Presenilin-1 Mutation L271V Results in Altered Exon 8 Splicing and Alzheimer's Disease with Non-cored Plaques and No Neuritic Dystrophy*

John B. J. Kwok,* Glenda M. Halliday, b,c William S. Brooks, b,d Georgia Dolios,* Ohoshi Murayama,* Marianne Hallupp,* Renee F. Badenhop,* c James Vickers,* Rong Wang,* Jan Naslund,f Akihiko Takashima,* Samuel E. Gandy,* and Peter R. Schofield* a

From the aGarvan Institute of Medical Research, Darlinghurst, Sydney 2010, Australia, bPrince of Wales Medical Research Institute, Randwick 2031, Australia, cUniversity of New South Wales, Sydney 2052, Australia, dCentre for Education and Research on Ageing, University of Sydney and Concord Repatriation General Hospital, Concord 2139, Australia, eDepartment of Human Genetics, Mount Sinai School of Medicine, New York, New York 10029, fNeurotec, Section of Experimental Geriatrics, Karolinska Institute, Stockholm S-141 86, Sweden, gLaboratory for Alzheimer's Disease, Brain Science Institute, Riken, Saitama 350-01, Japan, hDepartment of Pathology, University of Tasmania, Hobart, 7001, Australia, and iFarber Institute for Neuroscience, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

The mutation L271V in exon 8 of the presenilin-1 (PS-1) gene was detected in an Alzheimer's disease pedigree. Neuropathological examination of affected individuals identified variant, large, non-cored plaques without neuritic dystrophy, reminiscent of cotton wool plaques. Biochemical analysis of L271V mutation showed that it increased secretion of the 42-amino acid amyloid-β peptide, suggesting a pathogenic mutation. Analysis of PS-1 transcripts from the brains of two mutation carriers revealed a 17–50% increase in PS-1 transcripts with deletion of exon 8 (PS-1Δexon8) compared with unrelated Alzheimer's disease brains. Exon trapping analysis confirmed that L271V mutation enhanced the deletion of exon 8. Western blots of brain lysates indicated that PS-1Δexon8 was overexpressed in an affected individual. Biochemical analysis of PS-1Δexon8 in COS and BD8 cells indicated the splice isoform is not intrinsically active but interacts with wild-type PS-1 to generate amyloid-β. Western blots of cell lysates immunoprecipitated with anti-Tau or anti-GSK-3β antibodies indicated that PS-1Δexon8, unlike wild-type PS-1, does not interact directly with Tau or GSK-3β, potential modifiers of neuritic dystrophy. We postulate that variant plaques observed in this family are due in part to the effects of PS-1Δexon8 and that interaction between PS-1 and various protein complexes are necessary for neuritic plaque formation.

The presence of senile plaques is one of the key neuropathological features of familial as well as sporadic forms of Alzheimer's disease (AD). 1 Senile plaques are extracellular deposits composed mainly of the amyloid-β peptide (Aβ), which is cleaved by a series of secretases from the amyloid precursor protein (APP) (1). Mutations in any of three genes, APP, presenilin-1 (PS-1), or presenilin-2 (PS-2), give rise to early onset familial AD (EOFAD). Mutations in the PS-1 gene are associated with severe neuropathology. Typically, the brains of affected individuals have a large number of diffuse as well as cored neuritic plaques that are deposited in the cerebral cortex and in regions not normally involved in AD, such as the cerebellum (2). Moreover, there is intense Tau pathology with neuritic dystrophy around the plaques and neurofibrillary tangles (3, 4). However, four mutations in PS-1, an in-frame deletion of the exon 9 sequence (PS-1Δexon9) (5, 6), a two-amino acid deletion (ΔI83ΔM84) (7), a P436Q (8), and a E280G (9), have been shown to be associated with a variant "cotton wool" plaque pathology. Brains from individuals carrying these specific mutations have extensive deposition of large spherical plaques that lack distinctive cores and neuritic pathology. Biochemical analysis of cells transfected with PS-1 cDNAs, carrying any of these mutations, secrete exceptionally high levels of Aβ1–42 (8). This suggests that PS-1 has a role not only in determining the levels of different Aβ species but also in the morphology of the plaques and neuritic dystrophy.

