

The *APOE* Gene Locus in Frontotemporal Dementia and Primary Progressive Aphasia

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Objective: To investigate the role of the apolipoprotein E (*APOE*) locus in frontotemporal dementia (FTD) and primary progressive aphasia (PPA).

Design: Case-control study.

Setting: Neurology clinic, Rome, Italy.

Patients: Eighty-six patients with a clinical diagnosis of sporadic FTD, including 32 patients with a clinical diagnosis of PPA, and 99 nondemented cognitively intact control subjects.

Main Outcome Measures: Genotype analysis of the 3 single-nucleotide polymorphisms rs449647, rs769446, and rs405509 in the promoter region of the *APOE* gene and of the 2 single-nucleotide polymorphisms rs429358 and rs7412 forming the common apoE polymorphism.

Results: Significant associations with FTD were observed for genotypes rs449647 A/T (odds ratio [OR], 2.1; 95% confidence interval [CI], 1.0-4.5), rs769446 T/C (OR, 4.4; 95% CI, 1.9-10.2), and *APOE* $\epsilon 3/\epsilon 4$ (OR, 4.1; 95% CI, 1.6-10.9). Significant associations with PPA were also observed for genotypes *APOE* $\epsilon 3/\epsilon 4$ (OR, 22.5; 95% CI, 1.2-229.4) and $\epsilon 4/\epsilon 4$ (OR, 7.5; 95% CI, 2.6-21.6). Significant associations with FTD were observed for haplotypes A-C-G-C-C (OR, 5.6; 95% CI, 1.4-21.5) and T-T-T-C-C (OR, 5.2; 95% CI, 1.1-24.0). Significant associations with PPA were also observed for haplotypes A-T-T-C (OR, 0.4; 95% CI, 0.2-0.9) and A-T-T-C-C (OR, 5.2; 95% CI, 1.4-19.3).

Conclusion: Although the physiological role of apoE in FTD and PPA needs further investigations, our results suggest an involvement of the *APOE* gene locus in the genetics of FTD and PPA.

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F RONTOTEMPORAL DEMENTIA (FTD) is the second most common cause of dementia in persons older than 65 years.¹ This condition, which is a more common cause of late-onset dementia than previously recognized, includes familial and sporadic cases,² thus indicating the importance of genetic factors in this condition.³ From a clinical and pathological point of view, sporadic FTD is heterogeneous, characterized by focal degeneration of the cerebral cortex that may present different behavioral and cognitive disturbances, particularly deficits of linguistic functions.⁴ In primary progressive aphasia (PPA), the most relevant clinical endophenotype associated with FTD, language impairment is progressive, whereas other cognitive functions remain relatively spared for at least the first 2 years of the course.⁵

Various investigations⁶⁻¹⁴ have suggested an unclear involvement of the apolipoprotein E (*APOE*) gene (GenBank

AF261279.1) in FTD and PPA. More recent studies¹⁵ suggest that the *APOE* $\epsilon 2/\epsilon 4$ genotype may be a risk factor for PPA, particularly in women.¹⁶ However, to our knowledge, none of these studies have investigated the involvement of the *APOE* gene locus as a whole in FTD and PPA.

The aim of this study was the analysis of the 3 major single-nucleotide polymorphisms (SNPs) in the promoter region of the *APOE* gene and the analysis of the common apoE polymorphism to determine the possible involvement of the *APOE* gene locus as a genetic risk factor for FTD and PPA.

METHODS

PATIENTS RECRUITMENT

From January 1, 2003, through December 31, 2008, a total of 885 unrelated subjects (409 men and 476 women; mean [SD] age, 72.5 [8.6] years), consecutively referred for suspected dementia to the Neuropsychology Unit at the Department of Neurology of the Catholic University School of Medicine (Rome, Italy), were

enrolled in the study. All subjects were white, with most individuals of Central and Southern Italy ancestry, and the group did not include people of Jewish, Eastern European, or Northern African descent.

STANDARD PROTOCOL APPROVAL, REGISTRATION, AND PATIENT CONSENT

This was a prospective multicenter cohort study fulfilling the Declaration of Helsinki, the Good Clinical Practice, and the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.¹⁷ Approval was obtained from the local ethics committees on human experimentation. Written informed consent for research was obtained from each patient or from relatives/legal guardians in the case of critically disabled demented patients.

