LUND UNIVERSITY

Thrombotic microangiopathy mimicking membranoproliferative glomerulonephritis

Brackman, Damien; Sartz, Lisa; Leh, Sabine; Kristoffersson, Ann-Charlotte; Bjerre, Anna; Tati, Ramesh; Fremeaux-Bacchi, Veronique; Karpman, Diana

Published in:
Nephrology Dialysis Transplantation

DOI:
10.1093/ndt/gfr422

2011

Link to publication

Citation for published version (APA):
Brackman, D., Sartz, L., Leh, S., Kristoffersson, A.-C., Bjerre, A., Tati, R., Fremeaux-Bacchi, V., & Karpman, D. (2011). Thrombotic microangiopathy mimicking membranoproliferative glomerulonephritis. *Nephrology Dialysis Transplantation*, *26*(10), 3399-3403. https://doi.org/10.1093/ndt/gfr422

Total number of authors:
8

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Download date: 18. May. 2025

Thrombotic microangiopathy mimicking membranoproliferative glomerulonephritis

Damien Brackman[1*], Lisa Sartz[2], Sabine Leh[3], Ann-Charlotte Kristoffersson[2], Anna Bjerre[4], Ramesh Tati[2], Veronique Frémeaux-Bacchi[5], Diana Karpman[2*]

1. Department of Pediatrics, Haukeland University Hospital, Bergen, Norway
2. Department of Pediatrics, Clinical Sciences Lund, Lund University, Sweden
3. Department of Pathology, Haukeland University Hospital, Bergen, Norway
4. Department of Pediatrics, Oslo University Hospital, Oslo, Norway
5. Assistance Publique-Hopitaux de Paris, Hopital Europeen Georges-Pompidou, Service d'Immunologie Biologique, Paris, France

* Corresponding authors:

Diana Karpman
Department of Pediatrics
Clinical Sciences Lund
Lund University
22185, Lund
Sweden
e-mail: diana.karpman@med.lu.se
Telephone: + 46 46 2220747 / fax: + 46 46 2220748

and

Damien Brackman
Department of Pediatrics
Haukeland University Hospital
Bergen, Norway
e-mail: damien.brackman@helse-bergen.no

Abstract

A four year-old-boy presented with proteinuria and developed progressive renal failure over six years. In the patient's family five individuals were affected with atypical hemolytic uremic 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 MCP (Y155D) and C3 (R713W and G1094R) were detected in all affected family members. The patient also had transient auto-antibodies to factor H. The findings suggest that aHUS and glomerulopathy resembling membranoproliferative glomerulonephritis may have a common molecular background

Keywords

Complement, C3, hemolytic uremic syndrome, membranoproliferative glomerulonephritis.

Background

Hemolytic uremic syndrome is defined as non-immune microangiopathic hemolytic anemia, thrombocytopenia and renal failure. A subtype, termed atypical HUS (aHUS), is associated with activation of the alternative pathway of complement (1). Mutations have been identified in complement regulators factor H, 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 (1)).

Membranoproliferative glomerulonephritis (MPGN) is a distinct renal disease presenting with hematuria, proteinuria, hypertension and impaired renal function. MPGN is sub-classified 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 four-year old boy was investigated at the Department of Pediatrics, Section of Pediatric Nephrology of the Haukeland University Hospital in Bergen. He is referred to as Patient III1. 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 hematuria 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 supplementary Table 3 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 (supplementary Table 3, 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 treated with four anti-hypertensive 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). Glomerular basement membranes were thickened with double contours. Arterioles and smaller arteries showed media hypertrophy. Immunohistochemistry showed, as in previous biopsies, labeling 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 anti-hypertensive medications. The laboratory values for the first time indicate ongoing hemolysis (undetectable haptoglobin, elevated lactic dehydrogenase, elevated reticulocyte counts and reduced hemoglobin). 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, two years older, that developed HUS at 4 months-of-age following a vaccination (Patient II1). Upon hospitalization he exhibited bloody diarrhea. Hospital records indicate hemolytic anemia and thrombocytopenia and the patient succumbed within 48 hours. 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 II1 and II2. He developed transient anemia 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 8 episodes of HUS since she was 4-months-old. Six episodes of HUS occurred by the age of 21 months at which time regular plasma infusions were instituted after which she has suffered 2 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.

