Worldwide distribution of *PSEN1* Met146Leu mutation

A large variability for a founder mutation

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ABSTRACT

Objective: Large kindreds segregating familial Alzheimer disease (FAD) offer the opportunity of studying clinical variability as observed for presenilin 1 (*PSEN1*) mutations. Two early-onset FAD (EOFAD) Calabrian families with *PSEN1* Met146Leu (ATG/CTG) mutation constitute a unique population descending from a remote common ancestor. Recently, several other EOFAD families with the same mutation have been described worldwide.

Methods: We searched for a common founder of the *PSEN1* Met146Leu mutation in families with different geographic origins by genealogic and molecular analyses. We also investigated the phenotypic variability at onset in a group of 50 patients (mean age at onset 40.0 ± 4.8 years) by clinical, neuropsychological, and molecular methodologies.

Results: EOFAD Met146Leu families from around the world resulted to be related and constitute a single kindred originating from Southern Italy before the 17th century. Phenotypic variability at onset is broad: 4 different clinical presentations may be recognized, 2 classic for AD (memory deficits and spatial and temporal disorientation), whereas the others are expressions of frontal impairment. The apathetic and dysexecutive subgroups could be related to orbital-medial prefrontal cortex and dorsolateral prefrontal cortex dysfunction.

Conclusions: Genealogic and molecular findings provided evidence that the *PSEN1* Met146Leu families from around the world analyzed in this study are related and represent a single kindred originating from Southern Italy. The marked phenotypic variability might reflect early involvement by the pathologic process of different cortical areas. Although the clinical phenotype is quite variable, the neuropathologic and biochemical characteristics of the lesions account for neurodegenerative processes unmistakably of Alzheimer nature. *Neurology*® 2010;74:798-806

GLOSSARY

AD = Alzheimer disease; EOFAD = early-onset familial Alzheimer disease; FAD = familial Alzheimer disease; H-E = hematoxylin-eosin.

Alzheimer disease (AD) is a common degenerative disorder of unknown etiology, believed to involve a combination of genetic and environmental factors. About 47% of families with early-onset familial AD (EOFAD) have been attributed to *PSEN1* mutations (<http://molgen-www.ua.ac.be/ADmutations>). Since the early 1970s, we have been studying 2 large Calabrian EOFAD kindreds: the N family¹ and the TO family,² both instrumental for the cloning of *PSEN1* gene.³ A shared extended haplotype containing the *PSEN1* gene and the Met146Leu (ATG/CTG) mutation was shown to be identical by descent,³ thus confirming a posteriori the common origin of the families.⁴ Both Calabrian kindreds encompass branches inde-

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pendently identified in different geographic places and at different times. The EOFAD pedigree described in 1963⁵ was later genealogically linked to the N family¹ and the C family,⁶ whereas the FJ01 family ascertained in Milan in 1990 was linked to the TO family in 1993.⁷ Emigrated branches dispersed throughout the world suggest that all *PSEN1* Met146Leu (ATG/CTG) families (or apparently sporadic cases) could belong to the Calabrian kindreds, thus indicating that this mutation is a private and founder one.

Clinically, the *PSEN1*-EOFAD phenotype presents with a picture of cognitive disorder typical of AD. However, instances of frontotemporal dementia⁸⁻¹² have been reported, even if mutations in Progranulin might explain some of these atypical cases.¹³

The aims of the study were 1) to demonstrate the genealogic and genetic links among the different *PSEN1* Met146Leu (ATG/CTG) families reported in the literature or investigated by the authors and 2) to investigate the phenotypic variability with specific reference to clinical symptoms.

METHODS Families. We included 3 EOFAD families with the *PSEN1* Met146Leu (ATG/CTG) mutation: the 2 Calabrian kindreds¹⁻⁴ and the recently identified Naples family.

N and TO families. The N and TO families are considered as a unique EOFAD population reconstructed from the present to the 17th century, over 11 generations and consisting of 138 affected subjects (49 personal examination, 13 neuropathologically confirmed, 36 clinical records, 40 reported affected by history) and 15 obligate carriers. Age at onset is 41.8 ± 5.8 years; age at death is 49.9 ± 5.8 years; duration is 7.3 ± 4.1 years (1–16); segregation ratio is 0.67; sex ratio M:F is 1:1.32.

