Homozygous carriers of *APP* A713T mutation in an autosomal dominant Alzheimer disease family

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ABSTRACT

Objective: To report, for the first time, a large autosomal dominant Alzheimer disease (AD) family in which the *APP* A713T mutation is present in the homozygous and heterozygous state. To date, the mutation has been reported as dominant, and in the heterozygous state associated with familial AD and cerebrovascular lesions.

Methods: The family described here has been genealogically reconstructed over 6 generations dating back to the 19th century. Plasma β -amyloid peptide was measured. Sequencing of causative AD genes was performed.

Results: Twenty-one individuals, all but 1 born from 2 consanguineous unions, were studied: 8 were described as affected through history, 5 were studied clinically and genetically, and 8 were asymptomatic at-risk subjects. The A713T mutation was detected in the homozygous state in 3 patients and in the heterozygous state in 8 subjects (6 asymptomatic and 2 affected).

Conclusions: Our findings, also supported by the β -amyloid plasma assay, confirm (1) the pathogenic role of the *APP* A713T mutation, (2) the specific phenotype (AD with cerebrovascular lesions) associated with this mutation, and (3) the large span of age at onset, not influenced by *APOE*, *TOMM40*, and *TREM2* genes. No substantial differences concerning clinical phenotype were evidenced between heterozygous and homozygous patients, in line with the classic definition of dominance. Therefore, in this study, AD followed the classic definition of a dominant disease, contrary to that reported in a previously described AD family with recessive *APP* mutation. This confirms that genetic AD may be considered a disease with dominant and recessive traits of inheritance. *Neurology*® 2015;84:2266–2273

GLOSSARY

A β = β -amyloid; **AD** = Alzheimer disease; **ADL** = activities of daily living; **AMC** = affected mutation carrier; **APP** = amyloid precursor protein; **ASMC** = asymptomatic mutation carrier; **CVL** = cerebrovascular lesion; **FDG** = fluorodeoxyglucose; **HIS** = Hachinski Ischemic Score; **IADL** = instrumental activities of daily living; **MMSE** = Mini-Mental State Examination; **NMC** = non-mutation carrier; **PolyPhen-2** = Polymorphism Phenotyping 2; **PSEN1** = presenilin 1; **PSEN2** = presenilin 2; **SIFT** = sorting intolerant from tolerant.

Heterozygous mutations in the amyloid precursor protein (*APP*) gene are associated with different phenotypes, such as autosomal dominant early-onset Alzheimer disease (AD),^{1–3} cerebral amyloid angiopathy,⁴ autosomal dominant early-onset AD with cerebral amyloid angiopathy,⁵ and hereditary cerebral hemorrhage with amyloidosis.⁶ Two recessive mutations, causing disease in the homozygous state, have also been identified^{7,8} in 2 different families. Heterozygous *APP* mutations affecting codons from 714 to 717 alter γ -secretase cleavage causing an increase of the β -amyloid (A β)42/A β 40 ratio.^{9,10} In AD families, A β 42 elevated levels have been reported among symptomatic carriers of presenilin mutations,¹¹ and A β 42 and A β 42/A β 40 ratio levels were higher in unaffected familial AD mutation carriers compared with the unaffected individuals without mutation belonging to the same AD families.¹² First-degree relatives of patients with late-onset AD without any known mutations have also been found to have an increase of

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plasma A β 42.¹³ The *APP* A713T mutation has been reported with dominant inheritance and in heterozygosis, associated with familial AD with both early and late onset and cerebrovascular lesions (CVLs).^{5,14,15} All Italian patients with the A713T mutation^{5,15} shared a common DNA haplotype, suggesting that this mutation originated from a putative ancestor,¹⁶ probably living centuries ago in Calabria (Southern Italy), where a high prevalence of this mutation has been observed.¹⁶

We report the A713T mutation, for the first time to our knowledge, both in homozygosis and heterozygosis in a family (not genealogically related to the previously described families) affected by autosomal dominant AD with CVLs.

METHODS Pedigree reconstruction and family members.

