American Journal of Pathology, Vol. 152, No. 4, April 1998

Short Communication

Light and Electron Microscopic Immunolocalization of Presenilin 1 in Abnormal Muscle Fibers of Patients with Sporadic Inclusion-Body Myositis and Autosomal-Recessive Inclusion-Body Myopathy

Valerie Askanas,* W. King Engel,*
Chih-Chao Yang,* Renate B. Alvarez,*
Virginia M.-Y. Lee,† and Thomas Wisniewski‡

From the USC Neuromuscular Center, Department of Neurology, University of Southern California School of Medicine, Good Samaritan Hospital, Los Angeles, California, the Department of Pathology & Laboratory Medicine,† University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, and the Department of Neurology,‡ New York University Medical Center, New York, New York*

Sporadic inclusion-body myositis (s-IBM) is the most common progressive muscle disease of older persons. The muscle biopsy demonstrates mononuclear cell inflammation and vacuolated muscle fibers containing paired helical filaments and 6- to 10-nm fibrils, both resembling those of Alzheimer disease brain and Congo red positivity. The term hereditary inclusion-body myopathies (h-IBMs) designates autosomal-recessive or autosomal-dominant disorders with muscle biopsies cytopathologically similar to s-IBM but without inflammation. Vacuolated muscle fibers of both s-IBM and the h-IBMs contain accumulations of several "Alzheimer-characteristic proteins" including β-amyloid protein and β-amyloid precursor protein, and their paired helical filaments are composed of phosphorylated tau. We used six well characterized antibodies against several residues of presenilin 1 (PS1) to immunostain muscle biopsies of 12 patients with s-IBM, 5 patients with autosomal-recessive inclusion-body myopathy, and 16 normal and disease controls. Seventy to eighty percent of the vacuolated muscle fibers of both s-IBM and autosomal-recessive inclusion-body myopathy had inclusions that were strongly PS1-immunoreactive, which by immunoelectron microscopy localized mainly to paired helical filaments and 6- to 10-nm filaments. None of the control biopsies had PS1-positive inclusions characteristic of the s- and h-IBM abnormal muscle fibers. Mutations of the newly discovered PS1 gene are responsible for early-onset familial Alzheimer disease (AD), and PS1 is abnormally accumulated in sporadic and familial AD brain. Our study provides the first demonstration of PS1 abnormality in non-neural tissue and in diseases other than AD and suggests that the cytopathogenesis in AD brain and IBM muscle may share similarities. (Am J Pathol 1998, 152:889–895)

Sporadic inclusion-body myositis (s-IBM) is the most common muscle disease beginning in persons age 55 years or older.[1] It is of unknown etiology and pathogenesis, and leads to severe disability. Light microscopic features of the s-IBM muscle biopsy include various degrees of mononuclear-cell inflammation, muscle fibers with characteristic vacuoles on Engel-Gomori trichrome staining,[2] and atrophic muscle fibers.[1] On a given section, 60 to 80% of the vacuolated muscle fibers contain foci positive with Congo red, thioflavine S, or crystal violet.[1,3–5]

Hereditary inclusion-body myopathies (h-IBMs) encompass several syndromes, each characterized by progressive muscle weakness but having somewhat different clinical manifestations.[1] h-IBMs are inherited either as an autosomal-recessive or autosomal-dominant trait.[1] Abnormal genes have not yet been identified. In autosomal-recessive h-IBM characterized by relative sparing of quadriceps muscle, linkage to chromosome 9p1-q1 has been established,[6–8,9] but the abnormal gene has been not yet identified; this h-IBM has been designated as AR1-IBM.[8] The clinical onset of h-IBM is usually in the

Supported in part by grants from the National Institutes of Health and Muscular Dystrophy Association (V. Askanas). C.-C. Yang was supported by the Taiwan National University Hospital.

Accepted for publication January 22, 1998.

Address reprint requests to Dr. Valerie Askanas, USC Neuromuscular Center, 637 South Lucas Avenue, Los Angeles, CA 90017-1912.

second or third decade or sometimes earlier.[1] Muscle biopsies of the h-IBMs contain vacuolated muscle fibers similar to s-IBM but with no inflammation. To emphasize the hereditary aspect and lack of inflammation, we introduced the term "hereditary inclusion-body myopathy".[10] In contrast to s-IBM, in h-IBM patients most of the vacuolated muscle fibers lack foci of positivity with Congo red (even with the enhancement technique[11]) or crystal violet.

