Brief report

Factor H, membrane cofactor protein, and factor I mutations in patients with hemolysis, elevated liver enzymes, and low platelet count syndrome

Fadi Fakhouri,1 Mathieu Jablonski,1 Jacques Lepercq,2 Jacques Blouin,3 Alexandra Benachi,4 Maryvonne Hourmant,5 Yves Pirson,6 Antoine Durrbach,7 Jean-Pierre Grünfeld,1 and Bertrand Knebelmann,1 and Véronique Frémeaux-Bacchi3,8

1Université Paris Descartes, Assistance Publique–Hôpitaux de Paris (AP-HP), Hôpital Necker, Department of Nephrology, and Inserm U845, Paris, France; 2Department of Gynecology and Obstetrics, Hôpital Saint-Vincent de Paul, Paris, France; 3AP-HP, Department of Immunology, Hôpital Européen Georges Pompidou, Paris, France; 4Department of Gynecology and Obstetrics, Hôpital Necker, Paris, France, 5Department of Nephrology, Centre Hospitalier Universitaire (CHU), Nantes, France; 6Department of Nephrology, Université Catholique de Louvain, Brussels, Belgium; 7Department of Nephrology, CHU Bicêtre, Le Kremlin Bicêtre, Paris, France; and 8Centre de Recherche des Cordeliers, Inserm Unité Mixte de Recherche en Santé (UMRS) 872, Paris, France

The HELLP syndrome, defined by the existence of hemolysis, elevated liver enzymes, and low platelet count, is a serious complication of pregnancy-related hypertensive disorders and shares several clinical and biologic features with thrombotic microangiopathy (TMA). Several recent studies have clearly shown that an abnormal control of the complement alternative pathway is a major risk for the occurrence of a peculiar type of TMA involving mainly the kidney. The aim of this study was to screen for complement abnormalities in 11 patients with HELLP syndrome and renal involvement. We identified 4 patients with a mutation in one of the genes coding for proteins involved in the regulation of the alternative pathway of complement. Our results suggest that an abnormal control of the complement alternative pathway is a risk factor for the occurrence of HELLP syndrome. (Blood. 2008;112:4542-4545)

Introduction

The HELLP syndrome, defined by the existence of hemolysis, elevated liver enzymes, and low platelet count is a serious complication of pregnancy-related hypertensive disorders. Hypertensive disorders are one of the most frequent complications of pregnancy. Their pathophysiology remains partially understood and several underlying mechanisms have been suggested and may not be exclusive.1 Endothelial dysfunction mediated by excess placenta-derived soluble VEGF receptor 1 and placenta-derived soluble TGF-beta coreceptor is emerging as a prominent component in pre-eclampsia pathogenesis.2,3 However, it remains unknown why only a minority of pregnant women (~ 1%) develop HELLP syndrome, which shares several clinical and biologic features with thrombotic microangiopathy (TMA).4 TMA is a disorder characterized by the occurrence of thrombi in the microvasculature of several organs, leading to thrombocytopenia, mechanical hemolytic anemia, and various organ failure. TMA may be classified in 3 main subtypes: ADAMTS13 deficiency related-TMA, complement dysregulation related-TMA, and a third category of TMA of unknown mechanism (ie, indeterminate TMA).5 As previous studies had shown that HELLP syndrome was not associated with a complete deficiency in ADAMTS13 activity,6 we sought to determine whether HELLP syndrome was rather associated with complement dysregulation.

Study design

Patients

Between January 2006 and July 2007, we identified 11 consecutive patients with HELLP syndrome who presented to 4 French and Belgian nephrology centers. HELLP syndrome was defined by a lactate dehydrogenase level higher than 600 IU/L, an aspartate aminotransferase level higher than 70 U/L, and a platelet count lower than 150 × 10^9/L. Informed consent was obtained from all patients in accordance with the Declaration of Helsinki. Complement analysis was done as part of the usual workup regularly performed in all patients with TMA seen in nephrology departments. The study was approved by the ethics committee of Necker-Enfants Malades (Paris, France).

Complement assays

EDTA plasma samples were obtained. Plasma concentrations of factor H (FH) and factor I (FI) were measured by enzyme-linked immunosorbent assay (ELISA); C4, C3, and factor B (FB), by nephelometry. Membrane cofactor protein (MCP) expression was analyzed on granulocytes using anti-CD46 phycoerythrin (PE)–conjugated antibodies (Serotec, Oxford, United Kingdom). All coding sequences for complement factor H (CFH), complement factor I (CFI), and membrane cofactor protein (MCP) genes were sequenced as previously described.7-9

Results and discussion

The clinical and biologic characteristics of the 11 patients are summarized in Table 1.