The pedigree of the *PEC* family has been reconstructed over 6 generations from the present time back to the 19th century (subject II-2 was born in the year 1823) through the systematic collection of data from municipal documents (births, deaths, and marriage acts since 1809).

To avoid bias in the collection of data, we followed apparently unaffected as well as affected branches of the pedigree to verify consanguinities at any level. Any subject transitively linked with the proband(s) by ascent, descent, or marriage relationship was considered as belonging to the pedigree. A genealogical search was conducted in a village in Calabria (*Car*). The data obtained were stored in a database containing thousands of subjects linked through transitive filiation-marriage relationships and maintained and updated at the Regional Neurogenetic Centre since 1973.

Forty-nine people were identified; among them, 21 subjects (13 males, 8 females) over the last 3 generations were identified as at risk or affected by dementia. Twenty of these 21 individuals were born from 2 consanguineous unions (subjects 1, 2, 3, and 4 in the IV generation) (figure 1). Eight persons were reported to be affected by dementia through history (5 males, 3 females), and 5 were studied clinically and genetically (5 males, average age at onset 70.2 ± 5.2 years, range 62–76 years). Eight asymptomatic at-risk subjects (3 males, 5 females, average current age 56.4 ± 7.3 years, range 47–66 years) were also clinically and genetically investigated (figure 1).

Control subjects. A control group, comprising 200 cognitively healthy controls (mean age 60.9 ± 8.72 years; Mini-Mental State Examination [MMSE] score 28 ± 3.7) and 200 unrelated patients with AD who had a family history of dementia (mean onset age 71.9 ± 7.4 years) belonging to the Calabrian population, was screened to exclude that the *APP* A713T variation was a common polymorphism.

Standard protocol approvals, registrations, and patient consents. This study was performed in accordance with the Declaration of Helsinki. All participating family members, or their legal guardians, and all participating control group subjects gave written informed consent to be enrolled in the study.

Clinical assessment. All living patients underwent a detailed clinical assessment involving medical history, physical, and routine laboratory examinations, including serum folate, vitamin B₁₂, thyroid function, and syphilis serology. Activities of daily living (ADL) and instrumental activities of daily living (IADL) were assessed in all patients. Vascular risk factors such as hypertension, hypertriglyceridemia, hypercholesterolemia, cardiomyopathy, and diabetes were also systematically ascertained.

A detailed neuropsychological battery validated in previous studies was used.¹⁷ The battery consisted of an extensive assessment of all cognitive functions. The NINCDS-ADRDA (National Institute of Neurological and Communicative

Figure 1 Pedigree of the PEC family

Double bars represent consanguineous union; diagonal lines indicate deceased family members.

Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association),¹⁸ McKeith,¹⁹ Lund-Manchester group criteria,²⁰ and NINDS-AIREN (National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences) criteria²¹ were used to make differential diagnosis. The Hachinski Ischemic Score (HIS) was measured²² in each case.

Brain morphologic and functional imaging (CT-MRI-SPECT, fluorodeoxyglucose [FDG]-PET) were performed in available affected subjects.

Genetic analyses. Genetic screening was conducted in all the available family members (5 affected and 8 asymptomatic at-risk subjects). DNA was extracted from peripheral leukocytes using standard phenol-chloroform procedures. Coding exons^{3–12} of presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) genes and exons 15–18 of the *APP* gene were amplified using previously described primers.^{3,23,24} Bidirectional sequencing was performed on the ABI3130 automated sequencer (Applied Biosystems, Foster City, CA) using the Big Dye kit (PerkinElmer, Waltham, MA). A second internal set of primers was designed by Primer Premier software (Premier Biosoft, Palo Alto, CA), to amplify exon 17 of *APP* gene, to rule out the possibility that a primer binding site polymorphism could have prevented the amplification of normal alleles and thereby given a false indication of homozygosity. The potential functional impact of the A713T mutation was predicted using Polymorphism Phenotyping 2 (PolyPhen-2) (<http://genetics.bwh.harvard.edu/pph/>), sorting intolerant from tolerant (SIFT) (http://sift.jcvi.org/www/SIFT_enst_submit.html), and MutationTaster (<http://www.mutationtaster.org/>).

