IgA nephropathy associated with a novel N-terminal mutation in factor H

Roland Schmitt[1], Rafael T. Krmar[2], AnnCharlotte Kristoffersson[1], Magnus Söderberg[3¶], Diana Karpman[1*]

[1] Department of Pediatrics, Clinical Sciences Lund, Lund University, Lund Sweden

[2] Division of Pediatrics, Department for Clinical Science, Intervention and Technology, Karolinska Institutet, Karolinska University Hospital, Huddinge, Sweden.

[3] Department of Pathology, Karolinska University Hospital, Huddinge, Sweden.

* Corresponding author:
Diana Karpman
Department of Pediatrics
Clinical Sciences Lund, Lund University
22185 Lund
Sweden
Tel: +46-46-2220747
Fax: +46-46-2220748
diana.karpman@med.lu.se

¶, Current address: AstraZeneca R&D, Södertälje, Sweden

Abstract

Most patients with IgA nephropathy exhibit complement deposition in the glomerular mesangium. Certain cases of IgA nephropathy have been associated with reduced levels of factor H. A recent study could not demonstrate mutations at the C terminal of factor H. We describe a novel heterozygous mutation in factor H, position A48S (nucleotide position 142 G>T, alanine>serine), detected in exon 2 of a 14 year old girl with IgA nephropathy. The patient exhibited reduced levels of C3 and factor H, the latter suggesting that the mutation affected factor H secretion. The patient developed initial signs and symptoms of glomerulonephritis at the age of 9 years but presented again at the age of 14 years with weight gain, renal failure, nephrotic-range proteinuria and malignant hypertension. Blood tests suggested the development of microangiopathic hemolytic anemia (MAHA) but the renal biopsy was mostly indicative of chronic changes associated with IgA nephropathy as well as vascular changes associated with malignant hypertension. Immunofluorescence exhibited depositis of IgA, C3 and IgM. Screening of the factor H gene revealed, in addition to the mutation, three heterozygous hemolytic uremic syndrome-associated risk polymorphisms (-257 c/t, 2089 a/g and 2881 g/t) which may have increased the patient's susceptibility to the occurrence of MAHA triggered by malignant hypertension. Thus the combined clinical picture of IgA nephropathy and MAHA may have been partly related to the alterations in factor H.

Keywords: IgA nephropathy, factor H, complement, child

Introduction

IgA nephropathy (IgAN) is characterized by glomerular deposits of aberrantly glycosylated IgA1 and complement proteins [10,17]. Complement components deposit mainly in the mesangium and include C3, C4d, C4-binding protein, factor H, mannose-binding lectin, C5b-9 and properdin [1,7,8,13,15,17,20,21]. Polymeric IgA may activate both the alternative and lectin pathways of complement [11,16,17] and studies have suggested that complement activation during IgAN involves the alternative and lectin pathways [17]. Mechanisms by which complement activation occurs during IgAN are not fully understood. It has been suggested that inadequate complement regulation could lead to complement activation in vivo and progressive glomerular disease [7].

Factor H is the main fluid phase regulator of the alternative pathway of complement. Dysfunction of factor H has been associated with certain renal diseases such as atypical hemolytic uremic syndrome (HUS) and membranoproliferative glomerulonephritis as well as the ophthalmological condition termed age-related macular degeneration [reviewed in 27]. Factor H is deposited in the kidneys during IgAN [1]. Urinary levels of factor H have been found to be increased and related to disease activity [26]. A recent study investigated 46 patients with IgAN and found normal factor H levels and no mutations in the C terminal of factor H, the region responsible for host cell recognition [6].

In this study we present a girl who primarily developed IgAN followed several years later by malignant hypertension and microangiopathic hemolytic anemia (MAHA). The latter led us to investigate the patient's factor H levels which were found to be low. A novel mutation was detected at the N terminal of factor H. In addition to the mutation, three polymorphisms, associated with increased risk for HUS, were detected in the factor H gene.

