The APOE ε2 Allele Increases the Risk of Earlier Age at Onset in Machado-Joseph Disease

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Objective: To investigate a modulating effect of the apolipoprotein E (APOE) polymorphism on age at onset of Machado-Joseph disease (MJD).

Design: We collected blood samples from 192 patients with MJD and typed the APOE polymorphism.

Patients: The 192 patients with MJD included 59 from the Azores, 73 from mainland Portugal, and 60 from Brazil.

Setting: Academic research center.

Results: Cases with the ε2/ε3 genotype had an earlier onset compared with those with the ε3/ε3 or the ε3/ε4 genotype. In this series of patients, the presence of an APOE ε2 allele implies a decrease of nearly 5 years in the age at onset. When combining several other predictors in a general linear model, namely, the presence/absence of the APOE ε2 allele, with the size of the (CAG)n in expanded alleles, the model was significantly improved and the explanation of onset variance was raised from 59.8% to 66.5%. Furthermore, the presence of the ε2 allele was associated with an onset before age 39 years (odds ratio, 5.00; 95% CI, 1.18-21.14).

Conclusion: The polymorphism at the APOE gene plays a role as a genetic modifier of MJD phenotype.

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MACHADO-JOSEPH DISEASE (MJD), also known as spinocerebellar ataxia type 3, is an autosomal dominant neurodegenerative disorder of late onset caused by an expansion of (CAG)n in the coding region of ATXN3 (14q32.1; OMIM *607047), encoding for ataxin 3.1,2 Machado-Joseph disease is the most frequent type of spinocerebellar ataxia3 and reaches its highest worldwide prevalence in the Azores islands of Portugal.4 Wildtype ATXN3 alleles present 12 to 44 CAG repeats, whereas expanded alleles consensually have more than 52 repeated units.5,6 Patients with MJD have a clinically heterogeneous presentation with a mean age at onset of approximately 40 years, but extremes range from as young as 4 years7 to as old as 70 years.8 Variation in the age at onset is only partially explained (approximately 50%-75%)9,10 by the size of the (CAG)n tract in the expanded ATXN3 alleles. Familial factors may explain additional variance in age at onset,11,12 indicating that modifier genes may play a role. The hypothesis that other CAG-containing proteins could interact with the expanded ataxin 3 and influence the onset of MJD has been raised.13,14 An association between severity of fasciculations (minor signs in MJD) and the CAG length of the large spinocerebellar ataxia type 2 allele was found,14 but no influence on disease onset was detected.

Apolipoprotein E (apoE) is a ubiquitous protein involved in lipid storage, transport, and metabolism.15 The APOE gene (19q13.2) has 3 main alleles (ε2, ε3, and ε4), encoding for isoforms E2, E3, and E4 (which differ at positions 112 and 158).16,17 These differences alter the protein’s structure, influencing association with lipids and its binding to the receptors. Although apoE3 and apoE4 bind to low-density lipoprotein receptors with similarly high affinity, apoE2 has a 50- to 100-fold weaker affinity.18,19 In the central nervous system, apoE is secreted by astrocytes and is highly expressed in intracellular and extracellular spaces,20 constituting an important mediator of cholesterol and lipid transport in the brain (reviewed by Adibhatla and Hatcher21), especially of cholesterol transport from astrocytes to neurons. Furthermore, it has been suggested that
apopE isoforms differentially regulate synaptic plasticity and repair.22

The APOE ε4 allele has been consistently associated, for example, with increased risk23,24 and a lower onset in sporadic Alzheimer disease,24 increased risk of cognitive impairment,25,26 and a more unfavorable outcome after traumatic brain injury.27,28 On the other hand, the ε2 allele has been associated with a higher prevalence29 and earlier onset in Huntington disease,30 a finding that has been confirmed in several populations. The main goal of this work was to investigate the effect of the APOE ε2 allele on the age at onset of the MJD phenotype.

METHODS

Blood samples from 192 patients with MJD (59 from the Azores, 73 from mainland Portugal, and 60 from Brazil) were collected after informed consent. We extracted DNA from all samples using standard procedures. The size of the (CAG)n tract was determined following a method previously reported,34 and the APOE polymorphism was typed according to previously described conditions.35 For the total series of patients, data on the age at onset was collected as close as possible to the first patient reports of gait instability or diplopia (the 2 most consistent initial symptoms in MJD, according to the extensive study by Coutinho8). Patients with several years of disease progression were asked for the age at onset of the mentioned symptoms. The self-reported age was compared with the one stated by their close relatives (usually caregivers), and, whenever possible, additional information from previous records was also taken into account to get an age at onset as accurate as possible.