Genetic defects of complement regulatory proteins

Four patients carried heterozygous missense mutations in 1 of the 3 genes encoding for alternative complement regulatory proteins (Table 2). A mutation in the SCR 5 (pArg303Gln) on the CFH gene was identified in one patient (P1) who had a normal FH plasma level. Patients 2 and 3 who presented with a normal FI level, had a...
Table 1. Clinical and biologic characteristics of 11 patients with HELLP syndrome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y†</th>
<th>GA, w</th>
<th>BP, mmHg</th>
<th>Plts, G</th>
<th>ALAT, U/L</th>
<th>LDH, U/L</th>
<th>Scr, μM</th>
<th>Outcome</th>
<th>Peculiar features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 (0)</td>
<td>34</td>
<td>160/110</td>
<td>39</td>
<td>85</td>
<td>890</td>
<td>220</td>
<td>ESRD</td>
<td>P1 with a CFH mutation had a history of unexplained chronic renal failure (CrCl: 0.7 mL/sec) when she developed HELLP syndrome. Subsequently, she experienced 2 early (1 mo) losses of renal transplants due, on at least one occasion, to widespread intrarenal thrombosis, a feature highly suggestive of TMA.</td>
</tr>
<tr>
<td>2</td>
<td>21 (0)</td>
<td>28</td>
<td>180/120</td>
<td>78</td>
<td>186</td>
<td>855</td>
<td>93</td>
<td>CR</td>
<td>Uneventful second pregnancy, while on aspirin.</td>
</tr>
<tr>
<td>3</td>
<td>28 (3)</td>
<td>38</td>
<td>230/110</td>
<td>37</td>
<td>218</td>
<td>850</td>
<td>384</td>
<td>CR</td>
<td>Liver hematoma.</td>
</tr>
<tr>
<td>4</td>
<td>41 (2)</td>
<td>38</td>
<td>150/95</td>
<td>130</td>
<td>365</td>
<td>750</td>
<td>247</td>
<td>CRF</td>
<td>Altypical HUS at the age of 28 years requiring HD and PE.</td>
</tr>
<tr>
<td>5</td>
<td>31 (0)</td>
<td>27</td>
<td>170/100</td>
<td>&lt;100</td>
<td>85</td>
<td>830</td>
<td>115</td>
<td>CRF</td>
<td>Mild thrombocytopenia (110 × 10⁹/L) and undetectable haptoglobin were noted during 2 subsequent pregnancies (while on aspirin).</td>
</tr>
<tr>
<td>6</td>
<td>24 (0)</td>
<td>40</td>
<td>160/95</td>
<td>31</td>
<td>328</td>
<td>710</td>
<td>NA</td>
<td>CR</td>
<td>Liver hematoma.</td>
</tr>
<tr>
<td>7</td>
<td>32 (0)</td>
<td>24</td>
<td>160/100</td>
<td>30</td>
<td>92</td>
<td>945</td>
<td>NA</td>
<td>CR</td>
<td>Liver hematoma.</td>
</tr>
<tr>
<td>8</td>
<td>30 (2)</td>
<td>28</td>
<td>230/120</td>
<td>35</td>
<td>71</td>
<td>3400</td>
<td>HD</td>
<td>CR</td>
<td>Underwent PE.</td>
</tr>
<tr>
<td>9</td>
<td>36 (1)</td>
<td>37</td>
<td>180/95</td>
<td>16</td>
<td>580</td>
<td>6500</td>
<td>500</td>
<td>CRF</td>
<td>Underwent PE.</td>
</tr>
<tr>
<td>10</td>
<td>33 (0)</td>
<td>34</td>
<td>180/110</td>
<td>100</td>
<td>304</td>
<td>863</td>
<td>107</td>
<td>CR</td>
<td>Liver hematoma.</td>
</tr>
<tr>
<td>11</td>
<td>31 (3)</td>
<td>31</td>
<td>180/90</td>
<td>26</td>
<td>875</td>
<td>1000</td>
<td>HD</td>
<td>CR</td>
<td>Liver hematoma.</td>
</tr>
</tbody>
</table>

Patients with identified mutations in CFH, CFI, and MCP genes are shown in bold; patients with features of an abnormal control of the complement alternative pathway and no identified mutations in CFH, CFI, and MCP genes are shown in italics.

†Age at the onset of the HELLP syndrome.

The number of previous pregnancies is indicated in parentheses. Death of the newborn occurred in cases 2, 5, and 7.