By electron microscopy, characteristic features of the vacuolated muscle fibers are similar between s- and h-IBM; for example, there are cytoplasmic, 15- to 21-nm diameter paired helical filaments (PHFs) that strikingly resemble PHFs of Alzheimer disease (AD) brain.[5]

A remarkable feature of s- and h-IBM-vacuolated muscle fibers is that a group of proteins are abnormally accumulated in the brain of AD patients. Those include: β-amyloid protein (Aβ),[4] C- and N-terminal regions of β-amyloid precursor protein (βAPP),[12] apolipoprotein E,[13] ubiquitin,[14] and α_1-antichymotrypsin.[15] βAPP mRNA is also increased in s- and h-IBM vacuolated muscle fibers.[16] Like Alzheimer PHFs, IBM PHFs contain hyperphosphorylated tau; however, its expression is qualitatively different between s- and h-IBMs.[17]

Mutations in the recently discovered gene of presenilin 1 (PS1) on chromosome 14 account for the majority of familial early onset AD patients.[18–21] Despite lack of PS1 mutations in sporadic AD, abnormal accumulation of PS1 occurs in the brain of both sporadic and familial AD.[22–27]

To our knowledge, there are no reports concerning abnormality of PS1 in any non-Alzheimer human diseases.

We now report that PS1 is abnormally accumulated in vacuolated muscle fibers of s- and h-IBMs as revealed by light and electron microscopic immunocytochemistry. This study provides the first demonstration of PS1 abnormality in diseases other than AD and in a tissue other than the brain, namely human muscle.

Materials and Methods

Patients

Immunocytochemistry was performed on sections of diagnostic muscle biopsies obtained (with informed consent) from 34 patients, age 34–79 years, with the following diagnoses: s-IBM (n = 12); quadriceps sparing autosomal-recessive IBM (AR1-IBM) (n = 5); polymyositis (n = 5); amyotrophic lateral sclerosis (n = 5); morphologically nonspecific myopathy (n = 2); normal muscle (n = 4). Diagnosis of each patient was based on detailed clinical and laboratory studies, including 18 reaction histochemistries of the muscle biopsy.[28] Biopsies of all IBM patients showed muscle fibers with vacuoles on Engel-Gomori staining,[2] and 15- to 21-nm paired helical filaments by electron microscopy and by SMI-31 immunoreactivity.[29] All s-IBM patients had, in 60 to 80% of their vacuolated muscle fibers, Congo red positivity using fluorescence enhancement.[11]

Antibodies

Six well characterized polyclonal and monoclonal antibodies against several epitopes of PS1, including N-terminal, PS1 loop, and C-terminal domains, were used: 1) mouse monoclonal antibody X-81 reacting with N-terminal epitope of PS1, amino acids 1–81,[30] diluted 1:500; 2) rabbit polyclonal antiserum (α-PS1 loop) reacting with amino acids 263–407 of the PS1 loop,[31] diluted 1:200 to 1:600; 3) mouse monoclonal antibody against a glutathione S-transferase-fusion protein containing amino acid residues 12–69 of the PS1 N-terminal region (V. M.-Y. Lee, unpublished data), diluted 1:500; 4) rabbit polyclonal antiserum (anti-PS1–395) against C-terminal residues 395–410,[24] diluted 1:50 to 1:100; 5) rabbit polyclonal antiserum (anti-PS1–448) against C-terminal residues 448–467,[24] diluted 1:100 to 1:200; and 6) rabbit polyclonal antiserum (4627) against C-terminal residues 450–467,[30] diluted 1:500. The last three antibodies react with the C-terminal epitopes of PS1 and PS2.

Mouse monoclonal antibody SMI-31 (Sternberger Monoclonals, Inc., Baltimore, MD) directed against 200-kd-phosphorylated mammalian neurofilament but recognizing phosphorylated tau in IBM muscle and AD brain[17,29,32] was used to identify bundles of PHFs in double immunofluorescence stainings.

Light Microscopic Immunocytochemistry

Stainings were performed on 10-μm transverse sections of fresh-frozen muscle biopsies using peroxidase-anti-peroxidase (PAP) and fluorescence methods, as described.[4,5,12–17] Incubation in all primary antibodies, except SMI-31, was performed for 18 to 36 hours at 4°C and was followed by a 1-hour incubation with an appropriate secondary antiserum at room temperature.[4,5,12–15]

Controls for staining specificity were: 1) preabsorption of the primary PS1 antibodies with corresponding peptides and 2) omission of the primary antibody or its replacement with nonimmune serum. To block nonspecific binding of antibody to Fc receptors, sections were preincubated with 1:10-diluted normal goat serum as described.[4,5,12–15]

Immunoelectron Microscopy

This was performed using 10-μm unfixed frozen sections adhered to the bottom of 35-mm Petri dishes according to our technique.[4,5,12–15] In brief, after incubation for 36 hours at 4°C in a primary antibody, the sections were incubated in either gold- (78 hours at 4°C) or HRP- (1 hour at room temperature) conjugated secondary antiserum. Adjacent sections were processed for the PAP reaction. Subsequently, the sections were fixed in a 2% paraformaldehyde and 1.2% glutaraldehyde mixture, postfixed in osmium, and embedded in Epon *in situ* in the Petri dish according to our method for cultured muscle.[32] The embedded section in the dish was viewed under phase-contrast microscopy and compared with an adjacent cross-section that had been incubated with the

