Multiple Sclerosis 2004; **00:** 1–5
www.multiplesclerosisjournal.com

Genotypes at the APOE and SCA2 loci do not predict the course of multiple sclerosis in patients of Portuguese origin

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Multiple sclerosis (MS) is a demyelinating disease that affects about one in 500 young Europeans. In order to test the previously proposed influence of the APOE and SCA2 loci on susceptibility to MS, we studied these loci in 243 Portuguese patients and 192 healthy controls and both parents of 92 patients. We did not detect any significant difference when APOE and SCA2 allele frequencies of cases and controls were compared, or when we compared cases with different forms of the disease. Disequilibrium of transmission was tested for both loci in the 92 trios, and we did not observe segregation distortion. To test the influence of the APOE ε4 and SCA2 22 CAGs alleles on severity of disease, we compared age at onset and progression rate between groups with and without those alleles. We did not observe an association of the ε4 or the 22 CAGs alleles with rate of progression in our total patient population; allele ε4 was associated with increased rate of progression of MS in a subset of patients with less than 10 years of the disease. However, globally in the Portuguese population, the APOE and SCA2 genes do not seem to be useful in the clinical context as prognostic markers of this disorder.
Multiple Sclerosis *(2004)* **00,** 1–5

Key words: *allelic association; demyelinating disease; genetics; linkage disequilibrium; prognostic markers; spinocerebellar ataxia*

Introduction

Multiple sclerosis (MS) is a chronic primary demyelinating disease of the central nervous system (CNS) affecting one in 500 young Europeans. It is characterized by a wide range of clinical manifestations and classified in relapsing–remitting, primary progressive, secondary progressive, and benign forms.[1]

The higher concordance rate of MS in monozygotic twins (25%) versus dizygotic twins (3%)[2–4] strongly suggests that genetic factors are involved in the aetiology of this disorder; however, the number and type of genes involved and their mechanisms remain uncertain. In the interaction between susceptibility genes and environmental factors, none seem sufficient by itself to cause the disease.[1] Moreover, while some genes may be involved in the induction of the disease, others may have a role in its progression.[5]

Although several chromosomal regions have been proposed as possible locations of susceptibility genes, the MHC class II on chromosome 6 is the only consistent one: only the DR15 allele association, in northern Europeans,[6] and the association with DR4 in Sardinians,[7] have been reproducible. In the UK, regions 3p21-p14, 5q14-q15, 6p21, 7p21-p15, 17q22-q24, 19q13 and Xp21-p11 have also been proposed.[8,9] Other regions were suggested by the Canadian study (2p16, 3p14-p12, 3q21-q25, 5q14-q15, 6p21, 7p21-p15, 7q21-q22, 19q13 and Xp21-Xp11),[10] the study of the Multiple Sclerosis Genetics Group (2p16, 3q21-q25, 5q14-q15, 6p21, 7q11-q22 and 19q13)[11] and by the Finnish group (3q21-q25, 6p21, 17q22-q24 and 19q13).[12] Regions overlapping among all these are 3q21-25, 6p21 and 19q13. Whole genome screens for linkage disequilibrium in MS published by the UK[13] and German[14] groups suggested several interesting regions for MS susceptibility loci. Overlapping genomic regions between these two studies are on chromosomes 1p, 1q, 6p (HLA region), 17q and 19q.

The *APOE* and *SCA2* genes are involved in other neurodegenerative diseases and previous studies suggested they may play a role in susceptibility to MS. The *SCA2* gene, when containing a CAG repeat above 36 units

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Received 16 June 2003; revised 23 September 2003; accepted 3 November 2003

10.1191/1352458504ms998oa

in the coding region, causes an autosomal dominant spinocerebellar ataxia (SCA2).[15–17] It has been reported that the 22 CAG allele segregates preferentially with MS,[18] either due to a direct effect or to that of a gene in linkage disequilibrium with this locus.