The *APOE* genotype was assessed as previously described.²⁵ The *TOMM40* rs10524523 genotype was evaluated using specific primers and according to previously reported methods.²⁶ The *TREM2* rs75932628 (R47H) was evaluated using specific primers designed using Primer Premier software (Premier Biosoft) (primer sequences available on request). Bidirectional sequencing was performed as already described above for the *APP*, *PSEN1*, and *PSEN2* genes.

Biochemical assay. Plasma samples from 12 available family members were collected and frozen. Of these, 10 were mutation carriers (2 homozygotes and 8 heterozygotes); an additional 2 were not mutated and were born from a heterozygous mutation carrier. An aliquot of each plasma sample was sent on dry ice to the ISS laboratory in Rome for the plasma Aβ peptide dosage. Aβ40 and Aβ42 were measured using a sandwich ELISA by the β-Amyloid (1–40) JP 27713 kit from IBL and the INNOTEST β-Amyloid (1–42) kit from Innogenetics. Each sample was assayed in duplicate and all the samples were run on a single plate.

RESULTS Genetic results. Mutations in the *PSEN1* and *PSEN2* genes were excluded in all affected subjects; sequencing of the *APP* gene exon 17 revealed the previously reported A713T mutation (275329G>A, GenBank accession number D87675.1)^{5,14} in 11 subjects (6 asymptomatic and 5 affected) and was excluded in 2 at-risk relatives (asymptomatic subjects VI-7 and VI-8, females, current age 57 and 63 years) (figure 1). The mutation was found in the homozygous state in 3 patients (patients V-2, V-10, V-17, males, onset age 76, 70, and 70 years, respectively), heterozygous in 2 patients (patients V-5 and V-7, males, onset age 62 and 73 years, respectively), and in 6 asymptomatic at-risk individuals (3 males, 3 females, average current age 55.2 ± 8.0 years, range 47–66 years) (figure 1). In silico analyses predicted that the mutation is functionally “probably damaging” (PolyPhen-2 score of 1.000), “damaging” (SIFT score of 0.00), and “disease causing” (MutationTaster, probability value close to 1 indicates a high “security” of the prediction), respectively.

The *APP* variation was absent in the control group, comprising 800 chromosomes, belonging to Calabrian population.

APOE, *TOMM40* rs10524523, and *TREM2* rs75932628 genotypes are showed in table e-1 on the *Neurology*® Web site at Neurology.org.

Biochemical results. The comparison of plasma Aβ levels of asymptomatic mutation carriers (ASMCs) and non-mutation carriers (NMCs) belonging to the family showed that in ASMCs: (1) Aβ40 plasma levels were significantly lower ($p < 0.05$); (2) Aβ42 plasma levels were higher, although not significantly; and (3) the Aβ42/Aβ40 ratio was significantly higher ($p < 0.05$) (table 1).

Comparing NMCs and affected (both homozygotes and heterozygotes) mutation carriers (AMCs), the Aβ42/Aβ40 ratio was found to be significantly higher in the whole group of AMCs ($p < 0.05$) (table 1). Homozygous and heterozygous AMCs had no significant difference in plasma levels of Aβ40,

Table 1 Plasma biomarker values in unaffected NMCs belonging to the family compared with ASMCs and AMCs

	ASMCs (n = 6)	NMCs (n = 2)	p Value	AHOMCs (n = 2)	AHEMCs (n = 2)	AMCs (n = 4)	NMCs (n = 2)	p Value ^a
Plasma Aβ40, pM	178.2 ± 20.8	239.5 ± 33.2	0.02 ^b	305.6 ± 105.5	269.9 ± 5.7	288.3 ± 64.6	239.5 ± 33.2	0.39, NS
Plasma Aβ42, pM	9.6 ± 2.4	6.2 ± 0.0	0.11, NS	13.1 ± 3.8	10.0 ± 0.8	11.6 ± 2.9	6.2 ± 0.0	0.07, NS
Plasma Aβ42/Aβ40	0.05 ± 0.01	0.025 ± 0.007	0.02 ^b	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.025 ± 0.007	0.02 ^b

Abbreviations: AHEMC = affected heterozygous mutation carrier; AHOMC = affected homozygous mutation carrier; AMC = affected mutation carrier; ASMC = asymptomatic mutation carrier; NMC = non-mutation carrier; NS = not significant.

