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RESEARCH PAPER *Multiple Sclerosis* 2009; **15:** 299–303

An investigation of susceptibility loci in benign, aggressive and primary progressive multiple sclerosis in Northern Irish population

OM Gray[1], H Abdeen[2], GV McDonnell[1], CC Patterson[3], CA Graham[2] and SA Hawkins[4]

Objective To investigate the possibility that susceptibility loci in multiple sclerosis (MS) have a role in determining the disease outcome in Northern Ireland population.
Background The Genetic Analysis of Multiple Sclerosis in Europeans (GAMES) initiative and follow-up refined analysis identified 15 candidate susceptibility loci within the Northern Irish population for MS. We aimed to investigate the 12 most significant markers for their role in disease outcome.
Methods Cases with probable or definite MS (Poser criteria) were classified as benign onset (Kurtzke Expanded Disability Status Scale [EDSS] ≤ 3.0 at 10 years), aggressive (Kurtzke EDSS ≥ 6.0 by 10 years), or primary progressive MS. All cases were Caucasian of Northern Irish origin. DNA was extracted from venous blood, microsatellite markers were amplified using polymerase chain reaction and typed using fluorescent fragment analysis. Allele frequencies were compared statistically using a chi-squared test with allowance for multiple comparisons (critical $P < 0.0042$); significant markers were further analyzed by CLUMP (critical $P < 0.0014$).
Results Two microsatellite markers were significant: D3S1278 (Chr 3q13, $P < 0.001$) and tumor necrosis factor (TNF)-α (Chr 6p21, $P < 0.001$). A further three markers were significant in our preliminary analysis suggesting a trend toward impact on disease outcome; D4S432 (Chr 4p16, $P = 0.001$), D2S347 (Chr 2q14, $P = 0.003$), and D19S903 (Chr 19p13, $P = 0.003$).
Conclusions This is the first study to suggest a role for TNF-α in the disease outcome in MS. Larger replication studies need to be performed to assess the role of markers D4S432, D2S347, and D19S903. *Multiple Sclerosis* 2009; **15:** 299–303. http://msj.sagepub.com

Key words: genetics; multiple sclerosis; Northern Ireland; susceptibility genes

Introduction

Multiple sclerosis (MS) is a heterogeneous condition which at the extremes can have an extremely benign course, where patients remain independently mobile for many years, or it can have a fulminant course with rapid decline in function in the weeks following diagnosis. Certain clinical factors have a higher likelihood of a more benign course e.g. initial symptom being sensory or visual, younger age at onset, female sex, [1] and a long interval between the first two relapses [2]. However, in an individual patient, it is not possible to accurately predict the course of their illness at the time of diagnosis.

Several genes have been implicated in modifying the course or severity of MS. HLA-DR2 has been shown to favor the progression to MS in patients with clinically isolated syndromes [3] and is associated with earlier disease onset [4] and a relapsing remitting rather than progressive course [5]. The presence of apolipoprotein E-ε4 allele is thought to be an unfavorable prognostic factor [6,7], whereas the ε2 allele has demonstrated a more favorable effect [8]. Polymorphisms in interleukin (IL)-1ra allele-2 have been found to favorably influence

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disease outcome in four studies [5,9–11], however, a further two studies did not see such an effect [12,13]. Polymorphisms in IL-1ra allele-3 have been found to be favorable in a single study [12]. To date, no association has been found between tumor necrosis factor (TNF)-α polymorphisms and disease course or severity [14–18].

The Northern Ireland arm of the Genetic Analysis of Multiple Sclerosis in Europeans (GAMES) study and follow-up refined analysis identified 15 microsatellite markers which may locate susceptibility genes for MS in the Northern Ireland population [19,20]. The present study considered the 12 most significant of these markers in benign onset, aggressive, and primary progressive sub-groups. The aim of this study was to identify any susceptibility genes present in one of the cohorts and not in the others; therefore, it would be a possible marker for that disease outcome. Clinically, primary progressive patients present and behave in a way dissimilar from relapsing remitting patients, of whom 70% progress. Therefore, the most useful test in the clinical setting would be one that could predict a benign or aggressive course in relapsing remitting disease.

