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High apolipoprotein E4 allele frequency in FXTAS patients

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Purpose: Fragile X-associated tremor/ataxia syndrome is a late-onset neurodegenerative disorder that occurs in *FMR1* premutation carriers. It is well known that the apolipoprotein E ε4 allele is a risk factor for neurodegenerative disease. The main goal of this work was to evaluate the apolipoprotein E genotypes and allelic distribution among patients with fragile X-associated tremor/ataxia syndrome.

Methods: A total of 44 unrelated *FMR1* premutation carriers (22 presenting with fragile X-associated tremor/ataxia syndrome and 22 without fragile X-associated tremor/ataxia syndrome) were genotyped.

Results: All the apolipoprotein E ε4/4 genotype carriers detected (100%), and six of the seven apolipoprotein E ε4/3 genotype carriers (85.7%) are patients presenting with fragile X-associated tremor/ataxia syndrome symptoms, whereas only 40% of the apolipoprotein E ε3/3 genotype carriers belong to the fragile X-associated tremor/ataxia syndrome group. The results showed that the presence of the apolipoprotein E ε4 allele increases the risk of developing fragile X-associated tremor/ataxia syndrome (odds ratio = 12.041; $P = 0.034$).

Conclusion: On the basis of these results, we conclude that the presence of at least one apolipoprotein E ε4 allele might act as a genetic factor predisposing individuals to develop fragile X-associated tremor/ataxia syndrome.

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Key Words: apolipoprotein E; association; FXTAS; neurodegeneration

Fragile X-associated tremor/ataxia syndrome (FXTAS, OMIM no. 300623) is a late-onset neuropsychiatric degenerative disorder that occurs in *FMR1* premutation carriers (55–200 CGG repeats). Clinical symptoms, which appear in patients in their 50s or later, include action tremor, progressive cerebellar ataxia, peripheral neuropathy, autonomic dysfunction, cognitive decline, and dementia.[1–4] Magnetic resonance imaging in patients with FXTAS demonstrates mild to moderate cerebellar and brain atrophy, as well as white matter hyperintensities. In addition, hyperintensities in the middle cerebellar peduncles on T2 have been described as a characteristic finding in patients with FXTAS and therefore constitute a major diagnostic feature of the disorder.[3,5] It has been estimated that at least one-third of all *FMR1* premutation carriers will develop an FXTAS syndrome, although there is significant variability in the progression of neurological dysfunction.[2,6,7]

Apolipoprotein E (ApoE) is a lipoprotein that transports cholesterol and other lipids and lipid-soluble molecules into the central nervous system.[8–11] ApoE also modulates the inflammatory response to cellular damage in the brain.[12] The human *ApoE* gene shows polymorphic variation, and three alleles, designated as ApoE ε2, ε3, and ε4, are common in the general population.[13] Variant distribution of these alleles has been shown to be associated with a number of age-related diseases including atherosclerosis, cardiovascular disease, and neurodegenerative disorders.[14–16] Although the pathogenic mechanism involving ApoE in these diseases is still unclear, it has been demonstrated that the ApoE ε4 allele is a well-established genetic risk factor for neurodegenerative disorders including Alzheimer disease (AD), Parkinson disease, and other disorders in which dementia is present.[17–19] On the basis of this observation, we have evaluated the ApoE genotypes and allelic distribution among a *FMR1* premutation carrier cohort presenting with FXTAS. These data might contribute to uncover a new genetic risk factor for FXTAS and might be useful to identify new genes involved in the disease onset and progression.

METHODS

Subjects

A total of 44 unrelated *FMR1* premutation carriers (22 presenting with FXTAS symptoms and 22 without FXTAS clinical symptoms) were included. Samples from subjects belong to the Hospital Clinic of Barcelona and were molecularly diagnosed in the genetics laboratory of the same hospital. All participants were enrolled from families with members known to be affected with fragile X syndrome, and all of them are of Caucasian ethnicity. A classification on the basis of the gender and the age of the participants is summarized in **Table 1**. Although clinical data is scarce for some of the patients and we did not diagnose dementia in all of them, none of the cases included in the study had a diagnosis of AD. Overall, FXTAS encompasses patients who meet criteria in any of the three categories of involvement: definite, probable, and possible.[3] ApoE allele frequencies were compared with those of the control population reported by

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Adroer et al.[20] The study was accomplished in compliance with the Hospital Clinic ethics committee. Written informed consent was obtained from all the subjects before their participation.

