Association of a Presenilin 1 S170F Mutation With a Novel Alzheimer Disease Molecular Phenotype

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Objective: To report an ataxic variant of Alzheimer disease expressing a novel molecular phenotype.

Design: Description of a novel phenotype associated with a presenilin 1 mutation.

Setting: The subject was an outpatient who was diagnosed at the local referral center.

Patient: A 28-year-old man presented with psychiatric symptoms and cerebellar signs, followed by cognitive dysfunction. Severe β-amyloid (Aβ) deposition was accompanied by neurofibrillary tangles and cell loss in the cerebral cortex and by Purkinje cell dendrite loss in the cerebellum. A presenilin 1 gene (PSEN1) S170F mutation was detected.

Main Outcome Measures: We analyzed the processing of Aβ precursor protein in vitro as well as the Aβ species in brain tissue.

Results: The PSEN1 S170F mutation induced a 3-fold increase of both secreted Aβ42 and Aβ40 species and a 60% increase of secreted Aβ precursor protein in transfected cells. Soluble and insoluble fractions isolated from brain tissue showed a prevalence of N-terminally truncated Aβ species ending at both residues 40 and 42.

Conclusion: These findings define a new Alzheimer disease molecular phenotype and support the concept that the phenotypic variability associated with PSEN1 mutations may be dictated by the Aβ aggregates’ composition.

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MUTATIONS IN THE PRESENILIN 1 GENE (PSEN1) account for the majority of early-onset familial Alzheimer disease (FAD) cases. A common effect of PSEN1 mutations is the selective increased production of β-amyloid (Aβ) species ending at residues 42 (Aβ42), the C-terminal variant of Aβ peptides that has a stronger tendency to form aggregates. To explain this event, it has been proposed that the mutant PSEN1 alters the specificity of the γ-secretase to favor the production of Aβ42 at the expense of Aβ40. The relative increased production of the Aβ42 species is consequently believed to be the major cause of Aβ accumulation and deposition occurring in FAD.

However, FAD pedigrees associated with PSEN1 mutations show a wide variety of clinical and pathological phenotypes that do not simply depend on the extent of the Aβ42:Aβ40 ratio. For instance, neither the time of onset nor the clinical variability of FAD cases correlates with Aβ42 production, arguing that other factors influence the timing, severity, and distribution of Aβ accumulation in brain tissue. We describe a PSEN1 S170F mutation associated with a very early age at onset of clinical signs as well as unusual clinical, pathological, and molecular features.

REPORT OF A CASE

CLINICAL HISTORY

A 28-year-old man presented with delusions and lower limb jerks accompanied by intentional myoclonus and ataxia. No family history of neuropsychiatric diseases was reported. However, the available data on the family history were largely incomplete. A brain magnetic resonance imaging study...
documented a mild and diffuse cortical atrophy and a slight displacement (<5 mm) of cerebellar tonsils in the upper cervical canal without compression of surrounding structures. The latter finding was consistent with an asymptomatic Chiari type I malformation. At age 29 years, the patient was reported to have cognitive dysfunction that impaired his working activity. At age 31 years, a global impairment of all cognitive functions was noted. Neuropsychological testing revealed a decrease of attention, sensory-perceptual functioning, psychomotor activity, and memory (Wechsler Memory Scale score, 53; Milan Overall Dementia Assessment score, 64 [cognitively healthy score, mean ± SD, 96.6 ± 2.3]). A neurological examination disclosed an ataxic gait and intentional tremor. Electroencephalography demonstrated bilateral theta waves of 5 cycles/s in the parietal areas. Repeat magnetic resonance imaging showed cortical atrophy in the parietal and temporal lobes, and at that time, the presence of Chiari type I malformation was interpreted as questionable. A positron emission tomography with fluorodeoxyglucose F 18 study showed cortical hypometabolism in temporoparietal as well as in frontobasal regions. A complete examination of cerebrospinal fluid, including 14.3.3 protein reactivity, yielded results that were unremarkable. In spite of this, the possibility of an atypical prion disease was considered. A progressive worsening of cognitive functions occurred over the next few months. At age 33 years, the patient was bedridden, anarthric, and incontinent and had generalized hypertonus. A bronchopneumonia was the final cause of death at 35 years of age.