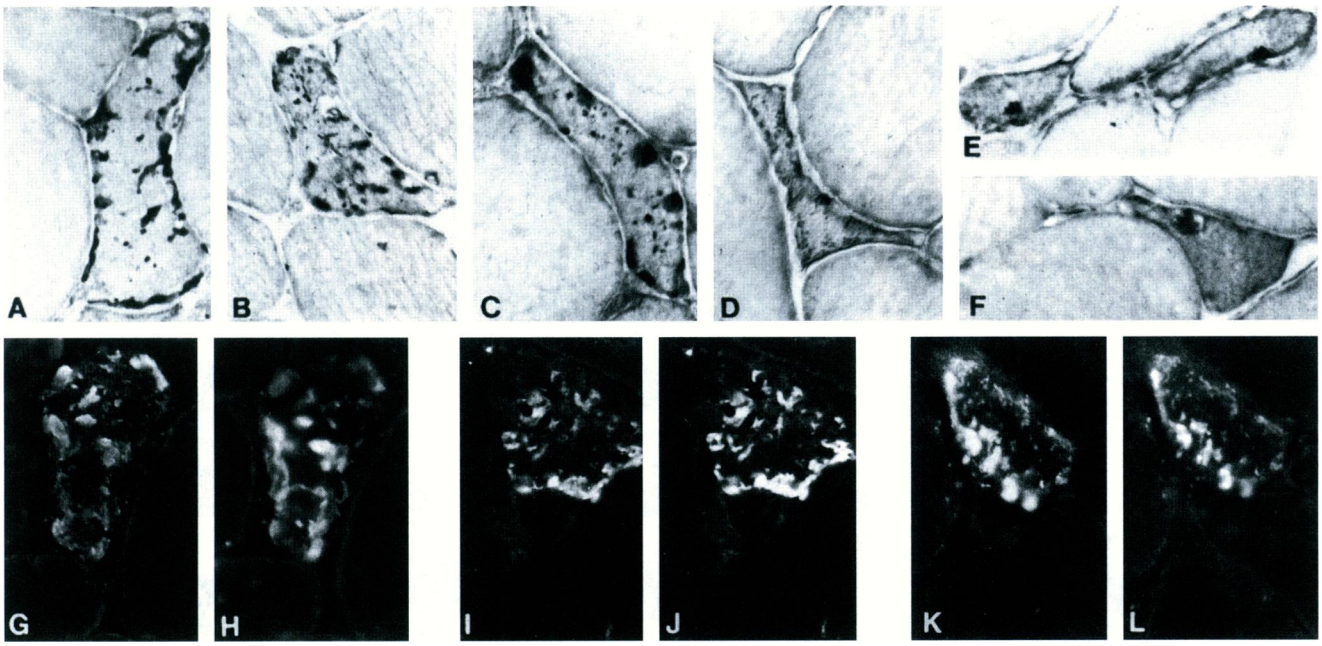

Figure 1. Light microscopic immunocytochemistry of s-IBM (**A** to **C** and **G** to **L**) and h-IBM (**D** to **F**) muscle biopsies. **A** to **F**: PAP reaction. **G** to **L**: Double-labeled immunofluorescence. Antibodies used: X-81 (**A, E,** and **G**); α1 loop PS1 (**B, D,** and **K**); monoclonal against a glutathione S-transferase-fusion protein (**C** and **E**); anti-PS1–448 (**E** and **I**); and SMI-31 recognizing phosphorylated tau (**H, J,** and **L**). With all antibodies in both s- and h-IBM, strongly immunoreactive inclusions of various sizes are present within the abnormal muscle fibers. By double-labeled fluorescence in each pair, respectively, (**G, H, I, J, K,** and **L**) there is a close colocalization between PS1 and SMI-31 immunoreactivities. Fluorescein (**G, I,** and **K**); Texas Red (**H, J,** and **L**). Magnification, ×600.

same antibody as for immunoelectron microscopy but stained with the PAP reaction. The identical vacuolated muscle fibers that contained PAP-positive inclusions were identified in the adjacent gold-labeled Epon-embedded section, were marked, and 1-mm diameter cores were drilled out.[33] From the cores, each containing at least one vacuolated muscle fiber, thin sections were cut, counterstained with uranyl acetate and lead citrate, and examined by electron microscopy.

Results

Light Microscopic Immunocytochemistry

About 70 to 80% of the vacuolated muscle fibers of both s- and h-IBM patients were immunoreactive with PS1 antibodies. PS1 immunoreactivity with all antibodies was in the form of well-defined, squiggly or plaque-like, strongly positive inclusions either within the vacuoles or in the vacuole-free cytoplasm (Figure 1). All antibodies gave exactly the same results except the antibody PS1–395, which gave only a very faint staining. This antibody is considered good for immunoblotting but not for tissue immunostaining (T. Wisniewski, unpublished observation). In s-IBM (Figure 1, A to C), the cytoplasm of most of abnormal fibers did not have diffusely increased immunoreactivity. However, in patients with h-IBM (Figure 1, D to F), some of the vacuolated muscle fibers had, in addition to the inclusions, a diffuse cytoplasmic PS1 immunoreactivity. In double-label fluorescence immunocytochemistry, there was close colocalization between the PS1 and SMI-31 immunoreactive deposits (Figure 1).