Molecular analysis

ApoE genotyping was performed by polymerase chain reaction amplification as described in previous studies.[21] Allele frequencies were estimated by counting the alleles.

Statistical analyses

Differences in age, gender, CGG repeat number, and the presence of the ApoE ε4 allele among patients with and without FXTAS were evaluated by multivariate logistic regression analysis. A nonparametric test (Mann–Whitney *U* test) was applied to examine whether the presence of the ApoE ε4 allele is related to the age of onset of the disease. Moreover, ApoE ε4 allele frequency in FXTAS was compared with those previously described in AD patients and control population.[20] *P* values <0.05 were considered statistically significant. Statistical analyses were performed using commercially available software (SPSS SmartViewer, version 18.0; SPSS, Chicago, IL).

Table 1 Classification of the *FMR1* premutated individuals enrolled in the study

	FXTAS	Non-FXTAS	Total
Men (*n*)	14	7	21
Age			
Mean	70.1	71	
SD	9.1	16.7	
CGG repeat			
Mean	81.3	79.6	
SD	20.3	20.1	
Women (*n*)	8	15	23
Age			
Mean	61.6	55.1	
SD	10.8	12.5	
CGG repeat			
Mean	90.9	80.9	
SD	36.5	24.8	

FXTAS, fragile X-associated tremor/ataxia syndrome.

RESULTS

A total of 22 patients with FXTAS and 22 patients without FXTAS were genotyped for the ApoE locus. The mean ± SD of age and the mean ± SD of CGG repeat number for the FXTAS group were 67 ± 10.4 and 84.8 ± 26.9, respectively. The mean age for the non-FXTAS group was 60 years ±15.5, and the mean value for CGG repeat number was 80.5 ± 22.9 for the non-FXTAS group. When comparing the two groups, there were no significant differences in age (odds ratio (OR) = 1.048; *P* = 0.069) or in CGG repeat number (OR = 1.013; *P* = 0.313). However, significant differences were found in gender (OR = 4.46; *P* = 0.042; 95% confidence interval (CI) = 1.14–17.5) (**Table 1**) and the presence of the ApoE ε4 allele (OR = 12.041; *P* = 0.034; 95% confidence interval = 1.21–119.7) (**Table 2**). ApoE genotypes from *FMR1* premutation carriers (FXTAS and non-FXTAS) are given in **Table 2**. The only ApoE 4/4 genotype carrier detected, and six of the seven ApoE 4/3 genotype carriers were patients presenting with FXTAS symptoms (31.8% of all patients with FTXAS). By contrast, 95.5% of patients without FXTAS were ApoE 3/3 genotype carriers as compared with 62.8% of patients with FXTAS. The ApoE allele frequencies for patients with and without FXTAS are given in **Table 3**. With respect to age of disease onset, Mann–Whitney *U* test showed no statistically significant differences among patients with FXTAS carrying ApoE ε4 allele (65.8 ± 11.9) and those not carrying this allele (67.5 ± 6.3; *P* = 0.596), ruling out an early age of onset on the basis of the presence of the ApoE ε4 allele.

ApoE ε4 allele frequencies were then compared with those previously reported for patients with AD (*n* = 88) and age-matched controls (*n* = 147) (**Table 3**). Of note, no significant differences were found when comparing FXTAS ApoE allele ε4 frequencies with those detected in patients with AD ($\chi^2 = 1.858$; degrees of freedom = 1; *P* = 0.2). By contrast, the comparison with the control population group showed significant differences ($\chi^2 = 7.78$; degrees of freedom = 1; *P* = 0.013).

Table 2 ApoE allele genotype and allele frequencies in *FMR1* premutation carriers

	FXTAS			Non-FXTAS		
Gender (*n*)	Women	Men	No. of subjects (*n*)	Women	Men	No. of subjects (*n*)
	8	14	22	15	7	22
Genotype						
4/4	1	0	1 (4.5)	0	0	0 (0)
4/3	2	5	6 (27.3)	1	0	1 (4.5)
4/2	0	0	0 (0)	0	0	1 (0)
3/3	4	10	14 (63.6)	14	7	21 (95.5)
3/2	1	0	1 (4.5)	0	0	0 (0)

Numbers in parentheses indicate frequencies.

