Mutations in Complement Factor I Predispose to Development of Atypical Hemolytic Uremic Syndrome

David Kavanagh,* Elizabeth J. Kemp,* Elizabeth Mayland,* Robin J. Winney,† Jeremy S. Duffield,‡ Graham Warwick,§ Anna Richards,* Roy Ward,∥ Judith A Goodship,* and Timothy H.J. Goodship*

*Institute of Human Genetics, University of Newcastle upon Tyne, United Kingdom; †Department of Renal Medicine, Royal Infirmary, Edinburgh, United Kingdom; ‡Renal Division, Brigham and Women’s Hospital, Boston, Massachusetts; §John Walls Renal Unit, University Hospitals of Leicester, Leicester, United Kingdom; and ∥Department of Immunology, Newcastle upon Tyne Hospitals NHS Trust, Newcastle upon Tyne, United Kingdom

Mutations in the plasma complement regulator factor H (CFH) and the transmembrane complement regulator membrane co-factor protein (MCP) have been shown to predispose to atypical hemolytic uremic syndrome (HUS). Both of these proteins act as co-factors for complement factor I (IF). IF is a highly specific serine protease that cleaves the α-chains of C3b and C4b and thus downregulates activation of both the classical and the alternative complement pathways. This study looked for IF mutations in a panel of 76 patients with HUS. Mutations were detected in two patients, both of whom had reduced serum IF levels. A heterozygous bp change, c.463 G>A, which results in a premature stop codon (W127X), was found in one, and in the other, a heterozygous single base pair deletion in exon 7 (del 922C) was detected. Both patients had a history of recurrent HUS after transplantation. This is in accordance with the high rate of recurrence in patients with CFH mutations. Patients who are reported to have mutations in MCP, by contrast, do not have recurrence after transplantation. As with CFH- and MCP-associated HUS, there was incomplete penetrance in the family of one of the affected individuals. This study provides further evidence that atypical HUS is a disease of complement dysregulation.


Hemolytic uremic syndrome (HUS) is characterized by the triad of thrombocytopenia, Coomb’s test–negative microangiopathic hemolytic anemia, and acute renal failure (1). HUS is classified as either typical when it is associated with a preceding diarrheal illness, which is most commonly caused by infection with Escherichia coli O157, or, less commonly, atypical, which is rarely associated with diarrheea. Atypical HUS may be sporadic or familial.

Recent findings suggest that atypical HUS is a disease of complement dysregulation. Mutations in the complement regulatory protein factor H (CFH) have been described in both sporadic and familial HUS (2–7). CFH is a serum protein that binds to C3b, accelerates the decay of the alternative pathway convertase (C3bBb), and acts as a co-factor for factor I (IF)-mediated proteolytic inactivation of C3b (8). The majority of mutations in CFH in atypical HUS are missense heterozygous changes that tend to cluster in the C-terminal end of the molecule, an area that is known to be important for binding to anionic surfaces and C3b (9). Such mutations result in impaired protection of host surfaces against complement activation (10,11).

Recent studies have shown that mutations in the membrane-bound complement regulator membrane co-factor protein (MCP; CD46) also predispose to atypical HUS (12,13). MCP acts as a co-factor for IF-mediated cleavage of C3b and C4b.

IF consists of a light and a heavy chain linked by a disulphide bond (Figure 1). There are four domains in the heavy chain: a factor I domain, a scavenger receptor domain, and two LDL receptor (LDLr-1 and LDLr-2) domains. There is one domain in the light chain, a serine protease (SP) domain (14). The IF gene is located on chromosome 4q25 and spans 63 kb (15). It comprises 13 exons, and there is a strong correlation between the exonic organization of the gene and the modular structure of the protein. IF is a highly specific serine protease and cleaves the α-chains of C3b and C4b. This function is dependent on co-factors: CFH for cleavage of C3b in the fluid phase (8) and MCP (16) on host cells. Inactivation of C3b and C4b prevents the formation of the C3/C5 convertases. IF cleaves C3b at two positions in the α-chain to yield iC3b and C3f, whereas it cleaves C4 on either side of the thioester to yield C4c and C4d.