Preabsorption with corresponding peptides abolished or markedly diminished the stainings.

None of the control biopsies had PS1-positive inclusions characteristic of the s- and h-IBM abnormal muscle fibers. Strong immunoreactivity of PS1 was present at the postsynaptic domain of NMJ in all biopsies — normal, disease control, and the IBMs (not shown).

In regenerating muscle fibers from patients with various muscle diseases, diffuse cytoplasmic PS1 immunoreactivity was often present (not shown).

Immunoelectron Microscopy

In both s- and h-IBM, ultrastructural immunoreactivities with all antibodies were remarkably similar. They were localized mainly to the PHFs (Figure 2). In addition, PS1 was also localized to 6- to 10-nm diameter amyloid-like fibrils and amorphous material (Figure 2). There was no PS1 immunoreactivity associated with the myofibrils, myelin-like whorls, various lysosomal inclusions, and other pathological and normal structures present in vacuolated muscle fibers from s- or h-IBM patients.

Discussion

PS1 is a newly discovered protein encoded by a gene on chromosome 14.[19] It has 467 amino acids with six to eight transmembrane domains.[34–36] The PS1 mRNA and protein are abundantly expressed in various tissues,[18,30,37] and the expression PS1 mRNA is developmentally regulated.[37] The physiological functions of PS1

