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Identification of Misdiagnosed Fronto-Temporal Dementia Using *APOE* Genotype and Phenotype-Genotype Correlation Analyses

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Abstract

Objective—Postmortem and genetic studies of clinically diagnosed Frontotemporal dementia (FTD) patients suggest that a number of clinically diagnosed FTD patients are actually "frontal variants" of Alzheimer's disease (fvAD). The purpose of this study was to evaluate this hypothesis by combining neuropathological data, genetic association studies *of APOE*, phenotype-*APOE* genotype correlations and discriminant analysis techniques.

Methods—Neuropathological information on 24 FTD cases, genetic association studies of *APOE* (168 FTD, 3083 controls and 2528 AD), phenotype-genotype correlations and discriminant techniques (LDA, logistic regression and decision trees) were combined to identify fvAD patients within a clinical FTD series.

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CONFLICT OF INTEREST
The authors confirm that this article content has no conflicts of interest.

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AUTHOR DISCLOSURES
Isabel Hernández has nothing to disclose.
Ana Mauleón has nothing to disclose.
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Results—Four of 24 FTLD patients (16.6%) met criteria for definite AD. By comparing allele and genotype frequencies of *APOE* in controls, FTD and AD groups and by applying the Hardy-Weinberg equilibrium law (HWE), we inferred a consistent (17.2%) degree of AD contamination in clinical FTD. A penetrance analysis for *APOE* ε4 genotype in the FTD series identified 14 features for discrimination analysis. These features were compared between clinical AD (n=332) and clinical FTD series (n=168) and classifiers were constructed usinglinear discriminant analysis logistic regression or decision tree techniques. The classifier had 92.8% sensitivity to FTD and 93.4% sensitivity to AD relative to neuropathology (global AUC=0.939, p<<0.001). We identified 30 potential fvAD cases (17.85%) in the clinical FTD sample.

Conclusion—The *APOE* locus association in clinical FTD might be entirely explained by the existence of "hidden" fvAD cases within an FTD sample. The degree of fvAD contamination can be inferred from *APOE* genotypes.

Keywords

Alzheimer's disease; apolipoprotein E4; diagnostic classification; frontotemporal lobe dementia; genetics

INTRODUCTION

Clinical discrimination between the frontal variant of Alzheimer's disease (fvAD) and true non-AD Frontotemporal dementia (FTD) is challenging. Several studies have tried to find a useful biomarker that discriminates AD, FTD or other dementias [1, 2]. Impaired language and executive functions are common in both FTD and AD [3] and "frontal" behavioral symptoms may also appear in AD [4]. The clinical manifestations of frontal system dysfunction observed in some AD patients are usually correlated with the presence of neurofibrillary degeneration in the frontal lobes [5].

Patients presenting with behavioral, language and executive system abnormalities in the context of a neurodegenerative disorder are usually diagnosed with FTD. FTD comprises a group of progressive neurodegenerative disorders sub-classified according to the clinical phenotype: behavioral variant (bvFTD), semantic dementia (SD), progressive non fluent aphasia (PNFA), corticobasal syndrome (CBS), progressive supranuclear palsy (PSP), and FTD with motor neuron disease (FTD-MND).The clinical spectrum of FTD ranges from behavioral symptoms until progressive aphasic syndromes, parkinsonism plus and / or motor neuron disease. They are often associated various syndromes in the same subject over the clinical [6]. Although FTD and fvAD share clinical signs and symptoms, FTD is usually associated with several different histopathological features, different abnormally aggregated proteins in target neurons and the presence of a range of germline mutations in several multiple genes completely different from those found in AD [7].

Although the *APOE* locus is the strongest genetic risk marker for sporadic AD [8–10] it is less clear whether there is a link between *APOE* and FTD [11–15]. Indeed, any observed associations could be explained partially (or even totally) if the FTD clinical series was contaminated by "hidden" AD cases [16]. That is, AD cases within an FTD series would tend to inflate the ε4 allele frequency, yielding an intermediate allele frequency between

population-based controls and AD. The purpose of the present study was to evaluate this hypothesis [17] by combining neuropathological data, genetic association studies of *APOE*, phenotype-*APOE* genotype correlations and discriminant analysis techniques. We estimated the proportion of AD pathology in our clinical FTD series and identified potential candidate fvAD patients.