Mutation located in exon 10 (p.Arg345Gln) and exon 4 (p.His183Arg) in CFI1 gene, respectively. Patient 4 with a MCP A304V mutation has been previously reported.11 All mutations had not been detected in 100 healthy subjects from the same ethnic background. The functional consequences of A304V mutation in MCP have been previously established, and this mutation clearly hinders the ability of the protein to control the complement alternative pathway (CAP).11 The functional consequences of the other mutations noted in our patients have not yet been established. However, all amino acid variants occur near domains that are important for C3b binding (P1, R303 in CFH gene and P3, H183 in CFI gene) or near a variant, which functional analysis revealed to have a marked defect in C3b cofactor activity (P2, R345 in CFI gene; Table 2). Moreover, 2 patients (P5 and P6) had low C3 and factor B level, and 1 patient (P7) had isolated low FB level in the absence of any identified mutation in CFH, CFI, or MCP genes. Further workup, including screening for mutations in factor B and C3 genes, was negative in these 2 patients. However, the detection of a low C3 or factor B level in these patients clearly indicates a dysfunctional regulation of the CAP.

Constitutional abnormalities in FH, FI, and MCP, 3 major regulatory proteins of the CAP, and less frequently acquired abnormalities (antibodies) in FH, have been reported in patients with a peculiar type of TMA involving mainly the kidney.12-14 The HELLP syndrome shares several clinical and biologic features with TMA, even though liver involvement, a hallmark of HELLP syndrome, is particularly rare in TMA.15-16 Our data clearly indicate a link between a deficient control of the CAP and the occurrence of HELLP syndrome. As previously reported in patients with atypical hemolytic uremic syndrome (aHUS), we identified patients with features of an abnormal activation of the CAP in the absence of identified mutations, which suggests the implication of other unknown genetic factors.

Placental tissue is a target of activated complement in physiologic and pathologic pregnancies.17-18 Thus, local control of the complement activation is required to reduce complement-induced tissue (mainly endothelial) lesions. The precise role of complement activation in the development of pregnancy-related hypertensive disorders remains unclear. As previously suggested in patients with aHUS, pregnancy per se may trigger the complement activation and hence complement-induced endothelial lesions. We hypothesize that in patients with hypertension (preeclampsia or related syndromes) and a pregnancy-related hypercoaguable state, the coexistence of mutations in complement regulatory proteins may ultimately lead to HELLP syndrome. However, hypertension may also be partly related to pregnancy-induced renal TMA. Indeed angiogenic/antiangiogenic imbalance, a hallmark of preeclampsia (especially decreased free VEGF and PIGF) could promote hypertension both directly and through TMA.

Moreover, 4 patients had either previous (P3, P4, and P8) or subsequent (P2) pregnancies without any HELLP syndrome, which suggests that an abnormal control of the CAP is a risk factor for HELLP and that other precipitating factors (ie, pregnancy-related hypertension) are required for the occurrence of this syndrome. As in patients with aHUS, it is difficult to estimate the risk of HELLP syndrome in patients with abnormal control of the CAP.

Our study has several limitations. It included a relatively small number of patients, and thus the frequency of complement...
abnormalities in patients with HELLP syndrome and the phenotype-genotype correlation need to be confirmed in larger series. Moreover, complement abnormalities in fetoplacental interface (mainly endothelial cells) due to the paternal allele expression of proteins have not been explored.

**Clinical characteristic of patients with HELLP syndrome and complement abnormalities**

HELLP syndrome is associated with an increased morbidity—including acute renal failure—and mortality.20,21 Our series included patients with renal involvement of variable severity. Acute renal failure, defined by a serum creatinine level higher than 70 μM, was present in 9 patients and hemodialysis was required in 2 patients. We cannot exclude that the frequency of CAP abnormalities may be higher in patients with HELLP and renal involvement compared with patients with HELLP and no renal involvement. However, acute renal dysfunction is frequent in HELLP syndrome, reported in a minimum of 3% to 8% of patients and up to 36% to 53% of cases.20-23 Moreover, the patients included in our study had clinical and biologic features, including liver involvement, similar to those previously reported in patients with HELLP syndrome.24,25 Finally, in contrast to the HELLP syndrome, liver involvement, a hallmark of HELLP syndrome, is uncommon in patients with aHUS.15 Thus, even though atypical HUS and HELLP syndrome are 2 distinct entities, our report indicates for the first time that HELLP syndrome may be part of the expanding spectrum of complement dysregulation-associated disorders. A classification of TMA-related disorders based on the underlying pathogenic mechanisms is more relevant than the terms used to describe these entities (HELLP syndrome, aHUS, etc).

Medical history of some of our patients with HELLP is remarkable for features of (recurrent in some cases) TMA. Patient 1 with a CFH mutation had a history of unexplained chronic renal failure when she developed HELLP syndrome. She subsequently lost 2 renal transplants due, on at least 1 occasion, to widespread intrarenal thrombosis, a feature highly suggestive of TMA. Two patients with an abnormal activation of the CAP in the absence of identified genetic mutations had a full-blown TMA (aHUS in P5) or features suggestive of TMA (decreased haptoglobin and thrombocytopenia during 2 subsequent pregnancies in P6). Our report underlines the fact that patients with an abnormal control of the CAP may present with various clinical manifestations of TMA. However, it remains unknown why complement abnormalities lead to different clinical disorders. In this regard, the case of the MCP A304V mutation is highly illustrative, as this mutation has been reported in patients with aHUS, a peculiar type of C3 deposits glomerulonephritis, and HELLP syndrome.11 Other genetic or environmental factors may modulate the clinical expression of a constitutional abnormality in the regulatory factors of the CAP, leading to a wide spectrum of complement dysregulation-associated disorders.