Apolipoprotein E (ApoE) is a 34 kDa protein involved in cholesterol metabolism, lipid transport and in neuronal development and regeneration.[19] The *APOE* gene has three main alleles, ε3 being the most frequent.[20–22] The ApoE ε4 isoform has a higher affinity to bind lipid molecules,[23] and has been associated with a higher risk of Alzheimer's disease[24,25] and cardiovascular disease,[26,27] but also with a worse outcome in MS;[28–32] this could be related to a lower capacity of repair (remyelination of lesions) or to a protection against axonal degeneration consecutive to these lesions. The *APOE* gene has been implicated as a susceptibility risk factor[29] and as affecting the progression of disability in MS.[28,30–32] We have now tested the hypotheses 1) that the *APOE* and *SCA2* loci may have a role in the susceptibility to MS, and 2) that specific alleles in these loci may correlate with severity and progression in the Portuguese population.

Material and methods

Subjects

We studied a sample of 243 unrelated patients (mean age = 38.3 ± 10.8 years; 170 females, 73 males) of Portuguese origin collected in different regions of the country, with definite diagnosis of MS according to the criteria of Poser *et al.*[33] The disease was classified as primary progressive (PP; $n = 34$) or other forms of the disease (OF; $n = 184$), of which 72.8% were with recurrences and remissions, 12.0% secondary progressive, 2.7% optical nevritis, 1.1% benign and 11.4% undefined (patients for whom it was impossible for the neurologist to clearly classify the disease course); for 25 cases we had no information about the form of the disease. Values of Expanded Disability Status Scale (EDSS), ranging from 0 (no impairment) to 10 (death from MS),[34] were obtained for 140 patients; 85.7% of these patients were undergoing β-interferon therapy. The progression index (PI)[35] gives a measure of increase in disability through time and is calculated by dividing the EDSS by disease duration in years. Both parents of 92 patients were also studied. We also studied 192 unrelated healthy controls (HC) (all excluded for MS by a neurologist) matched to the case by gender, age (±2 years) and region of origin. Written informed consent was obtained from all subjects.

Methods

Genomic DNA from all subjects was isolated from peripheral blood using the Gentra system (Puregene™). The *APOE* genotyping was performed by PCR-RFLP,[36] in an 8% nondenaturing polyacrilamide gel, using *Hha*I. The size of the $(CAG)_n$ at the *SCA2* locus was determined by PCR, as described;[16] PCR products (separated in a 6% denaturing polyacrylamide gel) were hybridized with a $(CAG)_{15}$ ^{32}P-labelled oligonucleotide probe.

Statistical analysis

Frequency of risk genotypes, i.e., at least one risk allele (ε4 for the *APOE* gene and homozygosity for the 22 allele for the *SCA2* gene) was compared between cases and controls, using the McNemar test. This was also used to compare frequencies of risk genotypes in patients and individually matched controls, for each clinical subgroup (PP or OF). Fisher's exact test was used to compare genotypes of cases with PP versus OF of MS. Disequilibrium of transmission was tested with the TDT-STDT program (v.1.1) in the 92 trios. To compare patients concerning clinical variables, and to test for differences between patient subgroups with a specific genotype, Student's *t*-test and Mann–Whitney tests were performed (Table 1).

Results

No significant differences were found between patient subgroups with a specific genotype with regard to the patients' mean age, disease duration and gender distribution (data not shown); we concluded these would not be confounding factors for the remaining analyses.

We found no significant differences when cases and controls were compared regarding the presence/absence of the allele ε4 of the *APOE* gene ($P = 1.0$) and homozygosity for the 22 CAGs allele at the *SCA2* locus ($P = 0.752$) (Table 2). Comparison of genotype frequencies for the *APOE* and *SCA2* genes, between cases with PP versus OF disease, and between each of these subgroups with matched controls, did not reveal any significant differences (Figure 1a and b). We also did not detect transmission disequilibrium for any allele at either loci in the 92 trios studied (data not shown).

We did not detect an influence of the risk genotypes on the severity of the disease, measured through the EDSS and PI values in the total sample. However, patients with a duration of disease less than 10 years and carrying the *APOE* ε4 allele ($n = 8$) presented a faster progression than those not carrying this allele ($n = 61$) (PI: 0.80 ± 0.5 and 0.49 ± 0.4, respectively; $P = 0.026$) (Table 1).