METHODS

Patients

All of the patients agreed to participate in the study and blood samples were obtained after they and/or their legal representatives provided written consent according to the GIPSY protocol approved by the ethics committee in the Hospital Clinic i Provincial (Barcelona, Spain). Informed consent was in accordance with Spanish biomedical laws (Law 14/2007, July 3rd, about biomedical research; Royal Decree 1716/2011, November 18th).

All patients were south-Europeans (Spanish lineage, two generation or more) recruited from the Fundació ACE Barcelona Alzheimer Treatment & Research Center. For *APOE* analysis and Hardy-Weinberg estimations data from 5779 unrelated individuals were used: 168 FTD, 2528 AD and 3083 population-based controls. The AD patients and population-based controls series have been previously described [18]. The 168 clinical FTD patients underwent a full neurological evaluation and were diagnosed either at baseline or during follow-up as clinical FTD cases by using international research criteria: bvFTD [19, 20], PNFA [21, 22], SD [23], PSP [24] and CBS [25]. Some FTD patients developed MND signs during follow-up. (Table 1-a and 1-b). Mutation screening of *MAPT* in FTD patients has been conducted in all FTD patients. We found 1 out of 168 carrying a *MAPT* mutations (0.5% of cases). We also identified a *C9ORF72* expansion in 3 out of 168 FTD (1.7%) [26]. Of note, patients carrying either *MAPT* or *C9ORF72* mutations were not excluded from this work. Other FTD genes have been not studied.

The Fundació ACE Memory Clinic diagnoses patients at a daily consensus conference among neurologists, neuropsychologists and social workers. Baseline signs and symptoms are acquired directly from the patient or from the primary caregiver. The patients were administered a neuropsychological battery [27] that included measures sensitive to orientation, attention, verbal learning and long-term memory, language, visuoperception, gnosis, praxis and executive functions. Neuroimaging studies were either CT and/or MR1 which is required for an FTD diagnosis [20]; in some cases SPECT scans were also available. The locus and extent of hemispheric atrophy was rated by visual inspection by an experienced neurologist, and independently confirmed by a neuroradiologist.

Neuropathology

Postmortem neuropathology examination was performed at the Neurological Tissue Bank (NTB) of the Biobanc-Hospital Clinic-IDIBAPS (Barcelona, Spain), after obtaining written informed consent from patients and/or next of kin. Brain was processed according to a standardized protocol. Histopathological evaluation was performed on multiple, formalin-fixed and paraffin embedded tissue blocks.

AD-related neurofibrillary pathology was staged according to the Braak & Braak [28, 29] classification and a diagnosis of definite AD was assigned applying the CERAD criteria [30, 31] based on the semiquantitative assessment of neuritic plaque density. Both were combined using the current consensus guidelines [32].

Germline DNA Extraction and *APOE* Analysis

We extracted DNA from frozen blood using the Nucleo-Spin Blood kit (Macherey-Nagel, Düren, Germany). The *APOE* ε4 allele was identified with commercial kits for *APOE* rs429358 (SNP112) and rs7412 (SNP158) from Roche Diagnostics (Germany). The *APOE* alleles were amplified using LightCyclerApoE Mutation Detection Kit (Roche diagnostics, Germany) and detected using real-time PCR technology (LightcyclerR 480 System, Roche Diagnostics, Germany) following the manufacturer's instructions. To check the quality of the results, different compound heterozygotes for *APOE* SNPs were verified in an independent research laboratory. FTD-control, AD-control and HWE statistical analyses were performed manually or using online tools (http://ihg.gsf.de/cgi-bin/hw/hwal.pl).

Penetrance Analysis of *APOE*ε4 Genotype in FTD Series

We divided the FTD patients into two groups based on the presence or absence of the ε4 allele, irrespective of the allele dosage (dominant model, i.e. *APOE* ε4+ and *APOE* ε4− subgroups) in order to identify the clinical, neuropsychological and neuroimaging variables (features) potentially associated with *APOE* ε4 carrier status in FTD patients. All baseline variables were analyzed as a function of *APOE* genotype categories using SPSS for Windows, v18.0 (SPSS Inc, Chicago, IL). To compare qualitative variables a standard Pearson's chi-square test was performed. To compare quantitative variables we used student's T-test or the Mann-Whitney U test (as appropriate). All of the clinical variables that showed a trend towards association ($p<0.1$; when comparing *APOE*ε4+ and ε4− subgroups), were retained for the discriminant analyses (Supplementary Table 1).