Methods

Case ascertainment and classification

Cases were interviewed and examined to confirm the diagnosis. Kurtzke Expanded Disability Status Scale (EDSS) [21] was calculated at attendance and retrospectively at 10 years from onset of first symptom. Individuals recruited gave written consent for genetic analysis. Only cases with benign, aggressive, or primary progressive MS were included in this study. Benign-onset MS was defined as a Kurtzke EDSS ≤ 3.0 at 10 years. Aggressive MS included all cases with Kurtzke EDSS ≥ 6.0 by 10 years. Primary progressive MS was defined as a progressive clinical history from onset of first symptoms, with no clear evidence of relapses or remissions. All cases were Caucasian of Northern Irish origin. The clinical characteristics of each group are documented in Table 1.

Diagnostic criteria

Those satisfying the Poser criteria for definite or probable MS [22] or the McDonald criteria for MS [23] were included. Clinically isolated syndromes were not included in this study.

DNA extraction

DNA was extracted from venous blood by standard methods. The DNA concentration of each sample was determined by spectrophotometry. Sample concentrations were adjusted to 50 ng/μl.

Polymerase chain reaction

The 12 microsatellite markers tested are detailed in Table 2. The polymerase chain reaction (PCR) was performed on 10 mcgl reaction volumes using a thermophilic DNA polymerase. The cycle reaction was performed on an Applied Biosystems (Forster City, CA, USA) 9700 thermal cycler according a standard protocol.

Capillary electrophoresis

The products from each PCR were electrophoresed on an Applied Biosystems 3100 Genetic Analyser. Resultant electropherograms were analyzed using GENESCAN (version 3.5) and GENOTYPER (version 3.7) software.

Statistical analysis

The sample size target of 200 per group was justified in the basis of comparing each allele against all the

Table 1 Clinical characteristics of benign onset, aggressive, and primary progressive cohorts

Characteristic	Benign onset	Aggressive	Primary progressive
No. of cases	181	167	136
F:M ratio	2.3:1	2.0:1	1.1:1
Mean age (years)	46.8	48.6	54.7
Mean age at onset (years)	27.4	33.3	42.1
Mean duration (years)	19.3	15.3	12.6
EDSS present			
Mean	3.3	6.6	5.8
Range	0.0–7.5	6.0–7.5	2.0–7.5
EDSS at 10 years			
Mean	1.8	6.3	5.6
Range	0.0–3.0	6.0–7.5	2.0–7.5

EDSS, Expanded Disability Status Scale.

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Table 2 Chi-squared test results used to screen 12 microsatellite markers

Microsatellite marker	Chromosomal location	Chi-squared statistic	Degrees of freedom (df)	Significance value (*P*)*
D15S1040	15q14	13.7	18	0.75
D3S1278	3q13	63.1	24	<0.001
D11S1998	11q23	11.3	8	0.18
D7S2537	7q21	25.3	14	0.03
D4S432	4p16	46.7	20	0.001
D20S892	20p12	13.3	16	0.65
D2S1779	2p14	12.1	12	0.44
D2S347	2q14	32.6	14	0.003
D19S583	19p13	12.3	14	0.58
D3S2432	3p24	24.9	20	0.21
D19S903	19q13	36.3	16	0.003
TNF-α	6p21	129.4	20	<0.001

*$P < 0.0042$ denotes a significant result.

rest combined. With 12 markers, three group comparisons and an average of 10 alleles per marker, a Bonferroni correction factor of $12 \times 3 \times 10 = 360$ was applied to the significance level for the sample size calculation. A study of 200 cases per group has approximately 80% power to detect a difference in allele frequencies of 5% in one group versus 15% in another group.