INCLUSION/EXCLUSION CRITERIA

Each patient underwent a complete clinical and instrumental evaluation, including neuropsychological, behavioral, and neuroimaging assessments. A schematic representation of the study design is summarized in **Figure 1**. Patients diagnosed as having probable sporadic Alzheimer disease (AD), according to guidelines of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer Disease and Related Disorders Association Work Group,¹⁸ were excluded from the study. Diagnosis of vascular dementia (VaD) was made in accordance with the criteria of the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences Work Group.¹⁹ Differential diagnosis between AD and VaD was based also on the Hachinski Ischemic Score²⁰ to address unclear AD/VaD diagnoses. In particular, scores of 4 or less were considered probable AD, whereas scores of 7 or more were considered VaD and excluded from the study. Scores of 5 or 6 were diagnosed as mixed dementia and excluded from the study. Diagnosis of dementia with Lewy body was made in accordance with the guidelines of the DLB Consortium.²¹ These patients were excluded from the study. A total of 70 patients who did not fulfill the specific diagnostic criteria at the clinical presentation of the disease were excluded from the study. Finally, a diagnosis of FTD, including frontal and temporal variants, as well as the PPA clinical subtype according to the Work Group on Frontotemporal Dementia and Pick's Disease²² and Mesulam,⁵ was given to 146 patients who were admitted to the study. At admission, 8 patients withdrew their consent to be included in the study.

DATA COLLECTION AND DIAGNOSIS OF FTD/PPA

All patients enrolled in the study underwent a 3-year follow-up (at 1-year intervals) to confirm the diagnosis of FTD and to exclude cases suggestive of autosomal dominant transmission. The complete examination at the baseline and at follow-up included (1) a detailed clinical history of patients and family collected by a structured interview and a clinical evaluation and review of medical records from the patient's general practitioners; (2) a complete neuroimaging documentation, including brain magnetic resonance imaging with a detailed study of hippocampus volumetry, and a search for focal brain atrophy by means of brain single proton emission computed tomography to investigate focal or diffuse cerebral metabolic hypoperfusion; (3) a standardized neuropsychological examination by the Mental Deterioration Battery,²³ including (a) the Mini-Mental State Examination,²⁴ (b) an evaluation of visual and verbal memory, constructional praxis, visual attention, inductive

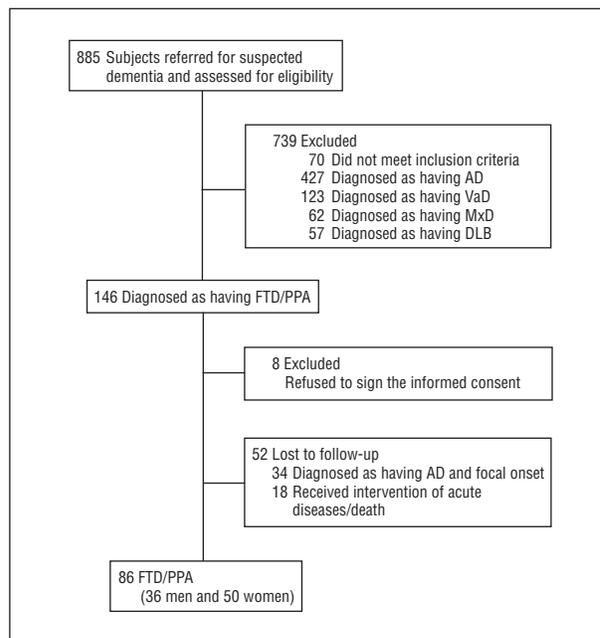


Figure 1. Schematic representation of the study design. AD indicates Alzheimer disease; DLB, dementia with Lewy body; FTD, frontotemporal dementia; MxD, mixed dementia; PPA, primary progressive aphasia; and VaD, vascular dementia.

