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A novel presenilin 1 mutation (L174 M) in a large Cuban family with early onset Alzheimer disease

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Abstract We studied a Cuban family with presenile dementia (autosomal dominant) consisting of 281 members within six generations, the proband descended from a Spanish founder. Mean age at onset was 59 years of age. Memory impairment was the main symptom in all patients, additionally, ischemic episodes were described in 4 (*n*=18) patients. Neuropathological examination of brain material (1 patient) revealed neuronal loss, amyloid plaques, and neurofibrillary tangles. Thirty DNA samples were genotyped (regions on chromosome 1, 3, 10, 12, 14, 17, 19, 20, and 21). A maximum Lod score of 3.79 at θ=0 was obtained for marker D14S43, located in a 9-cM interval in which all patients shared the same haplotype. Sequencing of the *PSEN1* gene revealed a heterozygous base substitution, C520A (exon 6), which is predicted to cause an amino acid change from leucine to methionine in the TMIII of the presenilin 1 protein. The mutation was found to co-segregate with the disease phenotype and the associated disease haplotype. The C→A change was not observed in 80 control chromosomes from the Cuban population. Leucine at position 174 is highly conserved among species and is identical in presenilin 1 and presenilin 2 proteins. We propose the *L174 M* mutation might lead to an abnormal N-terminal and probably C-terminal fragments and malfunction of the protein complex. In conclusion, we found a novel *PSEN1* mutation in a large family with clinical and pathological diagnosis of early onset familial Alzheimer disease, which may be relevant for other Hispanic populations.

Keywords Alzheimer disease · Familial Alzheimer disease · Presenile dementia · *PSEN1* mutation · *L174 M*

A.M. Bertoli Avella and B. Marcheco Teruel contributed equally to this work. Disclosure: all experiments comply with the current laws in The Netherlands.

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Introduction

Alzheimer disease (AD, MIM 1043300) is the most common type of dementia, characterized by progressive memory loss and deterioration of cognitive functions, resulting in a state of complete dependency, with severe implications for patients, relatives, and caregivers.

The complex etiology of AD comprises both genetic and environmental factors; sporadic and familial (FAD) forms of the disease have been described. The high similarity in clinical and neuropathological features between the early onset familial and "sporadic" late-onset AD suggests that similar pathophysiological factors are involved. Studies focusing on large families with the rare, early onset, autosomal dominant form of the disease led to the discovery that mutations in the amyloid precursor protein (APP) [1], presenilin 1 (*PSEN1*) [2], and presenilin 2 (*PSEN2*) [3, 4] genes cause AD. Although these autosomal dominant fami-

lies represent only a small percentage of the total AD cases, functional studies of the effects of the identified mutations have become an important way to dissect the causes and underlying disease mechanisms leading to AD.

Mutations in *PSEN1* are responsible for 6%–18% of the AD cases with early onset [5]. To date the majority of the described *PSEN1* mutations are missense mutations, giving rise to the substitution of a single amino acid (www.alzforum.org/members/resources/pres_mutations/ and molgen-www.uia.ac.be/admutations/). It has been speculated that most AD-related mutations result in a gain of function. Mutations in *PSEN1* alter the processing of βAPP by favoring the production of potentially toxic long-tailed β-amyloid (Aβ) peptides ending at residue 42 or 43 [6]. Presenilin 1 activity is also required for the cleavage of Notch1 at the plasma membrane and the release of the Notch1 intracellular domain [7]. Mutations in genes involved in Notch signaling also lead to late-onset neurological diseases such as CADASIL [8].

Although the provisional diagnosis of AD may be made on the basis of clinical symptoms, neuropathological confirmation is necessary to establish a definite diagnosis. AD neuropathology includes the presence in the extracellular space of amyloid plaques composed mainly of Aβ, deposition of amyloid in the wall of blood vessels, and the presence of intraneuronal neurofibrillary tangles (NFT), consisting of hyperphosphorylated microtubule-associated protein tau.

Only a few *PSEN1* mutations have been described in Latin-American [9, 10, 11] and Caribbean populations [12]. In Cuba, a prevalence for AD of 5.13% has been reported for the population over 60 years [13], but to date genetic studies for AD have not been performed. Here we describe a large Cuban family with a clinical and neuropathological diagnosis of AD carrying a novel *PSEN1* mutation, the first to be reported in the Cuban population.