The genealogic database reconstructed around the Calabrian kindreds contains approximately 50,000 individuals from the 17th century onwards.

Naples family. The apparent origin of the Naples family is in the town of TdG (near Naples) in 1800, where ancestors were traced by historical documents belonging to the family (figure 1). The proband (133611, IV-1), a 40-year-old man showing memory loss, attention and planning deficits, and partial insight, had been diagnosed with familial AD caused by a *PSEN1* Met146Leu (ATG/CTG) mutation at the Neurology Department of the University of Florence (appendix e-1 and table e-1 on the *Neurology*® Web site at www.neurology.org).

History revealed that 6 other members of the family over 4 generations developed dementia. One of them had been clinically diagnosed with Pick disease (appendix e-1).

Database searches of PubMed using the key words EOFAD-*PSEN1* mutations and of the AD&FTD Mutation Database (<http://www.molgen.ua.ac.be/ADmutations>) produced 7 articles in which EOFAD families (or single subjects) were associated

with the Met146Leu (ATG/CTG) mutation, subsequent to the first presentation of Calabrian kindreds.^{3,14-20} The authors of the articles were contacted to trace the family origins. Consent was obtained from the family members for this specific purpose.

Standard protocol approvals, registrations, and patient consent. This study was financially supported by the Italian Health Ministry projects: 1) DGRST no. 4/2760-P/I.9.ab, 2007; 2) RFP5-2006-7-334858, 2006, both with ethics committee approval. Written informed consent was obtained from all patients (or guardians of patients) participating in the study (consent for research).

Medical records belonging to affected deceased subjects are recorded in the archives of the Provincial Psychiatric Hospital.

Clinical trial identifier number and public trials registry are not applicable.

Genealogic methods. Data from municipal documents since 1809 and from parish registers dating back to 1606 were gathered to reconstruct family trees (appendix e-2).

To verify whether members of the Naples family, and of all affected subjects reported in the literature or their possible ancestors, were already present we searched the database for specific surnames in specific periods.

Genetic analysis. All available patients or their families provided informed written consent to participate in this study. Genomic DNA was extracted from blood buffy-coats using standard phenol-chloroform procedures.

Screening for *PSEN1* Met146Leu (ATG/CTG) mutation by sequencing, and screening for *APOE* genotype, tau haplotypes, and the prion protein gene (PRNP) Val129Met polymorphism by PCR–restriction fragment length polymorphism, was performed as described previously.²¹⁻²⁴

To determine whether the *PSEN1* Met146Leu mutation had been inherited from a common ancestor, haplotype analysis on one affected subject per family (N family 1193; TO family 16056; Naples family 133611) was performed. Six adjacent microsatellite markers, from centromere to telomere, D14S1002, D14S1028, D14S77, D14S1377, D14S999, and D14S53, located within a 6-Mb region on the physical map surrounding the *PSEN1* gene, were studied. In particular, D14S77 and D14S53 were previously used in genetic linkage studies.³ All microsatellites were selected when informative (heterozygosity >75%), with an interval between markers of about 1 Mb. Primer sequences for microsatellite markers are listed at www.ncbi.nlm.nih.gov and www.ensembl.org. Each marker was amplified by PCR with one fluorescent labeled primer using standard conditions for microsatellites. The PCR products were assessed on the ABI Prism Genetic Analyzer (Applied Biosystems) and analyzed with Genescan version 3.0 and Genotyper software version 2.1. Marker allele frequencies were established in 40 chromosomes from Italian controls unrelated to the patients. To estimate ancestry proportions for each individual and to determine whether the recurring mutation represented an independent mutational event or was due to a common ancestry, we analyzed the ancestry information content from a set of 17 very informative microsatellite markers on different chromosomes, analyzing them as independent alleles, using the program STRUCTURE, which is based on the Bayesian approach.²⁵

Neuropathologic study. The brains of patients 1768 and 1772 were obtained for neuropathologic investigation 5 hours after death. One hemisphere was fixed in 10% formalin; the other was dissected into coronal slices and immediately frozen at -80°C .