Discriminant Analyses

To identify the linear combination of features that best discriminated between the two groups of patients with pathologically confirmed FTD or AD, an discriminant analysis was carried out [33]. Fourteen pre-selected features from the clinical FTD database were used for classifier generation (direct approach) including two qualitative neuroimaging variables (hemispheric pattern atrophy and predominant brain atrophy), presence of six clinical variables at baseline (memory deficit; behavioral symptoms; language alteration; dyslipidemia, sucking reflex and gait alteration) and six neuropsychological variables at baseline (Boston naming; verbal comprehension; semantic verbal fluency; abstract reasoning; digits span forward and Poppelreuter test). The coding of each feature, the coefficients applied to calculate the classifier and the final discriminant function are detailed in Supplementary (Table 2).

The classifier was not biased based by the proportion of patients in each class (i.e., prior probability set to 0.5 for each class). To calculate the classifier coefficient for each variable and the constant for the canonical discriminant function, we used 332 randomly selected clinically diagnosed AD patients, 113 *APOE* ε4 negative FTD patients and 8 FTD patients

with unknown genotype (discovery set). We used only the data from *APOE* ε4 negative patients during this initial step because, we inferred a lower frequency of fvAD cases in the FTD *APOE* ε4 negative subgroup (13%) compared to *APOE*ε4 positive subgroup (29%). Any missing values from the features were replaced by the mean value observed in the whole series. 41 FTD *APOE* ε4 positive patients with available clinical data were used to conduct a replication analysis to check the reliability of the results. Classifier scores from 332 clinical AD and 168 clinical FTD (n=500) were used to calculate the area under curve (AUC) of the proposed classifier. AUC was generated using SPSS. This exercise was conducted only to provide information about classifier performance (not for true diagnostics purposes yet). Of note, SPSS automatically calculated the best cut-off for the proposed classifier in an unsupervised manner. Individuals with classifier values ≤−l were classified as FTD automatically by the system. Individuals with a classifier value >−l were classified as AD. Fourteen FTD cases with neuropathologically-confirmed disease and clinical data were used to estimate the sensitivity of the classifier to detect true FTD individuals.

Sensitivity Analysis

Other discriminant techniques were used to demonstrate the existence of "AD information" within FTD series. Specifically, to further re-assure that results were not technical artifacts inherent to LDA method limitations, we conducted a sensitivity analysis by using two additional statistical approaches. Briefly, the prognostic utility of selected variables was investigated by an unsupervised decision tree approach entering all selected variables. Two variables were automatically selected by the system: node 0: "predominant brain atrophy" divided in three sub-nodes. Node 1: temporal pole or fronto-temporal and parietal atrophy or unknown. Node 2: parietal atrophy (with or without language alterations; node 4 or 5) and node 3: temporal-parietal or hippocampal atrophy. Of note, training and validation samples were identical to those used in LDA. Sensitivity (fraction FTD) and specificity (fraction EA) values were produced. Data were considered significant when the P-value was below 0.05. Concordance between LDA and decision trees was calculated by using a simple 2×2 chi-square test among classified subjects. Concordant classification was obtained for 86.8% of individuals ($p=3.47\times 10^{-55}$) (Supplementary Table 5).

Finally, a binary logistic regression approach was conducted by using identical 14 features entered during discriminant analysis or decision tree experiments. By comparing predicted phenotypes by LDA or logistic regression, an identical classification of individuals was obtained for 92.4% of individuals ($p=7.87\times10^{-48}$). Concordance between decision tree classification and logistic regression was also measured (97.1%; $p=7.83\times10^{-67}$) (Supplementary Table 5).

Phenotype Genotype Correlations

The classifier algorithm identified 30 FTD patients (17.85%) that had classifier scores in the range of AD (i.e., score >−l). So, we conducted a new exhaustive phenotype analysis including demographic, clinical, neuropsychological and follow-up data of the FTD series by dividing it in two groups (genuine "FTD" and fvAD).

RESULTS

Histopathological examination of the 24 FTD patients revealed that 4 (16.6%) met criteria for Definite AD without any other pathological evidence or protein deposit related to an FTD phenotype. A summary of each FTD patient with inconsistent clinical and pathological findings is provided in Supplementary Table 3. The remaining cases were classified pathologically as FTLD-TDP [N=11(45.8%)], FTLD-TAU [N= 8 (33.3%)] and FTLD-FUS [N= 1 (4.1%)].