reasoning, and linguistic tasks, (c) the Frontal Assessment Battery,²⁵ and (d) a specific language assessment by the standardized Esame Neuropsicologico per l'Afasia (Italian neuropsychological aphasia evaluation).²⁶ A behavioral assessment was obtained using the Neuropsychiatric Inventory.²⁷ General rates' criteria for the assignment of FTD to temporal variant FTD and frontal variant FTD subgroups were (1) main clinical disorders at the onset, particularly linguistic disorders characterized by speech slowness, articulatory and word-finding difficulties (temporal variant FTD), and lack of awareness of disease or personality change (frontal variant FTD); (2) magnetic resonance imaging pattern of atrophy, particularly left perisylvian atrophy (temporal variant FTD) and prefrontal or frontotemporal atrophy or no cortical atrophy (frontal variant FTD); and (3) single proton emission computed tomography hypoperfusion, particularly left rolandic hypoperfusion (temporal variant FTD) and frontal or frontotemporal (polar or lateral) hypoperfusion (frontal variant FTD). During follow-up, 18 patients withdrew because of intervening acute disease (eg, cardiovascular or infectious disease) or death, whereas 34 subjects were diagnosed as having AD with "focal onset" during follow-up and were excluded from the study. No patients with semantic dementia were recognized. Thus, 86 unrelated white patients with a clinical diagnosis of FTD, including 32 patients with PPA, were enrolled in the study.

CONTROL SUBJECTS

Control subjects were part of our previous study investigating the role of the *APOE* gene promoter polymorphisms in AD.²⁸ Briefly, these subjects were selected from 268 unrelated white people, enrolled from central and southern Italy, who were consecutively evaluated at the Neuropsychological Unit of the Catholic University School of Medicine in Rome for suspected cognitive impairment. A complete examination, including (1) a detailed clinical history of patients and family collected by a structured interview, (2) a clinical evaluation and a review of medical records from the patient's general practitioners, (3) a

Table 1. Demographic and Clinical Characteristics of the Study Groups

Variable	FTD/PPA		FTD		PPA		Control Subjects Value (N=99)
	Value (N=86)	P Value	Value (n=54)	P Value	Value (n=32)	P Value	
Sex							
Male	36	.30	21	.18	15	.72	50
Female	50		33		17		49
Male sex, %	42	...	39	...	77	...	51
Age at onset, y ^a							
Mean (SD)	69.42 (8.44) ^b	.002 ^c	70.39 (8.30)	.002	67.78 (8.53)	.35	66.21 (7.31)
Range	51-85		51-85		52-82		50-91

Abbreviations: FTD, frontotemporal dementia; PPA, primary progressive aphasia.

^aThe age at patient evaluation coincided with the age at onset.

^bNonnormally distributed.

^cWilcoxon rank sum test with continuity correction.

complete neuroimaging documentation, and (4) a standardized neuropsychological examination including the Mini-Mental State Examination²⁵ and Clinical Dementia Rating Scale,^{29,30} was available for all subjects. Diagnosis of sporadic AD was made in accordance with the criteria of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer Disease and Related Disorders Association Work Group.¹⁸ Diagnosis of VaD was made in accordance with criteria of the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences Work Group.¹⁹ Diagnosis of dementia with Lewy body was made in accordance with the guidelines of the DLB Consortium.²¹ Of these subjects, 99 were diagnosed as cognitively intact (Mini-Mental State Examination score, ≥ 26 ; and Clinical Dementia Rating Scale score, 0) with no personal or familial history of psychiatric or cognitive impairment, alcohol abuse, or drug abuse. Clinical diagnosis of no cognitive impairment was also confirmed at follow-up after 12 months. All controls were white, with all individuals having Central and Southern Italy ancestry, and the group did not include people of Jewish, Eastern European, or Northern African descent.

GENETIC ANALYSIS

Genomic DNA was purified from fresh frozen blood samples by means of the salting-out method.³¹ The SNPs in the promoter region of the *APOE* gene as well as the common *APOE* gene polymorphism were determined as described elsewhere.^{28,32}