The range of functions of the wild-type PS-1 protein are still being defined, although a primary function may be to serve as the γ-secretase, which cleaves the APP molecule to generate Aβ40–41 and Aβ1–42 (10). PS-1 has also been shown to interact with various proteins, including Tau (10), GSK-3β (11, 12), and nicas- trin (13). Immunohistological examination of sporadic and familial AD brains reveals that PS-1 protein is associated with the amyloid cores of the senile plaques and dystrophic neurites (14, 15), as well as neurofibrillary tangles, the other neuropathological feature of AD (15, 16). Whether protein interactions with PS-1 are actively required for plaque formation, or neuritic dystrophy, has not yet been demonstrated. We report the genetic and functional analysis of a novel mutation (L271V) in the PS-1 gene in an EOFAD pedigree with a variant neuropathology. The missense mutation also results in the production of PS-1 that lacks exon 8. We show that exon 8 sequences of the PS-1 molecule interact with Tau and GSK-3β, a candidate protein kinase that can cat-

protein; APP, amyloid precursor protein; EOFAD, early onset familial AD; ELISA, enzyme-linked immunosorbent assay; GSK, glycogen synthase kinase; PS-1 and -2, presenilin-1 and -2; IP-MS, immunoprecipitation-mass spectrometry; RT-PCR, reverse transcription-PCR.

* This work was supported by Department of Veteran Affairs (Australia) Grant 9937441 (to J. B. J. K., W. S. B., and P. R. S.), National Health and Medical Research Council (Australia) grants, Project Fellowship Grant 113804 (to G. M. H.), Block Grant 99050 (to P. R. S.), Network Grant 983302 (to J. B. J. K., W. S. B., and P. R. S.), and National Institutes of Health Grant NIA AG10491 (to S. E. G. and R. W.).

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2 To whom correspondence should be addressed: Garvan Institute of Medical Research, 384 Victoria St., Darlinghurst, Sydney, New South Wales 2010, Australia. Tel.: 61-2-9295-8285; Fax: 61-2-9295-8281; E-mail: p.schofield@garvan.org.au.

3 The abbreviations used are: AD, Alzheimer's disease; Aβ, amyloid-β
amplification using RNA isolated from an affected pedigree member as mutation L271V was introduced into the PS-1 cDNA by oligonucleotide—mammalian expression vector, pRc/CMV (Invitrogen). The missense total RNA was isolated for exon trap analysis (17).

EXPERIMENTAL PROCEDURES

Clinical Description—The Tas-1 family includes 13 affected individuals over three generations in a pattern consistent with autosomal dominant inheritance (Fig. 1A). The clinical features and disease progression in the five affected individualsa were consistent with AD, with initial difficulties in activities of daily living followed by progressive memory, language, and visuospatial deficits leading to severe dementia over a span of several years. All developed myoclonus late in their illness. In contrast to some other families with variant plaques (5, 6, 8), none of the members examined had spastic paraparesis, although individual III.30 had pathologically brisk reflexes. The onset of symptoms ranged from 43 years of age to the early sixties (mean, 49 years); the age at death ranged from 52 to 65 years, with one affected individual (III.22) still living at 68 years.

Neuropathology—The brains of two affected pedigree members were obtained at autopsy for neuropathological examination (17). Standardized neuropathological criteria were used for diagnosis (18, 19).

Genetic Analyses—Intronic polymerase chain reaction (PCR) primers were used for amplification of PS-1 gene (20). PCR products were sequenced using Big Dye chemistry on an ABI377 sequencer (Applied Biosystems). Linkage analysis of the Tas-1 pedigree was performed using the MLINK program (21). A mutant allele frequency of 0.01 was used in the analysis.