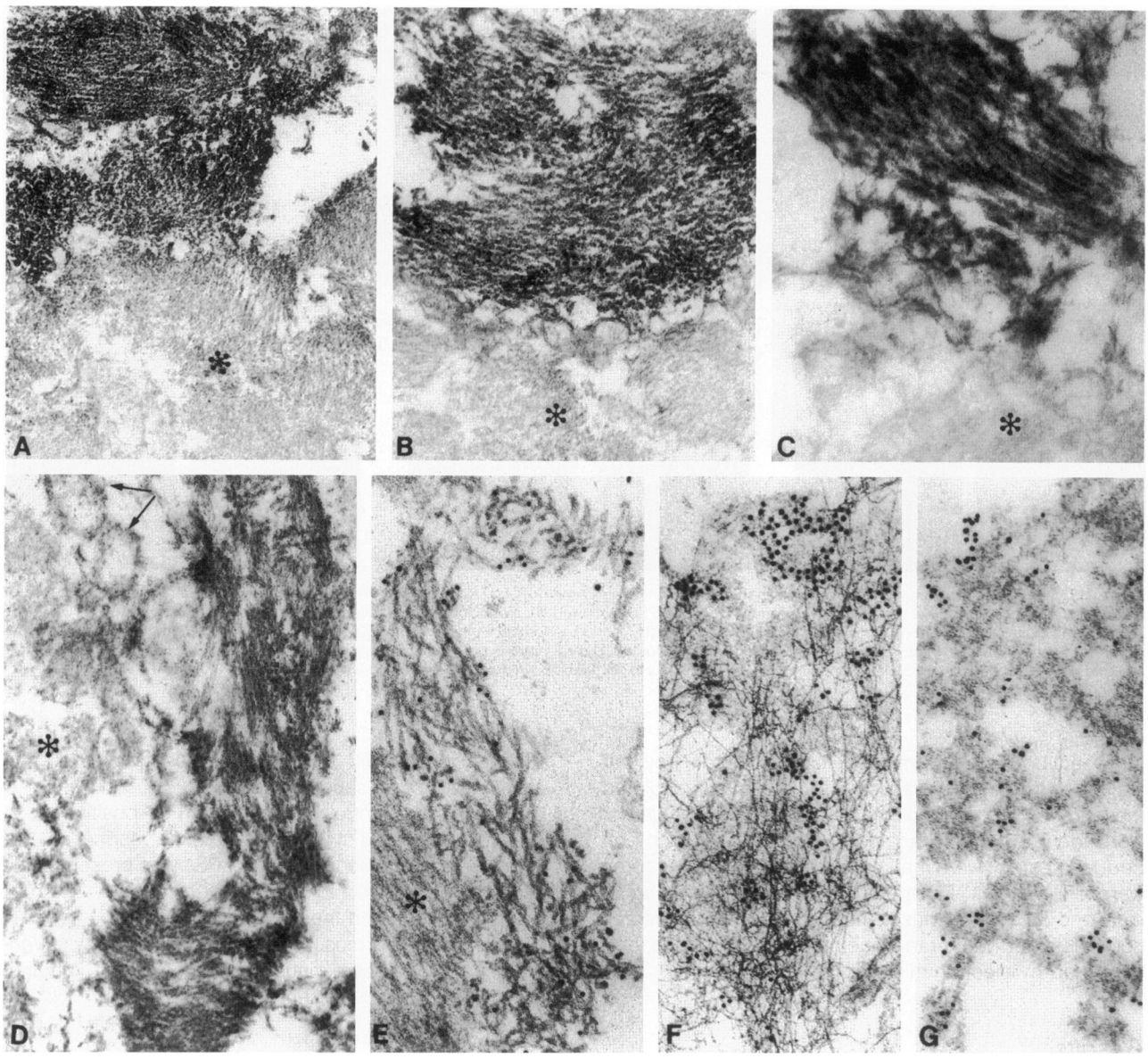

Figure 2. Immunoelectron microscopy of abnormal muscle fibers in hereditary (**A**) and sporadic (**B** to **G**) IBMs. Antibodies used: α1 loop PS1 (**A, D, F,** and **G**); X-81 (**B**); and monoclonal against a glutathione S-transferase-fusion protein (**C** and **E**). In **A** to **E**, immunoreactivity is localized strictly to the paired-helical filaments (in a cluster), whereas an adjacent portion of a normal myofibril (indicated by an **asterisk** in each photograph) and other structures (indicated by **arrows** in **D**) are not immunostained. **A** to **D:** Peroxidase reaction; **E, F,** and **G:** gold immunoelectron microscopy using 10-nm gold particles. In **F**, gold particles partially label 6- to 10-nm amyloid-like fibrils; in **G** they label amorphous material. Magnification, ×15,000 (**A**); ×21,000 (**B**); ×33,000 (**C**); ×26,000 (**D**); ×53,500 (**E**); ×43,000 (**F** and **G**).

are not yet known. Based on a high degree of homology to the *Caeorhabditis elegans* SEL-12 and SPE-4 proteins,[38] it has been proposed that PS1 may function as a channel or as a transmembrane transporter protein.[36]

In normal and disease-controls, we found PS1 immunoreactivity present at the postsynaptic domain of the neuromuscular junctions. Thus, PS1 is a normal neuromuscular junctional protein and may play a role in the trophic or excitatory interaction between the lower motor neuron and muscle fiber.

Missense mutations in the PS1 gene lead to the most common and aggressive form of familial Alzheimer disease. Despite the fact that there are no mutations of the PS1 gene in sporadic AD, the same abnormal accumulations of PS1 occur in the brains of both sporadic and familial Alzheimer patients in which PS1 is immunolocalized to the same structures, namely neurofibrillary tangles and senile plaques.[22–27]

Even though mutations of the PS1 gene experimentally cause increased production of Aβ−42 both *in vivo* and *in vitro*,[39–42] this mechanism cannot be responsible for Aβ abnormalities present in sporadic AD patients because they do not have PS1 mutations. However, the accumulations of PS1 in both sporadic and familial forms of AD suggest that it plays a role in a cerebral pathogenic cascade common to different groups of AD patients.

Our study demonstrates that PS1 is abnormally accumulated in muscle fibers of patients with sporadic and

with quadriceps-sparing autosomal-recessive IBM. In both groups, PS1 immunolocalizes to the same structures and with the same intensity. Even though three antibodies used in this study recognize both PS1 and PS2, the other three specifically recognize PS1[30,31] (V.M.-Y. Lee, unpublished). Therefore, based on our studies, in the IBMs we can access the status of PS1 but not of PS2.

It is of particular interest that in IBM muscle and AD brain, PS1 is immunolocalized mainly to the PHFs (which contain phosphorylated tau) and, to a lesser extent, amyloid-like fibrils (which contain Aβ). The mechanism(s) leading to the abnormal accumulation of PS1 within IBM muscle fibers is not known. It is not likely that a mutation of PS1, the product of chromosome-14 gene, is responsible for PS1 abnormality in autosomal-recessive IBM because in four genetically studied patients, the linkage was to the chromosome 9p1-q1[8] (DNA of the fifth patient has not yet been studied). It is also unknown whether PS1 abnormality in the IBMs precedes or is a consequence of other abnormalities. We propose that in the IBMs, PS1 abnormality may be secondary to abnormal accumulation of Aβ and possibly other βAPP epitopes because our previous studies demonstrated that abnormalities of βAPP precede other changes including congophilia.[4,5,12] Moreover, overexpression of the βAPP gene in normal cultured muscle is sufficient to induce IBM-characteristic mitochondrial abnormalities and other aspects of the IBM phenotype.[43,44]

Previously, it was shown that PS1 can form complexes with either βAPP or Aβ.[24,45,46] This phenomenon has been considered relatively specific to Aβ and βAPP as PS1 immunoreactivity is not associated with other biochemical types of amyloid deposits.[24] Since in the IBMs there are abnormal accumulations of Aβ and other βAPP epitopes, it is possible that PS1 is binding directly to them, because in the IBMs they[4,12] and PS1 localize to the 6- to 10-nm diameter fibrils and amorphous material. An additional or alternative possibility is that the pathological overaccumulation of βAPP, the whole molecule or its derivative (Aβ fragment), can influence posttranslational processing or degradation of PS1, such as leading to its becoming more "sticky" and resulting in its accumulation in IBM muscle (and in sporadic AD brain). (A similar role has been suggested for some of the amyloid-associated proteins, such as apolipoprotein E, and their interaction with Aβ.[47,48]) In support of this proposal are our preliminary *in situ* hybridization studies (Askanas and Sarkozi, 1997, unpublished), which indicate that PS1 mRNA is not increased in IBM abnormal muscle fibers. Likewise, PS1 mRNA levels were equal in normal and AD brain.[49]

In s- and h-IBMs, the prominent association of PS1 with the PHFs, which do not contain Aβ or βAPP epitopes, conceivably could be 1) a nonpathogenic adhesion of the PS1 to phosphorylated tau or another of the 10 known components already existing in the IBM PHFs[50] or 2) pathogenic adhesion of PS1 to one or more of those PHF components, such as tau, that causes the molecular transformation essential to the formation of the PHFs.