STATISTICAL ANALYSIS

Hypotheses regarding sex differences between groups were tested using the Fischer exact test. This analysis was made using the 2-Way Contingency Table Analysis available at The Interactive Statistical Calculation Pages.³³ Normalcy of continuous variables (age and age at onset) was verified using the Shapiro-Wilk normality test. Normally distributed variables were compared by means of the Welch Two-Sample *t* test, whereas nonnormally distributed variables were compared by means of the Wilcoxon rank sum test with continuity correction. The Pearson χ^2 test was used to verify the Hardy-Weinberg equilibrium for the investigated polymorphisms. Logistic regression analysis was used to estimate the adjusted odds ratios (ORs) and the 95% confidence intervals (CIs) in testing for possible association between the genotype and study groups, using age and sex as covariates. Reference genotypes for the associations were chosen because they were wild types (rs449647 A/A, rs405509 A/A, and *APOE* $\epsilon 3/\epsilon 3$) or because they were the most

common in the controls (rs769446 T/T). The a priori sample size estimation, setting a type I error rate of .05 and a statistical power of 60%, called for a sample size of 72 case patients and 72 controls. The logistic regression analysis was performed with SPSS, version 10.1.3 (SPSS Inc, Chicago, Illinois). The other analyses were performed with R, version 2.10.0.³⁴ The Haploview 4.1 genetic software package³⁵ (MIT/Harvard Broad Institute, Cambridge, Massachusetts) was used to estimate the frequencies of *APOE* gene haplotypes and to compare the estimated haplotype frequencies among the study groups. In all analyses, results for which the *P* value was smaller than .05 were declared significant.

RESULTS

Demographic and clinical characteristics of patients and controls are reported in **Table 1**. Considering the whole sample of patients, no difference was observed in sex distribution with respect to controls ($P = .30$). Conversely, a significant difference was observed in the mean age at onset of the 2 groups. Patients were significantly older than controls (69.42 [8.44] years vs 66.21 [7.31] years; $P = .002$). Considering only patients with FTD, no difference was observed in sex distribution with respect to controls ($P = .18$). Conversely, a significant difference was observed in the mean age at onset of the 2 groups (70.39 [8.30] years vs 66.21 [7.31] years; $P = .002$). Considering only patients with PPA, no differences were observed in sex and age at onset with respect to controls.

According to these differences, the analysis of genotypes distribution at the *APOE* gene locus adjusted for sex and age is summarized in **Table 2**. Considering the whole sample of patients, the analysis of rs449647 reveals a significant difference in the distribution of the A/T genotype, which was overrepresented in FTD/PPA compared with controls (53.49% vs 37.37%; $P = .02$). Thus, the A/T genotype of rs449647 seems to be associated with FTD/PPA (OR, 2.073; 95% CI, 1.102-3.898). Also, the analysis of rs769446 revealed a significant difference in the distribution of the T/C genotype, which was overrepresented in FTD/PPA compared with controls (37.21% vs 14.14%; $P < .001$). Thus, the T/C genotype of rs769446 seems to be associated with FTD/PPA (OR, 3.906; 95% CI, 1.865-8.182). No difference was observed in the distribution of rs405509 genotypes. Significant differences

Table 2. Genotypes Distribution at the APOE Gene Locus in FTD and PPA

Variable	FTD/PPA (N=86)			FTD (n=54)			PPA (n=32)			Controls (N=99)
	No. (%)	P Value	OR (95% CI)	No. (%)	P Value	OR (95% CI)	No. (%)	P Value	OR (95% CI)	
rs449647 (50 100 404 Bases From pter) (A⁴⁹¹→T)										
A/A	32 (37)		1 [Reference]	20 (37)		1 [Reference]	12 (38)		1 [Reference]	57 (58)
A/T	46 (53)	.02	2.073 (1.102-3.898)	30 (56)	.04	2.163 (1.035-4.519)	16 (50)	.12	1.983 (0.838-4.696)	37 (37)
T/T	8 (9)	.08	2.989 (0.878-10.170)	4 (7)	.21	2.560 (0.598-10.961)	4 (12)	.06	3.994 (0.921-17.322)	5 (5)
rs769446 (50 100 468 Bases From pter) (C⁴²⁷→T)										
T/T	53 (62)		1 [Reference]	33 (61)		1 [Reference]	20 (62)		1 [Reference]	83 (84)
T/C	32 (37)	<.001	3.906 (1.865-8.182)	20 (37)	<.001	4.399 (1.887-10.253)	12 (38)			14 (14)
C/C	1 (1)	.61	0.532 (0.046-6.127)	1 (2)	.85	0.788 (0.067-9.201)				2 (2)
rs405509 (50 100 676 Bases From pter) (A²¹⁹→C)										
A/A	26 (30)		1 [Reference]	19 (35)		1 [Reference]	7 (22)		1 [Reference]	25 (25)
A/C	41 (48)	.75	0.888 (0.434-1.818)	20 (37)	.17	0.554 (0.240-1.279)	21 (66)	.31	1.6790 (.623-4.528)	45 (45)
C/C	19 (22)	.21	0.591 (0.260-1.344)	15 (28)	.22	0.561 (0.225-1.399)	4 (12)	.32	0.509 (0.133-1.951)	29 (29)
APOE Gene Polymorphism: rs429358 and rs7412 (50 103 781 and 50 103 919 Bases From pter) (C^{3,937}→T and C^{4,075}→T) (Arg¹¹²→Cys and Arg¹⁵⁸→Cys)										
ε2/ε2										
ε2/ε3	8 (9)	.81	0.888 (0.340-1.818)	5 (9)	.66	0.771 (0.247-2.412)	3 (9)	>.99	0.999 (0.251-3.972)	16 (16)
ε2/ε4	5 (6)	.056	8.740 (0.946-80.770)	1 (2)	.51	2.642 (0.144-48.506)	4 (12)	.008	22.570 (1.220-229.438)	1 (1)
ε3/ε3	42 (49)		1 [Reference]	29 (54)		1 [Reference]	13 (41)		1 [Reference]	73 (74)
ε3/ε4	28 (33)	<.001	5.745 (2.405-13.720)	16 (30)	.003	4.195 (1.613-10.907)	12 (38)	<.001	7.535 (2.619-21.674)	9 (9)
ε4/ε4	3 (3)			3 (6)						