Patient and family member

A currently 18 year old Caucasian girl was admitted to Karolinska University Hospital at the age of 9 years with macroscopic hematuria and proteinuria after a severe tonsillitis. Her serum creatinine was slightly elevated at 67 µmol/L (normal reference value < 60 µmol/L). Within the following two months she recovered with persistent microscopic hematuria but no proteinuria after which she was lost to follow-up. She was readmitted at the age of 14 years with a history of weight gain during a few months as well as progressive fatigue, weakness, headache and blurred vision during the days before admission. There was no history of diarrhea. Upon admission her blood pressure was 250/150 mmHg, she had altered sensorium and was in respiratory distress. She exhibited severe oliguric renal failure with high levels of creatinine (1360 µmol/L, reference value < 90) and BUN. In addition, laboratory values showed low serum albumin (31 g/l, reference value: 40-51 g/L), hemolytic anemia (hemoglobin 65 g/L (110-160), lactate dehydrogenase 16.5 µkat/L (< 6.2)) and thrombocytopenia (69 x 10^9/l, 150-400). C3 was low 0.52 g/L (0.67-1.43), C3dg elevated 11.5 (< 5mg/L) and C4 normal. Urinalysis revealed microscopic hematuria and nephrotic-range proteinuria.

Serologic analysis for anti-nuclear antibodies, anti-double stranded antibodies, anti-phospholipid antibodies, anti-neutrophil cytoplasmic antibodies, anti-glomerular basement membrane antibodies, hepatitis B and C, HIV were all negative. Fundoscopic exam revealed papilledema, exudates and retinal hemorrhages. She was treated with continuous veno-venous hemofiltration, anti-hypertensive medications and pulses of methyl-prednisolone. Ophthalmologic and cardiovascular involvement as well as hemolytic anemia remitted and blood pressure normalized.

Renal biopsy showed 17 glomeruli, 11 exhibited total sclerosis (Figure 1A), and two crescents (Figure 1B). The remaining exhibited mesangial proliferation (matrix and cells). There was no evidence of thickening or double contours of the glomerular basement membrane. Tubules showed marked atrophy with mononuclear infiltrates in the interstitium. There were no visible thrombi in the renal blood vessels, but arterioles displayed myointimal proliferation in a concentric pattern typical for “onion-skin” lesions (Figure 1C). Immunofluorescence showed intense mesangial deposits of IgA (Figure 1D) and to a lesser degree IgM and C3 (not shown). The electron microscopy sample did not contain glomeruli. She did not regain renal function and underwent a successful renal transplant donated by her father 16 months later. She has not had a recurrence of IgA nephropathy or MAHA since transplantation in June 2007.

Serum and whole blood in EDTA tubes were obtained from the patient and her father. The project was performed with the informed written consent of the patient and her parents and the approval of the Ethics committee of the Medical Faculty, Lund University.

Materials and Methods

Factor H levels and mutation analysis

Factor H levels were measured by rocket immunoelectrophoresis as previously described [22]. Factor H size was detected by immunoblotting [22]. Extraction of genomic DNA and sequencing of the factor H gene were performed as described [22].

ADAMTS13

ADAMTS13 activity in plasma was detected by a modified collagen binding assay as previously described [9].

Results

Factor H levels were repeatedly low at 50 % (reference value: 69-154) upon admission at the age of 14 years, and 52 % three years later, after transplantation. Immunoblotting revealed a weak factor H band at 150 kD (data not shown) indicating normal size.

Genomic DNA from the patient and her father were screened for mutations in the factor H gene. A novel heterozygous mutation in exon 2, corresponding to short consensus repeat (SCR) 1, was found at G142T leading to replacement of alanine by serine: A48S. In addition, three heterozygous polymorphisms were identified in the factor H gene: -257 c/t (promoter region), 672 a/g A2089G in exon 14 (silent) and G2881T: E936D in exon 19. These polymorphisms have been previously described as risk-associated with HUS [3]. The patient's father did not bear the mutation but had all three heterozygous polymorphisms. DNA was not available from the patient's mother. ADAMTS13 function was normal.

Discussion

A novel mutation at the N terminal of factor H is described in a girl with evidence of IgAN and one episode of MAHA in conjunction with malignant hypertension. The mutation is located in SCR 1 of factor H. Factor H is a co-factor of factor I in cleaving C3b. The cofactor and complement-regulating domain of factor H is ascribed to SCRs 1-4 which bind C3b. This region is active in decay acceleration, displacing factor B from the C3 and C5 convertase [12]. Low levels of factor H have been previously described in certain patients with IgAN [23,24,25]. The patient exhibited low levels of factor H at separate time points suggesting that the heterozygous mutation interfered with secretion of the product of the mutated allele. The mutation is in proximity of a cysteine residue at codon 52 (http://www.fh-hus.org/) possibly altering a disulphide bridge and/or the stability of SCR1. Thus we suggest that the mutation may affect complement regulatory functions and may partially block secretion of factor H from cells as has been demonstrated for other N terminal mutations in factor H, mostly associated with membranoproliferative glomerulonephritis [5, 22].