TISSUE AND IMMUNOHISTOCHEMICAL STUDY METHODS

At autopsy (6 hours post mortem), one half of the brain was fixed in buffered formalin and the other half was frozen at −80°C. Formalin-fixed samples of frontal, parietal, temporal, and occipital cortices; hippocampus; cerebellum; and basal ganglia were processed for histologic studies according to established methods. Eight-micrometer-thick paraffin sections were deparaffinized, rehydrated, and treated with 88% formic acid for 5 minutes at room temperature. Sections were incubated overnight using antibodies against α-synuclein (LB509; Zymed, San Francisco, Calif) (1:500), prion protein (3F4; gift of Richard Kascak, PhD, Staten Island, NY) (1:500), Aβ (4G8; Signet Laboratories, Dedham, Mass) (1:200), Aβ40 (1A10; IBL, Gunma, Japan) (1:500), Aβ42 (21F12; Elan Inc, San Francisco) (1:2000), and tau (AT8; Innogenetics, Gent, Belgium) (1:100). Subsequent antibody detection was carried out using a biotinylated goat antimouse antibody or biotinylated horse antirabbit antibody (Amersham BioSciences, Buckinghamshire, England) (1:200) and the Vectastain ABC Elite Kit (Vector Laboratories, Burlingame, Calif). Diaminobenzidine tetrahydrochloride was used to visualize the immunoreactivity. Double immunohistochemical studies were carried out using a rabbit polyclonal against calbindin (D-28k; Swant Pharmaceutical, Bellinzona, Switzerland) (1:10000) and a mouse monoclonal antibody against Aβ42 (21F12). Sections were pretreated with 88% formic acid for 20 minutes at room temperature, rinsed, and processed with the EnVision double stain kit (DakoCytomation, Carpinteria, Calif). Blocks of frontal cortex from 10 sporadic Alzheimer disease (AD) cases (aged 59-82 years; postmortem interval, 4-8 hours) provided by the brain bank of Case Western Reserve University were used as controls.

GENETIC ANALYSIS METHODS

Genomic DNA was extracted from 25 mg of cerebellar cortex using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). Polymerase chain reaction (PCR) and direct sequencing of the coding exons of PSEN1 were performed following standard procedures by BMR–Bio Molecular Research, CRIBI Biotechnology Centre and Department of Biology, University of Padua, Padua, Italy. The coding region of the PSEN1 gene was amplified using intronic primers (exons 3-12) by PCR and direct cycle sequencing. Polymerase chain reaction products were sequenced using an ABI 3730XL DNA sequencer using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit; data were analyzed using Genotype 2.5 software (Applied Biosystems, Framingham, Conn). Polymerase chain reaction products of exon 6 were analyzed by denaturing high-performance liquid chromatography in 94 healthy subjects. The apolipoprotein E (ApoE) genotype was determined using restriction isotyping. After PCR amplification, ApoE sequences were digested with HinfI and the resulting fragments were separated on a 3.5% MetaPhor Agarose gel (Cambrex Bio Sciences, Rockland, Me). The relatives of the patient were not available for genetic analysis.