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 presumably 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 auto-antibodies to CFH were detected upon debut of disease but not five years later. The MCP mutation was found in two unaffected family members and the C3 mutations were found in one unaffected family member. The mutations and their presumed functional consequences are presented in Figure 2 and supplementary Table 4, as well as the supplementary results and discussion.

Discussion

In this report we describe a child with progressive renal failure and pathology exhibiting a membranoproliferative pattern. Other family members presented with features of aHUS. All 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 hemolysis 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 labeling is not found in thrombotic microangiopathy but usually associated with systematic lupus erythematosis, C1q nephropathy (6) 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 from thrombotic microangiopathy to MPGN (7-9). This group of disorders could also encompass the more recently described C3 glomerulopathy with isolated C3 deposits (10). However, the strong C1q labeling 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.

Acknowledgments

This study was supported by grants from The Swedish Research Council (K2010-65X-14008-10-3 to DK), 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 DK). Diana Karpman is the recipient of a clinical-experimental research fellowship from the Royal Swedish Academy of Sciences. The Queen Silvia Jubilee Fond to LS. Agence Nationale de la Recherche (COMPTISS and FCTH), Assistance Publique-Hôpitaux de Paris (Programme Hospitalier de Recherche Clinique (AOM05130/P051065 and AOM08198) and by AIRG France (to VF-B). A preliminary version of the manuscript appeared in the Ph.D. thesis of Dr. Lisa Sartz.

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 III 1 and Professor Martin Olsson, Department of Transfusion Medicine, Lund University, for control samples.

Transparency declaration

Veronique Frémeaux-Bacchi was consultant for and gave lectures for Alexion Pharmaceuticals during 2010.

Diana Karpman was the national coordinator in Sweden of the multi-center trial of Eculizumab (Alexion Pharmaceuticals) in patients with atypical hemolytic uremic syndrome.

References

1. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; 361: 1676-1687
2. Fremeaux-Bacchi V, Miller EC, Liszewski MK *et al.* Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. *Blood* 2008; 112: 4948-4952
3. Licht C, Fremeaux-Bacchi V. Hereditary and acquired complement dysregulation in membranoproliferative glomerulonephritis. *Thromb Haemost* 2009; 101: 271-278
4. Vaziri-Sani F, Holmberg L, Sjoholm AG *et al.* Phenotypic expression of factor H mutations in patients with atypical hemolytic uremic syndrome. *Kidney Int* 2006; 69: 981-988
5. Fremeaux-Bacchi V, Moulton EA, Kavanagh D *et al.* Genetic and functional analyses of membrane cofactor protein (CD46) mutations in atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2006; 17: 2017-2025

6. Vizjak A, Ferluga D, Rozic M *et al.* Pathology, clinical presentations, and outcomes of C1q nephropathy. *J Am Soc Nephrol* 2008; 19: 2237-2244
7. Pickering MC, Cook HT. Translational mini-review series on complement factor H: renal diseases associated with complement factor H: novel insights from humans and animals. *Clin Exp Immunol* 2008; 151: 210-230
8. Servais A, Fremeaux-Bacchi V, Lequintrec M *et al.* Primary glomerulonephritis with isolated C3 deposits: a new entity which shares common genetic risk factors with haemolytic uraemic syndrome. *Journal of medical genetics* 2007; 44: 193-199
9. Skerka C, Licht C, Mengel M *et al.* Autoimmune forms of thrombotic microangiopathy and membranoproliferative glomerulonephritis: Indications for a disease spectrum and common pathogenic principles. *Mol Immunol* 2009; 46: 2801-2807
10. Fakhouri F, Fremeaux-Bacchi V, Noel LH, Cook HT, Pickering MC. C3 glomerulopathy: a new classification. *Nat Rev Nephrol*; 6: 494-499