Plasma Aβ values are expressed as mean ± SD. The AMC subjects are further divided in AHOMCs and AHEMCs.

^a Comparison between NMC and AMC subjects.

^b Significant values.

A β 42, or A β 42/A β 40 ratio (table 1), thus showing the lack of a “dose effect” of the mutation.

Comparing the mean A β 42/A β 40 ratio according to the genotype in NMCs, ASMCs, and AMCs, we found a trend toward an increase of the ratio in NMCs and ASMCs, and a decreased ratio in AMCs compared with ASMCs (figure 2).

Main clinical and neuroimaging features of subjects carrying the mutation. Homozygous patients. V-2. Patient V-2, a male, showed disease onset at age 76 years with memory disorder, followed by disorientation to time and place and progressive loss of independence (figure 1). At that time, he scored 23 of 30 on the MMSE, insight was present, and neurologic examination was normal. HIS measured 3. He was successively lost at follow-up, and died at 84 years of bowel cancer.

V-17. This male patient, still living, aged 84 years, showed the first signs of disease at age 70 years. At that time, he had deficits in episodic memory, but awareness was referred to as preserved. After 7 years, he was disoriented to time, showed prosopagnosia, and progressively lost his independence. Currently, he is markedly apathetic and repeatedly picks up rubbish on the streets and brings it home during the day. MMSE score is 14.5/30; ADL 5/6, and IADL 0. Brain functional and morphologic imaging is shown in figure 3.

V-10. Memory disturbances and attention were the main symptoms developed by this male at age 70 years. The course of the disease worsened with

behavioral problems, such as apathy and verbal aggressiveness toward his family. At the age of 73 years, MMSE score was 18.3/30, ADL 6/6, and IADL 5/5. Neurologic examination was unremarkable. HIS was 0. He is currently 75 years old. CT scan evidenced diffuse cortical atrophy, which was more pronounced in parietal posterior areas, and hypodensities in the white matter. FDG-PET showed a large area of hypoperfusion, which was asymmetric and prevalent in the left parietal and temporomesial cortex. Left lingual gyrus was more compromised.

Heterozygous patients. V-5. He is currently 89 years old. Disease onset was at 62 years with memory loss; after 2 to 3 years he showed prosopagnosia and spatial-temporal disorientation (figure 1). At 75 years, changes of personality were evident; he became easily irritable and emotionally flattened. After 1 year, MMSE score was 23.7/30, ADL 6/6, and IADL 3/5. He was unable to make small purchases, use the telephone, and manage money or his personal therapy. Neurologic examination was normal and HIS was 2. Neuropsychological assessment evidenced mild memory impairment in short-term verbal skills and slight deficit in logical-deductive reasoning. A SPECT examination evidenced a slight parieto-temporal hypoperfusion. Over time, the patient became stubborn and capricious; he had strange behavior and was opposite toward his wife, arguing with her continuously. At age 85, he was still able to sleep at home alone and during the day he relied on his daughters for home management. MMSE score was 23.4/30. At 87 years, he began to neglect personal hygiene and he worsened in behavioral disinhibition, urinating outdoors, regardless of the presence of people, and collecting waste from rubbish bins and taking it home. MRI is shown in figure 3. Two years later, he was moribund, disoriented to time and space, and understood only simple verbal messages. He obtained the following scores: MMSE 20.4/30, IADL 1/5, and ADL 3/6. He was able to use cutlery, did not need help to get out of bed, and controlled sphincters.

V-7. He developed memory loss at 73 years. After 4 years, he still had an amnesic mild cognitive impairment (MMSE score 26.7; Clinical Dementia Rating 0.5; visuoconstructional abilities and Rey figure copy are impaired). ADL are unimpaired, neurologic examination is normal, and HIS = 2. SPECT examination is shown in figure 3.