Abbreviations: APOE, apolipoprotein E; CI, confidence interval; FTD, frontotemporal dementia; OR, odds ratio; PPA, primary progressive aphasia.

were observed in the genotype distribution of the common APOE gene polymorphism. The ε3/ε4 genotype appears overrepresented in FTD/PPA compared with controls (32.56% vs 9.09%; $P < .001$). Thus, the ε3/ε4 genotype seems to be associated with FTD/PPA (OR, 5.745; 95% CI, 2.405-13.270). No APOE ε2/ε2 and ε4/ε4 genotypes were observed in this group, and thus in the FTD and PPA subgroups. Considering only patients with FTD, the analysis of rs449647 revealed a significant difference in the distribution of the A/T genotype, which was overrepresented in FTD compared with controls (55.56% vs 37.37%; $P = .04$). Thus, the A/T genotype of rs449647 seems to be associated with FTD (OR, 2.163; 95% CI, 1.035-4.519). The analysis of rs769446 also revealed a significant difference in the distribution of the T/C genotype, which was overrepresented in FTD compared with controls (37.04% vs 14.14%; $P < .001$). Thus, the T/C genotype of rs769446 seems to be associated with FTD (OR, 4.399; 95% CI, 1.887-10.253). No differences were observed in the distribution of rs405509 genotypes. Significant differences were observed in the genotype distribution of the common APOE polymorphism. The ε3/ε4 genotype appears to be overrepresented in FTD compared with controls (29.63% vs 9.09%; $P = .003$). Thus, the ε3/ε4 genotype seems to be associated with FTD (OR, 4.195; 95% CI, 1.613-10.907). Considering only patients with PPA, no significant differences were observed in the distribution of the rs449647, rs769446, and rs405509 genotypes with respect to controls. Significant differences were observed in the genotype distribution of the common APOE polymorphism. The ε2/ε4 genotype appeared overrepresented in PPA compared with controls (12.50% vs 1.01%; $P = .008$). Thus, the ε2/ε4 genotype seems to be associated with PPA (OR, 22.570; 95% CI, 1.220-229.438). Similarly, the ε3/ε4 genotype

was overrepresented in PPA compared with controls (37.50% vs 9.09%; $P < .001$). Thus, the ε3/ε4 genotype seems to be associated with PPA (OR, 7.535; 95% CI, 2.619-21.674).