RT-PCR Analysis of PS-1 mRNA—Total RNA was extracted from cell lines or frozen brain tissue using the SV Total RNA Isolation System (Promega). 2 μg of RNA was reverse-transcribed using the Superscript II RT enzyme (Invitrogen) and a poly(dT) primer (Invitrogen) followed by PCR using the primers PS1 exon7F (5′-TCCTGGAATGGAAGGTTGGCTAC-3′) and PS1 exon8R (5′-CTTTGAGCTTCCCAGGTCTCT-3′). The relative ratio of PCR products with and without exon 8 sequences was determined semiquantitatively by PCR amplification using 0.2 μg of cDNA template and 32P end-labeled PS1 exon7F/PS1 exon8 primer (17).

Exon Trapping Analysis—PCR products, which contained either the wild type or the L271V mutation in exon 8 along with 62 and 99 bp of 5′ and 3′ flanking intronic sequence, were subcloned into the exon trap vector pBSL3 (Invitrogen). HEK293 and COS-7 cells were transfected with the exon trap constructs using the Fugene 6 reagent (Roche Molecular Biochemicals). Cells were collected 48 h post-transfection, and combined conditioned medium was collected after 24 h. 500 μl of conditioned medium was assayed for the presence of the L271V mutation and the disease phenotype. Linkage analysis of the Tas-1 pedigree was performed using the SignalSelect β-amyloid ELISA kits (BIOSOURCE Int.). For the assay of truncated β species, transfection medium was removed and replaced with 2 ml of medium containing reduced growth medium (0.2% fetal calf serum)/10-cm plate, and conditioned medium was collected after 24 h. 500 μl of conditioned medium was assayed for the presence of β species by immunoprecipitation/mass spectrometry (IP-MS) β assay (23). For the luciferase-based assay of γ-secretase activity in BDB (PS-1/PS-2 knockout) cells, PS-1 cDNAs were transiently transfected into cells and assayed for activity after 24 h as described (24).

Metabolic Labeling and Immunoprecipitation of PS-1 Isoforms in Transfected COS-7 Cells—COS-7 cells in six-well plates were transfected with PS-1 cDNAs as described above. After 48 h, cells were starved for 2 h in methionine- and serum-free COS medium. Cells were metabolically labeled with 200 μCi of [35S]methionine for 30 min and then chased with COS medium with excess unabeled methionine (0.8 mM) for 4 or 6 h (25). Cells were lysed in 1× lysis buffer (90 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1× complete mixture protease inhibitor (Roch Molecular Biochemicals)) and 0.01% Tween 20 and immunoprecipitated as described (11) using a rat monoclonal antibody against the amino terminus of PS-1 (Chemicon). Protein lysates were heated to 50 °C for 10 min prior to electrophoresis on a 10% SDS-PAGE and transfer to a nitrocellulose membrane (Transblot transfer medium, Bio-Rad).

Western blot Analysis of PS-1 Isoforms—COS-7 cells were transfected with PS-1 cDNAs as described above. After 48 h, cells were pelleted and lysed in 1× lysis buffer and 0.1% Triton X-100. Crude membrane extracts of brain tissue were also made (11). Protein lysates were heated to 50 °C for 10 min prior to electrophoresis on a 7.5% SDS-PAGE and transfer to a nitrocellulose membrane. A rat monoclonal antibody (Chemicon), a mouse monoclonal antibody (NT1) raised against residues 1–25 of PS-1 (26) and a rabbit polyclonal antibody (Ab14) raised against residues 1–25 of PS-1 (27) were used to detect PS-1 protein. Co-immunoprecipitation experiments to detect interactions between different PS-1 isoforms and Tau or GSK-3β were performed as described previously (11).

RESULTS

PS-1 Mutation (L271V) in Tas-1 Pedigree—An affected individual (III.28) was examined for nucleotide substitutions in the PS-1 gene. As shown in Fig. 1B, the electropherogram traces indicate a C to T mutation that substitutes a valine for a leucine at codon 271 (L271V). This mutation is situated within exon 8 of the PS-1 gene. An additional 17 pedigree members, including four affected individuals, were examined for the presence of the L271V mutation. The affected individuals were found to carry the mutation, consistent with L271V being the pathogenic mutation within this pedigree. Linkage analysis of the co-segregation of the L271V mutation and the disease phenotype revealed a positive logarithm of odds ratio; score (LOD = 1.8, θ = 0).

