Thrombotic microangiopathy mimicking membranoproliferative glomerulonephritis

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Abstract

A 4-year-old boy presented with proteinuria and developed progressive renal failure over 6 years. In the patient’s family, five individuals were affected with atypical haemolytic uraemic syndrome (aHUS) but not the patient. Renal biopsies (n = 3) showed glomerular basement membrane thickening with double contours, endothelial swelling and deposits of C3 and C1q. Electron microscopy revealed mesangial and subendothelial electron-dense deposits. Complement mutations in membrane cofactor protein (Y155D) and C3 (R713W and G1094R) were detected in all affected family members. The patient also had transient autoantibodies to factor H. The findings suggest that aHUS and glomerulopathy resembling membranoproliferative glomerulonephritis may have a common molecular background.

Keywords: complement; C3; haemolytic uraemic syndrome; membranoproliferative glomerulonephritis; eculizumab

Background

Haemolytic uraemic syndrome is defined as non-immune microangiopathic haemolytic anaemia, thrombocytopenia and renal failure. A subtype, termed atypical haemolytic uraemic syndrome (aHUS), is associated with activation of the alternative pathway of complement [1]. Mutations have been identified in complement regulators factor H (CFH), factor I, membrane cofactor protein (MCP) and complement factors C3 [2] and factor B. Deletions were found in factor H-related proteins 1 and 3, often associated with anti-factor H antibodies. In addition, mutations have been found in thrombomodulin (mutations reviewed in ref. 1).

Membranoproliferative glomerulonephritis (MPGN) is a distinct renal disease presenting with haematuria, proteinuria, hypertension and impaired renal function. MPGN is subclassified based on the localization of immune deposits consisting of IgG and/or C3 [3]. MPGN Types I and III are considered to be immune complex-mediated diseases, whereas Type II, also known as dense deposit disease, is associated with complement activation via the alternative pathway [3].

Both aHUS and MPGN can thus be associated with activation of the alternative pathway of complement. Certain cases have been described in which individuals present with a combined clinical picture of aHUS and MPGN Type I [4], indicating that these conditions may have a common molecular background.

Case report

A 4-year-old boy was investigated at the Department of Paediatrics, Section of Paediatric Nephrology of the Haukeland University Hospital in Bergen. He is referred to as Patient III. He was asymptomatic at the time of primary investigation and had not previously exhibited any signs or symptoms of renal disease but underwent a medical examination because his younger sister had an episode of aHUS. The initial examination revealed a clinically healthy child with normal blood pressure. Urinalysis revealed proteinuria, microscopic haematuria and casts. Blood and urine tests are summarized in supplementary Table 1.

Complement levels taken when the patient was first examined showed normal levels (supplementary Table 2). Ultrasound of the kidneys showed mildly increased parenchymal echogenicity. A renal biopsy was performed. The results are presented in Table 1 and Figure 1A–C. The biopsy showed glomerular basement membrane (GBM) thickening with double contours, mesangial cell proliferation, endothelial cell swelling and deposits of C3, IgG and IgM. Electron microscopy revealed subendothelial and mesangial electron-dense deposits as well as mesangial cell interposition and podocyte foot effacement. The patient was treated with an angiotensin-converting enzyme inhibitor and an angiotensin receptor blocker and remained asymptomatic.

At the age of 6, his creatinine levels started to rise and he exhibited increased proteinuria. He underwent a second...
renal biopsy (Table 1; Figure 1D). As in the first biopsy, thickening of the GBM, with double contours, and narrowing or occlusion of glomerular capillaries were noted. In addition, mild tubular atrophy and interstitial fibrosis were demonstrated. Blood samples were repeatedly assessed during symptom-free periods and during infections but no laboratory parameters indicated HUS.

At the age of 6.5 years, the patient developed hypertension and by the age of 8 years, he was being treated with four antihypertensive medications. He was stable on this treatment until just before he turned 10 when his creatinine and proteinuria increased and glomerular filtration rate decreased (supplementary Table 1). He underwent a third renal biopsy at 10 years (Figure 1E–G). The biopsy showed global sclerosis of 12/17 glomeruli and the remaining glomeruli exhibited mesangial expansion due to increased matrix and cells. Endothelial cells were swollen and glomerular capillaries were thickened or occluded. One thrombus was noted in a capillary (Figure 1E). GBMs were thickened with double contours. Arterioles and smaller arteries showed media hypertrophy. Immunohistochemistry showed, as in previous biopsies, labelling with C3 and C1q (Figure 1F). Electron microscopy showed electron-dense precipitations in capillary lumina, mesangial cell interposition and podocyte foot process effacement (Figure 1G).

Currently, at the age of 10 years, the patient’s clinical condition has deteriorated with decreased renal function and increased blood pressure refractory to treatment with a combination of five antihypertensive medications. The laboratory values for the first time indicate ongoing haemolysis (undetectable haptoglobin, elevated lactic dehydrogenase, elevated reticulocyte counts and reduced haemoglobin). The direct antiglobulin test is negative. Proteinuria has decreased and platelet counts remain normal. Treatment with regular infusions of eculizumab (humanized monoclonal anti-C5 antibody; Alexion) was initiated in January 2011. The initial dose was 600 mg intravenously once a week for 4 weeks followed by 600 mg every other week.