Figure legends

Figure 1: Pathological findings in Patients III 1 and II 1

A – C: Patient III 1, first renal biopsy taken at the age of 4 years. **A:** Glomerulus with slight mesangial expansion and thickened capillary walls with double contours (PAS staining). **B:** Immunohistochemical staining for C3 (anti-C3 from Dako, Glostrup, Denmark) showing marked labelling of capillary walls. (**A - B:** original magnification x 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 III 1 second renal biopsy at age 6 years. Hypercellular glomerulus with thickened glomerular basement membranes 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 x 200, inset x 1000). **E - G**: Patient III 1 third renal biopsy at age 10 years. Panel **E** shows thickening of the glomerular basement membrane, occluded capillaries (arrowhead) and one thrombus in a glomerular capillary (see arrow). Panel **F** shows C1q labeling of capillary walls (anti-C1q from Dako). Panel **G**: Ultrastructure showing electron-dense precipitations in capillary lumina (arrowheads) and duplication of the glomerular basement membrane (arrow). Activated endothelial cells contain many organelles. Effaced podocyte foot processes (scale bar 2 µm). **H**: Patient II 1, postmortem renal tissue. Glomerulus with intracapillary thrombi and swollen endothelial cells (trichrome stain, thrombi are red, erythrocytes yellow). **E, F, H**: original magnification x 400.

Figure 2: Pedigree of the investigated family

MCP: membrane cofactor protein. CFH ab: Anti-factor H (CFH) antibodies. The affected individuals in two generations are marked in red. Mutations: C3: G1094R + R713W and MCP: Y155D. ΔCFHR1/3: heterozygous deletion of CFHR1 and CFHR3. Serum anti-CFH antibodies detected in patient III 1 at debut but not five years later. Patients III 2 and III 7 were tested and did not have serum anti-CFH antibodies.

Table 1: Pathological findings in patient III1

		Light microscopy glomerular pathology					Immunohistochemistry				Electron microscopy	Figure
Biopsy	**Age (yrs)**	**GBM thick-ening and double contours[a]**	**Mesan-gial cell prolifera-tion**	**Lobu-lation**	**Micro-thrombi**	**Endo-thelial cell swelling**	**C3[b]**	**C1q**	**IgG**	**IgM**		
1st biopsy	4	+	+	-	-	+	++	+++	+/-	++	Mesangial and subendothelial EDD, mesangial cell inter-position. Podocyte foot process effacement	1A-C
2nd biopsy	6	+	+	-	-	+	+	++	-	+/-	Mesangial cell interposition. Podocyte foot process effacement	1D
3rd biopsy	10	+	+	-	+	+	++	+++	+/-	+	Electron dense precipitates in capillary lumina. Mesangial cell interposition. Podocyte foot process effacement	1E-G

GBM: Glomerular basement membrane. EDD: electron dense deposits. [a], Tram-tracks. [b], Granular deposition along glomerular capillary walls.

Table 2: Molecular characteristics of genetic alterations in patients

Complement protein	Mutation or deletion	Codon	Protein	Phenotype	Reference
MCP	Y155D	565 T>G	Tyr155Asp	Reduced cell-surface expression of MCP. No detectable C3b or C4b binding activity and negligible cofactor activity.	5
C3	G1094R	3346 G>C	Gly1116Arg	Adjacent mutation (D1093N) showed reduced binding to MCP and, to a lesser degree, CFH.	2
C3	R713W	2203 C>T	Arg735Trp	No documented abnormality. Normal C3 binding to CFB, CFH, MCP and soluble CR1.	2
CFHR1/3	ΔCFHR1/3[a]			Associated with the presence of anti-CFH antibodies.	11

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. [a], This deletion has also been detected in the healthy population.

References

11. Zipfel PF, Edey M, Heinen S *et al.* Deletion of complement factor H-related genes CFHR1 and CFHR3 is associated with atypical hemolytic uremic syndrome. *PLoS Genet* 2007; 3: e41