It is of particular interest that s-IBM and all recessive and dominant forms of the h-IBMs have specific pathological features in common including: vacuolar degeneration of muscle fibers; filamentous inclusions composed of paired-helical filaments containing phosphorylated tau; and accumulations of βAPP epitopes, apolipoprotein E, other AD-characteristic proteins,[1] and now PS1. Two questions are 1) despite their presumably different etiologies, what causes their common AD-like pathological features and 2) do they result from the same ultimate pathogenic cascade occurring in all forms of the IBMs, as we have suggested.[1,9] The same questions relate to AD. Despite three different genetic defects (identified thus far) causing early-onset familial AD and the apolipoprotein E genetic risk factor existing in some late-onset sporadic AD patients, all forms of AD have virtually the same brain pathology,[51] suggesting that there may be an ultimate common pathogenic cascade in the familial and sporadic ADs.[51]

Moreover, accumulation of PS1 deposits in abnormal muscle fibers of the IBMs enhances the possibility that pathogenic cellular mechanisms in IBM muscle and in Alzheimer disease brain may have similarities.

The proteins pathologically accumulated that are common to the s- and h-IBMs might indicate abnormalities that are key events in their putatively common pathogenic cascade or may reflect other yet unknown events that are the crucial underlying pathogenesis of their muscle fiber weakening, atrophy, vacuolization, and destruction. The same possibilities apply to sporadic and hereditary AD, regarding neuronal deterioration. These possibilities need to be explored.

Conclusions

Our study indicates that abnormal accumulation of PS1 occurs in diseased normal muscle and is not unique to AD brain. Normal and pathological human muscle, including use of cultured normal and genetically abnormal muscle such as h-IBM,[52] can provide living human tissue more readily accessible than brain for a wide range of molecular studies of PS1 synthesis and processing.

Additional studies will be required to determine the precise origin and pathogenic steps of abnormal PS1 accumulation in IBM muscle. Such analysis could lead to a better understanding of PS1 in s-IBM and the h-IBMs and perhaps be directly relevant to Alzheimer disease brain.

Acknowledgments

We thank Drs. Dennis J. Selkoe and Gopal Thinakaran for the generous gifts of antibodies X-81, 4627, and αPS1 loop and their corresponding peptides. Dr. Gopal Thinakaran provided helpful comments on the manuscript. Maggie Baburyan provided excellent photographic assistance.