We observed the expected ε4 allele differences when comparing AD and controls (44.8% vs. 18.5% respectively; *APOE* ε4 allele Odds Ratio = 3.2; 95% Confidence Interval (C.I.) = [2.839–3.511] ; p<<.001). To calculate potential AD contamination in the FTD series, we compared the ε4 allele frequencies between FTD, AD and the population-based controls (Table 2). Two assumptions were made: a) the ε4 allele frequency does not deviate from the HWE law expectation and b) any increase of ε4 allele frequency in FTD would be due to contamination by AD cases. There was a significant "excess" of ε4 carriers in the FTD series when compared with population based controls (26.2% vs. 18.5% carriers; Allele Odds Ratio=1.5 (95% CI = [1.104–2.093]), *p*=.009). The observed allele frequency inflation in the clinical FTD cases (i.e., 26.2 − 18.5 = 7.7%) was used to deduce the fraction of fvAD individuals not carrying this allele. Taking into account that 44.8% of AD individuals were ε4+ (Table 2), we inferred the fraction of fvAD non-carriers contaminating the FTD series by using a simple rule of three (assuming HWE) which results in 9.5% of the FTD series. The global estimation of "hidden" AD cases within our clinical FTD series was obtained by summing the excess of ε4 carriers observed and the corresponding estimate of non-carriers assuming HWE (i.e., 7.7+9.5=17.2%). The genetics-based estimation suggests that about 29 FTD cases may, in fact, be fvAD patients. This result is not statistically different from that observed in the neuropathological series (16.6%) (*p*=.69; Fisher's exact test).

A penetrance analysis was conducted to identify phenotype differences between *APOE*ε4+/− FTD subgroups (Supplementary Table 1). Fourteen variables (features) had moderate association (*p*< .1) with genotype (Table 3). A numeric classifier using selected features was constructed using discriminant analysis techniques and was applied to 500 individuals (332 clinical AD vs 168 clinical FTD; 14 of these with pathological confirmation). The classifier resulted in 91.9% sensitivity (90.7% cross-validation) to AD (global AUC= .939, p<<.001), and correctly classified 92.8% of the postmortem FTD cases (13/14 pathological FTLD with whole phenotype available for discriminant analysis). Similar results were obtained by using decision tree or binary logistic regression approaches (supplementary Table 5)

Of note, the classifier displayed a consistent, lower sensitivity for FTD ε4 carriers (82.7%) and non-carriers (80.5%) compared to AD (both groups were separately analyzed) (supplementary Table 5). Thirty FTD patients (17.85%) were assigned as fvAD, which is consistent with our previous estimation from the pathological series (16.6%; *p*= .64) and HWE methods (17.2%; *p*= .88) which also predicted a similar number of fVAD cases (n=29).

In order to identify clinical differences, the series was split based on the results of the classifier (fvAD versus genuine FTD) (Table 4). The FTD patients who were classified as fvAD had a higher frequency of change of diagnosis during the course of the disease (31% versus 16.9%; *p*=.01). In addition, there were statistically significant differences between groups in terms of the presence of memory complaints or absence of behavioral symptoms at baseline (Table 4). The MMSE score did not vary between the two groups (ANOVA, eta^2= .001. p>.05), nor did multiple neurological and neuropsychological variables differ. Neuroimaging data suggested that differences in predominance of hemispheric atrophy for each of the groups might be useful for selecting fvAD candidates.

Given that FTD is more common in presenile age (especially the behavioral variant phenotype), we also separated the clinical series (N = 168) into older and younger than 65 years. We analyzed the effect of age at onset of symptoms on the clinical variables, regardless of ApoE4 genotype. (Supplementary Table 4)

The bvFTD phenotype was more often observed in those below 65 years. Patients below 65 years had a higher percentage of family history of dementia (55.8% *p*= .028). Younger patients showed more behavioral alteration at the onset of symptoms (64.1 % *p*= .005). Besides, memory impairment was the most frequent symptom in the older ones (46.3% *p*= . 012). Moreover the pathological background showed that dyslipidemia (42.3%, *p*= .012), heart disease (21.3%, *p* = .031) and osteoarthrosis (29.4%, *p*<.001) were more frequent in the older population and this is not surprising taking into account that this clinical findings increases with age. There were no further significant differences on neurological variables. Regarding the neuropsychological variables, only the SKT test and the clock test were significant in the older group. Of note, neither the neurological examination variables nor the neuroimaging variables discriminated by age at symptoms onset.