Fremeaux-Bacchi et al. (17) recently reported the finding of IF mutations in atypical HUS. We screened 76 patients with atypical HUS for IF mutations and identified two cases of heterozygous IF deficiency, which, like CFH-associated HUS, were associated with incomplete penetrance and disease recurrence after transplantation.

Materials and Methods

Patients

We studied 76 patients with atypical HUS; of these, six were known to have a CFH mutation (3,9) and two an MCP mutation (12). In 17 of
the 76, at least one other family member was affected. Ethical approval for the study was given by the Northern and Yorkshire Multi-Centre Research Ethics Committee.

Case Reports

Patient A (Figure 2A, II:1) presented with acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia at the age of 32, during the third trimester of her first pregnancy. A renal biopsy confirmed HUS. Complement assays were not performed at her initial presentation. Plasma exchange was not undertaken, renal function did not recover, and hemodialysis was commenced. Seventeen months later, she received a cadaveric renal transplant with excellent initial function. Immunosuppression was with cyclosporin A and prednisolone. After 2 mo, there was deterioration in transplant function, and biopsy showed recurrent HUS. The transplant was removed, and she returned to hemodialysis. For the subsequent 18 yr, she has been treated with both peritoneal dialysis and hemodialysis. During this time, she has had only two episodes of severe infection. She had an episode of severe peritonitis while on peritoneal dialysis, necessitating catheter removal. This was complicated by persistent intra-abdominal sepsis. There was also an episode of methicillin-resistant Staphylococcus aureus septicemia related to a hemodialysis catheter. There has been no infection with an encapsulated organism.

Patient B (Figure 2B, II:4) presented at the age of 31 with hematuria and proteinuria. Plasma creatinine at that time was normal, and a renal biopsy was not undertaken. Two years later, he presented again with general malaise and oliguria. Plasma creatinine was 1023 μmol/L, and lactate dehydrogenase (LDH) was 1320 IU/L. Hemoglobin was 7.6 g/dL, platelet count was 61 × 10^9/L, and there was evidence of hemolysis on a blood film. C3 on presentation was 0.3 g/L (normal range, 0.73 to 1.4 g/L), and C4 was 0.2 g/L (normal range, 0.12 to 0.3 g/L). Histologic findings on renal biopsy were consistent with HUS. He was initially treated with cyclophosphamide 150 mg daily, prednisolone 60 mg daily, and plasma exchange (eight daily exchanges with 40 ml/kg fresh-frozen plasma replacement fluid). With this, there was an improvement in both the LDH level and the platelet count. However, after plasma exchange was stopped, the LDH level increased and the platelet count fell; despite reinstating plasma exchange, he did not recover renal function.

Two years later, he received a live related transplant from his brother. Immunosuppression consisted of prednisolone, cyclosporin, and azathioprine. The transplant functioned immediately, and creatinine fell to 105 μmol/L. One year after transplantation, his plasma creatinine rose to 157 μmol/L. Biopsy showed acute rejection, which responded to three daily intravenous pulses of methylprednisolone. Twenty months after transplantation, there was another episode of acute graft dysfunction, but biopsy on this occasion showed recurrent HUS. He was treated with plasma exchange (23 exchanges with 40 ml/kg fresh-frozen plasma replacement fluid), but despite this renal function continued to deteriorate with evidence of ongoing hemolysis. Graft nephrectomy was undertaken, and the hemolysis resolved. For the past 6 yr, he has continued to be treated with hemodialysis. Since his initial presentation with HUS, he has had only one severe infection, a S. viridans peritoneal dialysis–related peritonitis, which settled with antibiotics.

His sister (Figure 2B, II:3) presented with end-stage renal failure and malignant hypertension at the age of 9. A biopsy was not undertaken. Subsequently, she received two cadaveric renal transplants, both of which failed rapidly. She died in 1973 at the age of 12. No tissue samples were available for analysis. Both parents are deceased.