Fixed specimens of several areas of cerebral hemispheres, brainstem, and cerebellum were embedded in paraffin for histology that was carried out on 10- μm -thick sections stained by hematoxylin-

Figure 1 Pedigree

Extended pedigree representing known affected subjects of all families with Met146Leu mutation and magnification of Naples family pedigree. E = explanted brain; PH = patients hospitalized in psychiatric hospital.

eosin (H-E), treated by Thioflavine S, and immunostained for A β (antibody: pan β Biosource, 1:1,000), phosphorylated tau (P-tau) (AT8 Innogenetics, 1:1,000), the tau isoforms with 3 (RD3, Upstate Biotechnology, 1:3,000) or 4 microtubule-binding repeat domains (RD4, Upstate Biotechnology, 1:100), as well as α -synuclein (clone 4D6, Signet, 1:5,000). Immunostaining was visualized by the EnVision Plus/Horseradish Peroxidase System for rabbit and mouse immunoglobulins (DakoCytomation) with 3-3'-diaminobenzidine as chromogen.

Sarcosyl-insoluble tau was extracted as previously indicated²⁶ and immunoblotting was performed with antiserum BR133.²⁶

Analysis of the clinical variability. Fifty patients (mean age at onset 40.0 ± 4.8 years, of which 18 were alive; 38% female) out of 148 patients with *PSEN1* Met146Leu mutation (138 from Calabrian kindreds, 7 from Naples, and 3 from the previously reported Australian family²⁰) with reliable data regarding history and clinical picture were selected for the study of the variability of the clinical phenotype at onset and during the initial stages of the disease. Twenty-seven patients were personally studied by the authors through a complete neurologic evaluation, neuropsychological battery,²⁷ and morphologic and functional brain imaging. Twenty-three patients were selected on the basis of completeness of clinical records. For deceased Calabrian patients, a complete survey of the records of the Provincial Psychiatric Hospital archives, encompassing data from 1881 to 1980, was performed. A checklist including demographic (age at death) and clinical data (age at onset, duration, cognitive and behavioral symptoms relying on Lund and Manchester clinical criteria²⁸) was filled out for all patients.

Statistical analyses. Hierarchical cluster analysis²⁹ was applied to establish whether subgroups existed. Cross-tabulation tables with χ^2 test were performed to calculate the association between variables. Quantitative data were compared with independent *t* tests between subgroups. Descriptive statistics and all analyses were performed using SPSS 11.5 software. Structure 2.2 program was used to analyze microsatellites frequencies.²⁵ For all analyses, significance was $p < 0.05$.

RESULTS Genealogic links. A genealogic link was established with all the subjects carrying *PSEN1* Met146Leu mutation reported in the literature (figure 1, appendix e-1, and figure e-1).

The reconstituted Calabrian kindreds plus the Naples family and the Australian family (table 1) comprises 148 affected persons and 19 obligate carriers from the 17th century onwards.

Molecular link. A common shared DNA haplotype was identified among Calabrian kindreds and the Naples family, thus suggesting that the Met146Leu mutation occurred in a common ancestor (appendix e-1). None of the controls had the same haplotype identified in the subjects from N and the Naples family. Patient 16056, belonging to the TO family, previously linked to the N family,³ showed a unique allelic pattern for the D14S53 marker. This microsatellite marker is the marker most distant from *PSEN1* gene and it is possible that a single recombination event occurred in this region. Unfortunately, we cannot estimate the exact number of generations separating them from the common founder because of the limited number of available Naples family members.

Neuropathologic study. The weights of the 2 brains examined were 970 and 1,230 g. Gross examination showed diffuse brain atrophy. Microscopic examination identified substantial A β and P-tau immunoreactivity (IR) in both brains. A β amyloid deposition was present in the neuropil of the cerebral cortex, mesial temporal structures, striatum, thalamus, brainstem, and cerebellar cortex (figure 2, A, D, and F) and in the wall of intraparenchymal and leptomeningeal vessels (figure 2C). P-tau IR could be detected in nerve cell bodies and neurites raising tangles and neuropil threads that were substantially present in the mesial temporal structures, in the neocortex, and, to lesser an extent, in striatum, thalamus, substantia nigra, and tegmental nuclei (figure 2, B and E). Specifically, abundant neuritic plaques, neurofibrillary tangles, and neuropil threads were observed by RD3 antibody (figure 2G); several deposits of "Pick-like bodies" were also stained by RD4 (figure 2H), indicating that they were not proper Pick bodies that contain only tau with 3 repeats. H-E staining revealed severe nerve cell loss and astrogliosis, particularly at the level of mesial temporal structures and the frontotemporal cortex.