IMMUNOBLETTING AND MASS SPECTROMETRY ANALYSIS OF Aβ SPECIES IN BRAIN TISSUE

Soluble Aβ was extracted from the water-soluble fraction of the frontal cortex using a method previously described. Tissues were homogenized in 10 vol of saline buffer (50 mM Tris buffer pH 7.6, 5 mM EDTA, 150 mM sodium chloride) containing protease inhibitors and centrifuged at 100 000g for 1 hour. Soluble Aβ was immunoprecipitated from the supernatants (1 mL/100 mg of tissue) adjusted to 1× radioimmunoprecipitation assay (RIPA) buffer (150 mM sodium chloride, 1% Nonidet P-40 [Amersham Biosystems], 0.5% cholic acid, 0.1% sodium dodecyl sulfate, 50 mM Tris buffer pH 8 with protease inhibitors) with 4G8, a monoclonal antibody that recognizes Aβ17-24. For the analysis of Aβ associated with the insoluble fraction, the pellet resulting from ultracentrifugation was extracted with 70% formic acid at 37°C for 10 minutes (1 mL for 500 mg of tissue). Brain-insoluble fractions were then directly analyzed by immunoblotting following precipitation with methanol. Immunoprecipitated soluble Aβ and 50 μL of precipitated insoluble Aβ were separated on 10% to 18% Tris buffer–Tricine gels and detected by immunoblotting with the monoclonal antibody 4G8, as well as with antibodies specific for the N terminal by pyrogallamate at residue 3 (α- py3) and residue 11 (α-py11). Antibodies specific for Aβ42 and Aβ40 were also used.

Mass spectra were acquired in positive ion mode on a QSTAR XL (Applied Biosystems) quadruple time-of-
flight mass spectrometer equipped with a matrix-assisted laser desorption ionization ion source. The 100 000g supernatant from the frontal cortex homogenate, after preclearing the serum immunoglobulins with 40 µL/mL of protein G agarose, was immunoprecipitated with the monoclonal antibody 4G8 (3 µL/mL) in RIPA buffer for 1 hour at 4°C. Then 10 µL of magnetic beads (Dynal AS, Oslo, Norway) covalently coupled with antimouse IgG were added to the sample and rocked overnight in the same conditions. The magnetic beads were washed twice with RIPA, twice with double-distilled water, and suspended in 10 µL of double-distilled water. Two microliters of this slurry were then incubated briefly with 2 µL of the matrix α-cyano-4-hydroxycin-
namic acid (10 mg/mL) (Aldrich Chemical Co, Milan, Italy) and dissolved in hydrochloric acid 0.05M acetonitrile and water in a 6:4 ratio. One microliter of the incubation mixture was placed on the sample plate with 1 µL of the matrix and evaporated at room temperature. The UV laser wavelength was 337 nm and the power ranged from 8.8 µJ to 13.2 µJ with a pulse rate of 20 Hz.

**CELL TRANSFECTION AND EVALUATION OF SECRETED Aβ AND Aβ PRECURSOR PROTEINS**

Total RNA was extracted from the tissue using the TRIzol method (Invitrogen, Carlsbad, Calif) and complementary DNA (cDNA) was synthesized starting from 3 µg of RNA using random primer. PSEN1 mutant S170F was amplified by PCR (forward 5’-GCT CCA ATG ACA GAG TTA CCT GC-3’, reverse 5’-GAA ACA TCC ATG GGA TTC TAA CCG-3’). Polymerase chain reaction product was purified from the agarose gel and cloned using a PCR cloning kit (Qiagen GmbH) according to the manufacturer’s protocol. The clone PSEN1 mutant S170F was identified and confirmed by directly sequencing the entire cDNA clone; the PCR product was subcloned into pcdNA3.1 plasmid (Invitrogen). PSEN1 wild-type and mutant M146V, cloned into pcdNA3.1 plasmid, were kindly provided by Luciano D’Adamio, PhD, Albert Einstein College of Medicine, New York, NY. Human embryonic kidney 293T cells, stably expressing wild-type human amyloid precursor protein (APP), were cultured in Dulbecco modified Eagle medium supplemented with 10% (volume-volume ratio) fetal bovine serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin, 2mM glutamine, and 5 µg/mL of puromycin (Euroclone, Milano, Italy) and maintained at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air. Cells were plated on T75 flasks and grown until 70% to 80% confluence. Transient transfection of wild-type and mutant PSEN1 cDNA (S170F and M146V) was carried out with poly-ethyleneimine (Sigma, St Louis, Mo) at 12 µL/20 µg of DNA according to the manufacturer’s instructions. Cells transfected with a pcdNA3.1 expression vector served as a negative control. The efficiency of transfection was evaluated as PSEN1 protein levels by immunoblotting. After 12 hours of transfection, the media were collected for Aβ analysis. The amounts of secreted Aβ40 and Aβ42 were evaluated by sandwich enzyme-linked immunosorbent assay (IBL). Cell culture supernatants were diluted 1:40 in enzyme immunoassay buffer and processed using kits specific for both Aβ species, following the indications of the manufacturer. Both assays show a linear reactivity within the range of concentration of 7 to 1000 pg/mL for both Aβ species. For β-amyloid precursor protein (βAPP) analysis, 50 µg of supernatants was collected 12 hours on transfection and resolved on 7.5% sodium decyl sulfate–polyacrylamide gel electrophoresis gels, transferred to polyvinyl difluoride membranes (Amer sham Biosciences), and probed with antibody specific for βAPPs (IBL).