Complement Assays

Convalescent EDTA plasma samples from patients with factor I mutations were obtained and stored at −80°C. C3 and C4 levels were measured by rate nephelometry (Beckman Array 360). CFH and IF levels were measured by radioimmunodiffusion (Binding Site, Birmingham, UK).

DNA Analysis

Direct sequencing of all IF exons and the promoter was undertaken in all 76 patients. Genomic DNA was prepared from peripheral blood according to standard procedures. Genomic DNA was amplified by PCR using intronic oligonucleotides. The minimal promoter region identified by Paramaswara et al. (18) was also amplified using oligonucleotides flanking the region. The sequences of the oligonucleotides,
annealing temperature, and magnesium concentration for each PCR reaction are shown in Table 1.

PCR products were purified by treatment with shrimp alkaline phosphatase (Amersham, Little Chalfont, UK) and Exonuclease (New England Biolabs, Beverly, MA). Direct DNA sequencing was performed using Megabase Dynemic ET (Amersham) on a Megabase1000 (Amersham). Sequence analyses were performed using Sequencher software (Gene Codes Corp., Ann Arbor, MI). To clarify the mutation in patient B, we cloned the PCR product spanning exon 7 using the pGEMTeasy Cloning Kit (Promega, Madison, WI) and sequenced it as above. Nucleotide and amino acid numbering was according to the published cDNA sequence (accession number M25615) (19). Nucleotide 1 is located 29 nucleotides before the start of the peptide signal sequence. The start codon of the sequence after the peptide signal was used as the first amino acid of the protein. This numbering corresponds to that used by Fremeaux-Bacchi et al. (17). Western Blots

Serum samples were stored at −80°C. Samples first were treated with an Albumin and IgG removal kit (Amersham Biosciences). They then were heated to 95°C in nonreducing sample buffer (Pierce, Rockford, IL) for 5 min. Protein electrophoresis was performed on 12% SDS-PAGE before transfer to nitrocellulose (Hybond ECL; Amersham). The membrane then was stained with a mouse anti-human factor I mAb (MCA507; Serotec, Oxford, UK), which recognizes the 56-kD heavy chain (20).

Results

Patient A had a heterozygous substitution c.463 G>A, which results in a premature stop codon W127X in exon 3 (Figure 3). Her unaffected father (I:1) has the same change. This change was not identified in 106 control subjects. IF levels in both patient A (29.2 mg/L; normal range 38–58 mg/L) and her father (33.7 mg/L) were low. C3 levels in patient A (0.87 g/L; normal range 0.73 to 1.4 g/L) and her father (1.4 g/L) were normal. The CFH level in patient A was normal (0.50 g/L; normal range 0.35 to 0.59 g/L). Mutation screening of CFH (3) and MCP (12) did not reveal a mutation in either of these genes.