Neuropathologically, AD could be staged 6 in all the patients according to the staging of neurofibrillary changes by Braak and Braak.³⁰ Following the distribution of A β deposits, AD could be staged IV in patient 1772 and V in patient 1768, according to I–V staging by Braak and Braak.³⁰

Immunoblotting of sarcosyl-insoluble tau extracted from parietal, frontal, and temporal cortex showed the 60, 64, 68, and 72 kDa bands characteristic of Alzheimer cases. Dephosphorylation of

Table 1 Demographic characteristics of the families with the Met146Leu mutation^a

Families	N_TO	Naples	Australian	All kindreds
Patients	138	7	3	148
Generations	11	6	11	11
Affected generations	6	4	2	6
Postmortem examination	13	0	2	15
Obligate carriers	15	0	4	19
Age at onset, y	41.7 $\pm$ 5.8	40.9 $\pm$ 5.2	40.0 $\pm$ 0.0	41.6 $\pm$ 5.7
Age at death, y	49.9 $\pm$ 5.8	47.0 $\pm$ 4.1	46.0 $\pm$ 1.2	49.7 $\pm$ 5.7
Duration	7.3 $\pm$ 4.0	6.8 $\pm$ 4.1	7.0 $\pm$ 1.4	7.6 $\pm$ 4.1
Segregation ratio	0.67	0.54	0.45	0.55
Sex ratio M:F	1:1.23	1:1	1:2	1:1.24

^aValues are n or mean $\pm$ SD.

(A–F) Neuropathologic changes were represented by parenchymal A β deposits (A, frontal cortex; D, putamen; F, inferior olive: anti-A β 42 antibody), neurofibrillary tangles and neuropil threads (B, frontal cortex; E, putamen: AT8 antibody), congophilic angiopathy (C, temporal cortex, anti-A β 40 antibody). (G–H) Immunohistochemical staining showing Tau immunoreactivity in the brain of patients 1768 (G, cortex: RD3 antibody) and 1772 (H, cortex: RD4 antibody). (I) Sarcosyl-insoluble tau from parietal (lane 1), temporal (lane 2), and frontal (lane 3) cortex of the patient appears as 3 major bands at 72, 68, and 64 kDa and a minor band at 60 kDa after immunoblotting with antiserum BR133. Sarcosyl-insoluble tau from AD brain is shown as comparison (lane 4). The human recombinant Tau is reported as marker (lane 5). Bar in A = 25 μ m, same magnification in A, B, D, E, and F. Bar in C = 50 μ m. Bar in G, H = 30 μ m.

sarcosyl-insoluble tau with alkaline phosphatase showed the presence of all 6 tau isoforms in each cortical area investigated (figure 2I).

Analysis of clinical variability. Cluster analysis, applied to the symptoms presented by the 50 patients at onset and during the first 2 years of the disease, identified 4 subgroups (table 2): 2 showed a cognitive onset (subgroup 2 and subgroup 4, globally 58% of patients, 24% female), and the remaining 2 subgroups (subgroup 1 and subgroup 3, 42% of patients, 57% female) presented with a behavioral onset (figure e-2). Within the subgroups having cognitive onset, subgroup 2 (19 affected subjects, 38%, age at onset 40.2 ± 4.8 years, age at death 46.1 ± 5.5 years) classically first presented

the disease with memory loss (2-amnesic) while subgroup 4 (10 affected subjects, 20%, age at onset 41.1 ± 5.7 years, age at death 48.4 ± 2.5 years) initially presented with temporal and spatial disorientation (4-disoriented).