Estimated haplotype frequencies spanning a 3-kb block at the APOE gene locus are summarized in **Table 3**. In considering the entire sample of patients, haplotype H1 was underrepresented in FTD/PPA compared with controls (0.203 vs 0.314; $P = .02$). Thus, it seems protective against FTD/PPA (OR, 0.554; 95% CI, 0.344-0.891). Similarly, haplotype H2 was also underrepresented in FTD/PPA compared with controls (0.199 vs 0.304; $P = .02$). Thus, it seems protective against FTD/PPA (OR, 0.570; 95% CI, 0.353-0.921). Conversely, haplotype H7 was overrepresented in FTD/PPA compared with controls (0.058 vs 0.016; $P = .03$). Thus, it seems at risk for FTD/PPA (OR, 3.881; 95% CI, 1.145-13.104). Similarly, haplotype H8 was overrepresented in FTD/PPA compared with controls (0.053 vs 0.009; $P = .01$). Thus, it seems at risk for FTD/PPA (OR, 6.376; 95% CI, 1.405-28.772). The haplotype H10 was also overrepresented in FTD/PPA compared with controls (0.041 vs 0.008; $P = .04$). Thus, it seems at risk for FTD/PPA (OR, 5.285; 95% CI, 1.099-25.271). Finally, haplotype H12 was overrepresented in FTD/PPA compared with controls (0.031 vs 0.003; $P = .03$). Thus, also this haplotype seems to be at risk for FTD/PPA (OR, 10.460; 95% CI, 1.145-94.802). Considering only patients with FTD, haplotype H8 was overrepresented in FTD compared with controls (0.071 vs 0.013; $P = .008$). Thus, this haplotype seems to be at risk for FTD (OR, 5.689; 95% CI, 1.495-21.540). Similarly, haplotype H10 was overrepresented in FTD compared with controls (0.048 vs 0.010; $P = .03$). Thus, this haplotype also seems to be at risk for FTD (OR, 5.221; 95% CI, 1.125-24.079). Considering only patients with PPA, haplo-

Table 3. Estimated Frequencies of Haplotypes Spanning 3 kb at the APOE Gene Locus in FTD and PPA

Haplotype ^a	FTD/PPA vs Controls				FTD vs Controls				PPA vs Controls			
	FTD/PPA	Controls	P Value	OR (95% CI)	FTD	Controls	P Value	OR (95% CI)	PPA	Controls	P Value	OR (95% CI)
H1 ATGTC	0.203	0.314	.02	0.554 (0.344-0.891)	0.215	0.319	.05	0.584 (0.339-1.006)	0.184	0.302	.07	0.522 (0.262-1.044)
H2 ATTC	0.199	0.304	.02	0.570 (0.353-0.921)	0.213	0.296	.12	0.644 (0.372-1.115)	0.184	0.315	.04	0.491 (0.247-0.980)
H3 TTGTC	0.081	0.104	.46	0.763 (0.379-1.538)	0.078	0.104	.45	0.726 (0.320-1.651)	0.107	0.110	.96	0.977 (0.404-2.369)
H4 TTTTC	0.110	0.076	.26	1.506 (0.748-3.032)	0.111	0.080	.36	1.441 (0.664-3.131)	0.109	0.070	.31	1.639 (0.648-4.163)
H5 TCTTC	0.071	0.034	.11	2.180 (0.855-5.549)	0.085	0.035	.06	2.579 (0.965-6.884)	0.042	0.032	.71	1.319 (0.341-5.128)
H6 ATGTT	0.022	0.052	.13	0.408 (0.131-1.271)	0.017	0.047	.18	0.344 (0.078-1.518)	0.027	0.064	.25	0.395 (0.089-1.763)
H7 ATTCC	0.058	0.016	.03	3.881 (1.145-13.104)	0.037	0.013	.17	2.891 (0.674-12.364)	0.085	0.017	.009	5.274 (1.430-19.390)
H8 ACGCC	0.053	0.009	.01	6.376 (1.405-28.772)	0.071	0.013	.008	5.689 (1.495-21.540)	0.023	0.007	.25	3.631 (0.483-27.261)
H9 ACGTC	0.034	0.021	.44	1.650 (0.491-5.536)	0.020	0.015	.75	1.334 (0.265-6.727)	0.049	0.023	.30	2.140 (0.545-8.431)
H10 TTTCC	0.041	0.008	.04	5.285 (1.099-25.271)	0.048	0.010	.03	5.221 (1.125-24.079)	0.036	0.008	.10	4.883 (0.787-30.194)
H11 ATGCC	0.029	0.011	.20	2.720 (0.627-11.748)	0.037	0.013	.17	2.891 (0.674-12.364)	0.025	0.011	.42	2.282 (0.374-13.961)
H12 TTGCC	0.031	0.003	.03	10.460 (1.145-94.802)								
H13 ATTTT	0.014	0.016	.89	0.890 (0.190-4.172)	0.020	0.026	.77	0.787 (0.181-3.428)				
H14 ACTTC	0.014	0.015	.96	0.952 (0.199-4.549)	0.013	0.015	.88	0.854 (0.144-5.085)	0.017	0.016	.96	1.065 (0.160-7.140)
H15 ACTCT									0.031	0.005	.09	6.355 (0.815-49.204)

