ORIGINAL ARTICLE

Primary glomerulonephritis with isolated C3 deposits: a new entity which shares common genetic risk factors with haemolytic uraemic syndrome

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Introduction: Abnormal control of the complement alternative pathway (CAP) (factor H, factor I and membrane cofactor protein (MCP) deficiencies) is a well established risk factor for the occurrence of haemolytic uraemic syndrome (HUS). In some instances, HUS may be associated with an unusual glomerulonephritis with isolated C3 deposits (glomerulonephritis C3). We determined whether HUS and glomerulonephritis C3 share common genetic susceptibility factors.

Methods: We identified 19 patients with glomerulonephritis C3. We measured levels of circulating complement components, performed assays for the detection of C3 nephritic factor (C3NeF) and screened factor H, factor I and MCP coding genes for the presence of mutations.

Results: Patients were divided in two groups based on renal pathology findings: group I (n = 13) had typical features of type I membranoproliferative glomerulonephritis (glomerulonephritis C3 with membranoproliferative glomerulonephritis (MPGN)) and group II (n = 6) was characterised by mesangial and epimembranous C3 deposits in the absence of mesangial proliferation (glomerulonephritis C3 without MPGN). Mutations in complement regulatory genes were detected in 4/6 patients with glomerulonephritis C3 without MPGN (heterozygous mutations in factor H gene (two patients) with low factor H antigenic level in one case, heterozygous mutations in factor I gene (two patients)) and in only 2/13 patients with glomerulonephritis C3 with MPGN (heterozygous mutations in factor H gene (one patient) and double heterozygous mutation in CD 46 gene (one patient)). In contrast, C3NeF was present in 5/13 patients with glomerulonephritis C3 with MPGN and in 2/6 patients with glomerulonephritis C3 without MPGN, one of whom had a factor H mutation.

Conclusion: HUS and glomerulonephritis C3 without MPGN share common genetic risk factors. Constitutional or acquired dysregulation of the CAP is probably associated with a wide spectrum of diseases, ranging from HUS to glomerulonephritis C3 with MPGN.

Glomerular nephropathies with immune deposits may be characterised, on the basis of immunofluorescence analysis, by the composition of the deposits. Depending on the presence or absence of immunoglobulins, two main types are recognised. Deposits containing immunoglobulins and classical pathway components are the hallmark of immune complex mediated diseases: primary membranoproliferative glomerulonephritis (MPGN) type I and MPGN secondary to hepatitis C infection or to immune diseases. Conversely, deposits containing exclusively complement C3 and C5b-C9 without immunoglobulins or classical pathway components are indicative of complement alternative pathway (CAP) activation. Glomerular nephropathies with isolated complement C3 deposits can be observed in different situations: MPGN type II, also called dense deposits disease,[1] and acute poststreptococcal glomerulonephritis.[2] In these two diseases, abnormal activation of the CAP takes place, apparently linked to identified CAP activators: respectively, the C3 nephritic factor (C3NeF), an autoantibody to CAP C3 convertase in MPGN type II,[1] and a streptococcal peptide deposited in the glomerulus in acute poststreptococcal glomerulonephritis.[2–4] Homozygous deficiencies in factor H have been reported in rare cases of type II MPGN.[5 6]

In addition to these two forms of glomerulonephritis, we have observed a peculiar type of glomerulonephritis characterised by overt isolated mesangial C3 deposits (glomerulonephritis C3), manifest by light microscopy study. Glomerulonephritis C3 is clearly different from MPGN type II because of the absence of dense intramembranous deposits within the glomerular and the tubular basement membrane, and from acute poststreptococcal glomerulonephritis because of the absence of exudative lesions and the presence of mesangial deposits.[7] The disease usually recurs in renal allografts with identical lesions to that in the native kidney which suggests the role of a host factor.[8] The observation of the occurrence of C3 mesangial deposits[9] in Cfh deficient mice created by gene targeting in which factor H gene has been inactivated (factor $H^{-/-}$) support a role for abnormal control of the CAP in glomerulonephritis C3. Factor H downregulates the activity of the CAP by increasing the rate of dissociation of the AP convertase C3bBb and by acting as cofactor for the serine protease factor I, which cleaves C3b. In addition, factor H can inactivate membrane bound C3b through its binding to endothelial cells.[10] The membrane cofactor protein (MCP) is a cell membrane associated protein which also acts as cofactor for factor I. Glomerulonephritis C3 can be associated in rare instances with haemolytic uraemic syndrome (HUS).[11] As genetics studies have shown that mutations in CFH, IF and MCP genes