References

1. Askanas V, Engel WK: Sporadic inclusion-body myositis and hereditary inclusion-body myopathies. Current Neurology, vol 16. Edited by SH Appel. Illinois, Mosby-Year Book, Inc., 1996, pp 115–144
2. Engel WK, Cunningham GG: Rapid examination of muscle tissue: an improved trichrome method for fresh-frozen biopsy sections. Neurology 1963, 13:919–923
3. Mendell JR, Sahenk Z, Galtes T, Paul L: Amyloid filaments in inclusion-body myositis. Arch Neurol 1991, 48:1229–1234
4. Askanas V, Engel WK, Alvarez RB: Light-and electronmicroscopic localization of β-amyloid protein in muscle biopsies of patients with inclusion-body myositis. Am J Pathol 1992, 141:31–36
5. Askanas V, Engel WK, Bilak M, Alvarez RB, Selkoe DJ: Twisted tubulofilaments of inclusion-body myositis muscle resemble paired helical filaments of Alzheimer brain and contain hyperphosphorylated tau. Am J Pathol 1994, 144:177–187
6. Mitrani-Rosenbaum S, Argov Z, Blumenfeld A, Seidman CE, Seidman JG: Hereditary inclusion-body myopathy maps to chromosome 9p1–q1. Hum Mol Genet 1996, 5:159–163
7. Ikeuchi T, Asaka T, Saito M, Hajime T, Higuchi S, Tanaka K, Saida K, Uyama E, Mizusawa H, Fukuhara N, Nonaka I, Takamori M, Tsuji S: Gene locus for autosomal-recessive distal myopathy with rimmed vacuoles maps to chromosome 9. Ann Neurol 1997, 41:432–437
8. Middleton LT, Christodoulou K, Askanas V, Engel WK, McFerrin J, Kyriakides T, Zamba E, Papadopoulou E: Molecular genetics of autosomal-recessive hereditary inclusion-body myopathy (AR-IBM). Ann Neurol 1997, 42:414
9. Askanas V: New developments in hereditary inclusion-body myopathies. Ann Neurol 1997, 41:421–422
10. Askanas V, Engel WK: New advances in inclusion-body myositis. Curr Opin Rheumatol 1993, 5:732–741
11. Askanas V, Engel WK, Alvarez RB: Enhanced detection of Congo red-positive amyloid deposits in muscle fibers of inclusion-body myositis and brain of Alzheimer disease using fluorescence technique. Neurology 1993, 43:1265–1267
12. Askanas V, Alvarez RB, Engel WK: β-amyloid precursor epitopes in muscle fibers of inclusion body myositis. Ann Neurol 1993, 34:551–560
13. Mirabella M, Alvarez RB, Engel WK, Weisgraber KH, Askanas V: Apolipoprotein E and apolipoprotein E messenger RNA in muscle of inclusion-body myositis and myopathies. Ann Neurol 1996, 40:864–872
14. Askanas V, Serdaroglu P, Engel WK, Alvarez RB: Immunolocalization of ubiquitin in muscle biopsies of patients with inclusion-body myositis and oculopharyngeal muscular dystrophy. Neurosci Lett 1991, 130:73–76
15. Bilak M, Askanas V, Engel WK: Strong immunoreactivity of α_1-antichymotrypsin co-localizes with β-amyloid protein and ubiquitin in vacuolated muscle fibers of inclusion-body myositis. Acta Neuropathol 1993, 85:378–382
16. Sarkozi E, Askanas V, Johnson SA, Engel WK, Alvarez RB: β-amyloid precursor protein mRNA is increased in inclusion-body myositis muscle. NeuroReport 1993, 4:815–818
17. Mirabella M, Alvarez RB, Bilak M, Engel WK, Askanas V: Difference in expression of phosphorylated tau epitopes between sporadic inclusion-body myositis and hereditary inclusion-body myopathies. J Neuropathol Exp Neurol 1996, 55:774–786
18. Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin J-F, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HAR, Haines JL, Pericak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St. George-Hyslop PH: Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 1995, 375:754–760
19. Hardy J: Amyloid, the presenilins, and Alzheimer's disease. Trends Neurosci 1997, 20:154–159
20. Selkoe DJ: Alzheimer's disease: genotypes, phenotype, and treatments. Science 1997, 275:630–631
21. Haass C: Presenilins: genes for life and death. Neuron 1997, 18:687–690
22. Wisniewski T, Palha JA, Ghiso J, Frangione B: S182 protein in Alzheimer's disease neuritic plaques. Lancet 1995, 346:1366
23. Murphy Jr GM, Forno LS, Ellis WG, Nochlin D, Levy-Lahad E, Poorkaj P, Bird TD, Jiang Z, Cordell B: Antibodies to presenilin proteins detect neurofibrillary tangles in Alzheimer's disease. Am J Pathol 1996, 149:1839–1846
24. Wisniewski T, Dowjat W, Permanne B, Palha J, Kumar A, Gallo G, Frangione B: Presenilin-1 is associated with Alzheimer's disease amyloid. Am J Pathol 1997, 151:601–610
25. Busciglio J, Hartmann H, Lorenzo A, Wong C, Buamann K, Sommer B, Staufenbiel M, Yankner BA: Neuronal localization of presenilin-1 and association with amyloid plaques and neurofibrillary tangles in Alzheimer's disease. J Neurosci 1997, 17:5101–5107
26. Levey AI, Heilman CJ, Lah JJ, Nash NR, Rees HD, Wakai M, Mirra SS, Rye DB, Nochlin D, Bird TD, Mufson EJ: Presenilin-1 protein expression in familial and sporadic Alzheimer's disease. Ann Neurol 1997, 41:742–753
27. Huynh DP, Vinters HV, Ho DHD, Ho VV, Pulst S-M: Neuronal expression and intracellular localization of presenilins in normal and Alzheimer disease brains. J Neuropathol Exp Neurol 1997, 56:1009–1017
28. Engel WK: Muscle biopsies in neuromuscular diseases. Pediatr Clin North Am 1967, 14:963–995
29. Askanas V, Alvarez RB, Mirabella M, Engel WK: Use of anti-neurofilament antibody to identify paired-helical filaments in inclusion-body myositis. Ann Neurol 1996, 39:389–391
30. Podlisny MB, Citron M, Amarante P, Sherrington R, Xia W, Zhang J, Diehl T, Levesque G, Fraser P, Haass C, Koo EHM, Seubert P, St. George-Hyslop P, Teplow DB, Selkoe DJ: Presenilin proteins undergo heterogeneous endoproteolysis between Thr_{291} and Ala_{299} and occur as stable N- and C-Terminal fragments in normal and Alzheimer brain tissue. Neurobiol Dis 1997, 3:325–337
31. Thinakaran G, Borchelt DR, Lee MK, Slunt HH, Spitzer L, Kim G, Ratovitsky T, Davenport F, Nordstedt C, Seeger M, Hardy J, Levey AI, Gandy SE, Jenkins NA, Copeland NG, Price DL, Sisodia SS: Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. Neuron 1996, 17:181–190
32. Ksiezak-Reding H, Dickson DW, Davies P, Yen S-H: Recognition of tau epitopes by anti-neurofilament antibodies that bind to Alzheimer neurofibrillary tangles. Proc Natl Acad Sci USA 1987, 84:3410–3414
33. Gorycki M, Askanas V: Improvements of the technique of electronmicroscopy of the cultured cells. Stain Technol 1977, 52:249–254
34. Doan A, Thinakaran G, Borchelt DR, Slunt HH, Ratovitsky T, Podlisny M, Selkoe DJ, Seeger M, Gandy SE, Price DL, Sisodia SS: Protein topology of presenilin 1. Neuron 1996, 17:1023–1030
35. Lehmann S, Chiesa R, Harris DA: Evidence for a six-transmembrane domain structure of presenilin 1. J Biol Chem 1997, 272:12047–12051
36. Li X, Greenwald I: Membrane topology of the C. elegans sel-12 presenilin. Neuron 1996, 17:1015–1021
37. Lee MK, Slunt HH, Martin LJ, Thinakaran G, Kim G, Gandy SE, Seeger M, Koo E, Price DL, Sisodia SS: Expression of presenilin 1 and 2 (PS1 and PS2) in human and murine tissues. J Neurosci 1996, 16:7513–7525
38. Levitan D, Greenwald I: Facilitation of lin-12-mediated signaling by sel-12, a Caeorhabditis elegans S182 Alzheimer's disease gene. Nature 1995, 377:351–354
39. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe DJ, Younkin S: Secreted amyloid β-protein similar to that 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. Nat Med 1996, 2:864–870
40. Lemere CA, Lopera F, Kosik KS, Lendon CL, Ossa J, Saido TC, Yamaguchi H, Ruiz A, Martinez A, Madrigal L, Hincapie L, Arango L JC, Anthony DC, Koo EH, Goate AM, Selkoe DJ, Arango V JC: The E280A presenilin 1 Alzheimer mutation produces increased Aβ42 deposition, and severe cerebellar pathology. Nat Med 1996, 2:1146–1150
41. Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada C-M, Kim G, Seekins S, Yager D, Slunt HH, Wang R, Seeger M, Levey AI, Gandy SE, Copeland NG, Jenkins NA, Price DL, Younkin SG, Sisodia SS: Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1–42/1–40 ratio in vitro and in vivo. Neuron 1996, 17:1005–1013
42. Duff K, Eckman C, Zehr C, Yu X, Prada C-M, Perez-tur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L,

