellular localization of PS2 protein do not appear to be affected by *PS2* mutations. Studies on the normal and abnormal roles of presenilins have proceeded along several lines: (i) homologous genes: the *C. elegans sel-12* gene was identified as part of the Notch pathway, suggesting that presenilins have a developmental role in signal transduction; (ii) interactions with known components of AD pathology, specifically APP and Aβ; (iii) direct in vitro and in vivo studies of presenilins, which have identified at least two pathways of presenilin processing; (iv) apoptosis – a fortuitously discovered function of *PS2* which could be relevant to neuronal death. In many of these areas, PS1 has been more extensively studied than *PS2*, possibly because it is a commoner cause of early-onset FAD, and its association with a more virulent form of the disease suggests that its effects may be more pronounced and easier to detect. The following discussion relates specifically to experimental evidence regarding *PS2* (PS1 is reviewed by Kovacs and Tanzi in this issue).

PS2 and the Notch pathway

PS1 and *PS2* are both highly homologous to the *C. elegans sel-12* gene, which was identified as a modulator of *lin-12*, a member of the Notch receptor family [23]. Notch cell surface receptors are mediators of signal transduction pathways important in specifying cell fate, and the *sel-12/PS* homology suggested that presenilins may have an important role in neuronal development [23]. *Sel-12* hypofunctioning mutants have a defective egglaying phenotype (Egl), which was found to be rescued by human wild-type *PS1* and *PS2*, providing evidence that in at least some respects these genes are functional homologues of *sel-12* [55]. Further studies have all focused on *PS1*. *PS1* missense mutations, but not an exon-skipping mutation, had a diminished capacity to rescue *sel-12* mutants [55], and mouse PS1 knockouts have an embryonically lethal phenotype reminiscent of Notch knockouts [56]. *PS2* has not yet been studied in these regards.

While *C. elegans* is a powerful tool for studying presenilin biology, the role of developmental presenilin functions in AD is yet to be determined. Rescue of *sel-12* mutants may not be representative of the mutational effect in AD, since the exon-skipping mutation which causes AD is not different from the wild-type in this assay. Also, while the diminished rescue capacity of missense mutations demonstrates a mutational effect, this represents a loss of function, whereas the autosomal dominant inheritance of *PS* mutations suggests they are most likely to act through gain of deleterious function. This has been demonstrated for Aβ interactions and apoptosis (see below). In addition, the lethality of *PS1* mouse knockouts suggests that *PS1* and *PS2* are not functionally redundant during embryonic development [56].

PS2p/Aβ interactions

Aβ is a 40–43-aa polypeptide derived from the APPp, whose deposition in amyloid plaques is one of the fundamental features of AD pathology [57]. Aβ is generated by a series of proteolytic steps ending with cleavage at residue 40–43 by an unidentified γ-secretase, and its longer (42–43 aa), more aggregable form is the predominant species deposited in AD brains (reviewed in ref. 57). Mutations in the gene coding for APPp have been shown to cause a selective increase in Aβ_{42-43} production which is thought to accelerate the disease process [58, 59]. In this context presenilin mutations were also hypothesized to alter Aβ processing, either directly or indirectly.

In vivo studies in carriers of *PS* mutations have provided clear evidence of a PSp/Aβ interaction. Compared with controls, Aβ_{42-43} levels were significantly higher in plasma and in primary fibroblast culture media of *PS1* and *PS2* mutation carriers [60]. However, in brains of six subjects with the *PS2* VG mutation, the overall amount of Aβ_{42-43} and Aβ_{40} did not differ from that seen in sporadic AD cases and was significantly less than that observed in brains of *PS1* mutation carriers [13].

In vitro, the effects of *PS2* mutations on APP processing were investigated by cotransfecting APP in various cell lines with wild-type or mutant *PS2* complementary DNAs (cDNAs). Cotransfection with wild-type or mutant *PS2* did not result in any significant differences in the steady-state levels of immunoprecipitated APPp or its α- or β-secretase metabolites [61]. Total Aβ and Aβ_{42-43} production were unaffected by cotransfection with wild-type *PS2*, but increased significantly in cells transfected with the *PS2* N141I mutant, which showed a 5–10-fold increase in Aβ_{42-43} levels and a 2–4 fold increase in the Aβ_{42-43}/Aβ_{total} ratio [48, 61]. These results strongly suggest that mutated *PS2* alters γ-secretase activity in a manner favouring production of the longer, amyloidogenic form of Aβ. How this effect is achieved is currently being investigated.