Abbreviations: C3NeF, C3 nephritic factor; CAP, complement alternative pathway; HUS, haemolytic uraemic syndrome; MCP, membrane cofactor protein; MPGN, membranoproliferative glomerulonephritis

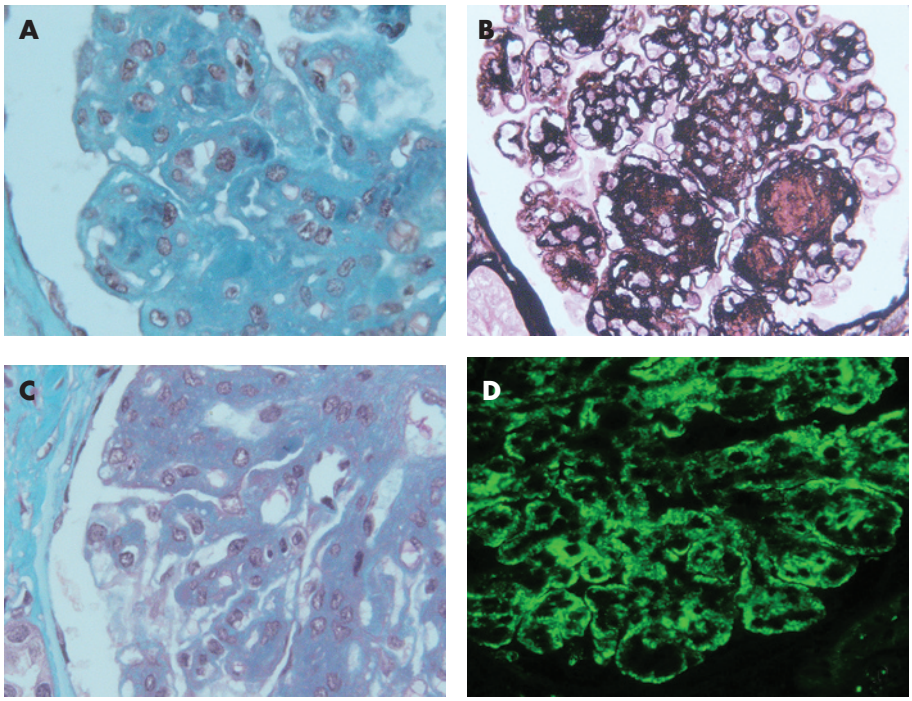

Figure 1 Illustrative cases of glomerulonephritis with isolated C3 deposits with membranoproliferative glomerulonephritis (glomerulonephritis C3 with MPGN). (A) Patient No 6. By light microscopy study (light green trichrome, ×400), glomerulonephritis C3 with MPGN was characterised by mesangial cellular proliferation, increase in mesangial matrix and subendothelial, mesangial and epimembranous deposits. (B) Patient No 6. By light microscopy study (Jone's staining, ×400), renal biopsy shows accumulation of the extracellular matrix in the intercapillary space, with double contours. (C) Patient No 2. By light microscopy study (light green trichrome, ×400), glomerulonephritis C3 with MPGN. (D) Patient No 4. Immunofluorescence study (×400) reveals granular C3 deposits in mesangial area and along the glomerular basement membrane.

predispose to HUS,[6 12–15] we screened for these mutations in 19 patients with glomerulonephritis C3, in the absence of HUS.