The results represent the means of values obtained from 3 different experiments normalized for the amount of transfected PSEN1. The values were statistically analyzed by analysis of variance with Bonferroni as a posttest.

**IMMUNOHISTOCHEMICAL STUDY RESULTS**

The antibody 3F4 showed no deposits of prion protein. The α-synuclein reactivity was absent. The antibody 4G8, which recognizes Aβ17-24, revealed abundant amyloid plaques in all neocortical areas, hippocampus, basal ganglia, thalamus, and midbrain (Figure 1A). In the cerebellum, diffuse plaques occupied the molecular layer, whereas compact plaques were observed in the inner portion of the granular layer (Figure 2A). The antibody 4G8 detected an intense amyloid angiopathy affecting parenchymal and meningeal arteries of the cerebrum and cerebellum (Figure 1A and Figure 2A). Seventy percent of compact and diffuse plaques reacted with the antibody specific for Aβ42 only (Figure 1B), whereas the remaining ones showed reactivity for both Aβ42 and Aβ40 (Figure 1C). The majority of vessels reacted with the Aβ42-specific antibody (Figure 1C). About 50% of vessels showed both Aβ42 and Aβ40 reactivity.

The antibody AT8, which recognizes a phosphorylation site of tau protein, revealed neurofibrillary pathology in the neocortex, hippocampus, basal ganglia, thalamus, and midbrain; this was in the form of variously shaped neurofibrillary tangles, neuropil threads, and neuritic plaques (Figure 3). Cerebellar neurons were free of neurofibrillary pathological features.

Double immunohistochemical study using 21F12, which recognizes Aβ42 and anticalbindin D-28k, revealed extensive deposition of Aβ in the molecular layer and a loss of Purkinje cell dendrites (Figure 2B). Loss of Purkinje cell dendrites was more severe than that observed in a case of late-onset AD (Figure 2C).

**GENETIC ANALYSIS RESULTS**

Direct sequencing of the PCR fragments demonstrated a C→T transition in exon 6 resulting in a Ser (TCT) to Phe (TTT) mutation at codon 170 of PSEN1 (S170F) (Figure 4). The denaturing high-performance liquid chromatography–altered pattern of the S170F mutation was not observed in 94 healthy controls. The patient was homozygous for ApoE allele ε3.

**ANALYSIS OF SECRETED Aβ AND βAPPs**

The Aβ42 and Aβ40 levels in the media of human embryonic kidney 293T cells stably expressing wild-type hu-
man APP and transiently transfected with wild-type and mutant PSEN1 cDNA are shown in Figure 5A. The PSEN1 M146V mutation that is associated with a typical early-onset AD phenotype determined a selective 44% increase of secreted Aβ42 species only, leading to an augmented Aβ42/Aβ40 ratio (0.13 vs 0.07) (Figure 5A), as expected with PSEN1 mutations. By contrast, the PSEN1 S170F mutation produced a significant increase of both Aβ40 (181%) and Aβ42 (181%) species resulting in a total Aβ augmentation of 2.8-fold compared with PSEN1 wild-type transfected cells (P<.01 and P<.05, respectively) (Figure 5A). To our knowledge, such increase of production of both Aβ species has never been reported in cellular models bearing mutant PSEN1 associated with FAD. In the same cellular model, we observed a significant 60% increase of βAPPs in the presence of the PSEN1 S170F mutation compared with wild-type PSEN1 (P<.01) (Figure 5B). A 40% increase of βAPPs occurred also with the PSEN1 M146V mutation (P<.05) (Figure 5B).