DISCUSSION

There are three main findings from the present study. First, based on neuropathological analysis, approximately 17% of the clinically diagnosed FTD patients met neuropathological criteria for definite AD. Second, it was possible to create a classifier algorithm using both genotype and phenotype information to identify those clinical FTD patients who were actually more likely to be AD. Third, when comparing the variables of these “hidden” AD cases within the FTD sample, they were more likely to be classified as clinical FTD than fvAD.

Although the problem of “hidden” AD in clinical series of FTD patients is well known, this is the first time that it has been possible to create a classifier that was able to identify individuals within a sample of clinical FTD who were at very high risk for having AD. Small studies have been inconsistent in terms of finding an association between APOE and FTD [11, 12, 13, 14, 34–40] although neuropathological series generally fail to demonstrate any link between the ε4 allele and FTD [41]. In addition, a recent GWAS using exclusively neuropathological TDP-43+ (FTLD-TDP) cases [11, 42] failed to isolate any significant signal around the APOE locus. Thus, our data are fully consistent with the idea that the ε4 allele is not associated with FTD risk, and any apparent increases in allele frequency are

entirely due to misdiagnosed AD cases within the clinical FTD series. However, last interpretation has some limitations. For example, there are not GWAS data for FTLD-TAU or FTLD-FUS. Further research is needed in order to guarantee that any apparent increases in allele frequency are entirely due to misdiagnosed AD cases within the clinical FTD series.

Clinicians and researchers must be cautioned that despite the "hidden" AD cases within the group with FTD, it would not be appropriate to eliminate patients with a ε4 allele from either clinical series or research studies of FTD. To do so would remove approximately two-thirds of the genuine FTD cases. Therefore, the most fruitful approach would be to use a statistical classifier to identify those individuals within the FTD sample who were most likely to be "hidden" AD cases, and to treat them as a separate group for analysis, as shown here. While this does not completely eliminate the problem of the contamination of the FTD series, it may help to minimize the impact of that contamination on the outcomes of interest.

The identification of individuals who were diagnosed with FTD but had AD is important since the misdiagnosis can result in the failure to provide appropriate medication for the patient, or even potentially the prescription of inappropriate medications [43]. The behavioral phenotype, alone, is not sufficient to disentangle these two clinical syndromes, but using both genetic and neuro-imaging markers along with the clinical signs/symptoms, has the potential to improve patient classification and thus case management. Indeed, it may be that among patients diagnosed with FTD who are ε4 carriers, it would be important for them to be able to have more specific neuroimaging assessment, using either FDG metabolic scans or *in vivo* amyloid imaging to help to clarify the diagnosis.

It is vital to emphasize the need for a reliable estimate of ε4 allele frequency; a large series of AD and controls (e.g., n > 1000) is an essential component of this kind of research. We used estimates based on more than eleven thousand chromosomes (5611 individuals) to calculate fvAD contamination. The precision of the ε4 frequencies facilitated the inference of fvAD (17.2%) that resulted in almost identical rates compared to the postmortem data (16.6%).

The penetrance and discriminant analyses were used to indirectly demonstrate that the information contained in clinical variables differentiating *APOE*ε4 positive and negative FTD patients may also differentiate AD cases and genuine FTD (postmortem) with relatively good precision (AUC 93.9%).

However, the proposed classifier needs independent replication and could be improved by extending the discriminant analysis to the whole clinical database and by including age at onset and *APOE* genotype. Of note, *APOE* was only used in the first phase of the study (penetrance analysis), but not in the discriminant analysis which led to the score. In addition the results of the discriminant average scores do not vary with the age of onset of the disease. So, we feel that by adding *APOE* or age at onset to the discriminant analysis we cannot improve discrimination too much. Further research increasing sample size and using deep phenotyping would be necessary to improve obtained discriminant function.