MATERIALS AND METHODS

Thirty two biopsies with glomerulonephritis C3 were referred to the Department of Pathology, Necker Hospital, Paris, France, between 1971 and 2004 and our analysis was restricted to cases for whom detailed clinical data, follow up and consent for genetic study were available. We included 19 patients with a definite diagnosis of glomerulonephritis C3 established at renal biopsy and without symptoms of HUS. Glomerulonephritis C3 is defined by isolated C3 deposits on immunofluorescence with no Ig deposits, and by the absence of dense intramembranous C3 deposits. Relevant clinical and biological data were collected through review of medical charts. Determination of C3 level was performed at the time of diagnosis in 18 patients before any immunosuppressive treatment. In the only remaining patient (patient No 12), measurement of C3 level was performed during follow up in the absence of any previous or concomitant immunosuppressive treatment. All laboratory tests were carried out as part of the routine follow up of these patients. Informed consent of patients (or parents of children) was obtained before DNA analysis.

Definitions

Creatinine clearance was estimated using the Cockroft–Gault formula. Stages of kidney disease were classed according to the K/DOQI clinical practice guidelines.[16]

Assays for complement components and genetic screening

All immunological and genetic analyses were performed at the reference laboratory for the investigation of the complement system (Hôpital Européen Georges Pompidou, France). EDTA plasma samples were obtained from all patients and stored at −80°C. Plasma protein concentrations of C3, factor H and factor I were measured as described previously.[6 17] Membrane expression of MCP (CD46) was analysed on granulocytes using a FACS Calibur flow cytometer (Becton-Dickinson, Heidelberg, Germany). Fluorescence staining was performed using anti CD46 PE conjugated monoclonal antibodies (IgG1; Serotec, Cergy, France). C3 nephritic factor (C3NeF) activity was determined as described previously[18] by assessing the ability of purified IgG from plasma to stabilise the cell bound C3b, Bb convertase.

Direct sequencing of all *CFH*, *IF* or *MCP* exons was undertaken in all 19 patients. Information on the primers and reaction conditions of polymerase chain reaction has been reported previously (Dragon Durey and VFB) and can be provided on request (veronique.fremeaux-bacchi@egp.aphp.fr). To determine whether a mutation was also present in a control collective and therefore more likely be a rare polymorphism than a deleterious mutation, we studied a control population consisting of a panel of 100 locally recruited healthy subjects. These controls were analysed for the presence of all mutations identified in this study using the same technique (200 chromosomes).

RESULTS

Pathological and clinical data

The 19 patients had a peculiar type of glomerulonephritis characterised by overt isolated mesangial C3 deposits (glomerulonephritis C3). They were divided in two groups based on renal pathology findings. In group I (n = 13), renal biopsy disclosed typical features of type I MPGN (glomerulonephritis C3 with MPGN) with mesangial proliferation, subendothelial, mesangial and, less frequently, epimembranous deposits, diffuse ''double contours'' aspect (fig 1) and accumulation of mesangial matrix with a nodular aspect in four cases (patient Nos 4, 6, 7 and 8). In group II (n = 6), renal biopsy showed a peculiar pattern of mesangial and epimembranous deposits (glomerulonephritis C3 without MPGN) without subendothelial C3 deposits or mesangial proliferation (fig 2).

In all cases, immunofluorescence showed isolated C3 deposits (figs 1, 2) while dense intramembranous deposits with ribbon-like aspects were not detected by light microscopy. C1q and IgG staining was negative. The absence of dense intramembranous deposits was confirmed in two cases (patient Nos 18 and 10) by electron microscopy (fig 2). In these two