The pathological findings were indicative of IgAN due to intense mesangial deposition of IgA. Membranoproliferative glomerulonephritis was ruled out due to lack of typical changes such as glomerular basement membrane thickening with double contours. The clinical history, with a glomerulonephritis in association with pharyngeal infection at the age of 9 years, indicates that the primary lesion was IgAN. The combined clinical picture of IgAN and HUS has been reported [4,14] in association with chronic advanced IgA nephropathy and malignant hypertension as was evident in our patient. Malignant hypertension in itself has been associated with MAHA (reviewed in [2]). This may be due to endothelial cell injury with narrowed microvasculature and enhanced shear stress [18]. MAHA may have developed in this patient secondary to progressive IgAN and malignant hypertension but the presence of

three HUS-associated polymorphisms in factor H [3] could have contributed to this process. Recently patients with IgAN have been investigated regarding allele frequency and these three polymorphisms were not associated with IgAN [6]. However, the presence of these polymorphisms in an IgAN patient with malignant hypertension and vascular damage may be a predisposing factor reducing complement regulation and precipitating MAHA. This raises the ethical issue of if a patient with a factor H mutation, and three factor H polymorphisms associated with increased risk to develop HUS, should undergo renal transplant. In patients with HUS and factor H mutations the risk of HUS recurrence after renal transplant, leading to graft loss, is high [19]. The primary diagnosis in the patient described herein was IgAN and she developed MAHA as a secondary phenomenon due to malignant hypertension. As N terminal factor H mutations have not been explored in a larger cohort of IgAN patients it is, as yet, unclear if these genetic alterations can increase the risk of IgAN recurrence after renal transplantation.

There may be several mechanisms for complement activation in IgAN via both the alternative and lectin pathways. Although we describe only one patient with an N terminal mutation in factor H, we suggest that the mutation and the three polymorphisms in factor H may have contributed to complement dysregulation and C3 deposition in the glomeruli.

Conflicts of interest

The authors declare that they do not have any conflicts of interest and no financial relationships that might have influenced the present work.

Acknowledgements

This study was supported by grants from The Swedish Research Council (K2010-65X-14008-10-3), The 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), funding from Region Skåne (to RS and DK); grants from Kristianstad Högskola and the Samariten Foundation (to RS). Diana Karpman is the recipient of a clinical-experimental research fellowship from the Royal Swedish Academy of Sciences.

References

1. Bene MC, Faure GC (1987) Composition of mesangial deposits in IgA nephropathy: complement factors. Nephron 46: 219
2. Benz K, Amann K (2010) Thrombotic microangiopathy: new insights. Curr Opin Nephrol Hypertens 19:242-247.
3. Caprioli J, Castelletti F, Bucchioni S et al (2003) Complement factor H mutations and gene polymorphisms in haemolytic uraemic syndrome: the C-257T, the A2089G and the G2881T polymorphisms are strongly associated with the disease. Hum Mol Genet 12: 3385-3395
4. Chang A, Kowalewska J, Smith KD et al (2006) A clinicopathologic study of thrombotic microangiopathy in the setting of IgA nephropathy. Clin Nephrol 66: 397-404
5. de Cordoba SR, de Jorge EG (2008) Translational mini-review series on complement factor H: genetics and disease associations of human complement factor H. Clin Exp Immunol 151: 1-13
6. Edey M, Strain L, Ward R et al (2009) Is complement factor H a susceptibility factor for IgA nephropathy? Mol Immunol 46: 1405-1408
7. Endo M., Ohi H, Satomura A (2001) Regulation of in situ complement activation via the lectin pathway in patients with IgA nephropathy. Clin Nephrol 55, 185-191
8. Espinosa M, Ortega R, Gomez-Carrasco JM et al (2009) Mesangial C4d deposition: a new prognostic factor in IgA nephropathy. Nephrol Dial Transplant 24: 886-891
9. Gerritsen HE, Turecek PL, Schwarz HP et al (1999) Assay of von Willebrand factor (vWF)-cleaving protease based on decreased collagen binding affinity of degraded vWF: a tool for the diagnosis of thrombotic thrombocytopenic purpura (TTP). Thromb Haemost 82: 1386-1389

