The development of atypical haemolytic-uraemic syndrome is influenced by susceptibility factors in factor H and membrane cofactor protein: evidence from two independent cohorts


Background: In both familial and sporadic atypical haemolytic-uraemic syndrome (aHUS), mutations have been reported in regulators of the alternative complement pathway including factor H (CFH), membrane cofactor protein (MCP), and the serine protease factor I (IF). A characteristic feature of both MCP and CFH associated HUS is reduced penetrance and variable inheritance; one possible explanation for this is that functional changes in complement proteins act as modifiers.

Objective: To examine single nucleotide polymorphisms in both CFH and MCP genes in two large cohorts of HUS patients (Newcastle and Paris).

Results: In both cohorts there was an association with HUS for both CFH and MCP alleles. CFH and MCP haplotypes were also significantly different in HUS patients compared with controls.

Conclusions: This study suggests that there are naturally occurring susceptibility factors in CFH and MCP for the development of atypical HUS.

HUS, haemolytic-uraemic syndrome; aHUS, atypical haemolytic-uraemic syndrome; CFH, complement factor H; HUS, haemolytic-uraemic syndrome; MCP, membrane cofactor protein; SNP, single nucleotide polymorphism.
CFH and MCP haplotypes in atypical HUS

RESULTS

The genotype and allele frequency for the two cohorts is shown in table 3. In both cohorts there was an association between CFH alleles and HUS. The same CFH SNPs were analysed in both cohorts. There was also an association between MCP alleles and HUS in both cohorts. The results for the one SNP (c.2232T→C) analysed in both cohorts showed a strong association (p < 0.001) in the Paris cohort but failed to reach a significance value of <0.01 in the Newcastle cohort (p = 0.012).

The allele frequency in those with and without known mutations in CFH, MCP, and IF is shown in table 4. For CFH in the Newcastle cohort c.257T c.2089G, c.2881T were associated with atypical HUS in those not known to have a mutation but not in those known to have a mutation. For CFH in the Paris cohort c.257T c.2089G, c.2881T were associated with atypical HUS only in those known to have a mutation. For MCP the association was present in both those with and those without an identified mutation for all the SNPs in the Paris cohort. In the Newcastle cohort c.1912+638A was associated with atypical HUS in those without a mutation.

The haplotypes generated by FUGUE are shown in tables 5–7. For both the Newcastle and the Paris cohort there was a strong association (p < 0.001) in the Newcastle cohort but failed to reach a significance value of <0.01 in the Paris cohort.
significant difference for both CFH and MCP haplotype frequency in the HUS patients and controls. The genotype and haplotype results for both cohorts are internally consistent in that an increase in frequency of the rarer allele and haplotype for both MCP and CFH was associated with HUS.

DISCUSSION

In this study we showed a significant difference in two independent cohorts of atypical HUS patients in both allele frequency and haplotypes for two complement regulatory genes, CFH and MCP. Mutations in both these genes have been described in aHUS patients. However, the inheritance...
and penetrance seen with mutations in both these is variable. For instance in two families with the same MCP mutation (S206P), only homozygotes are affected in one whereas in the other heterozygotes are affected. Moreover, the series reported to date show that the penetrance of CFH associated HUS is approximately 50%. This suggests that other factors are modifying the inheritance and penetrance. Both CFH and MCP belong to a cluster of genes located at 1q32 which are involved in complement regulation, the so called RCA (regulators of complement activation) cluster. Other members of this group include decay accelerating factor (DAF), complement receptor 1 (CR1), C4 binding protein (C4BP), and five factor H related proteins (FHR1–5). To date mutations have only been found in CFH and MCP but it is possible that genetic variability in these other regulators could be acting as modifiers for the development of HUS.

