## SHORT REPORT

# Allele frequencies of the third component of complement (C3) in MS patients

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**Abstract**
**No difference was found in the allele frequency of C3 (third component of complement) in 129 multiple sclerosis (MS) patients compared with both 69 controls or with similar reported controls from the published literature. An association cannot be confirmed between C3 and MS.**

Extensive genetic polymorphism of the third component of complement (C3) has been found in humans.[1] [2] Gene products are recognised by their relative electrophoretic mobilities in prolonged agarose-gel electrophoresis.[1] The use of calcium in the electrophoresis buffer in appropriate concentrations has been shown to cause C3 to migrate slower than transferrin and usually more slowly than beta lipoprotein.[3] The allotypes, which are inherited in an autosomal codominant manner, appear to differ in net surface charge at pH 8·6, but show no difference in complement activity, molecular size or ability to bind calcium.[1]

Rheumatoid factor positive rheumatoid arthritis,[4] atherosclerotic vascular disease[5] and hepatitis[4] are associated with the fast (F) allele of C3. More recently Jans and Sorensen[6] reported that a group of 60 multiple sclerosis (MS) patients were found to contain a significantly higher frequency of the fast allele than did 1066 controls. Because of accessibility to a large number of MS patients, we felt that a definitive answer could be obtained on the possible association between MS and C3 polymorphisms.

## Methods

### Diagnosis
Only white MS patients who were diagnosed as clinically definite[7] or laboratory supported probable MS[8] were included in this study. All of the patients in this study were selected at random from the MS clinic in London, Ontario.[9] The control subjects were white, over the age of twenty five and had negative neurological histories. No participants in this study were related.

The ratio of males to females in the MS patient group was 1:1·8, which is the same ratio observed for the entire clinic population of 1400. The ratio of males to females in the control group was 1:1.

Serum and plasma samples were collected

and frozen (−70°C) within three hours of collection from neurologically normal individuals and MS patients who were members of the MS clinic. A commercial preparation of the third component of human complement (C3) (Cedarlane, Canada) at a final concentration of 0·4 mg/ml was used as the positive control.

Typing for the third component of complement was performed by agarose gel electrophoresis as described elsewhere.[1] The bridge buffer was barbital buffer of pH 8·6 and ionic strength of 0·05, containing 0·0018M calcium lactate. Samples were thawed immediately before analysis. Gels were run at a constant voltage of 20 V/cm for 3·5 hours at 4°C, fixed in a 30% methanol, 20% acetic acid, stained with Coomassie Blue and destained in 30% methanol, 20% acetic acid. The position of C3 polymorphism was verified by an indirect immunoperoxidase overlay assay.

Comparison of the allele frequencies of MS patients to those of the controls was performed using a $2 \times 2$ contingency Chi square.[10] The expected phenotype frequencies were calculated by applying the Hardy-Weinberg distribution ($a^2 + 2ab + b^2 = 1$) to the sum of the cases and controls. Odds ratio with 95% confidence intervals were calculated for the three phenotypes in cases versus controls.

## Results
A total of 200 individuals were typed for C3 alleles, of whom two (one member of the control group and one of the MS patients) had an uncommon allele; subsequently these samples were not included in the study. The results of the typing are shown in table 1. The frequency of $C3^F$ allele was 0·1957 and 0·2210, while the frequency of $C3^S$ allele was 0·8043 and 0·7790 in the control and MS populations respectively. The distribution of the three common phenotypes (SS, FS, FF) within the

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*Table 1 Distribution of alleles of the third component of complement (C3) in MS patients (n = 129) and normal controls (n = 69)*

| C3 Phenotype | Normal controls | MS patients | Total |
|---|---|---|---|
| SS | 46 (42·85)[1] | 76 (80·10) | 122 (122·95) |
| FS | 19 (23·05) | 49 (43·10) | 68 (66·15) |
| FF | 4 (3·10) | 4 (5·80) | 8 (8·90) |
| Total | 69 | 129 | 198 |

[1]Expected values, given in brackets, were determined using Hardy-Weinberg distribution as applied to the total phenotype frequency.

*Table 2    Odds ratios with 95% confidence intervals for SS, FS and FF phenotypes in MS patients (n = 129) and normal controls (n = 69)*

| Allele | Cases | Controls | OR | 95%CI |
|---|---|---|---|---|
| SS | 76/129 | 46/69 | 0·717 | (0·279 to 1·155) |
| FS | 49/129 | 19/69 | 1·612 | (0·586 to 2·638) |
| FF | 4/129 | 4/69 | 0·520 | (0·217 to 1·546) |

control group compared with the MS patients was not significantly different (Chi square = 2·782; 2 df; 0·10 < P < 0·25). Assuming that the C3 system is in a Hardy-Weinberg equilibrium, good agreement was found between observed and expected values (Chi square = 0·150; 1 df; 0·50 < P < 0·75). The odds ratios with 95% confidence intervals for the three phenotypes in cases versus controls are shown in table 2.

## Discussion

Recent evidence supports an important role for genetic factors in MS susceptibility.[11] Inheritance of susceptibility appears to be polygenic with good evidence that MHC[12] and possibly T cell region[13] loci each contribute part of the overall susceptibility. A surprisingly large number of population associations have been described in MS. As yet, in no case has the relationship of these to pathogenesis been demonstrated. It is suspected that some associations reflect population stratification (Bulman DE, MS Thesis, University of Western Ontario, 1986) which is well illustrated for Gm allotypes.[14]

The results given in the tables suggest that there is no association between any C3 allele and MS. An earlier report, however, had suggested a modest association between MS and C3$^F$.[6] There are differences between these two studies in the size of the patient and control groups. Jans and Sorensen typed 60 patients and 1066 blood donors (controls). In this study 129 patients and 69 neurologically normal controls were typed and no difference in allele frequency was found. Similarly there was no difference between the MS patients in this study and the larger control group of Jans and Sorensen (Chi square = 5·416; p > 0·05). Finally, the allele frequencies in the London, Ontario MS population (100% white) of 0·7790 and 0·2210 for C3$^S$ and C3$^F$ respec-

tively, are extremely close to the frequencies 0·77 and 0·22 reported for the white population.[15] The reason for the discrepancy between the studies is unclear but it may be due to the smaller patient group of the earlier study or it may be due to ethnic variation within the control group. Because the gene frequency of C3$^F$ is approximately 20%, homozygotes for the fast allele of C3 would represent only 4% of the population, thus requiring a relatively large patient sample size for definitive results.

Our results suggest that there is no association between MS and any particular allele of C3. It is possible that the reported association reflects yet another population association secondary to differential MS patient and control stratification.

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