Table 1. Primer sequences and PCR conditions

<table>
<thead>
<tr>
<th>Primer Sequence</th>
<th>Annealing Temperature (°C)</th>
<th>MgCl2 (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promoter</td>
<td>F-tcttcacctggagtaacagattga 56.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-ttgccgactatagttggcattg</td>
<td></td>
</tr>
<tr>
<td>Exon 1</td>
<td>F-tctctctggtatccagaaacaaaa 56.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-ttgccgactatagttggcattg</td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>F-ccagaccacacacaaacaca     56.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-ggcaaccccctgtaccttttta 56.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Exon 3</td>
<td>F-tcgtgtatgcttcaagctct    56.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-tgatgcacatactttttctcttag</td>
<td></td>
</tr>
<tr>
<td>Exon 4</td>
<td>F-tcgtgcccaagctgtaactcc    56.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-gcaaagagcgtcaactcat</td>
<td></td>
</tr>
<tr>
<td>Exon 5</td>
<td>F-ccctcataagccaggtttagtga  58.9</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-ggcattgtgcttaacaaaaataa</td>
<td></td>
</tr>
<tr>
<td>Exon 6</td>
<td>F-tttctgtactaaagaggaagcaaa 56.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-agtttctgcaatgtcaccact</td>
<td></td>
</tr>
<tr>
<td>Exon 7</td>
<td>F-aatagggtgcctagagcttttca 56.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-gctttggatttatttcttaattc</td>
<td></td>
</tr>
<tr>
<td>Exon 8</td>
<td>F-catgctctggggtttttga       58.9</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-tccgatttttttaaggaacttcta</td>
<td></td>
</tr>
<tr>
<td>Exon 9</td>
<td>F-tccagccctgctctgctgctgg  61.0</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-ttgtggtggccctggcattt</td>
<td></td>
</tr>
<tr>
<td>Exon 10</td>
<td>F-tgccccaagacagccaaat      61.0</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-ttgtggtggccctggcattt</td>
<td></td>
</tr>
<tr>
<td>Exon 11</td>
<td>F-tttcttggaatggttcttttctag 56.4</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>R-ttggtcttctctgtgctgctgag</td>
<td></td>
</tr>
<tr>
<td>Exon 12</td>
<td>F-cctctctctctctctctctctct 58.9</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-tctgtgctctgctgctgctgctg</td>
<td></td>
</tr>
<tr>
<td>Exon 13a</td>
<td>F-agatgctgtgctctgctgctg    52.5</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-agaagccagctctctctctctct</td>
<td></td>
</tr>
<tr>
<td>Exon 13b</td>
<td>F-gccctctgtaagagctgctgtca 61.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>R-ggcataaactctgctctgctgtgac</td>
<td></td>
</tr>
<tr>
<td>Exon 13c</td>
<td>F-tgcccaaatattatttgactgga 56.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>R-cttggaatgctcatctgctgctg</td>
<td></td>
</tr>
</tbody>
</table>
in patient A. Western blot of serum from patient A did not show any abnormal bands (Figure 4).

Patient B had a single base pair deletion (922delC) in exon 7, which results in nine amino acid substitutions followed by a premature stop codon (Figure 5). This change was not identified in 100 control subjects. IF (29 mg/L; normal range 38 to 58 mg/L) and C3 (0.3 g/L; normal range 0.73 to 1.4 g/L) levels were low. C4 (0.2 g/L; normal range 0.12 to 0.45 g/L) and CFH (0.50 g/L; normal range 0.35 to 0.59 g/L) levels were normal. Mutation screening of CFH (3) and MCP (12) did not reveal a mutation in either of these genes in patient B. Western blot of serum from patient B did not show any abnormal bands (Figure 4).

Discussion

We report two HUS patients with IF mutations, both of which led to heterozygous premature termination codons. Possible effects of these changes include nonsense mediated decay of the RNA transcript or impaired secretion of the protein, both of which are consistent with the low levels of serum IF observed and the absence of an abnormal band on Western blot.

Our report confirms the recent findings of Fremeaux-Bacchi et al. (17), who described two patients with heterozygous IF premature stop codons associated with half-normal IF levels. They also described a patient with a heterozygous missense mutation, which results in an amino acid change (D506V) in the serine protease domain, the functional significance of which remains to be established.

The first case of IF deficiency was described by Alper et al. (21) in 1970. Since then, a total of 31 cases have been described. These patients all have had virtual complete deficiency of IF, presumably as a result of mutations in both IF alleles. The clinical manifestations are present from early childhood with increased susceptibility to recurrent infection with encapsulated microorganisms (Neisseria meningitidis, S. pneumoniae, and Haemophilus influenzae). This is secondary to functional C3 deficiency as a result of uncontrolled activation of the alternative pathway. C3 opsonizes encapsulated microorganisms, enhancing phagocytosis (22). The two patients in our study showed no evidence of increased susceptibility to infection. In these previous reports, no clinical phenotype was reported in the majority of family members who were found to have partial IF deficiency.