We tested conformity with the Hardy-Weinberg equilibrium using the exact probability without bias. An exact test of differentiation evaluated differences in APOE genotypic frequencies among the 3 groups of patients, as well as between the patients’ groups and the corresponding populations of origin (previously published for the populations of the Azores).33 mainland Portugal,36,37 and Brazil38,39. All analyses were performed using the Arlequin software package.40

Age at onset for the 3 most frequent APOE genotypes was adjusted for the mean number of CAG repeats in the expanded ATXN3 allele after fitting a linear regression model. Differences in the adjusted age at onset between APOE genotypes were analyzed using the t test calculator of OpenEpi, version 2.3.1 (http://www.openepi.com). We used multivariate linear regression analyses to test the effect of several variables on age at onset: number of CAGs in expanded and normal alleles, presence or absence of the APOE ε2 allele, population of origin, and sex. To account for kinship among some patients of the Azorean series, we also applied a generalized estimating equation model. The risk of developing MJD before age 39 years (mean age at onset for the present series) among patients with the APOE ε2 allele was estimated as an odds ratio using logistic regression analysis, with onset before age 39 years vs at 39 years or older as the dependent variable. All analyses were performed using commercially available software (SPSS, version 15.0).41

RESULTS

The APOE genotypic frequencies were in conformity with Hardy-Weinberg expectations. No significant differences were detected in the genotypic frequencies among the groups of patients (from the Azores, mainland Portugal, or Brazil) or between the patients’ groups and the corresponding populations of origin.

A summary of descriptive statistics for the MJD patients undergoing evaluation is given in the Table. When we adjusted age at onset for the (CAG)n size, patients with the ε2/ε3 genotype had an earlier onset than the other 2 groups (Table). This difference was statistically significant between the ε2/ε3 and ε3/ε3 genotypes (2-tailed t test, \( P = .02 \)) but did not reach statistical significance between the ε2/ε3 and ε3/ε4 genotypes (2-tailed t test, \( P = .10 \)).

The (CAG)n size in the expanded ATXN3 alleles is
known to be inversely correlated with the age at onset of MJD (present series, $r = -0.77\ [P < .001]$). Given the earlier onset observed for APOE ε2/ε3 genotype, the presence or absence of the APOE ε2 allele was tested in a general linear model in addition to the (CAG)$_a$ size in expanded alleles. When the APOE ε2 status was taken into account (given the impossibility to dissociate the effect of the ε2 from that of the ε4 allele, the 3 patients with ε2/ε4 genotype were excluded), the percentage of explanation of the onset variance significantly increased from 59.8% to 60.9% ($F = 6.46\ [P = .01]$). In this series of patients, the presence of APOE ε2 decreased the age at onset by nearly 3 years. When we added the number of CAGs in normal ATXN3 alleles, the model was not significantly improved; however, the population background (the Azores, mainland Portugal, and Brazil) ($F = 19.51\ [P < .001]$) and sex of patients ($F = 8.26\ [P = .005]$) ameliorated the outcome of the APOE ε2 allele in the model ($F = 8.71\ [P = .004]$), improving the explanation of onset variance to 66.5%. Even taking into account the fact that the subspecies of patients from the Azores contained related patients, the effect of the APOE ε2 allele was still statistically significant (Wald $\chi^2 = 7.12\ [P = .008]$). When patients were divided according to mean age at onset ($<39$ vs $\geq 39$ years), an association was found between the presence of the ε2 allele and an earlier onset (odds ratio, 5.00 [95% CI, 1.18-21.14]).

**COMMENT**

The present results indicate that the APOE ε2 allele influences the MJD phenotype, increasing the risk for earlier onset. When the ε2 allele status was accounted for (in addition to the CAG repeat size in expanded alleles), an apparent discrepancy between the approximately 5-year earlier onset in ε2 allele carriers and the minimal (but statistically significant) improvement of only about 1% in the explanation of onset variance was detected. This observation probably occurs because the number of patients with the ε2 allele was not very large ($n = 20$). Notwithstanding, in our series of patients, the risk of developing MJD before age 39 years is 5 times higher in carriers of the ε2 allele compared with the risk in noncarriers.

The APOE ε4 allele is associated with an increased risk for Alzheimer disease, whereas the ε2 allele may be protective. In contrast, having at least 1 copy of the ε4 allele may protect against age-related macular degeneration or may delay vision loss, whereas having at least 1 copy of the ε2 allele may increase the risk for this disease or for an earlier onset. The APOE ε2 allele has also been associated with an earlier onset of Parkinson disease and Huntington disease. This is in agreement with the effect observed in MJD. Some MJD patients may present a Parkinson disease–like phenotype, which may indicate a shared neuropathologic mechanism. In Huntington disease, the influence of the APOE genotype is still controversial; nevertheless, in agreement with our results, Keohoe and coworkers found that male patients with the ε2/ε3 genotype had an earlier onset than those with other APOE genotypes. In the face of the present results, and taking into account the postulated differential efficiency of different apoE isoforms in cholesterol transport, one can speculate that apoE2 may be less efficient, leading to earlier neuronal damage and MJD onset.

Using the rat as a model, Rapp et al postulated that neurons and astrocytes express different apoE receptors. Astrocytes preferentially express the low-density lipoprotein receptors in contrast to neurons, for which the principal receptor is low-density lipoprotein receptor–related protein. In hippocampal astrocytes, the efficiency of apoE3- and apoE4-mediated cholesterol uptake is similar, whereas it is reduced for apoE2. This low affinity of apoE2 for low-density lipoprotein receptors in astrocytes could contribute to the altered homeostasis of cholesterol in the brain, which may ultimately be associated with the earlier manifestation of MJD in ε2 allele carriers.

These results support a role of APOE as a modulator of MJD phenotypic variability, in addition to the known effect of the CAG tract size in the expanded allele.

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REFERENCES