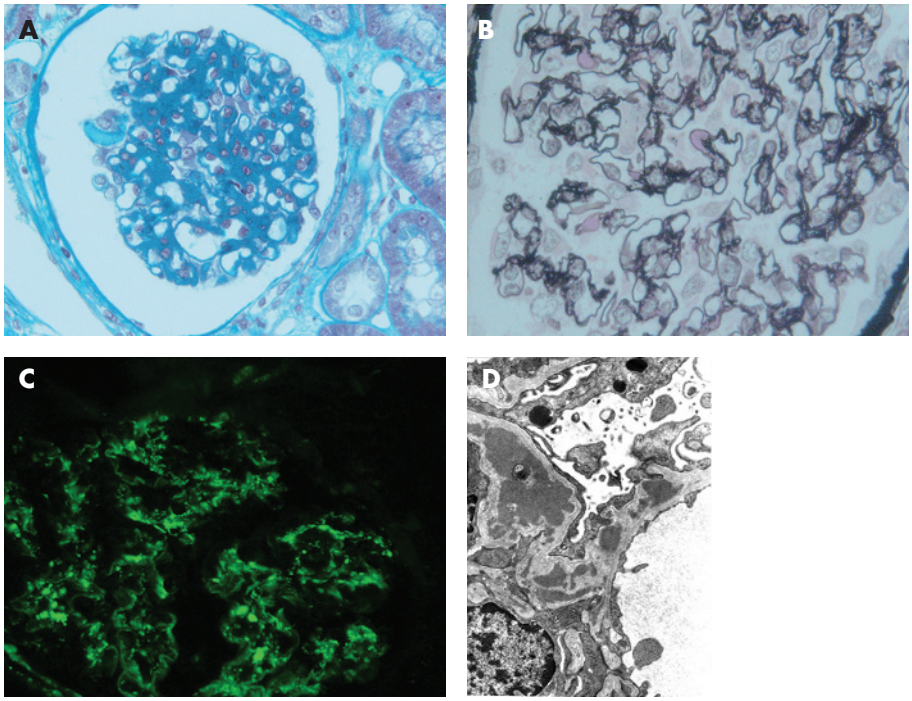

Figure 2 Illustrative cases of glomerulonephritis with isolated C3 deposits without membranoproliferative glomerulonephritis (glomerulonephritis C3 without MPGN). (A) Patient No 18. By light microscopy (light green trichrome, ×400), renal biopsy shows mesangial deposits without mesangial proliferation. (B) Patient No 16. By light microscopy study (Jone's staining, ×450), renal biopsy reveals mesangial and epimembranous deposits without mesangial proliferation. (C) Patient No 17. Immunofluorescence study (×400) shows granular C3 deposits in the mesangial area and along glomerular basement membrane without ribbon-like aspects. (D) Patient No 18. Electron microscopy study (×15000) shows mesangial and epimembranous deposits without mesangial proliferation and without ''dense deposits'' in glomerular basement membranes.

cases, mesangial deposits as well as ''humps'' were present in the absence of dense deposits in the basement membrane.

Patient clinical and biological data for the two groups are shown in tables 1 and 2. There were 10 women and nine men, all caucasian. Median age at onset was 29.9 years (range 7–70), and median follow up was 12.3 years (range 0.4–34.0). Renal symptoms at diagnosis included the following: hypertension in nine cases, stage 1 kidney disease in seven cases, stage 2 kidney disease in seven cases, stage 3 in three cases, stage 4 in one case and stage 5 in one case. Median proteinuria was 3.3 g/day (0.2–9.0), with no proteinuria in one case, a nephrotic syndrome in three cases and microhaematuria in 12 cases. Seological test for hepatitis C was negative in all patients.

Renal disease tended to be more severe in patients with glomerulonephritis C3 with MPGN compared with those with glomerulonephritis C3 without MPGN, with a higher median proteinuria at diagnosis (1.7 *v* 0.3 g/day) and a higher percentage of patients reaching ESRD (3/16 *v* 0/6) (table 2).

Five patients received steroid treatment (patient Nos 4, 6, 9, 13 and 17) and eight (patient Nos 4, 6, 7, 11, 13, 14, 16 and 18)