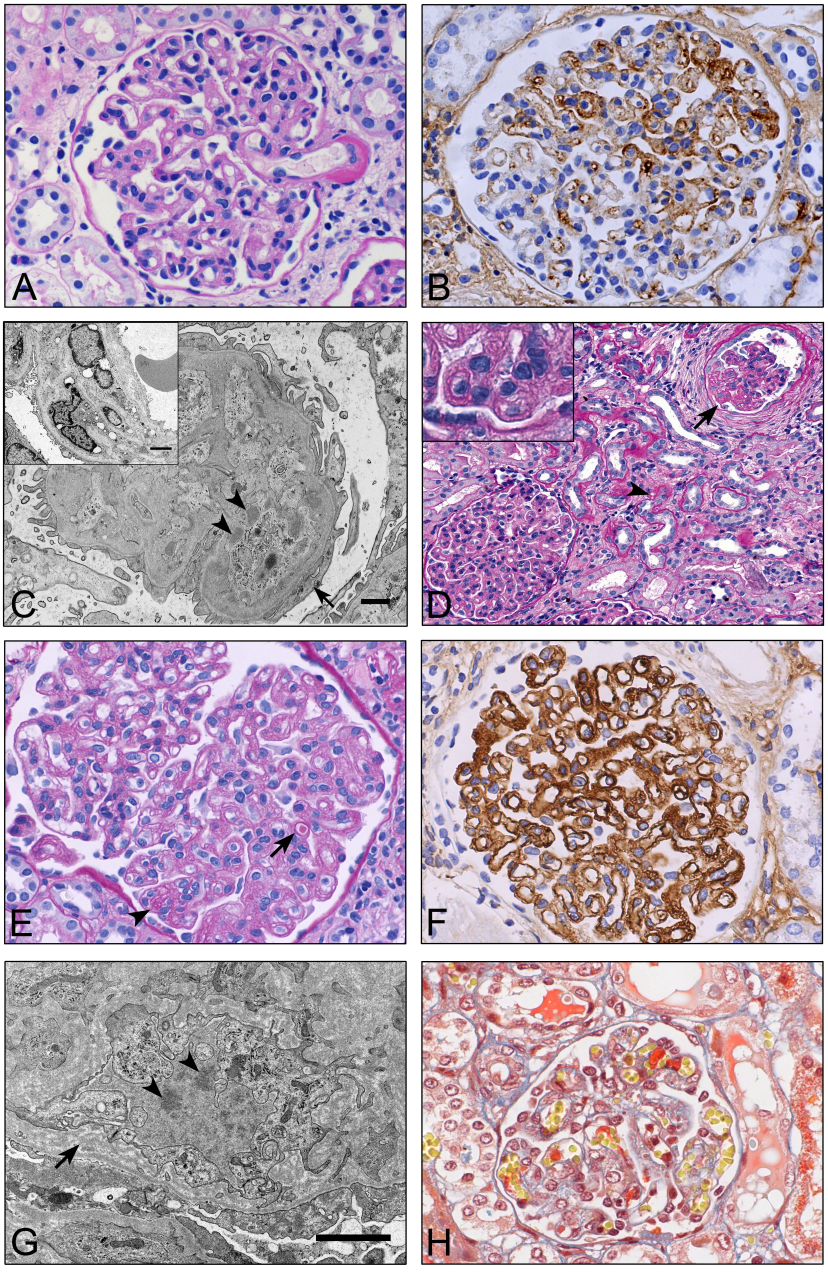

Figure 1

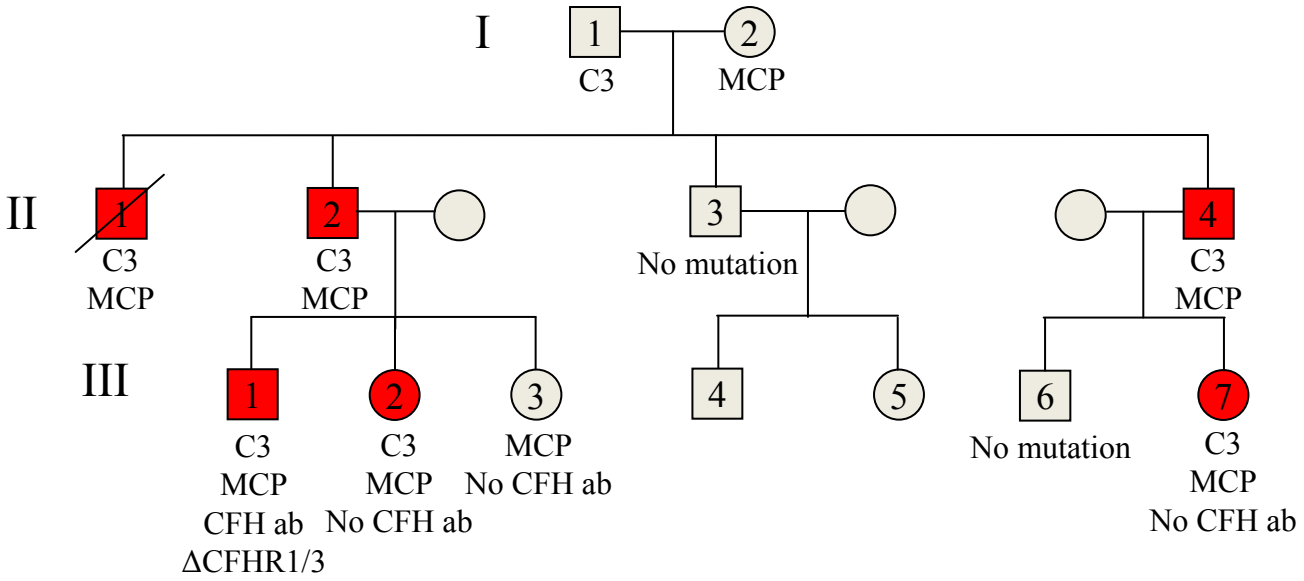
I
1
C3
2
MCP
II
1
C3
MCP
2
C3
MCP
3
No mutation
4
C3
MCP
III
1
C3
MCP
CFH ab
ΔCFHR1/3
2
C3
MCP
No CFH ab
3
MCP
No CFH ab
4
5
6
No mutation
7
C3
MCP
No CFH ab

Figure 2

Supplementary Methods

DNA extracts were obtained from Patients II1, II2, II4, III1, III2 and III7 and their unaffected family members. DNA extracts from an apparently healthy cohort of adult blood-donors (n=100) were screened as controls. The study was performed with the approval of the Ethics Committee of the Medical Faculty at Lund University (protocol numbers 731-04 and 323-06) and with the written informed consent of all patients, their parents and the parents of Patient 4. DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) as previously described (1) and from spleen (Patient II 1) using the QIAamp DNA FFPE tissue kit (Qiagen). Exons of *CFH* (2), *CFI* (3), *MCP* (4) and *C3* (primers available upon request) were bidirectionally sequenced using the Big dye terminator kit (Applied Biosystems, Foster City, CA) and analyzed on an Applied Biosystems DNA Analyzer, model 3730. Multiplex ligation-dependent probe amplification was used to quantify the number of allele copies of the *CFHR1* and *CFHR3* genes as previously described (5). The *C3* and *MCP* gene mutations were sequenced in all family members except individuals III 4 