Alleles were typed by fluorescent fragment analysis. Peak height was quantified to identify the major allele. Allele frequencies were initially compared between the three patient subgroups using a chi-squared test for 3 x k tables (which was considered valid because all expected values were ≥2.0). In these initial analyses, *P* values were deemed significant at the level $P < 0.0042$ (0.05 of 12). Markers showing significant difference were compared for differences between benign and aggressive, benign and primary progressive, and aggressive and primary progressive disease by CLUMP analysis [24] using the T1-statistic with a *P* value obtained using 10,000 permutations. In these comparisons, a *P* value of <0.0014 (0.05 of 12×3) was deemed significant. Finally, the CLUMP T3-statistic was used to identify which alleles were overrepresented in one group relative to another. This statistic compares each allele with all other alleles combined whilst taking into account the number of alleles at the locus. *P* values were obtained using 100,000 permutations and a *P* value of <0.0014 was taken as significant.

Results

A total of 484 patients with MS were recruited in this study as follows: benign onset-181 cases; aggressive-167 cases; and primary progressive-136 cases. The patient characteristics of each group are documented in Table 1. As expected, the benign onset and aggressive groups were of a younger age, had a significantly higher female to male ratio, and were younger at onset of first symptom than the primary progressive group. The mean Kurtzke EDSS at 10 years were 1.8 for benign-onset cases (range 0.0–3.0), 6.3 for the aggressive cases (range 6.0–7.5), and 5.6 for the primary progressive group (range 2.0–7.5).

Table 2 shows that five of 12 microsatellite markers were significant in the initial screening using the 3 x k chi-squared tests with the significance level set at the $P < 0.0042$ level: D3S1278 (Chr 3q13, $P < 0.001$), D4S432 (Chr 4p16, $P = 0.001$), D2S347 (Chr 2q14, $P = 0.003$), D19S903 (Chr 19p13, $P = 0.003$), and TNF-α (Chr 6p21, $P < 0.001$). Direct comparisons in pairs of the three groups by CLUMP T1-statistic were performed as detailed in Table 3. Markers D4S432, D2S347, and D19S903 did not show any statistical differences between benign onset, aggressive, and primary progressive groups at the $P < 0.0014$ significance level.

The marker D3S1278 had significant differences between primary progressive and aggressive groups ($P < 0.0001$) but not between benign onset and aggressive groups ($P = 0.11$) or benign onset and primary progressive groups ($P = 0.004$). The CLUMP T3-statistic confirmed a difference between the primary progressive and aggressive groups in the 213 and 215 allele frequencies as shown in Figure 1A; there is a shift toward allele frequency 215 in the primary progressive group.

The CLUMP T1-statistic showed that the TNF-α marker was significant between benign onset and aggressive groups ($P < 0.0001$) and benign onset and primary progressive groups ($P < 0.0001$) but not aggressive and primary progressive groups ($P = 0.002$). The CLUMP T3-statistic confirmed these differences as shown in Figure 1B with a difference between benign onset and aggressive groups in allele frequencies 249 and 251 and also 267 and 269. In the benign onset vs. primary progressive group, a difference was apparent for allele frequencies 249 and 251, with a shift toward 251 in the benign onset group.

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Table 3 CLUMP (T1) analysis performed on pair-wise comparisons of the benign, aggressive, and primary progressive groups on the significant markers

Marker comparison	Significance value (*P*)*
D3S1278	
Benign onset vs. Aggressive	0.11
Benign onset vs. PP	0.004
Aggressive vs. PP	<0.0001
D4S432	
Benign onset vs. Aggressive	0.002
Benign onset v PP	0.07
Aggressive v PP	0.03
D2S347	
Benign onset vs. Aggressive	0.003
Benign onset vs. PP	0.31
Aggressive vs. PP	0.10
D19S903	
Benign onset vs. Aggressive	0.003
Benign onset vs. PP	0.06
Aggressive vs. PP	0.16
TNFα	
Benign onset vs. Aggressive	<0.0001
Benign onset vs. PP	<0.0001
Aggressive vs. PP	0.002

PP, primary progressive.
*$P < 0.0014$ denotes a significant result.