Patients and methods

Family description

A large Cuban family with presenile dementia was ascertained through patient IV-26 (Fig. 1A) descending from a founder (I-1) of Spanish origin (Canary Islands) who settled in Cuba in the early nineteenth century. We interviewed 50 people to complete family history, genealogy, and clinical data. The pedigree consists of 281 family members within six generations, 22 patients were reported, and a disease description was obtained for 18 patients, of whom 6 were alive and 4 were available for clinical examination. No pathological studies had been performed. Medical records were available from patient IV-26 and III-26. Segregation of the disease in the family was consistent with an autosomal dominant mode of inheritance with high penetrance (Fig. 1A). Informed consent was obtained for both clinical examination and venous puncture for blood collection. The research project was approved by the Ethics Committee at the Higher Institute of Medical Sciences in Havana, Cuba.

Clinical studies

Two independent neurologists examined 4 patients and 6 at-risk family members, some of them with memory complaints; 9 were admitted to hospital for a complete examination. Blood and cerebrospinal fluid tests, electroencephalography (EEG), brain computed tomographic (CT) scan, and neurophysiological tests were performed. Neuropsychological studies, which consisted of a battery of several tests: Mini-Mental State examination [14], Wechsler Memory Scale, Word List Memory test, and Wechsler Adult Intelligence Scale (WAIS) [15], were applied. Follow-up of some patients was necessary and a second evaluation was performed after 1 year. The NINCDS-ADRDA criteria [16] were used to establish the clinical diagnosis.

Pathology

Patient (III-26) died at the age of 73 years during the course of this research. Neuropathological studies such as classical hematoxylin/eosin staining, Bielschowsky silver impregnation, Luxol fast blue, Congo red, and trichromic Masson techniques were carried out on brain tissue (temporo-occipital region fixed in paraformaldehyde). In addition immunohistochemical studies using antibodies against presenilin 1 N-terminal (dilution 1:50, from Chemicon International), presenilin 1 C-terminal (1:100, from Santa Cruz Biotechnology), Aβ (1:100), and ubiquitin (1:500, DAKO), AT8 (1:40, Innogenetics, SA), PHF (a gift from P. Davies), α-synuclein (1:1,000), and prion protein (1:100, Chemicon) were performed. Working conditions were implemented as recommended by the manufacturers. Diagnosis based on pathology findings was according to CERAD criteria [17].