Discussion

The European population is highly heterogeneous: genetic differences occur across relatively small distances and genetic clines for some markers of susceptibility to MS already identified also appear to correlate with distribution of the disease.[1] Different genetic backgrounds and environments, which could be interacting among them, may cause susceptibility to MS in different populations or even within the same population. Genes that play a role in

Table 1 Demographic data and *APOE* and *SCA2* genotypes among all MS cases

Demographic/clinical data	*Total*	*APOE*			*SCA2*		
		ε4+	*ε4−*	*P value*	*22/22+*	*22/22−*	*P value*
n	243	38	201	167	50		
F/M (%)	70.0/30.0						
Age (mean ± SD)	38.3 ± 10.8						
Age at onset (mean ± SD)	29.0 ± 9.7	30.4 ± 11.3	28.8 ± 9.4	0.373[a]	8.5 ± 9.2	31.1 ± 9.6	0.103[a]
Disease duration (mean ± SD)	9.3 ± 7.0	10.8 ± 8.6	8.9 ± 6.3	0.128[a]	9.2 ± 7.0	9.4 ± 6.4	0.883[a]
EDSS (*n*; mean ± SD)	140[b]; 2.0 ± 1.3	24; 2.0 ± 1.1	114; 2.0 ± 1.3	0.937[a]	103; 2.0 ± 1.3	26; 1.9 ± 1.0	0.794[a]
PI (*n*; mean ± SD)	129[b]; 0.4 ± 0.4	19; 0.4 ± 0.5	108; 0.3 ± 0.3	0.935[c]	95; 0.3 ± 0.3	25; 0.3 ± 0.4	0.936[c]
PI (*n*; mean ± SD)							
DD > 10 years	60; 0.15 ± 0.1	11; 0.13 ± 0.09	47; 0.15 ± 0.1	0.336[c]	45; 0.16 ± 0.1	12; 0.13 ± 0.04	0.876[c]
DD < 10 years	69; 0.52 ± 0.4	8; 0.80 ± 0.5	61; 0.49 ± 0.4	0.026[c]	50; 0.49 ± 0.4	13; 0.51 ± 0.4	0.812[c]

n, number of cases; MS, multiple sclerosis patients; F, female; M, male; DD, disease duration.
[a] Students's *t*-test.
[b] Total *n* is independent of *APOE* and *SCA2* genotyping.
[c] Mann–Whitney test.

Table 2 Comparisons of genotype frequencies for *APOE* and *SCA2* genes between MS cases and controls

	McNemar's test			*Fisher's exact test*
	MS/HC	*PP/HC*	*OF/HC*	*PP/OF*
APOE ε4+	$\chi^2 = 0$ $P = 1.00$	$\chi^2 = 0.57$ $P < 0.48$	$\chi^2 = 0.46$ $P < 0.53$	$P = 0.26$
SCA2 22/22+	$\chi^2 = 0.1$ $P = 0.75$	$\chi^2 = 0$ $P = 1.00$	$\chi^2 = 0.12$ $P < 0.75$	$P = 0.54$

susceptibility to MS in given populations may not have the same role in others.[5] It is thus important that studies be replicated in other population, and even with different samples within the same population.

Our study on Portuguese MS patients could not confirm the previously reported influence of the *APOE* gene on susceptibility to this disease,[29] which has also been excluded in various studies in other populations,[28,31,32,37–40] including linkage studies in a Finnish population, which suggested that 19q13.2-q13.3, where the gene *APOE* is localized, was not a candidate region for susceptibility to MS.[41] The 22 CAGs *SCA2* alleles did not seem to segregate preferentially with MS in our sample, as had been reported in patients from the UK.[18] Our data are in agreement with later studies in a Dutch population.[42] Therefore, neither of these two loci could be related to susceptibility to MS in Portuguese patients; this was confirmed for the total patient population, as well as for each clinical subtype considered (PP and OF). The *APOE* and *SCA2* risk alleles were not over-represented in any of the clinical subgroups of MS, in contrast to previous studies by Hogh *et al*.,[29] where a preferential representation of the *APOE* ε4 allele was detected in patients with the PP form.