ANALYSIS OF Aβ SPECIES IN BRAIN TISSUE

In the water-soluble fraction of the frontal cortex, following immunoprecipitation and immunoblotting, the monoclonal antibody 4G8, specific for Aβ1-40/42, recognized 3 bands of 4.5 kDa (B1), 4.2 kDa (B2), and 3.5 kDa (B3) (Figure 6). The 3 bands correspond to the full-length Aβ1-42/40 (B1) and N-terminally truncated species (B2 and B3), as previously described. B2 and B3 correspond to 2 N-terminally truncated Aβ peptides having glutamate converted in pyroglutamate at position 3 (Aβpy3-42/40) and at position 11 (Aβpy11-42), as ascertained using epitope-specific antibodies (Figure 6). The B2-B1 ratio was 2.5 and 3.2 in the frontal cortex and cerebellar cortex (immunoblot not shown), respectively. The B2-B3 ratios were more than 2-fold higher than the average of 14 sporadic AD cases (Figure 6). The same pattern of Aβ species was detected in immunoblots of the insoluble fraction of the frontal cortex, as well as in the other cerebral lobes (not shown). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, carried out on proteins immunoprecipitated with monoclonal antibody 4G8 from water-soluble fractions, confirmed the presence of Aβ1-40/42, Aβpy3-42, and Aβpy11-42, as well as of other N-terminally truncated peptides (Figure 7). The extent of the peaks did not reflect the real relative amount of the Aβ species because of a suppression effect exerted on Aβpy3-42 by the full-length Aβ1-42 molecule, an effect previously described with other peptides. Indeed, an overrepresentation of the full-length Aβ is observed when Aβ1-42, Aβpy3-42, and Aβpy11-42 are mixed together in equal amount and examined with mass spectrometry (not shown). In the water-soluble fraction, the analysis with matrix-assisted laser desorption ionization time-of-flight mass spectrometry revealed N-terminally truncated species ending at residue 40 (py3-40; 4-40; 8-40) that were not recovered in sporadic AD brains (Figure 7).

Figure 4. Direct sequencing showed a C→T transition in exon 6 resulting in a Ser (TCT) to Phe (TTT) mutation at codon 170 of PSEN1.
Figure 5. β-Amyloid (Aβ) 40 and Aβ42 and Aβ precursor proteins (pAPPs) evaluation. A, Human embryonic kidney 293T cells transfected with the PSEN1 S170F mutation show a significant increased secretion of both the Aβ40 and Aβ42 species. The PSEN1 M146V mutation caused a selective increase of Aβ40 only. *P<.05, †P<.01. B, The PSEN1 S170F mutation induced in the medium of human embryonic kidney 293T cells a 60% increase of pAPPs. A 40% increase of pAPPs was observed with the PSEN1 M146V mutation. *P<.05, †P<.01. wt indicates wild type. The error bars indicate standard error.