DNA studies

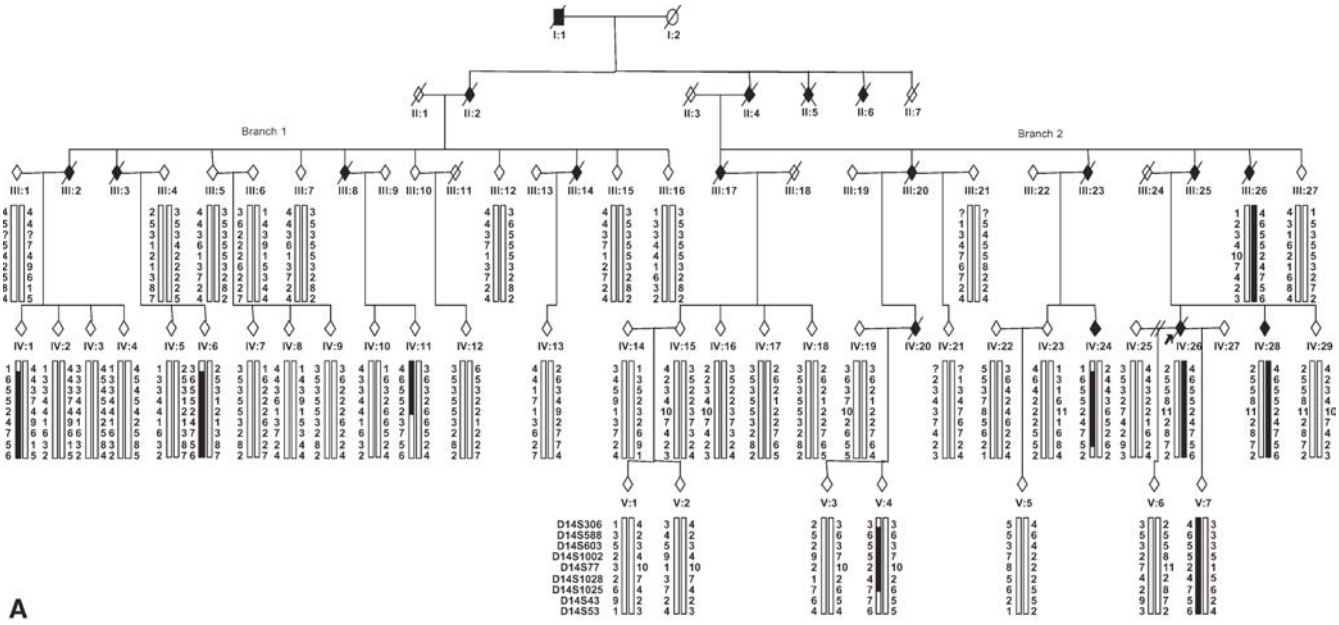
Blood samples were collected from 76 family members. DNA was isolated following standard procedures [18]. Thirty samples were tested with fluorescently labeled markers (short tandem repeat polymorphisms) from the CHLC Human screening set/Weber version 6, covering AD candidate regions on chromosomes 1, 10, 12, 14, 19, and 21 [1, 3, 19, 20, 21, 22]. Additional regions where other dementia-related loci have been localized on chromosome 3 [23], 17 [24], and 20 [25] were also tested. Genomic DNA (20 ng) was amplified in 7.5 µl PCR reaction, using 1× GeneAmp PCR Gold buffer, 1.5 mM $MgCl_2$, 10 pmol of each primer (forward primer labeled with FAM, TET, or HEX), 250 µM dNTPs, and 0.4 units of AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR products were pooled and loaded on an ABI 377 automatic DNA sequencer (6.25% denaturing FMC Long Ranger acrylamide gel, filterset C, and GS-500XL Tamra as size standard). Data were analyzed using Genescan 3.1 and Genotyper 2.5 (updated) software from Applied Biosystems.

Haplotypes for every region were constructed based on the minimal number of recombinations. Additional markers from the Genethon linkage map [26] were tested for the chromosome 14q24.3 region.

Genotypes for *APOE* were also determined following the one-stage PCR method described elsewhere [27]. PCR products were digested with *Hha*I (10 units/µl, Life Technologies), fragments were separated on Excell Gels (Pharmacia) using the Multiphor electrophoresis system (Pharmacia), and visualized by silver staining. Gels were scored by two independent observers.

Linkage analysis

Simulation calculations using the SLINK and MSIM programs [28] showed an average Lod score of 2.17 and a maximum of 5.55 at θ=0, demonstrating the family had enough statistical power to detect genetic linkage. In our linkage analysis, AD was assumed to be an autosomal dominant disease with a gene frequency of 0.001. Mutation rate was set equal to 0 and equal recombination rates were assumed for males and females. Age-dependent penetrance was defined by five liability classes based on the ages at onset observed in this family: unaffected at risk (<40 years old): 0,



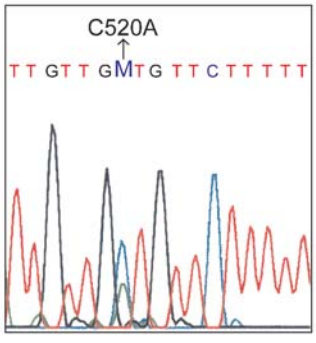


Fig. 1 **A** Pedigree of the family. Haplotypes corresponding to the chromosome 14q24.3 region are shown. Filled (*black*) symbols represent demented individuals. Gender and ages are not specified for reasons of confidentiality. A *diagonal line* across the symbol means deceased individual; *two diagonal lines* indicate suicide. Filled (*black*) bars indicate the disease haplotype. **B** Direct sequencing revealed a heterozygous base change C→A in exon 6 of the *PSEN1* gene

0, 0; 40–55 years old: 0.001, 0.5, 0.5; 56–70 years old: 0.01, 0.7, 0.7; 71–85years old: 0.01, 0.8, 0.8; married in and affected: 0, 0.9, 0.9. Equal allele frequencies for markers were assumed, since there were no data available from the Cuban population. Two-point linkage analysis was performed using the MLINK program from the LINKAGE package version 5.1. Marker order and genetic distances were used according to the Marshfield integrated linkage maps (www.marshfieldclinic.org/research/genetics/).