Table 1 Clinical and biological data in 19 patients with glomerulonephritis C3

Patient No	Age* (y)	Sex	HBP*	CrCl* (ml/min)	Puria* (g/day)	Huria*	Follow up (y)	CrCl at last follow up (ml/min)	Puria at last follow-up (g/day)	Histological type
1	20	F	+	117.4	7.7	+	7.0	11.2	4.5†	Group I
2	27	M	+	97.2	1.7	−	16.5	HD‡	1.4	Group I
3	26	M	−	115.3	1.5	−	30.0	86.8	4.7	Group I
4	11	F	−	65.3	6.5†	+	1.2	116.5	1.7	Group I
5	60	M	+	51.3	5.7†	+	0.5	46.3	0.9	Group I
6	42	F	+	68.2	1.3	+	9.0	39.2	1.5	Group I
7	22	M	+	122.6	0.3	+	14.0	64.8	0.4	Group I
8	41	M	−	91.6	6.6	−	10.0	28.6	2.8	Group I
9	10	M	−	10.2	8.2	−	5.5	HD‡	1.4	Group I
10	49	M	−	78.3	0.3	−	0.4	78.0	2.4	Group I
11	21	F	−	120.6	2.5	−	24.0	66.4	0.1	Group I
12	14	F	−	78.6	0.2	+	23.0	HD		Group I
13	7	F	+	66.6	9.0	+	1.5	54.0	0.3	Group I
14	9	F	−	44.8	0.7	+	2.5	71.7	0.8	Group II
15	26	M	+	81.8	0.3	−	23.0	79.5	0.0	Group II
16	56	F	+	49.8	0.0	+	5.0	40.3	0.0	Group II
17	70	F	−	28.7	0.3	+	1.5	14.6	5.2 †	Group II
18	37	M	+	97.2	6.2†	+	34.0	66.3	0.0	Group II
19	21	F	−	83.3	1.6	+	25.0	110.8	0.5	Group II

CrCl, creatinine clearance; F, female; FH, factor H; FI, factor I; Group I: GN C3 (glomerulonephritis with isolated C3 deposits) with MPGN (membranoproliferative glomerulonephritis); Group II: GN C3 without MPGN; HBP, high blood pressure; HD, haemodialysis; Huria, haematuria; M, male; Puria, proteinuria.
To convert CrCl in ml/min to ml/s, multiply by 0.01667.
*At diagnosis.
†Nephrotic syndrome.
‡Underwent renal transplantation.

Table 2 Main features of the groups of patients based on renal pathology data

	GN C3 with MPGN (n = 13)	GN C3 without MPGN (n = 6)
Age (y)*	26	26
Sex (F/M)	6/7	4/2
Puria (g/day)*	1.7	0.3
CrCl (ml/min)*	54	66.3
Follow up (y)*	9	5
ESRD	3/13	0/6
Decreased circulating C3 level	5/13	2/6
C3NeF	5/13	1/6†
FH, FI or MCP mutations	2/13	4/6

C3NeF, C3 nephritic factor; CrCl, creatinine clearance; ESRD, end stage renal disease; F, female; FH, factor H; FI, factor I; GN C3, glomerulonephritis with isolated C3 deposits; M, male; MPGN, membranoproliferative glomerulonephritis; Puria, proteinuria.
*Median values.
†Excluding patient No 15 with C3 NeF and FH mutation.

were treated with an angiotensin converting enzyme inhibitor. Outcome was very heterogeneous without any significant influence of treatment on renal function. At the last follow up, renal function remained normal in nine cases, was decreased in seven cases and three patients, all in the glomerulonephritis C3 with MPGN group, had end stage renal disease (patient Nos 2, 9, and 12). Patient Nos 2 and 9 underwent transplantation. Recurrence of glomerulonephritis C3 was observed on renal biopsy in patient No 9, one month after transplantation, with proteinuria (1.4 g/day) and normal renal function (CrCl 94.7 ml/min), while no recurrence was noted two months after transplantation in patient No 2 on renal transplant biopsy.

Complement component assessment

Plasma complement levels at the time of genetic analysis are shown in table 3.

Seven of the 19 patients presented with a decrease in C3 at the time of the investigation. Five of 16 patients in the glomerulonephritis C3 with MPGN group had low C3 levels compared with 2/6 patients in the glomerulonephritis C3 without MPGN group. In three patients, low C3 level was associated with decreased factor B plasma levels (patient Nos 9, 14 and 16), a finding suggestive of mild CAP activation. Absence of detectable CAP activation, with normal antigenic levels of C3 and factor B, was observed in 12 patients. C3 level was stable during follow up in 13 of 14 patients for whom sequential measurements of C3 were available. In one case (patient No 11), very low levels of C3 were noted at the time of diagnosis while C3 was normal 10 years later at inclusion in the study. All C4 values were normal, which confirm the absence of activation of the complement classical pathway.