Behavioral subgroup 1 (14 affected subjects, 28%, age at onset 39.6 ± 4.9 years, age at death 46.1 ± 5.5 years) presented the disease with apathy, emotional unconcern, and depression (1-apathetic). Subgroup 3 (7 subjects, 14%, age at onset 38.7 ± 3.7 years, age at death 49 ± 2 years) presented with deficit of planning, abstract reasoning, attention, critique, and judgment as well as loss of insight (3-dysexecutive). There was no significant difference among the 4 subgroups concern-

Table 2 Percentage symptoms in the 4 subgroups

Patients	1-Apathetic (n = 14), %	2-Amnesic (n = 19), %	3-Dysexecutive (n = 7), %	4-Disoriented (n = 10), %	p Value
Aphasia	0	21.1	28.6	0	0.072
Agnosia	0	5.3	14.3	10.0	0.472
Disinhibition	7.1	0	28.6	10.0	0.069
Distractibility	0	10.5	0	0	0.628
Psychotic disorders	0	10.5	28.6	10.0	0.187
Loss of memory	71.4 ^a	100 ^a	85.7 ^a	80 ^a	0.052
Temporal disorientation	21.4	10.5	57.1	90.0 ^a	0.000 ^b
Spatial disorientation	21.4	10.5	57.1	100 ^a	0.000 ^b
Verbal initiative reduction	7.1	0	28.6 ^a	0	0.034 ^b
Apraxia	14.3	0	71.4 ^a	0	0.000 ^b
Cognitive inertia ^c	21.4	26.3	100 ^a	0	0.000 ^b
Loss of insight	7.1	10.5	71.4 ^a	10.0	0.004 ^b
Apathy	92.9 ^a	0	57.1	10.0	0.000 ^b
Emotional unconcern	35.7 ^a	5.3	14.3	0	0.040 ^b
Depression	85.7 ^a	21.1	14.3	10.0	0.000 ^b
Anxiety	14.3	0	28.6	30.0	0.030 ^b

^aSymptoms showing a difference among the subgroups.

^bSignificant p value.

^cCognitive inertia encompasses deficit of planning, deficit of abstraction, deficit of attention, deficit of judgment; each of these symptoms was significant also when analyzed separately.

ing age at onset and age at death. FDG-PET of the Naples family proband is reported in figure 3.

No association was found between the different cluster groups and the polymorphisms analyzed ($p = \text{NS}$), data not shown.

DISCUSSION Our work demonstrates a common founder to all the EOFAD families reported worldwide segregating the *PSEN1* Met146Leu mutation. Consequently, we can consider this mutation as a “private mutation” shared, at least, among 148 affected subjects dispersed over several centuries and several continents and originating from Southern Italy.

A similar founder effect has been described for other recurrent mutations in *PSEN1* located in exon 4, exon 7, exon 12, and intron 4.^{21,31–34}

The founder effect could be explained through a selective advantage of some mutations that are not eliminated since they do not cause a reproductive disadvantage. Natural selection will favor these phenotypes and the novel trait will spread throughout the population. In the case of the *PSEN1* Met146Leu mutation, we can speculate that this gene mutation confers a selective advantage to survival, since it results in a significant higher segregation ratio (0.67) (table 1).

Demonstration of genetic identity among the *PSEN1* Met146Leu families is not trivial because it increases the size of this genetically homogeneous kindred, as far as we know the largest to date, which represents an interesting tool to study phenotype variability. In a previous work,³⁵ we compared Calabrian kindreds with an Argentinian EOFAD family carrying the same mutation: although there was a general concordance between the clinical and pathologic features of both pedigrees, a highly significant difference in the frequency of cerebellar signs was observed, probably due to the different genotypic variation possibly related with allosteric effects of DNA conformation on transcriptional regulators.³⁶ In the same direction, the identification of families with *PSEN1* mutation^{8–12} showing “frontal” phenotype raises the question of nosology and of genotype–phenotype relationships. In a number of these families^{8,9,12} the absence of neuropathology precludes a more accurate discussion and leaves doubt regarding the genotype–phenotype relationship. The family with *PSEN1* Gly138Val mutation clinically presenting with frontotemporal dementia¹⁰ shows no amyloid plaque pathology and a consistent tau accumulation similar to Pick bodies and Pick cells. The mutation affects a splice donor site and presenilin1 protein is reduced in brain.³⁷ Nevertheless, the presence of clinically affected subjects who do not show the mutation leave unresolved the issue between pathogenicity and disease.³⁸ Calabrian kindreds could offer the opportunity to analyze phenotype variability in a large number of genetically homogeneous affected subjects. Patients globally present with cognitive deficits, while visual hallucinations and agitation, myoclonus, extrapyramidal signs, and epileptic seizures occur later in the course of the disease. Nevertheless, when patients are investigated during the early stages of the disease, which is made possible by their previous “at risk” status, behavioral changes can be documented in a notable number of them.