This study included a correction for possible stratification of the population, since controls were individually matched to patients by gender, age and origin, whereas other studies have used data from unmatched control populations already described or randomly selected.[28,29,31,32,37–40] Population stratification can originate false associations, which may explain the results previously obtained with the *APOE* ε4 allele.[29]

In terms of MS severity and progression, no differences were found in the PI or EDSS values between carriers and noncarriers of ε4 in our total sample. When we considered patients with a disease duration less than 10 years ($n = 69$), however, we have detected an increased PI in carriers

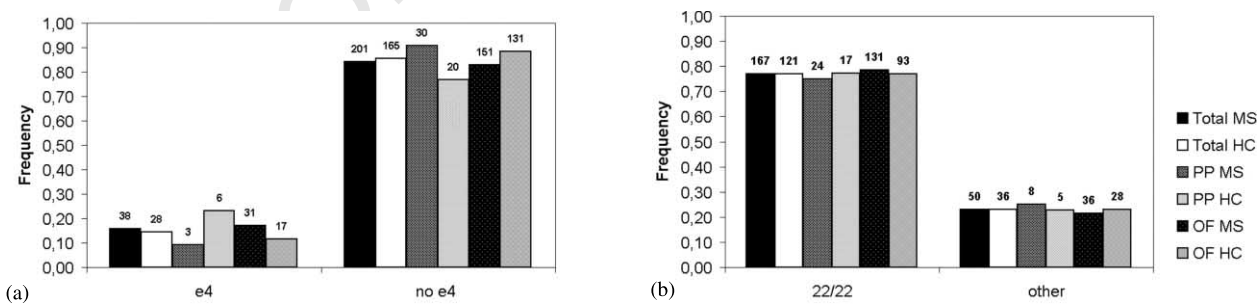

Figure 1 *Genotype frequencies for APOE* and *SCA2* loci. (a) *APOE* ε4+ versus *APOE* ε4− genotype frequencies and (b) *SCA2* 22/22+ versus *SCA2* 22/22− genotype frequencies, for both cases and controls. HC, healthy controls; MS, multiple sclerosis patients; PP, primary progressive; OF, other forms of disease. Numbers at the top of columns are number of individuals with that genotype.

of ε4. A similar finding was described in Danish patients,[29] and although this may be a spurious association due to the greater instability that characterizes the initial phase of the disease, when the EDSS evaluation is less reliable, it may also be the reflection of a stronger effect of the ε4 allele in the earlier stages of the disease. ApoE is known to play a role in the recapture of lipids from the myelin of damaged neurones and this function may be of particular relevance in the initial stages of MS pathogenesis. The role of the *SCA2* 22/22 genotype in MS progression was excluded here, for the first time, when we analysed the age at onset and severity parameters.

One of the questions that remains open in MS studies is which instrument is the most appropriate to quantify severity and rate of progression. The EDSS scale is non-linear and can be equivocal, providing a mean value that represents mostly the motor deficit; however, this is still the most widely used scale for MS. Cognitive deficits, as well as psychological and emotional dysfunction, may be related with the risk alleles studied but have not been assessed. In particular, it would be of great interest to study this correlation with the *APOE* gene, given its importance in the pathogenesis of dementia and the recently proposed association of the AA genotype, at the polymorphism -491 A/T in the promoter region of this gene, with a higher risk of cognitive impairment in MS patients.[43] Severity of MS can also be studied through MRI, measuring the load of cerebral lesions, which has been described to be more aggressive in *APOE* ε4 allele carriers.[30]

In conclusion, the role of the SCA2 gene in MS has been excluded in our population, whereas it remains possible that the APOE gene may have role in the progression of MS that is limited to the initial stages of the disease. This question needs to be addressed in a larger sample of patients with a short disease duration, using different severity measures, before definite conclusions can be drawn. To any extent, in our population, the association between *APOE* and the disease course is too weak to be of predictive value in the clinical practice.

Acknowledgements

We would like to thank Anabela Ferro for her co-operation since the beginning of this study, and Alda Sousa for helpful statistical discussion. We also would like to thank Jorge Rocha and Susana Seixas, who kindly provided us with positive controls for the *APOE* genotypes. This work was supported in part by Serono Portugal. Santos M supported by FCT, grant SFRH/BD/9111/2002.

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