and III 5. DNA from patient III 1 was further sequenced for the *CFH* and *CFI* genes as well as copy numbers of the *CFHR1* and *CFHR3* genes. Analysis of serum antibodies to CFH was performed by ELISA as previously described.(6)

References

1. Vaziri-Sani F, Holmberg L, Sjoholm AG *et al.* Phenotypic expression of factor H mutations in patients with atypical hemolytic uremic syndrome. *Kidney Int* 2006; 69: 981-988

2. Richards A, Buddles MR, Donne RL *et al.* Factor H mutations in hemolytic uremic syndrome cluster in exons 18-20, a domain important for host cell recognition. *American journal of human genetics* 2001; 68: 485-490
3. Fremeaux-Bacchi V, Dragon-Durey MA, Blouin J *et al.* Complement factor I: a susceptibility gene for atypical haemolytic uraemic syndrome. *Journal of medical genetics* 2004; 41: e84
4. Fremeaux-Bacchi V, Moulton EA, Kavanagh D *et al.* Genetic and functional analyses of membrane cofactor protein (CD46) mutations in atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2006; 17: 2017-2025
5. Dragon-Durey MA, Blanc C, Marliot F *et al.* The high frequency of complement factor H related CFHR1 gene deletion is restricted to specific subgroups of patients with atypical haemolytic uraemic syndrome. *Journal of medical genetics* 2009; 46: 447-450
6. Dragon-Durey MA, Loirat C, Cloarec S *et al.* Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2005; 16: 555-563