10. Giannakakis K, Feriozzi S, Perez M et al (2007) Aberrantly glycosylated IgA1 in glomerular immune deposits of IgA nephropathy. J Am Soc Nephrol 18: 3139-3146
11. Hiemstra PS, Gorter A, Stuurman ME et al (1987) Activation of the alternative pathway of complement by human serum IgA. Eur J Immunol 17: 321-326
12. Kuhn S, Zipfel PF (1996) Mapping of the domains required for decay acceleration activity of the human factor H-like protein 1 and factor H. Eur J Immunol 26: 2383-2387
13. Miyazaki R, Kuroda M, Akiyama T et al (1984) Glomerular deposition and serum levels of complement control proteins in patients with IgA nephropathy. Clin Nephrol 21: 335-340
14. Morita S, Sakai T, Okamoto N et al (1999) Hemolytic uremic syndrome associated with immunoglobulin A nephropathy: a case report and review of cases of hemolytic uremic syndrome with glomerular disease. Intern Med 38: 495-499
15. Rauterberg EW, Lieberknecht HM, Wingen AM, Ritz E (1987) Complement membrane attack (MAC) in idiopathic IgA-glomerulonephritis. Kidney Int 31: 820-829
16. Roos A, Bouwman LH, van Gijlswijk-Janssen DJ et al (2001) Human IgA activates the complement system via the mannan-binding lectin pathway. J Immunol 167: 2861-2868
17. Roos A, Rastaldi MP, Calvaresi N et al (2006) Glomerular activation of the lectin pathway of complement in IgA nephropathy is associated with more severe renal disease. J Am Soc Nephrol 17: 1724-1734
18. Ruggenenti P, Remuzzi G (1996) Malignant vascular disease of the kidney: nature of the lesions, mediators of disease progression, and the case for bilateral nephrectomy. Am J Kidney Dis 27: 459-475

19. Saland JM, Ruggenenti P, Remuzzi P et al (2009) Liver-kidney transplantation to cure atypical hemolytic uremic syndrome. J Am Soc Nephrol 20:940-949
20. Stangou M, Alexopoulos E, Pantzaki A et al (2008) C5b-9 glomerular deposition and tubular alpha(3)beta(1)-integrin expression are implicated in the development of chronic lesions and predict renal function outcome in immunoglobulin A nephropathy. Scand J Urol Nephrol 42: 1-8
21. Tomino Y, Sakai H, Nomoto Y et al (1981) Deposition of C4-binding protein and beta 1H globulin in kidneys of patients with IgA nephropathy. Tokai J Exp Clin Med 6: 217-222
22. Vaziri-Sani F, Holmberg L, Sjöholm AG et al (2006) Phenotypic expression of factor H mutations in patients with atypical hemolytic uremic syndrome. Kidney Int 69: 981-988
23. Watanabe S, Yamaguchi Y, Suzuki T et al (2001) Inherited factor H dysfunction and complement-associated glomerulonephritis in renal grafts of first and second transplantations. Clin Transplant 15 Suppl 5: 45-50
24. Wyatt RJ, Julian BA, Rivas ML (1991) Role for specific complement phenotypes and deficiencies in the clinical expression of IgA nephropathy. Am J Med Sci 301: 115-123
25. Wyatt RJ, Julian BA, Weinstein A et al (1982) Partial H (beta 1H) deficiency and glomerulonephritis in two families. J Clin Immunol 2: 110-117
26. Zhang JJ, Jiang L, Liu G et al (2009) Levels of urinary complement factor H in patients with IgA nephropathy are closely associated with disease activity. Scand J Immunol 69: 457-464

27. Zipfel PF, Heinen S, Jozsi M, Skerka C (2006) Complement and diseases: defective alternative pathway control results in kidney and eye diseases. Mol Immunol 43: 97-106

Figure legend

Fig 1: Histopathologic findings in the patient's renal biopsy

Renal biopsy showed global sclerosis in 11/17 glomeruli (**A**), crescents in two glomeruli (see arrow in panel **B**) and "onion-skin" lesions in arterioles (**C**). Immunofluorescence showed mesangial deposits of IgA (**D**).

Figure 1