Dewji and Singer have proposed that Aβ is a byproduct of transcellular binding between APPp and PS1p/PS2p. Following binding, the APPp/PSp complex would be internalized in vesicles where proteases would generate Aβ, and *PS* mutations would accelerate this process [39]. In support of this hypothesis they demon-

strated that DAMI cells transfected with *APP* formed large, specific aggregates with *PS2*/*PS1* transfectants, results compatible with intercellular binding [62]. However, events downstream of this interaction are yet to be demonstrated.

In order to determine if the effect of the presenilins on Aβ metabolism is due to a direct protein-protein interaction with APPp, Weidemann et al. searched for APPp/PS2p complexes in vitro. They transiently cotransfected *PS2* and *APP* into COS7 cells, and in a high-expression system found that approximately 10% of total APPp coprecipitated with anti-PS2p antibodies [52]. Xia et al. demonstrated similar coprecipitation of APPp and PS2p in stable transfectants with low expression levels and even at endogenous levels of expression in nontransfected CHO cells. [63] This suggests the observed APP/PS2p association is not an artifact of protein overexpression, but does not conclusively demonstrate that it occurs in intact cells. Furthermore, in both studies the N141I mutation did not alter APPp/PS2p complex formation [52, 63], indicating that either this direct system is not representative of the in vivo APPp/*PS2*p interaction described above, or that differences in APPp binding are subtle and require more sensitive means of detection. In a yeast two-hybrid system, no interactions were observed between various combinations of the hydrophilic domains of both APPp and the presenilins [64]. Although these findings do not rule out the possibility that direct interactions may occur through different domains, they imply that a more likely APPp/PSp interaction may be an indirect one.

PS2 and regulation of apoptosis

Apoptosis, or programmed cell death (PCD), is an active process of cellular suicide and is an attractive framework for studying a neurodegenerative disease. Although the primary mechanism of neuronal death in Alzheimer's disease has not yet been proven to be apoptotic, as opposed to necrotic, several lines of evidence implicate the deregulation of PCD in the AD pathway. Staining of AD brain sections has revealed TUNEL (terminal deoxynucleotide transferase nick-end labelling)-positive cells [65, 66], and the neurotoxic properties of β-amyloid have been shown to be due to increased apoptosis [67]. However, the involvement of *PS2* in PCD was an incidental finding: while screening for genes which could rescue mouse T-hybridoma (3DO) cells from T-cell receptor- and Fas-induced apoptosis, Vito et al. [68] isolated a partial mouse cDNA, designated *ALG-3*, which corresponded to the 103 C-terminal aas of the murine *PS2* gene. When transfected into 3DO or PC12 cells, this artificial cDNA was translated into a polypeptide which protected cells from induced PCD. Most interestingly, transfection of full-length *PS2* into these cells not only restored sensitivity to PCD but also accentuated apoptosis induced by a number of stimuli, including Aβ [68, 69]. Transfection of antisense *PS2*, on the other hand, blocked apoptosis. Together, this suggested that PS2p has a proapoptotic effect and that a *PS2*p C-terminal fragment (ALG-3) could act as a dominant-negative mutant of *PS2* [70, 71].

Janicki and Monteiro found that transient transfection of full-length *PS2* into HeLa cells caused a more than eightfold increase in the rate of cell death [35]. Dying cells were clearly identified as apoptotic due to their abnormal (condensed and shrunken) nuclear morphologies and TUNEL-positive staining. These findings were especially significant since *PS2* transfection was sufficient to cause noninduced apoptosis even though HeLa cells are usually highly resistant to PCD. Although no evidence of any proteolytic cleavage was observed in these experiments, by using deletion mutants the apoptotic-promoting domain was mapped to the N-terminal 166 aas of PS2p. This truncated polypeptide includes just the first two TMDs, and was sufficient to direct both localization to the ER and apoptosis [35].

The proapoptotic effect of *PS2* may be especially relevant to AD since it is enhanced by the N141I mutation. In PC12 cells mutant *PS2* caused constitutive apoptosis, [69], and in HeLa cells, mutant *PS2* transfectants exhibited greater rates of cell death than *PS2* wild type, even though expression of the mutant form was only 25% that of the wild-type protein [35]. Interestingly, although wild-type *PS1* has not been found to have proapoptotic effects, the L286V PS1 mutation sensitized PC12 cells to induced apoptosis [37]. Propensity to apoptosis is a possible mechanism which could have long-term negative effects on *PS2* and *PS1* mutation carriers, although the question of specific neuronal vulnerability would need to be clarified.