The family history is indicative of hereditary aHUS. The index patient has two sisters, currently 6 and 4 years old. The 6-year-old sister (Patient III2 in Figure 2) developed HUS following a respiratory tract infection at the age of 5 months but did not require dialysis and recovered with mild proteinuria. The father of Patients III1 and III2 developed HUS at the age of 10 years (Patient II2). He too did not require dialysis and recovered with no recurrences. Patient II2 had a brother, 2 years older, that developed HUS at 4 months age following a vaccination (Patient II1). Upon hospitalization, he exhibited bloody diarrhoea. Hospital records indicate haemolytic anaemia and thrombocytopenia and the patient succumbed within 48 h. Post-mortem examination of kidney tissue showed acute thrombotic microangiopathy with swollen endothelial cells and capillary thrombi (Figure 1H). Patient II4 is a younger brother of Patients III1 and II2. He developed transient anaemia and jaundice at the age of 10 months followed by HUS and proteinuria at 2 years of age. There have been no recurrences since. Patient II4’s daughter, Patient III7, currently 6 years old, has had eight episodes of HUS since she was 4 months old. Six episodes of HUS occurred by the age of 21 months at which time regular

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<table>
<thead>
<tr>
<th>Table 1. Pathological findings in patient III1</th>
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<tr>
<td>Light microscopy</td>
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<td>GBM thickening and double contours 4A-C</td>
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<tr>
<td>Glomerular foot process effacement 1A-C</td>
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<tr>
<td>Capillary lumina 1E-G</td>
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aEDD, electron-dense deposits. bTram-tracks. cGranular deposition along glomerular capillary walls.
plasma infusions were instituted after which she has suffered two recurrences in 2 years. Interestingly, the parents of Patients II1, II2 and II4 are cousins (Individuals I1 and I2 in Figure 2). They are unaffected but the paternal grandfather of Patients II1, II2 and II4 had recurrent bouts of jaundice with no liver disease. Complement levels of Patients II2, II4, III1, III2 and III7 are presented in supplementary Table 2. Complement levels were not available from Patient II1.

Fig. 1. Pathological findings in Patients III1 and III1. (A–C): Patient III1, first renal biopsy taken at the age of 4 years. (A) Glomerulus with slight mesangial expansion and thickened capillary walls with double contours (Periodic acid-Schiff staining). (B) Immunohistochemical staining for C3 (anti-C3 from Dako, Glostrup, Denmark) showing marked labelling of capillary walls. (A and B: original magnification ×400). (C) Ultrastructure showing a capillary with electron-dense deposits (arrowheads) in subendothelial position and effacement of podocyte foot processes (arrow). Inset shows mesangial cell interposition. Scale bar represents 1 μm (C) and 2 μm (C inset). (D) Patient III1, second renal biopsy at age 6 years. Hypercellular glomerulus with thickened GBMs and narrowed glomerular capillaries (inset). One sclerosing glomerulus with collapsed glomerular tuft, segmental sclerosis and thickening of Bowman’s capsule is visible (arrow). A group of tubules with reduced diameter and thickened basement membrane (arrowhead) indicating tubular atrophy (original magnification ×200, inset ×1000). (E–G): Patient III1, third renal biopsy at age 10 years. Panel E shows thickening of the GBM, occluded capillaries (arrowhead) and one thrombus in a glomerular capillary (see arrow). Panel F shows C1q labelling of capillary walls (anti-C1q from Dako). Panel G: ultrastructure showing electron-dense precipitations in capillary lumina (arrowheads) and duplication of the GBM (arrow). Activated endothelial cells contain many organelles. Effaced podocyte foot processes (scale bar 2 μm). (H) Patient III1, post-mortem renal tissue. Glomerulus with intracapillary thrombi and swollen endothelial cells (trichrome stain, thrombi are red, erythrocytes yellow). E, F and H: original magnification ×400.

Patients II1, II2 and II4 had recurrent bouts of jaundice with no liver disease. Complement levels of Patients II2, II4, III1, III2 and III7 are presented in supplementary Table 2. Complement levels were not available from Patient II1.
DNA sequencing was carried out as described in the supplementary methods. Patients II1, II2, II4, III1, III2 and III7 carry a previously described MCP mutation (Y155D) [5] and two C3 mutations: R713W (in Exon 17) and G1094R (in Exon 26). R713W has been previously described [2], whereas G1094R is a novel mutation situated one amino acid from a described mutation at position D1093N [2]. These C3 mutations were presumptively localized on the same allele due to the common pattern of inheritance and they were not found in DNA from 100 healthy controls. In addition, Patient III1 has a heterozygous deletion of CFHR1/3 and autoantibodies to CFH were detected upon debut of disease but not 5 years later. Patients III2 and III7 were tested and did not have serum anti-CFH antibodies.