Heterozygous asymptomatic subjects. All of these subjects ($n = 6$) are currently younger than the average age at onset of patients, thus they cannot be considered “escapees”; only one, VI-6, manifested signs of depression (figure 1).

The plot indicates A β 42/A β 40 ratio according to genotype in non-mutation carriers (NMCs), asymptomatic heterozygous mutation carriers (ASMCs), and affected mutation carriers (AMCs) belonging to the family.

DISCUSSION We report here, to our knowledge for the first time, 3 individuals affected by autosomal

Figure 3 Morphologic and functional brain imaging of *APP* mutation homozygous and heterozygous affected carriers

(A) Heterozygous patient. (Left) SPECT examination after 25 years from onset: fronto-parieto-temporal hypoperfusion. (Right) MRI FLAIR sequence 27 years after onset: severe atrophy is prevalent in parieto-temporal cortices and cerebrovascular lesions in the cortex. (B) Homozygous patient. (Left) SPECT examination after 14 years from onset: fronto-parieto-temporal hypoperfusion. (Right) T2-weighted MRI and FLAIR sequence 9 years after onset: cortical atrophy in fronto-parieto-temporal regions and cerebrovascular lesions. (C) Heterozygous patient. (Left) SPECT examination in an amnesic mild cognitive impairment case: fronto-parieto-temporal hypoperfusion. FLAIR = fluid-attenuated inversion recovery.

dominant AD, carrying the *APP* A713T mutation in the homozygous state. They were born to consanguineous parents and belong to a pedigree that also includes 2 affected and 6 at-risk heterozygous carriers of the same mutation. A false indication of homozygosity was ruled out using 2 sets of primers for *APP* gene exon 17 in the genetic sequencing analysis to exclude the remote possibility that a primer binding site polymorphism could have prevented the amplification of the normal allele. Moreover, the genealogical reconstruction showed that all the homozygous patients were born from consanguineous parents (IV-1 and IV-2, second cousins; IV-3 and IV-4, first cousins); it is clear, indeed, that both parents of the 3 homozygous patients were obligate mutation carriers. On these bases, the homozygosity of the 3 patients was unquestionably verified.

We confirmed that the *APP* A713T variant is a mutation (and not a common polymorphism), given that it was absent in a control group comprising 400 subjects belonging to the Calabrian population. However, the control sample size was not sufficiently large enough to exclude the variation as a very rare polymorphism.

Regarding the pathogenic nature of the *APP* A713T variant, in silico analyses predicted that the mutation is functionally “damaging” and “disease causing.” Furthermore, in this family, segregation of the mutation with disease is disclosed, given that all affected subjects carry the mutation in homozygous or in heterozygous state and given that heterozygous asymptomatic subjects are younger than the average age at onset of patients, thus they cannot be considered “escapees.” Moreover, comparing our data with findings from other *APP* mutations, A713T results in

a simultaneous decrease of A β 40 and increase of A β 42 plasma levels in ASMCs (table 1), similar to that observed for the pathogenic APP T714I mutation,⁹ in line with evidence that mutations at codon 714 appear to primarily affect γ 40-cleavage and cause a decreased secretion of A β 40.¹⁰ Our data from plasma show that the A β 42/A β 40 ratio increased in ASMCs compared with NMCs, as previously reported,¹² and then it decreases in AMCs (figure 2). The A β 42/A β 40 ratio was approximately 2-fold higher in the group of AMCs ($p < 0.05$) compared with the group of NMCs, as previously demonstrated in one patient with AD carrying the APP I716V mutation.²⁷ These observations are consistent with findings published in other longitudinal studies, suggesting that plasma levels of A β peptides increase in the preclinical phase of AD, successively declining with the progression of clinical dementia, reflecting the deposition of A β peptides in the brain.^{28–33}

No substantial differences concerning clinical phenotype and course were evidenced comparing heterozygous and homozygous patients (table e-2). In these affected persons, homozygosity for the APP A713T mutation does not aggravate the clinical picture of the disease, which is in line with the classic definition of dominance. This evidence is supported by the lack of a “dose effect” of the mutation in plasma levels of A β peptides. Of note is the very long course in both genotypes.