All patients except one (patient No 16) had normal factor H antigenic levels. Patient No 16 presented with CAP activation with mildly decreased C3 and factor B and half normal levels of plasma factor H. All others patients had normal factor H antigenic levels. Antigenic levels of factor I were normal in all patients. MCP expression was normal in all tested patients.

At the time of the investigation, C3NeF was detected in the IgG fraction isolated from plasma in 5/13 patients with glomerulonephritis C3 with MPGN and 2/6 patients with glomerulonephritis C3 without MPGN, one of whom had a factor H mutation. The absence of nephritic factor activity was defined by the lack of C3bBb stabilising activity of the patient's IgG, tested at increasing inputs up to 400 g/assay. In patient No 11, a first blood sample, obtained at the time of diagnosis, showed very low plasma levels of C3 (90 mg/l) with C3NeF activity. A second blood sample was collected 10 years later and showed normal levels of C3 and factor B. At this time, C3NeF activity was no longer detected in plasma. This patient did not receive any immunosuppressive treatment that could alter the assay results.

Molecular characterisation of mutations

The complete CFH, IF and MCP sequence was analysed in 19 patients with glomerulonephritis C3 by direct sequencing. Two previously reported mutations (R1210C in the CFH gene and A304 V in the MCP gene) and five new mutational events were found in six patients, as summarised in table 4. A unique

Table 3 Results of complement investigation, including C3, C4, factor B, factor H and factor I levels, expression of CD46 on peripheral blood mononuclear cell surfaces and assays for C3NeF

Patient No	C3* (660–1250 mg/l)	C4 (93–380 mg/l)	Factor B* (90–320 mg/l)	Factor H antigenic levels* (70–130%)	Factor I antigenic levels* (70–130%)	CD46 (600–1300)	C3NeF	Mutation	Histological type
1	**150**	327	108	112	104	836	+	–	Group I
2	1010	297	147	149	132	611	+	–	Group I
3	683	205	136	120	109	990	+	–	Group I
4	**398**	103	102	123	104	ND	+	–	Group I
5	681	329	97	136	87	1801	+	–	Group I
6	706	147	157	96	115	955	ND	FH	Group I
7	1190	296	193	147	168	769	–	CD 46	Group I
8	**645**	248	118	116	121	786	–	–	Group I
9	**653**	357	*87*	100	92	1332	–	–	Group I
10	**562**	108	98	100	92	1683	–	–	Group I
11	747	103	133	93	84	ND	–	–	Group I
12	769	312	167	124	129	ND	–	–	Group I
13	1130	226	ND	125	79	755	–	–	Group I
14	**384**	180	87	89	90	ND	+	–	Group II
15	794	188	98	103	93	772	+	FH	Group II
16	**616**	271	77	**51**	123	ND	–	FH	Group II
17	975	193	100	176	107	ND	–	FI	Group II
18	958	319	126	130	115	975	ND	FI	Group II
19	829	223	135	110	125	1279	–	–	Group II

C3NeF, C3 nephritic factor; FH, factor H; FI, factor I; Group I: GN C3 (glomerulonephritis with isolated C3 deposits) with MPGN (membranoproliferative glomerulonephritis); Group II: GN C3 without MPGN; MFI, mean fluorescence intensity; ND, not done.
Abnormal values are shown in bold.
*Normal values are indicated in parentheses.

Table 4 Molecular characterisation of the genetics defects

Patient no.	Codon change	Protein domain	Mutation characteristics
CFH gene			
15	R1210C	SCR20	Located in SCR20. Previously reported in several unrelated patients with HUS. Is associated with a reduced binding of FH to the central complement component C3b/C3d, as well as to endothelial cells.[10 19]
16	P76-X	SCR2	One nucleotide deletion in exon 2 leading to half antigenic levels of FH. Previously reported in more than 10 cases of HUS.
6	G650V	SCR 11	Located in SCR 11 close to the C3b binding domain. Other mutations in the same domain have been reported in two patients with atypical HUS.[6 20]
CFI gene			
17	A222G	LDLRA-1	Both mutations are located in two LDLr domains in the heavy chain of FI.
18	G243D	LDLRA-2	The LDLr domains are highly conserved cysteine-rich regions possibly involved in FI ligand binding. These mutations have been reported in 10 patients with HUS.[17 21 22]
MCP gene			
7	V181M + A304V	SCR 3	Two heterozygous mutations associated with normal expression of the protein at the surface of granulocytes. c.747G>A is located in the CCP-3 domain which is highly implicated in the active site of the protein (binding to C4b and C3b), as previously demonstrated using mutagenesis.[23] A304V has been reported in patients with HUS.[21]