Variant Neuropathology—Two affected members of the pedigree were examined neuropathologically. Subject III.28 died of pulmonary emboli at the age of 60 years, whereas III.32 died of bronchopneumonia aged 57. Macroscopically, both brains had...
considerable atrophy of the temporal and posterior white matter with enlargement of the lateral ventricles and narrowing of the corpus callosum. Subject III:28 had additional atrophy of the frontal and superior temporal gyri with some loss of white matter in the centrum semiovale. The locus coeruleus was depigmented in both cases. Microscopically, the most dramatic pathology was the large number of neocortical plaques, which resulted in a CERAD (Consortium to Establish a Registry for Alzheimer’s Disease) pathology grading of severe. These plaques were particularly prominent on the sections immunohistochemically stained for Aβ (Fig. 2, A and B) but were also visible as large diffuse plaques without neuritic dystrophy in the silver-stained sections (Fig. 2, C and D). These were reminiscent of the cotton wool plaques described in the Finnish EOFAD pedigree (5). In III:28, all plaques in all regions were diffuse and without neuritic dystrophy or cores (Fig. 2, A and C), whereas neuritic and cored plaques were observed in III:32 in the hippocampus (Fig. 2F). However, the large diffuse cotton wool plaques were the most numerous plaque type in both cases (Fig. 2, A and B). Despite the lack of neurites within the plaques, both cases had significant numbers of neurofibrillary tangles using either a silver stain or Tau immunohistochemistry (Fig. 2, E–H). These cases reached a neocortical stage for tangle formation (19), substantiating their clinical diagnosis as AD. Amyloid angiopathy was also prominent in both cases.

Biochemical Analysis of the L271V Mutation—To determine the effects of the PS-1 (L271V) mutation on the processing of APP, wild-type and mutant forms of the PS-1 cDNA were transiently co-transfected with APP(Swedish) cDNA into COS-7 cells, and the amount of secreted Aβ was measured by ELISA. The L271V mutation resulted in a significant 1.3-fold increase (p < 0.05, Student’s t test) in the secretion of Aβ1–42 compared with the wild-type sequence (Table I), in the same manner as another pathogenic missense mutation, L286V (28), which also showed a 1.3-fold increase in Aβ1–42 levels. Our assay also demonstrated that the PS-1Δexon 9 mutation results in an exceptionally high, 2-fold increase in production of Aβ1–42 compared with other PS-1 mutations (8) (Table I).

Specific mutations in the APP gene have been shown to alter the processing of APP to preferentially generate P3, the peptide cleaved at position 17 (Aβ17–40 and Aβ17–42), as well as a series of amino-terminally truncated Aβ species (29, 30). To determine whether the PS-1 L271V mutation altered processing of APP in a similar manner, the conditioned media from triplicate transfections were analyzed for the presence of the major P3 species or any truncated Aβ species by IP-MS (23). As shown in Fig. 3, PS-1 L271V mutation did not result in the generation of P3. Similarly, P3 was undetectable in the conditioned media of cells transfected with the PS-1Δexon 9 cDNA. Our assays were capable of detecting the full-length Aβ species corresponding to Aβ1–40 and Aβ1–42 in all of the PS-1 transfections. Moreover, the ratios of Aβ1–42/Aβ1–40 were consistent with the results obtained using an ELISA, with all mutant PS-1 cDNAs increasing the secretion of Aβ1–42 compared with the wild-type PS-1 (Table I).