Sequence analysis

All 12 exons from the *PSEN1* gene were sequenced by the use of intronic flanking primers as described previously [5]. For exon 2 (forward primer: 5′-TCGTGACAAATTAATACATTCC-3′) and exon 6 (forward 5′-CAGTCTGGGCGACAAAGTG-3′ and reverse 5′-TTAAATGATAGCTACACAGCA-3′) we designed new primers, further away from the exon-intron boundary (Genbank accession no. AH004968). We also sequenced part of the regulatory region, 2 kb upstream exon 1 (primer sequences published elsewhere [29]). Genomic DNA (50 ng) from 2 patients and 2 healthy Cuban controls was used as template for PCR reactions. Specific conditions were 1× PCR buffer GibcoBRL, 1.5 mM $MgCl_2$, 200 µM dNTPs, Taq DNA Polymerase 2 units per reaction, and 25 pmol of each forward and reverse primer in 50 µl of reaction volume. PCR product (5 µl) was checked on 1.5% agarose gel, the rest was purified (Qiagen kit) and used for a second reaction using the BigDye terminator kit (Applied Biosystems). Products were loaded on an ABI 377 Automatic DNA sequencer and analyzed with Factura and Sequence Navigator program (Applied Biosystems). Once a heterozygous base change or another variation was found in the index case, all patients and 4 healthy controls were sequenced.

Allele-specific oligonucleotide hybridization

Sixty family members and 40 unrelated Cuban controls were tested for the C→A change detected in exon 6 of the *PSEN1* gene. Allele-specific oligonucleotides [30] were designed for the normal 5′-ATTGTTG**C**TGTTCTT-3′ and mutated allele 5′-ATTGTTG**A**TGTTCTT-3′ and radioactively labeled with α-dATP [31]. PCR products from exon 6 were fixed on two nylon membranes and hybridized with the normal and mutated oligonucleotide respectively, under specific conditions (35°C 1 h). Filters were washed until a final stringency of 0.3× SCC and 0.1% sodium dodecyl sulfate (35°C) was reached. Membranes were exposed to X-ray films.

Results

Clinical studies

Clinical characteristics of 14 patients are shown in Table 1. Memory impairment was the main symptom shared by all patients. Mean age at onset was 59 years of age, with a mean disease duration of 9 years. Patient III-17 showed late-onset dementia (76–83 years), but with similar clinical characteristics to the other patients. No cases of presenile dementia have been reported among his descendants.

Table 1 Clinical description of patients (*DCF* deterioration of cognitive function)

	Patients													
Clinical characteristics	II-2[a]	II-4[a]	III-1[a]	III-3[a]	III-8[a]	III-14[a]	III-17[a]	III-23[a]	III-25[a]	III-26	IV-20[a]	IV-24	IV-26	IV-28
Age at onset/death (current)	65–80	61–68	55–72	63–68	51–56	59–67	76–83	58–69	63–72	67–73	53–62	51–(56)	48–59	50–(54)
Signs and symptoms														
Memory loss	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Impairment of daily activities	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Altered behavior	–	–	+	+	+	–	–	+	–	–	+	+	+	+
Depression	–	–	+	–	+	+	–	–	+	–	+	–	+	–
Anxiety	–	–	+	–	–	–	–	–	–	–	–	–	–	+
Aggression	–	–	+	+	+	–	–	–	–	–	–	–	–	–
Aphasia	+	+	–	+	+	+	+	+	+	+	+	–	+	–
Apraxia	–	–	–	+	–	+	+	+	–	+	+	–	+	+
Agnosia	+	+	–	–	+	+	+	+	+	+	+	–	+	–
Dementia	+	+	+	+	+	+	+	+	+	+	+	DCF	+	DCF
Ischemic episodes	–	–	–	–	–	–	–	+	–	+	+	–	+	–
Gait disorder	+	+	–	+	+	–	+	–	+	–	–	–	–	+
Seizures	–	–	–	–	–	–	–	–	–	–	+	–	+	–