Although AD and FTD can be differentiated using histopathological analysis the existence of the fvAD complicates the clinical identification of genuine FTD patients. From a clinical

point of view, the best predictor to identify hidden fvAD in FTD series is memory impairment (Table 4). Moreover, behavior changes in the genuine FTDs such apathy, desinhibition, or eating changes, are the first complaints referred by relatives. Besides, fvAD and FTD might have different neuroimaging atrophy patterns (Table 4).

The characterization of the phenotype of rare FTD syndromes is difficult and may affect the identification of valid genetic markers for FTD using GWAS strategies and other massive molecular techniques. Most important, the misclassification of AD patients as FTD might hamper the access to palliative or empirical therapies by these atypical AD cases. Consequently, the improvement of mathematical tools to comprehensively differentiate fvAD and FTD would be of interest not only for research but also for clinical purposes in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

FTD	Clinical Fronto-temporal dementia
fvAD	Clinical "frontal variant" Alzheimer's disease
AD	Alzheimer's Disease
FTLD	Histological Fronto-temporal Lobar degeneration

bvFTD	Behavioral variant Fronto-temporal dementia
PNFA	Progressive Non Fluent Aphasia
SD	Semantic Dementia
PSP	Progressive Supranuclear Palsy
CBS	Cortico Basal Syndrome
FTD-MND	Fronto-temporal Dementia with Motor Neuron Disease
APOE	Apolipoprotein E genotype
HWE	Hardy-Weinberg equilibrium law
LDA	Linear discriminant analyses
FTLD-TAU	Fronto-temporal Lobar degeneration with TAU inclusions
FTLD-TDP	Fronto-temporal Lobar degeneration with TDP-43 inclusions
FTLD-FUS	Fronto-temporal Lobar degeneration with FUS inclusions.

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Table 1

a. Demographic characteristics of study subjects.									
Diagnostic Groups at baseline								**Statisitic (p.value)[1]**	**D.f**
	vbFTD	**PNFA**	**SD**	**CBS**	**PSP**	**CI-ND***	**Other***		
N (%)	74 (44.0)	22(13.1)	24(14.3)	11(6.5)	11(6.5)	13(7.7)	13(7.7)		
Age at onset	66.5±10.2	68.6±8.7	68.4±11.6	67.6±7.5	68.3±8	70.9±4.9	67±9.2	0.513	6
Gender (%males)	43(58.1)	8(36.4)	18(75)	8(72.7)	7(58.3)	7(53.8	5(41.7)	9.36	6
Years evolution before diagnostic (average)	3.76±2.6	2.39±1.6	2.96±1.8	3.27±2.4	3.2±2.5	2±1	2.42±2.5	2.025	6
Family History N(%) Yes)	41(56.9)	9 (40.9)	6(25)	5 (45.5)	2(16.7)	9 (69.2)	3(25)	17.1^{2}	6
Education N(%) 6+ years	48(64.9)	15(68.2)	16(66.7)	6(54.5)	7(58.3)	4(30.8)	5(41.7)	8.25	6
ApoE$_4$ carriers frequency N(%)	22(29.7)	3(13.6)	5(20.8)	3(27.3)	2(18.2)	3(23.1)	6(46.2)	5.746	6

b. Evolutive phenotype of the groups.									
Evolutive phenotype N(%)	**Diagnostic Groups at baselineN(%)**								
	bvFTD	**PNFA**	**SD**	**CBS**	**PSP**	**CI-ND**	**Others**	**Total**	**ApoE$_4$ carriers frequency N(%)**
bvFTD	65(87.8)	1(4.5)	2(8.3)	0	0	8(61.5)	6(46.2)	82 (48.8)	25(30.5)
PNFA	1(1.4	13(59.1)	0	1(9.1)	0	1(7.7)	1(1.4)	17(10.1)	3(17.6)
SD	2(2.7)	1(4.5)	21(87.5)	0	0	2(15.4)	0	26(15.5)	5(19.2)
FTD-MND	1(1.4)	1(4.5)	0	0	0	1(7.7)	0	3(1.8)	1(33.3)
CBS	1(1.4)	2(9.1)	1(4.2)	9(81.8)	0	1(7.7)	4(30.8)	18(10.7)	5(27.8)
PSP	1(1.4)	2(9.1)	0	1(9.1)	11(100)	0	2(15.4)	17(10.1)	3(17.6)
AD	3(4.1)	2(9.1)	0	0	0	0	0	5(3.0)	2(40.0)
Total	74(44.0)	22(13.1)	24(14.3)	11(6.5)	11(6.5)	13(7.7)	13(7.7)	168	44(26.2)

vbFTD: behavioral variant Frontotemporal; PNFA: Progressive non Fluent Aphasia; SD: Semantic Dementia; CBS: Corlicobasal Syndrome;PSP Progressive Suplanudear Palsy: CI-ND: Cognitive Impairment non dementia:Other: Unspecific Dementia at baseline