Further implicating *PS2* in apoptosis is the discovery that in addition to the possible proteolysis described previously, PS2p is also cleaved by caspase-3 [71, 72]. Caspases, cysteine-aspartyl specific proteases, constitute a family of at least 10 proteases which regulate certain forms of apoptosis. Their activation is one of the final steps required for execution of PCD, whereas blocking their activity with inhibitors prevents neurons from undergoing apoptosis [73]. Following reports linking *PS2* to apoptosis, Kim et al. treated cells with zVAD, a broad-spectrum caspase inhibitor, and zDEVD, a more specific inhibitor of caspase-3 family proteases [72]. They found that both inhibitors blocked the generation of an alternative 20-kDa CTF in cells that were induced to undergo apoptosis, or overexpressed *PS2*. This inhibition was specific for the 20-kDa product, and did not affect the levels of other PS2p proteolytic species. A

potential caspase site, DSYDS, was identified in the hydrophilic loop at positions 326–330, and tested by making aa substitutions at either Asp[326] or Asp[329]. Either of these changes blocked the generation of the 20-kDa CTF. Loetscher et al. isolated and sequenced this CTF and confirmed that Asp[329]/Ser[330] was indeed the cleavage site [74]. Thus, the smaller (18–20-kDa) species of PS2p CTFs was shown to be a product of caspase-3 cleavage, distal to the presenilinase site which produces the ~23–25-kDa CTF.

In addition to apoptosis, caspase cleavage has also been shown to be sensitive to the *PS2* N141I mutation. Compared with wild-type *PS2*, in N141I transfectants the 20 kDa/26 kDa ratio was increased approximately threefold [72]. This could simply be a reflection of increased apoptosis in mutant transfectants, but it underscores the importance of studying independent effects of the 20-kDa caspase-derived CTF.

An interesting possibility is that this caspase-derived CTF is the physiological equivalent of the ALG-3 fragment, which acts as a dominant-negative inhibitor of apoptosis. A naturally occurring Met298-Ile448 polypeptide (PS2p short) which is generated in murine liver by alternative transcription, was indeed shown to protect transfected cells from Fas- and tumour necrosis α (TNFα)-induced apoptosis, in a manner analogous to that of ALG-3 [71]. In HeLa cells, a *PS2*cas construct, corresponding to the caspase-derived CTF, provided partial protection from induced apoptosis [71]. This would suggest a negative feedback loop in which apoptosis, mediated by the PS2p N-terminal domain, would result in production of the caspase-derived CTF, which itself may inhibit apoptosis. Cell fate could therefore be dictated by the balance between full-length PS2p (and/or the NTF) and caspase-derived CTF.

Conclusions

Presenilins are highly conserved in evolution and probably have multiple roles, not all necessarily related to AD. Delineation of differences and similarities between these highly homologous genes can provide valuable insights as to which functions are sufficient to cause the disease, although comparisons are incomplete since *PS1* has been investigated more extensively. The high degree of sequence homology between the presenilins dictates similar protein structures, and except for subtle differences, the expression pattern and subcellular localization of both presenilins are similar [28, 31, 40]. However, PS2p is probably expressed at lower levels [48] and is phosphorylated more significantly than PS1p [34], observations which point to differential regulation of these two genes. Work on the functions of presenilins has both validated previous hypotheses and led in new directions. An important outcome common to both *PS1* and *PS2* is confirmation of the pivotal role of Aβ deposition in AD, demonstrated in vivo by elevated plasma Aβ_{42-43} in carriers of presenilin mutations [60]. Determining the pathway by which these mutations result in altered APPp processing is a major focus for future research. *PS2*, but not *PS1*, has been found to have proapoptotic effects, which are enhanced by *PS2* mutations [37,70]. Caspase-derived PS2p fragments may participate in a feedback loop in which PS2p metabolism contributes to determination of cell fate [72], a delicate balance which is apparently perturbed by *PS2* mutations. Such pathways may also be related to Aβ, which has been shown to induce neuronal apoptosis in vitro. The presenilins are a clear starting point for at least some paths of AD pathogenesis, and current research suggests a number of relevant end-points such as Aβ deposition and apoptosis. Elucidating the routes in between will enhance our understanding of the sporadic form of the disease and may identify therapeutic targets.

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