Supplementary results and discussion

Two C3 mutations were identified in the family, R713W and G1094R. Based on studies previously performed on the adjacent C3 mutation D1093N, which exhibited reduced binding to MCP and, to a lesser degree, CFH (1), we assume that the G1094R mutation could similarly enable uninhibited activation of the alternative pathway to occur on cell surfaces. Interestingly, all patients in this family also had an MCP mutation, Y155D, shown to decrease cell-surface expression of MCP (2), which, in combination with reduced C3 binding to MCP, could exacerbate detrimental complement activation on host cells. Patient III7 had six recurrences of HUS before regular plasma infusions were instituted after which she only suffered two recurrences suggesting that the factor contributing to aHUS was not only cell-bound (MCP) but also circulatory (C3).

In addition to the mutations in C3 and MCP patient III1 also had a heterozygous deletion of *CFHR1/CFHR3* with transient auto-antibodies to CFH. The possibility that the latter alterations may have changed the phenotype in this individual cannot be excluded although it seems unlikely, as antibodies were not detected five years later in spite of disease progression. This family is further example of aHUS being a polygenic multifactorial disease. Susceptibility is complex and multiple hits, including, in addition to the genetic alterations, also triggering infections and vaccinations, contribute to the development of a full-blown phenotype. Indeed, in Patient II1, disease was precipitated after a vaccination and associated with bloody diarrhea and seizures.

References

1. Fremeaux-Bacchi V, Miller EC, Liszewski MK *et al.* Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. *Blood* 2008; 112: 4948-4952
2. Fremeaux-Bacchi V, Moulton EA, Kavanagh D *et al.* Genetic and functional analyses of membrane cofactor protein (CD46) mutations in atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2006; 17: 2017-2025

Supplementary Table 1: Laboratory parameters in Patient III 1

Age (years)		**4**	**5**	**6**	**7**	**8**	**9**	**10**
Creatinine (µmol/L)	Reference values 20 - 70	26	30	50	70-100	70-100	100-130[a]	200
Urea (mmol/L)	3.2 – 8.1	7.7	6	15	17.7	19.4	19.3	31
Albumin (g/L)	38 - 48	39	41	37	42	40	42	36
Hemoblobin (g/dL)	10.6 – 14.4	10.5	11.4	9.9	11.6	11.2	11.4	8.5
Reticulocyte count (10^{12}/L)	0.042 – 0.07	0.04	-	-	0.05	-	-	0.09
Platelet count (10^9/L)	200 - 400	425	465	339	276	300	304	235
LDH U/L	< 200	-	-	-	-	-	-	320, 440
Blood smear		Normal, no RBC fragmentation	-	-	-	-	-	Normal
Urine protein/creatinine (mg/mmol)	< 25	200-500	40-100	200-300	60	100-150	200	200, 60
Urine microscopy/HPF		5-15 RBCs, 0-5 hyaline or granular casts	-	> 5 casts	-	-	-	> 5 casts

LDH: lactic dehydrogenase. RBC: red blood cells, HPF: high-power field. [a], glomerular filtration rate was 24 ml/min/1.73m^2.

Supplementary Table 2. Complement levels in patients investigated in this study

Patient number	C3 (mg/l) (770-1380)	C4 (mg/l) (120-330)	CFB (mg/l) (59-164)	CFH (%) (69-154)	CFI (%) (60-152)	MCP[a] (600-1400)
II 2	520[b]	341	139	142	126	478
II 4	560[b]	262	112	125	116	692
III 1	796[c]	251	112	125	115	283
III 2	954	279	140	126	114	479
III 7	1146	426	222	127	114	433

CFB: factor B, CFH: factor H, CFI: factor I, MCP: membrane cofactor protein/CD46. [a], Depicted as mean fluorescence intensity (MFI) analyzed by flow cytometry. [b], Patients II 2 and II 4 presented with low levels of serum C3 during episodes of aHUS. C3 levels remained low for years even during remission. [c], C3 levels were repeatedly normal, C3 nephritic factor was negative. This patient exhibited normal serum C3 levels until the age of 10 years when levels just under normal reference values were detected.