L271V Mutation Increases Splicing Out of PS-1 Exon 8—Exonic mutations have previously been shown to alter the level of splicing of their cognate exons (31, 32). We examined RNA transcripts extracted from the brains of two mutation carriers (III:28 and III:32) for the presence of PS-1 splice isoforms by RT-PCR using primers that flanked exon 8. The level of the splice isoform was determined semiquantitatively (Fig. 4A). PS-1 RNA transcripts from the two affected mutation carriers were detected at a ratio of 4:5, with the wild-type PS-1 transcript at a ratio of 2:3. The splice isoform was not detected in the brains of other PS-1 carriers (Table II). Aβ secretion induced expression of by PS-1 cDNAs

| Table I |

| | ELISA | | IP-MS |
|---|---|---|---|
| | Aβ1–40 | Aβ1–42 | Aβ1–42/Aβ1–40 | Aβ1–42/Aβ1–40 |
| | pmol/ml | pmol/ml | ×100 | ×100 |
| wt | 1647 ± 377 | 264 ± 36 | 16.4 ± 2.1 | 13.8 ± 1.7 |
| L271V | 1229 ± 409 | 261 ± 49 | 22.6 ± 5.7 | 20.1 ± 2.1 |
| L286V | 1316 ± 271 | 279 ± 46 | 21.4 ± 1.7 | 25.6 ± 2.1 |
| Δexon9 | 1063 ± 356 | 356 ± 78 | 34.1 ± 3.9 | 31.1 ± 1.9 |
| Δexon8 | 1536 ± 236 | 239 ± 23 | 15.8 ± 1.4 | 18.9 ± 1.5 |

p < 0.05.
*p < 0.005.
**p < 0.0005.
were found to have ~17–50% increased levels of PS-1Δexon8 compared with RNAs isolated from two unrelated EOFAD brains (which do not have mutations in the PS-1 gene) and the neuroblastoma cell line SK-N-MC.

Exon trapping analysis (17) was used to examine the effects of the L271V mutation on the efficiency of splicing of PS-1 exon 8. Reverse transcription-PCR of exon trap products yielded two PCR products, which correspond to either the splicing in or the deletion of exon 8 (Δexon8). In both the HEK293 and COS-7 cell lines, the presence of the L271V mutation resulted in significantly more of the Δexon8 product compared with wild-type sequence (Fig. 4B). The increase in exon trap Δexon8 PCR products for the L271V mutation was determined semiquantitatively in both the HEK293 and COS-7 cells and was shown to be significantly increased by 35 and 70% (p < 0.05, Student’s t test), respectively, compared with wild-type sequence.

To confirm that PS-1Δexon8 was overexpressed in the brain of III:32, we performed Western blot analysis on the membrane fractions of proteins extracted from frontal cortical brain tissue in addition to COS-7 cells transfected with PS-1 cDNAs. As shown in Fig. 4C, a distinct 47-kDa polypeptide corresponding to PS-1Δexon8 was detected in the COS-7 cell lysate (transfected with the PS-1Δexon8 cDNA) and in the brain extracts of EOFAD2 and III:32 as detected by Western blot analysis using the NT1 antibody. The 47-kDa band can be distinguished from a 50-kDa band, which corresponds to the full-length wild-type PS-1 molecule. The same size bands were also detected when either the rat monoclonal PS-1 antibody or rabbit polyclonal PS-1 antibody (Ab14) were used for Western blotting (data not shown). Nonspecific bands (Fig. 4C) were detected in the brain extracts when the NT1 antibody was used but were not detected with the other two PS-1 antibodies.

Functional Analyses of PS-1Δexon8—We examined the stability of the PS-1Δexon8 isoform in metabolically labeled COS-7 cells transfected with PS-1 cDNAs. The PS-1Δexon9 molecule is normally rapidly degraded via the proteosome pathway (25). As shown in Fig. 5, A and B, newly synthesized full-length PS-1 molecules, uncleaved by the presenilinase, are rapidly degraded over a span of several hours. However, PS-1Δexon9 appeared to be the most stable of the PS-1 isoforms. Six hours after the cells were pulse-labeled, there was a 1.2–1.6-fold increase in the PS-1Δexon8 isoform compared with wild-type (p = 0.32, Student’s t test) and PS-1Δexon9 isoforms (p < 0.05, Student’s t test), respectively.