[a]Based on family history

The propositus, IV-26, started having problems at age 48 years when she suffered a cerebral ischemic episode. After recovering from unconsciousness (a few minutes), she had right hemiparesis in the face and body. The presence of any symptom before this episode could not be confirmed. Thereafter, she complained of memory loss, lack of interest, she started making mistakes at work, and due to progressive worsening of symptoms, she was admitted to hospital. The neuropsychological studies were altered, EEG showed slow base activity and bifrontal irritative areas, and head CT scan displayed cerebral and cerebellar atrophy and a hypodense lesion the in left parieto-temporal area. At age 53 years she was unable to recognize even her closer relatives, she was completely dependent on caregivers, and was bedridden until she died 6 years later.

In addition to the propositus, 5 relatives were reported as patients; 3 of them were available for clinical examination (III-26, IV-24, and IV-28). Patient III-26 had a well-established dementia with 5 years of disease evolution and was bedridden. Generalized cortical atrophy, ventricular enlargement, and hypodense regions suggestive of vascular lesions were observed on brain CT scan. The patient died 1 year later at age 73 years.

Patient IV-24 was seen for the first time after 1 year of disease evolution. Neuropsychological tests were clearly altered. Patient IV-28 had a subtle alteration in neuropsychological studies a few months after the onset of symptoms. Follow-up of patients IV-24 and IV-28 confirmed a progressive deterioration of cognitive functions with impairment of daily activities. The patients had concentration and calculation deficits, difficulties in immediate and delayed recall of recently presented material, decreased emotional response, impairment of spatial skills, delay of speech, and anosoagnosia. CT scans from patients IV-24 and IV-28 were normal at first-time examination. A second CT scan performed 2 years later (patient IV-28) showed signs of diffuse cortical atrophy. Other causes of dementia were excluded and the diagnosis of presenile dementia, probable AD was established.

Neuropathological studies

Microscopic examination of brain material (patient III-26) revealed neuronal loss and both diffuse and core neuritic plaques. The presence of amyloid plaques was confirmed by immunostaining with an Aβ antibody (Fig. 2 A and B). Cerebral amyloid angiopathy was also observed (Fig. 2B). The existence of abundant amyloid plaques was the most prominent feature. Tau immunohistochemistry (AT8 and PHF antibodies) revealed numerous NFT (Fig. 2C and D, respectively). Immunohistochemistry using antibodies against prion protein did not show any difference between normal controls and our patient. No inclusions were detected with the α-synuclein antibody. Pathological findings provided sufficient evidence to confirm the diagnosis of AD.

DNA studies

APOE *genotypes*

Forty-seven DNA samples were tested, 32 were homozygous ε3, 13 had the ε3/ε4 genotype, and 2 were homozygous ε4. Allele ε4 was only present in the second branch of the pedigree. Of 4 tested patients, 3 had the ε3/ε4 genotype.

Other genotypes

Genotypes for markers situated on chromosomal regions 1q31–42 (*PSEN2*), 10q24 (*AD6*), 12p11.23-q13.12