Refolo L, Zenk B, Hardy J, Younkin S: Increased amyloid-β42 (43) in brains of mice expressing mutant presenilin 1. Nature 1996, 383:710–713

43. Askanas V, McFerrin J, Baqué S, Alvarez RB, Sarkozi E, Engel WK: Transfer of β-amyloid precursor protein gene using adenovirus vector causes mitochondrial abnormalities in cultured normal human muscle. Proc Natl Acad Sci USA 1996, 93:1314–1319
44. Askanas V, McFerrin J, Alvarez RB, Baqué S, Engel WK: βAPP gene transfer into cultured human muscle induces inclusion-body myositis aspects. NeuroReport 1997, 8:2155–2158
45. Weidemann A, Paliga K, Dürrwang U, Czech C, Evin G, Masters CL, Beyreuther K: Formation of stable complexes between two Alzheimer's disease gene products: presenilin-2 and β-amyloid precursor protein. Nat Med 1997, 3:328–332
46. Xia W, Zhang J, Perez R, Koo EH, Selkoe DJ: Interaction between amyloid precursor protein and presenilins in mammalian cells: implications for the pathogenesis of Alzheimer disease. Proc Natl Acad Sci USA 1997, 94:8208–8213
47. Wisniewski T, Frangione B: Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. Neurosci Lett 1992, 135:235–238
48. Wisniewski T, Castaño EM, Golabek AA, Vogel T, Frangione B: Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. Am J Pathol 1994, 145:1030–1035
49. Johnston JA, Froelich S, Lannfelt L, Cowburn RF: Quantifications of presenilin-1 mRNA in Alzheimer's disease brains. FEBS Lett 1996, 394:279–284
50. Askanas V, Engel WK: Newest approaches to diagnosis and pathogenesis of sporadic inclusion-body myositis and hereditary inclusion-body myopathies, including molecular-pathologic similarities to Alzheimer disease. Inclusion-Body Myositis and Myopathies. Edited by V Askanas, G Serratrice, WK Engel. United Kingdom, Cambridge University Press, 1998 (in press)
51. Lippa CF, Saunders AM, Smith TW, Swearer JM, Drachman DA, Ghetti B, Nee L, Pulaski-Salo D, Dickson D, Robitaille Y, Bergeron C, Crain B, Benson MD, Farlow M, Hyman BT, St. George-Hyslop P, Roses AD, Pollen DA: Familial and sporadic Alzheimer's disease: neuropathology cannot exclude a final common pathway. Neurology 1996, 46:406–412
52. Alvarez RB, Fardeau M, Askanas V, Engel WK, McFerrin J, Tomé FMS: Characteristic filamentous inclusions reproduced in cultured innervated muscle fibers from patients with familial "inclusion-body myositis" (FIBM). J Neurol Sci 1990, 98:178