The PS-1Δexon8 splice isoform did not have an effect on the secretion of Aβ1–42 compared with wild-type cDNA when measured using an ELISA of transiently transfected cells (Table I). This finding is in agreement with previous studies of the effect of PS-1Δexon8 in stably transfected cell lines (33, 34). However, when we examined all of the Aβ species in the conditioned media using IP-MS, we found a small increase in the ratio of Aβ1–42/1–40 in the cells transfected with PS-1Δexon8 (0.19) compared with the wild-type cDNA (0.14) (Table I).
Moreover, two new peaks were detected in the mass spectra that correspond to carboxyl-terminally truncated Aβ species, Aβ1–19 and Aβ1–28. These Aβ species were not detected in the conditioned media of cells transfected with wild-type, L271V, or PS-1Δexon9 cDNAs (Fig. 3).

Measurement of total Aβ in COS cells transfected with either a lacZ control vector or PS-1 cDNAs was estimated by a combined ELISA of Aβ1–40 and Aβ1–42. As shown in Fig. 5C, PS-1Δexon8, in the same manner as the other PS-1 cDNAs, significantly increased Aβ secretion compared with cells expressing only endogenous PS-1 (which correspond to the lacZ control). This is similar to the results reported for stably transfected cell lines (34) and suggests that PS-1Δexon8 was capable of supporting the generation of Aβ. To determine whether PS-1Δexon8 has intrinsic γ-secretase activity, the splice isoform was transfected into BD8 (PS-1/PS-2 knockout) cells (24). In contrast to COS cells, which contain endogenous PS-1, the BD8 cells were unable to support the generation of Aβ in the presence of PS-1Δexon8, although the wild-type and mutant PS-1 cDNAs restored this activity to levels similar to that observed in COS cells with endogenous presenilins (Fig. 5D). Moreover, we noted, for the mutant PS-1 cDNAs, that an increase in the production of Aβ1–42 (Table I) was associated with an overall decrease in the secretion of total Aβ (Fig. 5, C and D).

Deletion mutants of PS-1 have defined a region spanning amino acids 250 to 298 that is thought to bind to both Tau and GSK-3β (11) and includes the sequence encoded by exon 8 (amino acids 257–289). Thus, the splice isoform PS-1Δexon8 would not be expected to interact with either Tau or GSK-3β. We examined whether this was the case for GSK-3β by co-immunoprecipitation of lysates from cells transfected with PS-1 cDNAs. As shown in Fig. 6A, wild-type PS-1 was detected by Western blot analysis in lysates immunoprecipitated with an anti-GSK-3β antibody. In contrast, no immunoprecipitated PS-1Δexon8 protein was detected. In the same manner, the PS-1 deletion mutant, which lacks amino acids 251–467 (PS-1N250), also failed to be immunoprecipitated by the anti-GSK-3β antibody. A similar result was obtained for the co-immunoprecipitation experiment between PS-1 and Tau (Fig. 6B). Finally, the co-immunoprecipitation experiment was repeated to determine whether PS-1Δexon9, which lacks amino acids residues 290–319, had the same effect as the PS-1Δexon8 isoform. As shown in Fig. 6C, PS-1Δexon9 did co-immunoprecipitate with GSK-3β. The binding affinity between the PS-1 protein and GSK-3β can be estimated semiquantitatively by the difference in levels of protein detected in the crude lysate and the immunoprecipitated fractions. Thus, PS-1Δexon9, like PS-1Δexon8, appears to bind with lesser affinity to GSK-3β than wild-type PS-1 (Fig. 6C).

**DISCUSSION**

We describe an EOFAD pedigree with a novel missense (L271V) mutation in the PS-1 gene that is associated with a variant neuropathology. Bielschowsky silver stain revealed that the majority of plaques in two affected individuals (III:28 and III:32) were large spherical deposits without defined cores or neuritic dystrophy (Fig. 2, C and D). The morphology of the plaques is reminiscent of the cotton wool plaques described in other studies (5–8). Two lines of evidence demonstrate that the PS-1 L271V mutation is the pathogenic variant within the Tas-1 pedigree. First, the mutation segregates with the EOFAD phenotype within the pedigree. Second, in vitro measurements of Aβ levels using either an ELISA or by IP-MS indicate that the L271V mutation increases Aβ1–42 secretion to comparable levels to other characterized PS-1 mutations. However, the simple elevation of Aβ1–42 secretion does not explain the presence of the variant plaques that occur in this pedigree.