Four clinical patterns could be recognized at onset. Besides the 2 subgroups presenting with the classic cognitive picture, i.e., memory loss or time and spatial disorientation, the other 2 subgroups are characterized by symptoms pointing to frontal lobe involvement. Interestingly, the 1-apathetic and the 3-dysexecutive subgroups clearly address the disruption of emotional-affective and cognitive processing described by Levy and Dubois³⁹ and relating to the orbital-medial prefrontal cortex and dorsolateral prefrontal cortex. The 2 patients who could be examined neuropathologically both grouped in the 1-apathetic subgroup. At the end stage of the disease, neuropathology showed severe nerve cell loss and astrogliosis in the frontotemporal cortex, but it

Axial slices of [^{18}F]FDG-PET scans of control (C) (male, 38 years) and patient (P). Images were normalized in the Montreal Neurological Institute (MNI) space using SPM2 (Wellcome Department of Imaging Neuroscience, London, UK). The images show relative tracer distribution. Compared to control, the patient shows a clear severe metabolic deficit in the temporoparietal, superior parietal, and posterior cingulate cortices. Slight hypometabolism is evident in the mesial temporal and prefrontal cortex. C: Results of SPM analysis highlight the location of hypometabolic deficit in our patient as compared to controls ($p < 0.02$ uncorrected) (patients vs 14 healthy controls, age range of controls: 27–70, age was considered in the statistical model of SPM as nuisance covariate).

cannot be established whether a predominant involvement of these cortical fields was present earlier in the disease progression. However, the importance of the neuropathology confirmed the unmistakable Alzheimer nature of the neurodegenerative process in these “frontotemporal” cases that display a high number of senile plaques, neurofibrillary tangles, and neuropil threads. Tau inclusions showed in some cases a round shape, but could not be defined as Pick bodies since they contained both tau with 3 and 4 repeats. Moreover, the analysis of sarcosyl insoluble tau from the brains was also consistent with AD showing the characteristic 4 tau bands ranging from 60–72 kDa.

The Naples family proband clustered in the 3-dysexecutive subgroup and FDG-PET showed hypometabolism in the prefrontal dorsolateral cortex together with the expected severe metabolic deficit in the temporoparietal areas. These data correlate with the impairment of executive functions and behavioral disturbances revealed by clinical and neuropsychological evaluation.

Visual inspection of charts of clinical course suggests that subgroup 4-disoriented (figure e-2) seems to carry a disease with a more favorable evolution since the patients are quite stable and they do not worsen during the 2 years of follow-up. On the contrary, 3-dysexecutive and 1-apatetic subgroups had a more aggressive course and within 2 years patients developed a global cognitive impairment (figure e-2). Incidentally, the 2 neuropathologic cases described here also had a very brief survival; death occurred after a mean duration of only 3 ± 1.4 years.

The different presentation of AD at onset is independent of the environment since it has been shown indifferently by Australian, Naples, and Calabrian kindreds. No molecular genetic differences arose from analyzed polymorphisms, although the number of patients was limited.

The wide phenotype variability at onset could be due to different localization of the early AD lesions in different cortical areas, as already suggested by brain imaging studies with Pittsburgh Compound in mild

AD,⁴⁰ or to different unknown genetic modifiers and is not dependent on the environment. Although the clinical presentation is highly variable, the neuropathologic phenotype accounts for a pure AD pathology, arguing against the coexistence of AD and frontotemporal dementia as a consistent event for this mutation.

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DISCLOSURE

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