ApoE, apolipoprotein E; FXTAS, fragile X-associated tremor/ataxia syndrome.

Table 3 Allele frequencies in patients with FXTAS, patients with AD, and age-matched controls

ApoE	FXTAS	Non-FXTAS	AD[a] (*n* = 88)	Control[a] (*n* = 147)
ε2 Allele	0.023	0.000	0.022	0.068
ε3 Allele	0.795	0.977	0.687	0.870
ε4 Allele	0.182	0.023	0.289	0.061

AD, Alzheimer disease; ApoE, apolipoprotein E; FXTAS, fragile X-associated tremor/ataxia syndrome.

[a]Data taken from ref. 20.

DISCUSSION

The ApoE ε4 allele is a well-known genetic risk factor for AD.[22] Some studies have shown that ε4 allele frequency is significantly increased among patients with AD and that this association might be related to a cognitive decline and a faster disease progression, contributing to the reduction of the median age for AD onset.[9,14,23] FXTAS is a late-onset neurodegenerative disorder molecularly characterized by increased levels of abnormal (expanded CGG repeat) *FMR1* mRNA and slightly reduced fragile X mental retardation protein levels. The presence of these elevated levels of *FMR1* mRNA led to the proposal of an RNA "toxic gain-of-function" model for FXTAS, in which the mRNA itself, with the abnormal CGG repeat tract, is causative of the neurological disorder.[2,3,24,25] Although AD and FXTAS have different clinical and neuropathological features, both disorders show protein aggregates, with a cytotoxic effect that leads to cell death or a disordered synaptic transmission.[25,26] AD is characterized by senile plaques that are predominantly composed of β-amyloid, an amino acid peptide cleaved from the amyloid precursor protein.[27] Considering the central role of ApoE, which includes transporting cholesterol into the central nervous system and helping to remove amyloid-β protein from the brain,[12] together with the fact that amyloid precursor protein mRNA is a target for fragile X mental retardation protein–mediated translational repression at the synapse,[27] it would seem likely that there is a biological connection among ApoE, the *FMR1* gene, and the FXTAS syndrome. Furthermore, the fact that ApoE variants have been associated with a large number of age-related and neurodegenerative disease and that no reports were available on the ApoE allelic frequencies in FXTAS patients, we found it necessary to investigate the relationship of ApoE with FXTAS. We therefore examined ApoE genotypes in relation to FXTAS among 44 *FMR1* premutation carriers (22 presenting with FXTAS and 22 without FXTAS). The comparison of the two groups (considering the CGG repeat number, age, and gender) showed no significant differences except with respect to gender (**Table 1**). This observation is in concordance with the fact that FXTAS penetrance has been found to be lower in female *FMR1* premutation carriers than in male carriers.[28]

Similar to what has previously reported in other neurodegenerative diseases,[9,14,29] we have found an association between the presence of ApoE ε4 and the risk of having FXTAS, evidencing a significantly higher risk for FXTAS among ε4 carriers (OR = 12.041; $P = 0.034$; 95% confidence interval = 1.21–119.7). By contrast, the ApoE ε2 allele, the allele that protects against AD, was not significantly found among individuals without FXTAS (**Table 2**). Furthermore, the ApoE genotype distribution detected among individuals with FXTAS resembles those found among Spanish patients with AD (**Table 3**).[20] Contrary to what has been published for AD,[14] no association has been found between the presence of the ApoE ε4 allele and the age of disease onset. Although the sample size is small, to our knowledge and considering that FXTAS is a rare disease and still poorly recognized among specialists, the cohort herein studied corresponds to one of the largest Spanish cohorts ever published.

In summary, on the basis of these results, we conclude that the presence of at least one ApoE ε4 allele, together with other factors, might act as a genetic factor predisposing individuals to develop FXTAS. However, further studies are required in order to clarify if this association is also found in other populations. The data herein reported provide a first approach that might help in unraveling other genes related to FXTAS pathology. A better understanding of the molecular underpinnings of FXTAS should shed light on therapeutic approaches that will combat neurodegeneration and improve cognitive and motor performance.

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DISCLOSURE

The authors declare no conflict of interest.

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