Our study presents the following limitations regarding the biochemical results in plasma: (1) it is possible that the small number of the APP A713T mutation carriers could have influenced the significance of the data, despite that these subjects with the mutation are among very few in the world; (2) the NMC subjects in this study included only 2 persons, given that we chose the available unaffected individuals without the mutation belonging to the present family; and (3) in this study, it was not possible to perform serial measurements of A β peptides before the onset and during disease progression, as is recommended in autosomal dominant AD families with ASMCs. Indeed, the diagnostic role of plasma as biomarker in these subjects remains to be further verified; we were unable for several reasons to measure the A β 42/A β 40 ratio in the CSF, largely recognized as the true biomarker.^{34–36}

In conclusion, we report for the first time a large autosomal dominant AD family in which a mutation in a causative gene is present in the homozygous and heterozygous state. Our findings confirm the pathogenic role of the APP A713T mutation and the large span of onset, already reported to range from 52 to 82 years,^{5,15} and not influenced by APOE, TOMM40 rs10524523, and TREM2 rs75932628 genotype, reported to be significantly associated with the risk of

AD^{37,38} (table e-1). It is likely that this large span of onset and disease course is attributable to a specific genetic background of modifier genes and/or to epigenetic effect, which in the future would be useful to investigate.

The phenotype of the PEC family, associated with this mutation, and showing AD with CVLs (evident at neuroimaging, but without neurologic signs), is not different from that of other affected subjects whom we previously described, probably being a distinctive feature, at least in the Calabrian population.¹⁶ Although neither in-depth genealogical reconstruction nor haplotype analysis were performed in the APP mutation carriers belonging to this pedigree, we hypothesize that they are related to families previously described and probably originated from the same common ancestor before the 19th century.¹⁶

In this study, AD behaves as a real dominant disease, similar to that reported for the rare homozygous CADASIL patients^{39,40} and differently from that reported in the AD family with the recessive APP A673V mutation,⁷ which causes disease only in the homozygous state, whereas heterozygous carriers were unaffected, consistent with a recessive trait of inheritance. All these data confirm that genetic AD may be considered a disease with dominant and recessive traits of inheritance, thus demonstrating the importance to search for recessive AD loci, for instance by analyzing homozygosity in consanguineous families and inbred populations.

Our biochemical data on A β peptide levels in plasma, despite the limitations mentioned above, could be considered in further research to better define their real diagnostic validity in the assessment of individuals at risk of developing AD. Identification of the APP A713T mutation in patients with this specific phenotype and with such a large span of age at onset further suggests that genetic epidemiology in large cohorts of familial late-onset AD with CVLs would increase the probability of identifying APP mutations that are still underestimated.

AUTHOR CONTRIBUTIONS

M.E. Conidi: design and conceptualization of the study, acquisition, analysis and interpretation of data, drafting of the manuscript. L. Bernardi: design and conceptualization of the study, acquisition, analysis and interpretation of data, drafting of the manuscript. G. Puccio: acquisition, analysis and interpretation of data. N. Smirne: acquisition, analysis and interpretation of data. M.G. Muraca: acquisition and analysis of data. S.A.M. Curcio: acquisition of data. R. Colao: acquisition of data. P. Piscopo: analysis and interpretation of data. M. Gallo: analysis of data. M. Anfossi: analysis of data. F. Frangipane: acquisition of data. A. Clodomiro: acquisition of data. M. Mirabelli: acquisition of data. F. Vasso: analysis of data. C. Cupidi: acquisition of data. G. Torchia: acquisition of data. R. Di Lorenzo: acquisition of data. P. Mandich: acquisition of data. A. Confaloni: design and conceptualization of the study, analysis and interpretation of data. R.G. Maletta: acquisition of data. A.C. Bruni: design and conceptualization of the study, analysis and interpretation of data, drafting and revising the manuscript.

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