Discussion

We have demonstrated that five microsatellite markers known to influence susceptibility to MS in the Northern Irish population may also have a role on disease outcome: D3S1278 (Chr 3q13, $P < 0.001$), D4S432 (Chr 4p16, $P = 0.001$), D2S347 (Chr 2q14, $P = 0.003$), D19S903 (Chr 19p13, $P = 0.003$), and TNF-α (Chr 6p21, $P < 0.001$). Although markers D4S432, D2S347, and D19S903 were significant only in our preliminary analysis, further analysis using CLUMP was unable to identify where the differences lay. These markers might usefully be assessed in a larger replication study in a different population. Marker D3S1278 was significant for comparison between aggressive and primary progressive disease; however, clinically this would be of limited value. TNF-α has been shown to be significant for comparison of benign onset with both aggressive and primary progressive disease. The authors would acknowledge that this study was not ideally powered.

The studies in TNF region of chromosome 6 are of particular interest because TNF-α and TNF-β are tandemly located within the HLA complex, suggesting that these genes may be in linkage disequilibrium with the HLA-DR loci that are associated with MS. TNF-α, in particular, has been repeatedly shown to confer susceptibility to MS in both linkage and association studies. Interestingly, in this study, the allele frequencies of the TNF-α marker proved to be significant between benign onset and aggressive groups ($P < 0.0001$) and benign onset and primary progressive groups ($P < 0.0001$). This would suggest that this marker may be the single most valuable predictor of disease outcome in MS in Northern Ireland population. This is in contrast with all previous studies which failed to demonstrate any association between TNF-α and disease course and severity [10–14].

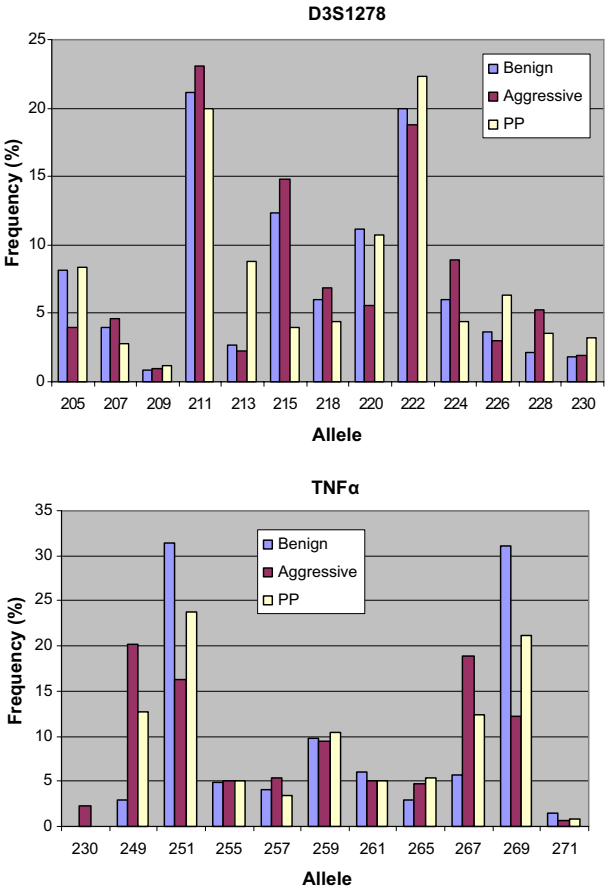

Figure 1 Allele frequencies for benign onset, aggressive, and primary progressive (PP) cohorts for (A) D3S1278 and (B) tumor necrosis factor (TNF).

Clinically, the most valuable test would be able to predict between benign and aggressive MS. A statistically significant difference between the benign onset and aggressive cohorts was identified with TNF-α ($P < 0.0001$). This would support that this marker as a potential future test to discriminate between patients who will follow a benign or aggressive clinical course. An unbiased assessment of the value of this marker would be best obtained from a replication study.

In conclusion, this study supports the concept that DNA analysis for known microsatellite markers may help in predicting the disease outcome in MS in the clinical setting. TNF-α showed significant differences between benign onset and aggressive MS in

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the Northern Irish population indicating that it is the most valuable predictor of disease outcome in this population. This study also suggests a trend in disease outcome with markers D4S432, D2S347, and D19S903 which may warrant further study.

Disclosure

The authors report no conflicts of interest.

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