### Discussion

In this report, we describe a child with progressive renal failure and pathology exhibiting a membranoproliferative pattern. Other affected family members were found to share complement mutations in C3 and MCP. This study suggests that differing clinical and pathological phenotypes of aHUS may have a common molecular basis. Thrombotic microangiopathy can thus develop even in the absence of clinical HUS.

Patient III1 did not exhibit clinical signs of aHUS although haemolysis without thrombocytopenia was detected 6 years after debut of disease. His biopsies showed a membranoproliferative pattern including C3 positivity. These findings are compatible with chronic thrombotic microangiopathy except for the presence of subendothelial electron-dense deposits. Electron-dense deposits are a peculiar feature in this patient usually not observed in thrombotic microangiopathy and making the distinction from MPGN Type I difficult. Furthermore, strong C1q labelling is not found in thrombotic microangiopathy but usually associated with systemic lupus erythematosus, C1q nephropathy [7] or MPGN Type I but the weak IgG staining suggests that the renal deposits were probably not mediated by immune complexes. The finding of membranoproliferative features is in line with other studies suggesting that there might be a continuous spectrum of morphological changes.

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**Table 2. Molecular characteristics of genetic alterations in patients**

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<tr>
<th>Complement protein</th>
<th>Mutation or deletion</th>
<th>Codon</th>
<th>Protein Phenotype</th>
<th>Reference</th>
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<tr>
<td>MCP</td>
<td>Y155D</td>
<td>565 T&gt;G</td>
<td>Tyr155Asp</td>
<td>Reduced cell-surface expression of MCP, No detectable C3b- or C4b-binding activity and negligible cofactor activity. [5]</td>
</tr>
<tr>
<td>C3</td>
<td>G1094R</td>
<td>3346 G&gt;C</td>
<td>Gly1116Arg</td>
<td>Adjacent mutation (D1093N) showed reduced binding to MCP and, to a lesser degree, CFH. [2]</td>
</tr>
<tr>
<td>C3</td>
<td>R713W</td>
<td>2203 C&gt;T</td>
<td>Arg735Trp</td>
<td>No documented abnormality. Normal C3 binding to CFB, CFH, MCP and soluble CR1. [2]</td>
</tr>
<tr>
<td>CFHR1/3</td>
<td>ΔCFHR1/3&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>Associated with the presence of anti-CFH antibodies. [6]</td>
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<sup>a</sup>CFB, factor B; CFH, factor H; MCP, membrane cofactor protein/CD46; CR1, complement receptor 1; CFHR, factor H-related protein. All genetic alterations, including ΔCFHR1/3, were heterozygous.

<sup>b</sup>This deletion has also been detected in the healthy population.
from thrombotic microangiopathy to MPGN [8–10]. This group of disorders could also encompass the more recently described C3 glomerulopathy with isolated C3 deposits [11]. However, the strong C1q labelling in the patient’s biopsies is not compatible with this diagnosis.

The presence of complement activation due to mutations in C3 and MCP enabled the pathological features to progress even in the absence of overt HUS. The clinical and pathological features overlap with MPGN Type I indicating that aHUS and MPGN may share a common molecular background. We thus conclude that this patient exhibited an unusual presentation of chronic thrombotic microangiopathy mimicking MPGN Type I.

Supplementary data
Supplementary data are available online at http://ndt.oxfordjournals.org.

Acknowledgements. This study was supported by grants from The Swedish Research Council (K2010-65X-14008-10-3 to D.K.), Torsten and Ragnar Söderberg Foundation, The fund for Renal Research, Crown Princess Lovisa’s Society for Child Care, Konung Gustaf V:s 80-årsfond, Fanny Ekdahl’s Foundation (all to D.K.). D.K. is the recipient of a clinical–experimental research fellowship from the Royal Swedish Academy of Sciences. The Queen Silvia Jubilee Fond to L.S. French Agence Nationale de la Recherche (R09097DS), Programme Hospitalier de Recherche Clinique (AOM05130/P051065) and French Association for Information and Research on Genetic Renal Diseases (AIRG) to V.F.-B. A preliminary version of the manuscript appeared in the Ph.D. thesis of Dr L.S.

The authors would like to thank Dr Marie-Agnes Dragon-Durey, Hopital Europeen Georges-Pompidou, Service d’Immunologie Biologique, Paris, for analysis of anti-CFH antibodies in Patient III1 and Professor Martin Olsson, Department of Transfusion Medicine, Lund University, for control samples.

Transparency declaration. V.F.-B. was consultant for and gave lectures for Alexion Pharmaceuticals during 2010. D.K. was the national coordinator in Sweden of the multi-center trial of Eculizumab (Alexion Pharmaceuticals) in patients with atypical hemolytic uremic syndrome.

Conflict of interest statement. None declared.

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Received for publication: 20.6.11; Accepted in revised form: 20.6.11