Two patients with complete IF deficiency have been reported to have renal disease. One patient presented with a multisystem inflammatory disorder characterized by hepatitis, pneumonitis, myositis, and histologic evidence of a microangiopathic vasculitis; he subsequently developed focal segmental glomerulosclerosis (23). The other patient had serologic evidence of systemic lupus erythematosus and diffuse proliferative glomerulonephritis on renal biopsy (24).

The molecular basis of complete IF deficiency in association with recurrent infection was described in two pedigrees by Vyse et al. (20). In one, a missense mutation resulted in a homozygous change (H400L); in the other, the proband was a compound heterozygote with H400L on one allele and a donor splice site change on the other.
Both of the patients described here had recurrence of HUS after transplantation, as did the deceased sibling of patient B. One of the patients reported by Fremeaux-Bacchi et al. (17) had recurrence in two transplants. Thus, all four transplants in patients with IF mutations reported to date have been lost to recurrent HUS. This is not unexpected as IF, like CFH, is predominantly synthesized by the liver and thus a renal allograft will not correct the underlying defect. From individuals reported in the literature and unpublished from our own cohort, the recurrence rate in those with CFH mutations and/or CFH deficiency is approximately 80% (2,4,6,7,25–30).

In contrast, the three patients who have MCP mutations and have received a transplant have had no recurrence of HUS (12). MCP is a transmembrane protein, and a renal allograft therefore should correct the underlying defect.

In patients with CFH and MCP mutations, C3 levels may be normal. In patients with asymptomatic partial IF deficiency, C3 levels have also been reported in some to be normal (31–35). In our study, one patient had normal C3 levels and the other had low levels. Therefore, a normal C3 does not rule out heterozygous IF deficiency. This was also seen in the patients who were reported by Fremeaux-Bacchi et al. (17).

In both our study and that of Fremeaux-Bacchi et al. (17), there is incomplete penetrance. This suggests that IF mutations, like CFH and MCP, do not directly cause a thrombotic microangiopathy but rather predispose to it. In this situation, endothelial activation secondary to injury is maintained by excess complement activation. Moreover, genetic variation in complement regulatory genes may modify disease penetrance in patients with IF, CFH, and MCP mutations. Caprioli et al. (7) reported an association between CFH alleles and HUS, and Esparza-Gordillo et al. (36) also recently reported that a specific single nucleotide polymorphism haplotype block spanning MCP is overrepresented in patients with atypical HUS. This may also explain why HUS has not previously been described in other families with IF deficiency. Another possibility is that what we and Fremeaux-Bacchi et al. have reported is an ascertainment artifact. We think that this is unlikely, as neither we nor Fremeaux-Bacchi et al. have found the same changes in >400 chromosomal analyses from regionally matched normal control subjects.

This study has important clinical implications. We suggest that an IF level be measured routinely in all patients who present with atypical HUS. It is important in patients who are being considered for transplantation that it be known whether they have a CFH, IF, or MCP mutation so that they can be informed appropriately of the risks for recurrence. We would not recommend live related transplantation in patients who are known to have either a CFH or an IF mutation unless the donor has been screened for the same mutation, as de novo HUS has been reported to occur in donors within a short period of time after surgery (26). Even if the living related donor is known not to carry the same mutation, the risk for recurrence in the recipient is likely to be as high as observed with cadaveric donors, a risk that some would consider to be unacceptably high for any transplant.

In conclusion, this study confirms that IF mutations leading to partial IF deficiency are associated with a predisposition to the development of atypical HUS. The description of mutations in IF, CFH, and MCP has established that atypical HUS is a disease of complement dysregulation. Understanding the molecular mechanisms that are responsible for this disease allows us now to examine the potential of complement inhibition as a means of therapy.

Acknowledgments
This study was supported by grants from the National Kidney Research Fund, the Northern Counties Kidney Research Fund, the Newcastle Healthcare Charity, the Peel Medical Research Trust, and the Robin Davies Trust. E.M. was supported by a Wellcome Trust Vacation Scholarship.

References