HUS, haemolytic uraemic syndrome; LDLr, low density lipoprotein receptor; MCP, membrane cofactor protein.
MCP gene, exon 11 encodes for the transmembrane domain 1. The amino acid numbering refers to the start codon of the sequence after the peptide signal (Met +34).
FI gene, the nucleotide number one is nucleotide located 29 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.
FH gene, the nucleotide number one is nucleotide located 73 before the ATG used as the first amino acid of the protein.

nucleotide substitution leading to a change in an amino acid localised in the SCR 11 (G650V) and in the SCR 20 (R1210C) was identified in two patients who presented with normal antigenic levels of factor H (patient Nos 6 and 15). Patient No 16, who presented with half levels of antigenic factor H, had a nucleotide substitution leading to the direct creation of a stop codon in the SCR 2 at position 76. The molecular abnormalities found in factor I gene led to a missense mutation located in exon 5 (A222G) and exon 6 (G243D) in patient Nos 17 and 18, respectively. Patient No 7 was compound heterozygous for two mutations located in exon 5 at position 181 (V181M) and in exon 11 which codes for the MCP transmembrane domain at position 304 (A304V).

Factor H, factor I or MCP mutations were detected in 4/6 patients in the glomerulonephritis C3 without MPGN group compared with 2/13 patients in the glomerulonephritis C3 with MPGN group (table 3). None of the mutations was detected in 100 normal individuals from the same ethnic background.

DISCUSSION

In the present study, we have reported a series of 19 patients with glomerulonephritis C3, associated with genetic abnormalities of the regulation of the alternative pathway (AP) in 70% of cases, and which may have a severe outcome.

Regulation of CAP activation depends on a complex system of circulating and/or membrane bound factors, including factor H, factor I and MCP. This regulatory process takes place both in the fluid phase and at the cell surface level, preventing systemic activation of the CAP and complement induced cell lesions, including renal endothelial cells. Several studies have established that uncontrolled activation of the CAP, as a result of factor H, factor I or MCP gene defects or anti-factor H antibodies, is a risk factor for HUS,[6 12–15] a disease characterised by damage to endothelial cells, erythrocytes and kidney glomeruli, probably through reduced complement regulatory activity at the endothelial cell surface.[19] In some rare forms of HUS, mesangial C3 deposits were reported.[11] Additional mutations with homozygous factor H deficiency have been associated with MPGN II.

We have observed several cases of an unusual primary glomerulonephritis characterised by isolated mesangial C3 deposits manifest on immunofluorescence. Some patients had typical features of type I membranoproliferative glomerulonephritis (GN C3 with MPGN) and others had mesangial and epimembranous C3 deposits in the absence of mesangial proliferation (GN C3 without MPGN). The recurrence in renal allografts of identical lesions to those in the native kidney proves the existence of a specific entity. Our data indicate that patients with C3 deposits can be divided in two distinct groups based on:

(a) renal pathology features which distinguish patients with typical type I MPGN (glomerulonephritis C3 with MPGN) from those with glomerulonephritis C3 without MPGN (that is, mesangial and epimembranous C3 deposits in the absence of mesangial proliferation). Renal disease tended to be more severe in patients in the first group.

(b) the type of CAP dysregulation that is associated with these glomerular nephropathies. Factor H, factor I or MCP mutations were more frequent in glomerulonephritis C3 without MPGN compared with glomerulonephritis C3 with MPGN patients in which C3NeF was more frequently detected.