[1]- X^2 for categorical data, F for continuous data (one-way ANOVA) D.f= degree of freedom 2 —$p < .05$

*: Patient initially not classified as FTD who envolved clinical FTD during follow up

bvFTD: behavioral variant Frontotemporal; PNFA: Progressive non Fluent Aphasia: SD: Semantic Dementia; FTD-MND: frontotemporal dementia with motor neuron disease: CBS: Corticobasal Syndrome;PSP: Progressive Suplanuclear Palsy; CI-ND: Cognitive Impairment non dementia Other: Unspecific Dementia at baseline.

Table 2

APOE locus genetic data available for this study

		FTD	AD	Controls
APOE (Haplogenotypes)	ε2ε2	1	5	17
	ε2ε3	9	117	317
	ε2ε4	4	41	34
	ε3ε3	114	1273	2178
	ε3ε4	37	947	508
	ε4ε4	3	145	29
	Total	168	2528	3083
APOE ε4 Allelic Frequency	(%)	14%	25.3%	9.7%
APOE ε4 carriers	(%)	26.2%	44.8%	18.5%
Hardy Weinberg Equilibrium (HWE)	(p-value; Pearson)	0.85	0.08	0.87

Table 3

Candidates discriminant variables derived from penetrance analysis.

Variable	APOE Status		Statistics	
	E4−	E4+	χ^2	t
Hemisferic Pattern atrophy (% (N))				
Lelf	34.7 (35)	8.8 (3)	0.013	
Right	10.9(11)	11.8(1)		
Symmetric	54.5 (55)	79.4 (27)		
Predominant Brain atrophy (% (N))				
Frontal	17.8(18)	32.4(11)	0.082	
Temporal	31.7(32)	20.6 (7)		
Parietal	3.0 (3)	11.8(4)		
Fronto-temporal	30.7(31)	23.5(8)		
Fronto-parietal	3.0(3)	5.9 (2)		
Global	13.9(14)	5.9(2)		
Initial symptom reported by caregiver (% (N))				
Memory	35(41)	50 (20)	0.094	
Behavior	41.4(46)	64.1 (25)	0.015	
Language	53.2 (59)	33.3(13)	0.033	
Pathological background (% (N))				
Dyslipemia	32.8(38)	47.5(19)	0.095	
Neurological exploration (% (N))				
Sucking	1(1)	5.7 (2)	0.092	
Normal gait	66.4 (75)	81.6(31)	0.076	
Altered gait	33.6(38)	18.4(7)		
Neuropsychological exploration. Quantitative evaluation (average)				
Visual Naming (15-BNT)	10.42±3.96	12.09±3.34		0.036
Verbal comprehemsion	4.91±1.3	5.53±0.96		0.006
Semantic verbal fluency	8.48±4.55	11.03±3.94		0.005
Similarities WAIS-III	5.49±3.50	7.34±3.60		0.016
Neuropsychological exploration. Qualitative evaluation impaired (% (N))				
Poppelreuter test	48.2(41)	21.2(7)	0.007	
Digit Span Forward WAIS-III	72.2 (65)	54.5(18)	0.064	

χ^2 = Pearson Chi-square

t= Levene for equality of variances

Table 4

Characteristics of patients as a function of diagnosis and classifier algorithm.