In summary, our results suggest that an abnormal control of the CAP is a risk factor for the occurrence of HELLP syndrome. The frequency and the phenotype-genotype correlation need to be confirmed in larger series. The treatment of HELLP syndrome, which includes steroids, plasma exchange, or fresh frozen plasma infusions, remains controversial.20 A better understanding of the pathogenic mechanisms underlying HELLP syndrome will certainly help clinicians in adapting treatment modalities to each patient.

**Table 2. Plasma complement component level and molecular genetic abnormalities found in 11 patients with HELLP syndrome**

<table>
<thead>
<tr>
<th>Patient</th>
<th>C3, mg/L</th>
<th>C4, mg/L</th>
<th>CFB, mg/L</th>
<th>CFH, mg/L</th>
<th>CFI, mg/L</th>
<th>MCP, MFI</th>
<th>Genetics abnormality</th>
<th>Mutations</th>
<th>Mutation characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>829</td>
<td>304</td>
<td>144</td>
<td>719</td>
<td>88</td>
<td>nd</td>
<td>CFH</td>
<td>pArg303Gln</td>
<td>Located in the SCR-1/5 fragment of FH, which is important for cofactor, C3b-binding, and decay-accelerating activity</td>
</tr>
<tr>
<td>2</td>
<td>1020</td>
<td>232</td>
<td>135</td>
<td>657</td>
<td>75</td>
<td>791</td>
<td>CFH</td>
<td>pArg345Gln (c1034G&gt;A)</td>
<td>Located near the previously reported mutation I322T; functional analysis revealed a marked defect in both C3b and C4b cofactor activity10</td>
</tr>
<tr>
<td>3</td>
<td>899</td>
<td>217</td>
<td>127</td>
<td>540</td>
<td>70</td>
<td>359</td>
<td>CFH</td>
<td>pHist183Arg (c548A&gt;G)</td>
<td>Located in the heavy chain domain that is important for C3b binding and the restriction of CFI10</td>
</tr>
<tr>
<td>4</td>
<td>912</td>
<td>199</td>
<td>125</td>
<td>652</td>
<td>77</td>
<td>966</td>
<td>MCP</td>
<td>pAla304Val</td>
<td>A304V mutation leads to deficiency in MCP to control the alternative pathway of complement activation on a cell surface11</td>
</tr>
<tr>
<td>5</td>
<td>664</td>
<td>169</td>
<td>85</td>
<td>555</td>
<td>54</td>
<td>1096</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>639</td>
<td>109</td>
<td>82</td>
<td>474</td>
<td>58</td>
<td>894</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>976</td>
<td>245</td>
<td>75</td>
<td>836</td>
<td>99</td>
<td>906</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1150</td>
<td>254</td>
<td>151</td>
<td>714</td>
<td>60</td>
<td>nd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>779</td>
<td>231</td>
<td>125</td>
<td>591</td>
<td>67</td>
<td>968</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1170</td>
<td>354</td>
<td>150</td>
<td>759</td>
<td>72</td>
<td>985</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1150</td>
<td>314</td>
<td>156</td>
<td>708</td>
<td>84</td>
<td>1246</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal values</td>
<td>660-1250</td>
<td>93-320</td>
<td>90-320</td>
<td>338-682</td>
<td>42-78</td>
<td>600-1500</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Patients with identified mutations in CFH, CFI, and MCP genes are shown in bold; patients with features of an abnormal control of the complement alternative pathway and no identified mutations in CFH, CFI, and MCP genes are shown in italics. Abnormal values of C3 and Factor B levels are shown in bold.

**Authorship**

Contribution: F.F., J.B., and V.F.-B. designed the study and performed the research; F.F., M.J., J.L., A.B., M.H., Y.P., A.D., J.-P.G., and B.K. were in charge of the patients; and F.F. and V.F.-B. wrote the article.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

From www.bloodjournal.org by guest on April 16, 2017. For personal use only.
References


Factor H, membrane cofactor protein, and factor I mutations in patients with hemolysis, elevated liver enzymes, and low platelet count syndrome

Fadi Fakhouri, Mathieu Jablonski, Jacques Lepercq, Jacques Blouin, Alexandra Benachi, Maryvonne Hourmant, Yves Pirson, Antoine Dürrbach, Jean-Pierre Grünfeld, Bertrand Knebelmann and Véronique Frémeaux-Bacchi