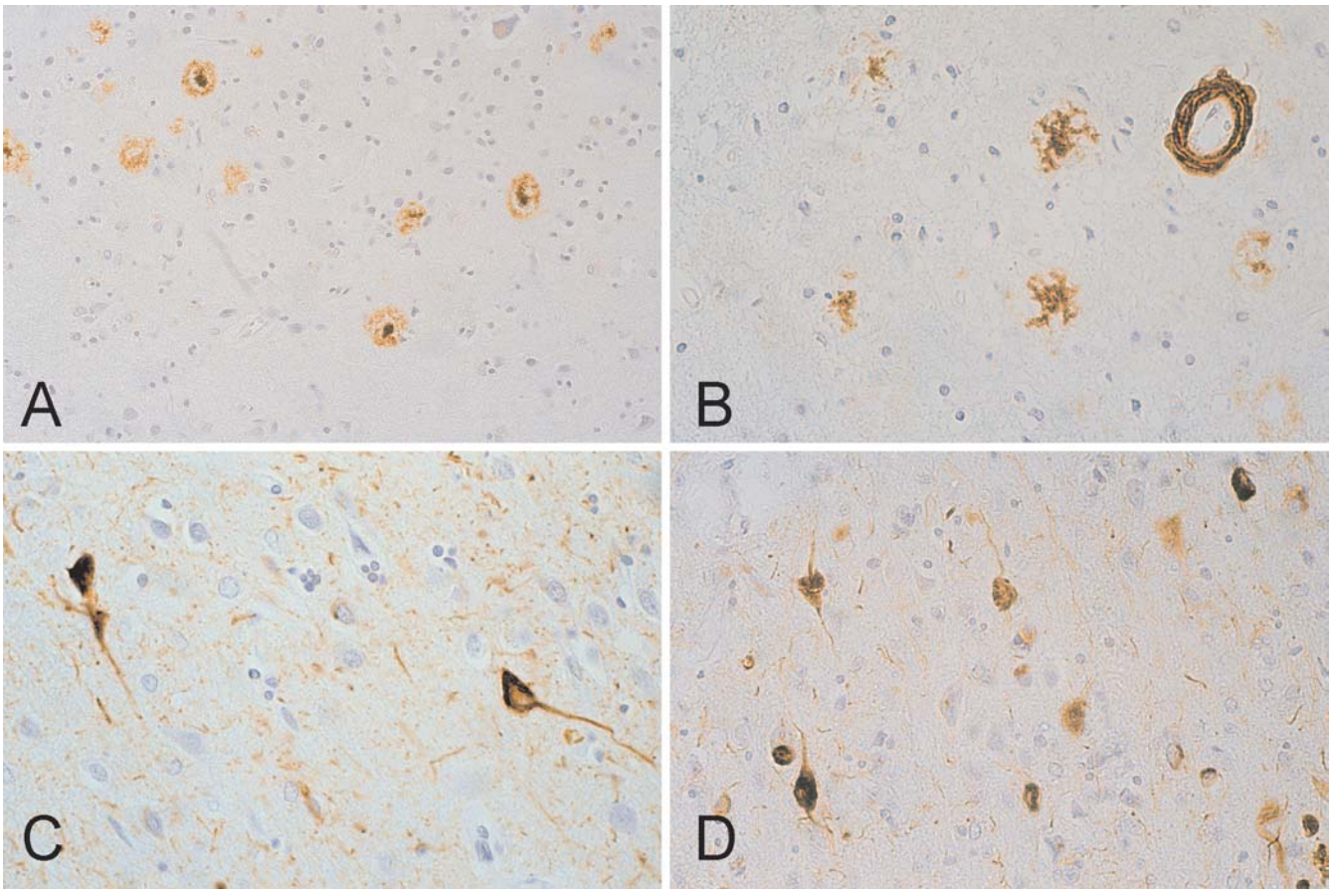


Fig. 2 Sections of temporal cortex of patient III-26, stained with β-amyloid antibody (**A** and **B**), showing numerous extracellular plaques and amyloid deposition in the wall of a blood vessel (**B**), magnification ×200. **C** and **D** Immunostaining with AT8 and PHF antibodies showed several neurofibrillary tangles, magnification ×400

(*AD5*), 14q24.3 (*PSEN1*), 19cen-q13.2 (*AD2*), and 21q21 (*APP*) were determined for 30 family members. In addition, other dementia-related regions 3p11.1-q11.2 (*DMT1*), 17q21.1 (*MAPT*), and 20pter-p12 (*PRNP*) were tested. Haplotype and linkage analysis allowed us to exclude the presence of the gene responsible for most of them.

Evidence supporting genetic linkage was only found for the chromosome 14q24.3 region, two-point linkage analysis revealed positive Lod scores for several adjacent markers, and a maximum Lod score of 3.79 was obtained for marker D14S43 at $\theta=0$.