	Diagnosed FTD			Diagnosed AD (AD)		
	Classified FTD (FTD)	Classified AD (fvAD)	Effect Size (FTD vsfvAD)		Effect Size (p-value) (AD versus fVAD)	Effect Size (p-value) (AD versus FTD)
Demographics						
N	138	30	Na	332	Na	Na
Age	70.6±9.8	72.8±7.3	Eta^2=0.005(0.3)	81.4±7.6	Eta^2=0.11(p<0.001)	Eta^2=0.29(p<0.001)
Education (% <6years)	37%	46%	OR=1.4(0.3)	48%	OR=0.94(0.8)	OR=0.63(0.02)
Sex (male %)	55.8%	60%	OR= 1.18(0.68)	25%	OR=4.3 (<0.001)	OR=3.6(<0.001)
Mini-Mental State Score	22.2±6.8	22.6±6.2	Eta^2=0.001(0.7)	19.2±3.9	Eta^2=0.04(<0.001)	Eta^2=0.067(<0.001)
Family History (% Yes)	37.2%	57.1%	OR=2.25(0.06)	46.6%	OR=1.5(0.28)	OR=0.68(0.08)
ApoE4 carriersfrequency N(%)	35(25.4%)	9(30%)	Na			
Phenotype						
Chief complaints at baseline						
Focal Signs	10%	3%	P(6df)=1.7E-5	0%	P(4df)=0.01	P(6df)=3E-34
Behavior	19.6%	0%		1.5%		
Gait disturbance	1.4%	0		0%		
Memory	34.1%	90%		89.2%		
Language	1.4%	0		0		
Other	33.5%	7%		9.3%		
Symptoms reported by the caregiver at the beginning of the process						
Depression	8.7%	0	P(8df)=6.8E-12	1.5%	P(6df)=0.8	P(8df)=9.5E-47
Job Loss	0.7%	0		0		
Focal Signs	15.2%	0		0.6%		
Behavior	29.8%	0		3.3%		
Gait disturbance	2.2%	0		0.6%		
Memory	20%	100%		90.7%		
Language	1.4%	0		0		

	Diagnosed FTD			Diagnosed AD (AD)		
	Classified FTD (FTD)	Classified AD (fvAD)	Effect Size (FTD vsfvAD)		Effect Size (p-value) (AD versus fVAD)	Effect Size (p-value) (AD versus FTD)
Others	22%	0		3.3%		
Predominant signs at baseline						
Agitation	5.0%	0%	OR=0.80(0.59)	2.1%	OR=0.92(1)	OR=2.4(0.12)
Bulimia	7.5%	0%	OR=0.80(0.21)	0%	Na	OR=0.25(<0.001)
Depression	24.2%	0%	OR=0.77(0.02)	24.5%	OR=0.9I(0.001)	OR=0.984(1.0)
Disorientation	26.7%	29.6%	OR= 1.15(0.81)	56%	OR=0.33(0.009)	OR=0.28(<0.001)
Hypersexuality	4.2%	0%	OR=0.81(0.58)	0%	Na	OR=0.26(0.001)
Delusions	10%	11.1%	OR=1.1(1)	10.1%	OR= 1.1 (0.74)	OR=0.99(1)
Inhibition	6.7%	0%	OR=0.8(0.35)	1.5%	OR=0.92(1)	OR=4.6(0.008)
Memory loss	71.7%	96.3%	OR= 10.2(0.005)	98.5%	OR=0.40(0.381)	OR=0.39(<0.001)
Focal sings	8.3%	3.7%	OR=0.42(0.69)	0.3%	OR= 12.5(0.14)	OR=29.6(<0.001)
Gait disorder	10.8%	0%	OR=0.79(0.12)	3.7%	OR=0.92(0.61)	OR=3.1(0.009)
Language disorder	46.7%	33.3%	OR=0.57(0.28)	25.1%	OR= 1.4(0.3 6)	OR=2.6(<0.001)
Neuroimaging data						
Symmetry Pattern						
Rt. Hemisphere	14.3%	4.8%	P(2df)=0.002	1.5	P(2df)=0.15	P(2df)=5.7E-14
Lt. Hemisphere	31.3%	0%		11.2		
Symmetric	54.5%	95.2%		87.3		
Atrophy Location						
Frontal	26.8%	0	P(5df)=5.8E-6	0.6%	P(7df)=4.5E-29	P(7df)=4.24E-64
Temporal Pole	34.8%	0		1.2%		
Parietal	4.5%	4.8%		2.4%		
FrontoTemporal	21.4%	66.7%		2.1%		
Global	8.9%	28.6%		49.5%		
Fronto Parietal	3.6%	0		0.3%		
Hippocampus	0	0		32.3%		
TemporoParietal	0	0		11.5%		

Note: p-values appear in bracket or alone on each ceil. P-vaiues below 0.0011 are declared significant (Bonferroni's Correction for multiple comparisons; 43 tests). Df=degree of freedom. Na=Not applicable.