The correlation between specific mutations in the APP and PS-1 genes and the distinctive neuropathology in affected individuals has led to the definition of two possible molecular mechanisms in the formation of cotton wool plaques. First, the APP T714I was postulated to play an integral role in the formation of the variant cotton wool form of senile plaques, through the generation of P3 and the amino-terminal truncated Aβ species (29). However, we did not detect the presence of P3 in conditioned media from cells transfected with the L271V cDNA. Furthermore, we were also unable to detect the presence of the truncated Aβ species in cells transfected with the PS-1Δexon9 cDNA, another PS-1 mutation associated with cotton wool plaques (5, 6). This suggests that, unlike the APP T714I mutation, cotton wool plaques found in PS-1 L271V mutation carriers are not attributable to the production of P3.
GSK-3 from COS-7 cells transfected with PS-1 cDNAs. Western blot analysis

However, levels of Aβ1–42 in the Cotton wool plaques in our pedigree, at least by the two mechanisms proposed above. Exonic mutations can have the unexpected consequence of altering the splicing of their cognate exons. For example, the exonic deletion ΔK280 mutation in the tau gene, MAPT (microtubule-associated protein Tau) have been shown to decrease the splicing in of exon 10 of the gene due to the disruption of a purine-rich exon splicing enhancer (32). We demonstrate that the L271V mutation has a similar effect on the alternative splicing of exon 8 of the PS-1 gene, possibly by a similar mechanism. RT-PCR and Western blot analysis of PS-1 expression in the frontal cortex of the two mutation carriers (II:28 and III:32) revealed a 17–50% increase in levels of PS-1Δexon 8 splice isoform compared with unrelated EOFAD brains and a neuroblastoma cell line (Fig. 4, A and C). The Δexon8 isoform is not proteolytically cleaved like the wild-type molecule (33, 34), which may effect its stability in vivo. This hypothesis was supported by our pulse-chase study of PS-1 isoforms, which demonstrated that PS-1Δexon8 was not as rapidly degraded as either the wild-type or PS-1Δexon9 isoform (Fig. 5B). Thus, the modest increase in the level of PS-1Δexon8 transcripts would lead to accumulation of higher levels of the Δexon8 isoform than the full-length wild-type molecule (Fig. 4C), and have functional consequences.

There has been debate concerning whether PS-1 is the actual γ-secretase catalytic enzyme or whether PS-1 forms part of a larger heteromeric complex that facilitates the positioning of γ-secretase and its substrates (35). Our analysis of γ-secretase activity associated with transfected PS-1 cDNAs using ELISA or the luciferase-based reporter system (Fig. 5, C and D) may provide some insight into this debate. First, the ELISA measurements of γ-secretase activity of the L271V and PS-1Δexon9 mutants indicated that an increase in the secretion of Aβ1–42 was associated with a concomitant decrease in the production of total Aβ (which consists mostly of Aβ1–40) (Fig. 5, C and D). This suggests that the mutations resulted in an alteration of PS-1 activity, such that the mutant PS-1 was more efficient in cleavage of Aβ1–42 than Aβ1–40. This is supported by the nonsteroidal anti-inflammatory drug, sulindac sulfide, which has been shown to inhibit selectively the production of Aβ1–42 while increasing the carboxyl-terminal truncated Aβ1–38 species (36). The ELISA indicated that PS-1Δexon8 might be capable of supporting the generation of Aβ (Fig. 5C), consistent with a similar study using stably transfected HEK cell lines (34). To remove the effects of endogenous presenilins on the ability of the PS-1 cDNAs to generate Aβ, this experiment was repeated in PS-1/PS-2 knockout cells (24). As shown in Fig. 5D, the PS-1Δexon8 isoform was unable to support the generation of Aβ. We inferred that in the COS-7 cells, PS-1Δexon8 must form oligomers with endogenous PS-1 to give rise to the modified γ-secretase activity. This hypothesis is supported by studies showing that oligomeric PS-1 is a high molecular weight complex in vivo (37) and that PS-1 molecules exist as homodimers with direct interactions between adjacent PS-1 amino-terminal fragments (38). The mechanism by which the unusual carboxyl-terminally truncated Aβ species (Aβ1–19 and Aβ1–28) are generated when PS-1Δexon8 is transfected into wild-type cells (Fig. 3) remains unknown. However, a recent IP-MS study using a series of specific γ-secretase inhibitors demonstrated that there was a wide number of apparently authentic cleavage sites for the enzyme, ranging from position 19 to 42 (39). The issues outlined above are unlikely to be resolved until the γ-secretase activity can be reconstituted in vitro.