Abbreviations: APOE, apolipoprotein E; CI, confidence interval; FTD, frontotemporal dementia; OR, odds ratio; PPA, primary progressive aphasia.
^aOnly haplotypes with an overall frequencies >1%.

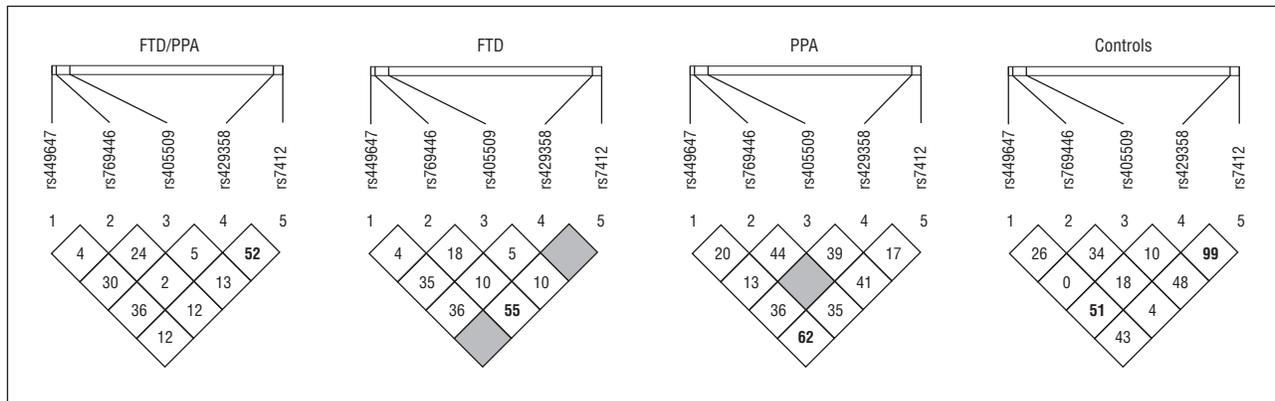


Figure 2. Schematic representation of r^2 linkage disequilibrium coefficient at the 3-kb block spanning the apolipoprotein E locus. FTD indicates frontotemporal dementia; PPA, primary progressive aphasia.

type H2 was underrepresented in PPA compared with controls (0.184 vs 0.315; $P = .04$). Thus, this haplotype seems to be protective for PPA (OR, 0.491; 95% CI, 0.247-0.980). Conversely, haplotype H7 was overrepresented in PPA compared with controls (0.085 vs 0.017; $P = .009$). Thus, this haplotype seems to be at risk for PPA (OR, 5.274; 95% CI, 1.430-19.390).

A schematic representation of the linkage disequilibrium coefficient r^2 is shown in **Figure 2**. No important differences were observed when the study groups were compared with each other. Only a minor difference was observed in FTD and PPA. In FTD, an increased linkage disequilibrium was observed between SNPs rs449647 and rs7412 ($r^2 = 0.032$) and between rs429358 and rs7412 ($r^2 = 0.016$), whereas in PPA, an increased linkage disequilibrium was observed between SNPs rs769445 and rs429358 ($r^2 = 0.077$).

COMMENT

The results of the present study, which was performed in a relatively large series of Italian patients with a clinical diagnosis of FTD and PPA, confirm previous find-

ings supporting a possible role of the APOE gene locus in the pathogenesis of sporadic FTD and PPA.⁶⁻¹⁶ Indeed, to our knowledge, this study is the first investigating the APOE gene locus as a whole in FTD and PPA. Although the APOE gene locus is not an X-linked locus, recent data reported an association of the $\epsilon 2/\epsilon 4$ genotype with PPA in females.¹⁶ For this reason, we rigorously adjusted our analysis for sex. Thus, we demonstrated that the associations of the rs449647 A/T and APOE $\epsilon 3/\epsilon 4$ with FTD and the association of APOE $\epsilon 2/\epsilon 4$ and $\epsilon 3/\epsilon 4$ genotypes with PPA are independent of sex.