We then saturated the region with additional markers from D14S52 to D14S617 covering 50 cM. Haplotypes from 45 family members are shown in Fig. 1. The 4 patients shared a common region spanning 9 cM from marker D14S588 to D14S43. Patient IV-24 showed recombination events in both telomeric and centromeric parts of the haplotype, allowing us to determine the upper and lower limit of the region shared by all 4 patients.

Several demented patients were reported to have psychiatric symptoms (aggression, restlessness, anxiety, depression), but did have haplotypes identical to the other patients.

Sequencing of PSEN1

According to several genetic maps, the *PSEN1* gene is located close to markers D14S1002 and D14S77 in the middle of our critical region. Since mutations in the presenilin genes are the most common cause of the presenile dementias with autosomal dominant inheritance, we performed direct sequencing of 12 exons of *PSEN1* and part of its regulatory region for both sense and antisense strands.

A heterozygous base substitution at position +520 from the ATG starting site, resulting in a C to A transversion, was found in exon 6 (Fig. 1B), which is predicted to cause an amino acid change from leucine to methionine in the transmembrane domain III of the presenilin 1 protein. We then included additional samples; all available DNAs from the family (60 individuals) and 40 unrelated Cuban controls were tested with allele-specific oligonucleotides. The mutation was found to co-segregate with the disease phenotype and the associated disease haplotype, and was present in 8 at-risk subjects (5 are shown in Fig. 1A), but not in other healthy individuals and spouses (96 chromosomes). The C→A change was not observed in any of the 80 control chromosomes from the general Cuban population.

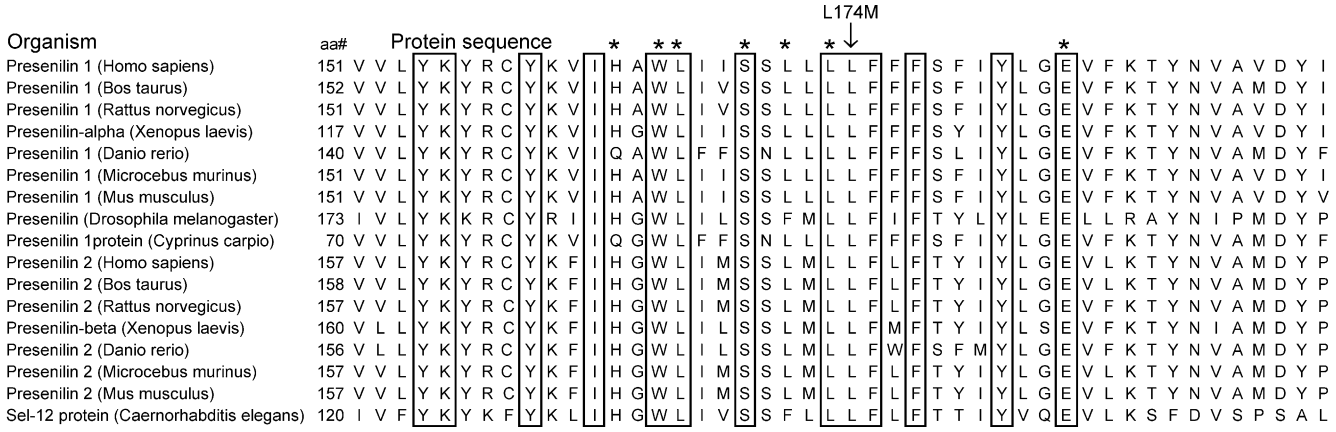


Fig. 3 Alignment of human presenilin 1, presenilin 2, and orthologous protein fragments. Highly conserved amino acids are shown in *boxes*. Leucine at position 174 is substantially conserved across several species. *Asterisks* indicate where other *PSEN1* mutations are located

Discussion

The clinical picture was compatible with AD except for the fact that 4 of 18 patients had vascular episodes such as transient ischemic crises. Cerebral amyloid angiopathy (CAA) is best known as a leading cause of lobar hemorrhages in the elderly and as a common finding in AD. It has also been implicated in the production of other cerebrovascular lesions [32]. The presence of CAA might explain the vascular episodes described in patients III-23, III-26, IV-20, and IV-26. Hypodense zones suggestive of vascular lesions were observed on CT scans from patients III-26 and IV-26; in addition, amyloid deposits were present in the wall of brain blood vessels (patient III-26), confirming the presence of CAA.