A key feature of the cotton wool plaques is the lack of neuritic dystrophy. Neuritic degeneration may depend on the level of Tau hyperphosphorylation (40), and GSK-3β appears to play a crucial role in this process, as GSK-3β conditional transgenic mice displayed increased Tau hyperphosphorylation (41). PS-1 has been proposed to interact with GSK-3β (11, 12) and Tau (11). In addition, PS-1 is co-localized to neuritic processes in AD brains (14–16). These studies consistently suggest a vital role for PS-1 in influencing the neuritic activity surrounding the senile plaques by bringing together GSK-3β and its substrate Tau during the formation of the plaques. Our co-immunoprecipitation experiments demonstrate that the PS-1Δexon8 splice isoform did not interact with GSK-3β or Tau (Fig. 6). This finding is supported by the observation that PS-1Δexon9, another mutation associated with plaques that lack neuritic dystrophy (5), also appears to have a lower affinity for GSK-3β as assessed by co-immunoprecipitation (Fig. 6C). Thus, we propose that the lack of neuritic dystrophy observed in the senile plaques of affected individuals within this pedigree (II:28 and III:32) is due to the
increased levels of the PS-1Δexon8 molecule. It would be of interest to determine whether the variable levels of neuritic dystrophy displayed in other pedigrees (6) might correlate with levels of PS-1Δexon8.

Cotton wool plaques have been associated in some AD pedigrees with the neurological disorder, spastic paraparesis (5, 6). However, it has been suggested that both cotton wool plaques and spastic paraparesis are variously expressed in these pedigrees as the outcome of phenotypic modifiers (6). Accordingly, some PS-1Δexon9 pedigrees have been reported to lack cotton wool plaques or spastic paraparesis (42, 43). The Tas-1 family is of interest because there is no indication of spastic paraparesis in the mutation carriers. The relationship of the phenotype to the specific nature of the mutation remains to be determined. The presentation of the neuropathology also differs from other pedigrees with cotton wool plaques. In several pedigrees with PS-1Δexon9 mutations, the distribution of core neuritic plaques and cotton wool plaques was fairly even (5, 6). In the Tas-1 pedigree, core neuritic plaques were found only in the hippocampus of one patient. This distribution of core and cotton wool plaques is similar to that described in a patient with an APP T714I missense mutation (27). However, the biological significance of this distribution of neuritic dystrophy remains unknown and may simply reflect the usual relatively high intensity of Tau pathology in the hippocampus of AD brains.

The EOFA D family, Tas-1, offers a unique opportunity to study the secondary processes after the initial overproduction of Aβ peptides by mutant PS-1. Our data provides the first example of a pathogenic PS-1 mutation that generates a molecule that cannot support Aβ production on its own but causes aberrant carboxyl truncated Aβ species to be generated when endogenous wild-type presenilins are present. Our data suggest a central role for PS-1 not only in determining the overall level of Aβ but also in specifying the morphology of plaques and neuritic dystrophy. Moreover, identification of factors that affect the splicing of PS-1 may also be of importance in understanding the pathogenesis of AD, as the PS-1Δexon8 isoform has biochemical properties that differ from those of wild-type, full-length PS-1. Correlation of the specific neuropathology of AD cases with genetic mutations will lead to a better understanding of the role that PS-1 plays in the disease process.

Acknowledgments—We thank Heather McCann and Heidi Cartwright for laboratory assistance. We are grateful to Dorit Donoviel for providing laboratory assistance. We are grateful to Dorit Donoviel for providing

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