Notably, in the analysis of genotypes at the APOE gene locus we found significant association only of heterozygotes, ie, the rs449647 A/T, rs769446 T/C, and $\epsilon 3/\epsilon 4$ genotypes with FTD, and the $\epsilon 2/\epsilon 4$ and $\epsilon 3/\epsilon 4$ genotypes with PPA. Although these associations might be because of an insufficient sample size or unknown specific features of our sample, the analysis may be considered quite solid. In fact, in an a posteriori estimation of the power of the analysis, we observed an effect size $h = 0.366$ and a power of approximately 58% in the association of the rs449647 A/T heterozygote with FTD, an effect size $h = 0.541$ and a power of approximately 86% in the association of the

rs769446 T/C heterozygote with FTD, whereas we observed an effect size $h=0.521$ and a power of approximately 87% in the association of *APOE* $\epsilon 2/\epsilon 4$ with PPA (h indicates parameter). The association of heterozygotes may be explained by molecular heterosis, because subjects heterozygous for a genetic polymorphism may show a significantly greater effect (positive heterosis) for a quantitative or dichotomous trait than do homozygotes for either allele.³⁶ The mechanisms of molecular heterosis, however, are still poorly understood. Nevertheless, the association of the rs449647 A/T and rs769446 T/C genotypes are specific for FTD, whereas the association of the *APOE* $\epsilon 2/\epsilon 4$ is specific for PPA. A trend toward a specific association of the rs449647 T/T genotype with PPA also may be suggested ($P=.06$). It must be noted that the possible presence of an unknown genetic factor at risk for FTD/PPA near the *APOE* gene locus, in linkage disequilibrium with the rs449647 A/T, rs769446 T/C, and *APOE* $\epsilon 2/\epsilon 4$ and *APOE* $\epsilon 3/\epsilon 4$ genotypes, cannot be excluded from this study. A potential limitation of our analysis is the large CIs associated with the *APOE* genotypes. However, the high ORs associated with these CIs make it difficult to draw negative conclusions. We also estimated the haplotype frequencies at the 3-kb block spanning the *APOE* gene locus by using the 3 SNPs in the promoter region of the *APOE* gene: rs449647 (50 100 404 bases from pter), rs769446 (50 100 468 bases from pter), and rs405509 (50 100 676 bases from pter) and the 2 SNPs forming the common *APOE* gene polymorphism³⁷: rs429358 (50 103 781 bases from pter) and rs7412 (50 103 919 bases from pter). Haplotypes H1 and H2 were the most frequent in the study groups, accounting for approximately 40% of all haplotypes. Notably, they were both AT- haplotypes, and AT- haplotypes account for more than 50% of all haplotypes in FTD, PPA, and cognitively intact controls. Among the several differences that we observed in haplotype distribution across the study groups, only in PPA did we observe haplotypes balancing their opposite effect, ie, “at risk” toward “protective” haplotypes, as commonly expected from haplotype analyses, whereas in FTD, only “at risk” haplotypes were observed. However, the association of haplotype H8 and H10 seems to be specific for FTD, and a trend toward a specific association of haplotype H1 ($P=.053$) and H5 ($P=.06$) with FTD also may be suggested. Conversely, the association of haplotypes H2 and H7 seems to be specific for PPA, and a trend toward a specific association of haplotypes H1 ($P=.07$) with PPA also may be suggested. It must be noted that these results were only estimations of the haplotype frequencies. Nevertheless, an a posteriori estimation of the power of the analysis at approximately 74% ($h=0.310$) for the association of haplotype H8 with FTD and at approximately 72% ($h=0.305$) for the haplotype H2 with PPA suggests that these results are quite solid.

Although the physiologic role of apoE in FTD and PPA needs further investigation, these data indicate the involvement of the *APOE* gene locus in the genetics of sporadic FTD and PPA. More studies on large samples of FTD and PPA, however, are needed to confirm whether the genetic analysis of the *APOE* gene locus may be useful in the differential diagnosis of sporadic FTD and PPA.

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