Factors such as the vasoactive character of Aβ, which can produce vasospasm in vitro at low concentrations [31], the secretion of Aβ by activated human platelets [33], and the hyaline necrosis surrounding the amyloid deposit in the vessel wall [34] have been postulated as possible mechanisms by which amyloid in the vessels can lead to vascular lesions.

Furthermore, a relationship between *APOE* ε4 allele and the presence of CAA and cerebrovascular pathology in AD has been suggested, since the *APOE* ε4 allele is a risk factor for the development of CAA in AD [35]. In this family, available patients with ischemic episodes also carry the *APOE* ε4 allele (patients III-26 and IV-26).

We found significant evidence for genetic linkage to chromosome 14q24.3. We were able to reconstruct haplotypes for 11 deceased patients using first-degree relatives; all of them had the same “disease haplotype”, except 1 patient (III-17) in which the reconstructed haplotype was different. This patient showed a much later disease onset (76 years old), while the observed range in the other patients was 48–67 years old. Unfortunately, only family history but no medical records were available. It might be that this patient had dementia due to other causes, and therefore could be a phenocopy. Coexistence of both early onset (with an associated *PSEN1* mutation) and late-onset forms of AD in the same family has been reported before [36].

Several heterozygous base changes were detected during *PSEN1* sequencing, but none of the sequence alterations was found to co-segregate with the disease except the C520A change. Several facts indicate the pathogenicity of this novel *PSEN1* mutation. The C520A change was present in all examined patients and in all relatives carrying the disease haplotype, although they were still below the age at onset of AD in the family. The C→A change is unlikely to be a common polymorphism, we did not find it in 80 control chromosomes (from the same population) or in unaffected relatives (96 chromosomes) that did not carry the disease haplotype.

L174 M is a conservative amino acid substitution since leucine and methionine are both nonpolar amino acids. Nevertheless, close to half of the *PSEN1* and *PSEN2* reported mutations also lead to the same type of amino acid change. The leucine at position 174 of the presenilin 1 protein is highly conserved among different species (Fig. 3) and between homologous proteins like presenilin 2 and sel-12, indicating that this position (TMIII) is important for protein function. Adjacent amino acid positions at 163, 165, 166, 169, 171, 173, and 184, where other mutations have been found, are also evolutionary conserved.

Under normal conditions presenilin 1 holoprotein is rapidly converted into two fragments, an amino terminal fragment (NTF) and a carboxy terminal fragment (CTF) [37]. Presenilin 1 fragments can associate into tightly bound complexes that may represent the principal form in which presenilin functions in cells [38]. Formation of these high molecular weight complexes composed of both presenilin 1 fragments may also explain why FAD-associated mutations within the N-terminal region of presenilin 1 (like *L174 M*) result in the hyperaccumulation not only of the NTF but also of the CTF. It has been proposed that the observed elevation in the accumulated amounts of mutant presenilin 1 NTFs and CTFs must be the result of enhanced endoproteolytic processing of mutant presenilin 1 protein and/or greater stability of mutant presenilin 1-derived fragments [39], which appears

to be associated with enhanced production of $A\beta_{42}$ and early onset FAD.

Another possible mode of action of the *L174 M* mutation might be that of an alternative translation start site. From the 82 mutations described in *PSEN1*, only *V94 M* in exon 4 [9] and the current mutation (exon 6) introduce a new methionine in the protein sequence.

Functional assays will be required to determine whether the *L174 M* mutation affects presenilin 1 protein activity for the cleavage of Notch1 at the plasma membrane and the release of the Notch1 intracellular domain.

In conclusion, we found a novel *PSEN1* mutation in a large family with clinical and pathological diagnosis of early onset FAD. This is the first genetic study on AD that identifies the involvement of the *PSEN1* gene in Cuba.

L174 M is the seventh mutation described in Latin-American and Caribbean populations; for some of them (Colombia and Dominican Republic) a founder effect has been described. We are currently in the process of collecting new familial and sporadic cases with early onset AD. It will be of interest to investigate the contribution of this mutation to other early onset AD cases within the Cuban population or in others of Hispanic origin.

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