Figure 6. Immunoblots of a water-soluble fraction of the frontal cortex of a case with a PSEN1 S170F mutation. A, The antibody 4G8 (Signet Laboratories, Dedham, Mass) recognizes 3 bands corresponding to the full-length 1-40/42 peptides (B1) and 2 N-terminally truncated species (B2 and B3). All 3 bands reacted to the antibody specific for β-amyloid (Aβ) 42 (α-42), B1 and B2 reacted also with the antibody specific for Aβ40 (α-40). B2 and B3 correspond to N-terminally truncated species, as ascertained with antibodies specific for the N terminal with pyroglutamate at residue 3 (α-py3) and residue 11 (α-py11). B, In the water-soluble fractions of the frontal cortex of a case with a PSEN1 S170F mutation, the ratio of B2 to B1 is 2.5 in comparison with 1.3, the average of 14 sporadic Alzheimer disease (AD) cases. The error bars indicate standard error.
We have reported an unusual AD clinical presentation characterized by cerebellar ataxia caused by a PSEN1 S170F mutation. This study confirms the wide spectrum of FAD associated with PSEN1 mutations and suggests that additional effects of mutant PSEN1 may exist. PSEN1 mutations express a variety of clinical and pathological phenotypes.13-15 Dementia can be present as the only characteristic or may be associated with spastic paraparesis or parkinsonian signs. Cerebellar ataxia as a prominent initial symptom has been described only in 1 FAD pedigree,16 in which a PSEN1 G384A mutation was later identified.17 In the present case, the cerebellar syndrome correlates with the severe β-deposition in the cerebellar cortex as well as a severe loss of Purkinje cell somata and Purkinje cell dendritic processes. A loss of Purkinje cells has been previously described in FAD cases that did not show cerebellar ataxia,18 suggesting that a threshold of Purkinje cell somata and dendrite loss has to be reached to produce signs of cerebellar dysfunction. Usually PSEN1 mutations exhibit high penetrance, whereas in the pedigree of our patient, a history of dementia is lacking. However, details on the familial history were too scarce to draw a conclusion.

The pathological features of the PSEN1 S170F mutation parallel the altered APP processing, which showed a pattern unusual for PSEN1 mutations. The mutation produced a 2.8-fold increase, in equal amounts, of both the Aβ42 and Aβ40 species. Concomitantly, the mutation determined an increase of secreted βAPPs, which are the direct product of β-secretase activity. These findings implicate that the type and the severity of the phenotype of genetically determined AD is dictated not only by the

Figure 7. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry spectra of β-amyloid (Aβ) species extracted from the water-soluble fractions of the frontal cortex. Various N-terminally truncated Aβ species ending at residue 40, which are absent in sporadic Alzheimer disease (AD), are detected in the PSEN1 S170F tissue.
Aβ42/Aβ40 ratio, a parameter used as an indicator of the effect of PSEN1 mutations,19 but also by an increase of both Aβ species. The pattern of APP processing induced by the PSEN1 S170F mutation suggests, as we previously proposed,20 that the β-secretase cleavage also is altered by some PSEN1 mutations.

The PSEN1 S170F mutation has been recently described in an early-onset FAD kindred, presenting with myoclonus and seizures, and pathologically associated with cortical Lewy bodies.21 The affected cases had an age at onset similar to our case but a different clinical and pathological phenotype. The different phenotypes expressed by the same PSEN1 mutation indicate that unknown individual genetic or environmental factors may influence the type of AD pathological features.

Another finding in this case is the accumulation of an extremely high amount of N–terminally truncated Aβ42/40 species as compared with the full-length Aβ. Various N–terminally truncated Aβ species exclusively ending at residue 42 are present in the cerebral cortex of individuals with sporadic AD.1,22 The most represented N–terminally truncated peptide is Aβ9-42, with glutamate in position 3 cyclized in pyroglutamate.7,22 At variance with sporadic AD, the present case also had N–terminally truncated Aβ40 species. It has been previously reported that N–terminally truncated Aβ42 species are significantly increased in the brain of subjects with FAD associated with PSEN1 mutations; the amount correlated with the severity of the disease, in terms of early onset and short course duration.20 Studies of cases with sporadic AD and non-pathological aging show that the ratio of Aβ9-42 to Aβ42 is proportional to the degree of neurodegeneration and that the mixtures of Aβ species specific to the 2 conditions are correlated with different properties of aggregation and toxicity.23 The present case suggests that the composition of Aβ species is related to the type and severity of the phenotype.

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REFERENCES