Caprioli et al have previously reported that the CFH alleles 2257T, c.2089G, and c.2881T are significantly more common in patients with atypical HUS. The results from both the Newcastle and Paris cohorts support this observation. They found that this was true both for patients with a CFH mutation and for those without. In the Newcastle cohort we found that 2257T, c.2089G, and c.2881T were more frequent in those without known mutations in CFH, MCP, and IF. In the Paris cohort, the reverse was seen in that 2257T, c.2089G, and c.2881T were more frequent in those with mutations. Caution must therefore be exercised in interpreting subgroup analysis such as this where numbers may be inadequate. It is not yet known whether c.2257C or c.2881G are functionally significant. c.2089A is a synonymous change. c.2257T is located in a putative NF–kB binding sequence of the CFH promoter and it is known that CFH expression is upregulated by interferon γ, providing a possible link. c.2881G changes a glutamate to an aspartate in CCP16 of CFH. We have now not only confirmed Caprioli’s observation but also extended it in two independent cohorts to show that genetic variability in MCP is also associated with atypical HUS. This confirms the recent finding by Esparza-Godilla et al that a specific SNP haplotype block which includes MCP was associated with aHUS. In the Paris cohort this strong association was present in both those with and those without known mutations in CFH, MCP, and IF. In contrast, the association was only seen in those with known mutations in the study of Esparza-Godilla. This discrepancy emphasises the need to be cautious in interpreting such data. In all, six MCP SNPs were examined in the two tables that follow.

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Factor H haplotypes for both cohorts</th>
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<tbody>
<tr>
<td></td>
<td>Newcastle</td>
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<tr>
<td>257C→T</td>
<td>c.2089A→G</td>
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<tr>
<td>257T→C</td>
<td>c.2881G→T</td>
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<td>257C→C</td>
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Newcastle: log likelihood ratio = 19.65. Permutations with higher ratio 0/1000. Paris: log likelihood ratio = 15.64. Permutations with higher ratio 779/10 000.

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<th>Table 6</th>
<th>MCP haplotypes for the Newcastle cohort</th>
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<tbody>
<tr>
<td>c.IVS12+638G→A</td>
<td>c.2232T→C</td>
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Log likelihood ratio = 9.252. Permutations with higher ratio 7947/100 000.

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<tr>
<th>Table 7</th>
<th>MCP haplotypes for the Paris cohort</th>
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<tbody>
<tr>
<td>c.IVS8–23T→G</td>
<td>c.IVS9-78G→A</td>
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Log likelihood ratio = 20.28. Permutations with higher ratio 7/1000.
cohorts; of these three are intronic, two are in the promoter, and one is in the 3’ UTR. It is possible that the promoter SNPs or the 3’ UTR SNP are functionally significant. In support of this is the recent study from Esparza-Gordillo which showed that MCP –261G disrupts a potentially functional CBF-1/ RBP-Jk binding site. Transient transfection showed that this was associated with a 25% lower transcriptional activity. It is also possible that a combination of factors within the haplotype block results in a functional effect. Alternatively, these markers may simply be surrogates for another untested SNP in the vicinity.

Conclusion
This study emphasizes the importance of variability in CFH and MCP as a modifier for the development of atypical HUS. The results suggest that complement regulatory genes in the RCA cluster are acting in a coordinated manner to prevent host cell damage and that perturbations of this network in the face of endothelial injury will lead to a thrombotic microangiopathy.

ACKNOWLEDGEMENTS
This study was supported by grants from the National Kidney Research Fund, the Northern Counties Kidney Research Fund, the Peel Medical Research Trust, and the Robin Davies Trust. VF-B was supported by an EMBO short term fellowship.

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Competing interests: none declared

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Received 10 January 2005
Revised version received 28 February 2005
Accepted for publication 4 March 2005

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The development of atypical haemolytic-uraemic syndrome is influenced by susceptibility factors in factor H and membrane cofactor protein: evidence from two independent cohorts

V Fremeaux-Bacchi, E J Kemp, J A Goodship, M-A Dragon-Durey, L Strain, C Loirat, H-W Deng and T H J Goodship

J Med Genet 2005 42: 852-856 originally published online March 22, 2005
doi: 10.1136/jmg.2005.030783

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