C3NeF is a circulating autoantibody that prolongs the half life of the CAP C3 convertase with increased resistance to factor H. The high incidence of C3NeF (and hence the high frequency of decreased C3 levels) in patients with glomerulonephritis C3 with MPGN is consistent with previous reports of a high incidence of C3NeF in type II MPGN[1 24] as well as in type I (42%) and type III MPGN (50%). The high incidence of C3NeF in our series is of interest. However, the direct pathogenic effect of C3NeF is a matter of debate and it could be an epiphenomenon. Interestingly, in patient No 7, C3Nef was associated with a mutation in factor H, which suggests that these abnormalities may coexist and that unusual types of factor H mutations may increase the risk of the occurrence and/or persistence of C3NeF.

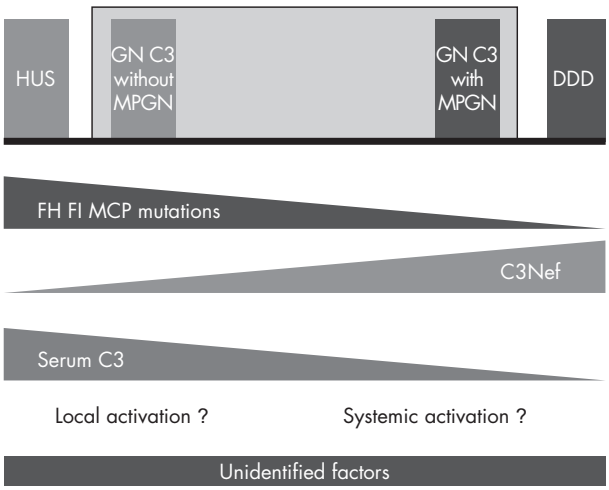

Figure 3 Schematic representation of the expanding spectrum of renal diseases associated with an abnormal control of the complement alternative pathway. The two types of glomerulonephritis reported in this study are defined by the presence of isolated C3 deposits (and no Ig), either glomerulonephritis with isolated C3 deposits with membranoproliferative glomerulonephritis (GN C3 with MPGN) or GN C3 without MPGN. Patients with haemolytic uraemic syndrome (HUS) and dense deposits disease (DDD) were excluded from this study. GN C3 without MPGN shares risk factor with HUS, namely mutations; GN C3 with MPGN, although different from DDD by the absence of dense deposits, was often associated with C3NeF. FH, factor H; FI, factor I; MCP, membrane cofactor protein; C3NeF, C3 nephritic factor.

Experimental data support a role for factor H mutations in the occurrence of C3 deposits.[9] Cfh deficient mice, created by gene targeting in which factor H gene has been inactivated (factor $H^{-/-}$), develop a type of glomerulonephritis C3 with MPGN. The introduction of a second mutation into the gene encoding complement factor B prevents C3 turnover in vivo and obviates the development of glomerulonephritis C3. Conversely, none of the heterozygous deficient (factor $H^{+/-}$) mice had histological evidence of glomerulonephritis C3 even though plasma C3 levels were depressed, which suggests that in this particular model factor H haploinsufficiency impairs normal C3 convertase control mechanisms but not complement regulatory activity at the cell level. Interestingly, Pickering *et al* have shown that prevention of C5 activation ameliorates glomerulonephritis in these mice.[25]

In humans, homozygous factor H deficiencies have been reported previously in rare cases of type II MPGN.[1 5 6] Heterozygous factor I deficiency was reported in more than 10 cases with sporadic HUS[17 22] and, interestingly, in one case report of immune complex glomerulonephritis with glomerular deposits of immunoglobulin and C3.[26] To date, MCP mutations have been published in more than 40 patients with familial HUS pedigrees[27–29] and no mutation in MCP has been associated with glomerulonephritis.

The functional consequences of three of seven mutations have been clearly demonstrated or are highly suspected based on previous reports of these mutations in HUS patients and/or the results of functional tests and mutagenesis experiments and/or the location of these mutations in well conserved proteins domains with well established physiological roles. More than 100 genetic mutations within the CFH, IF or MCP have been reported previously in patients with atypical HUS and most frequently the disorder either destabilises the structure and is associated with a quantitative deficiency (type I) or interferes with the functional activity of the proteins (type II). Mutation R1210C, reported in more than five pedigrees with atypical HUS, is associated with a reduced binding of factor H to surface attached C3b molecules and reduced complement regulatory activity at the cell surface.[10 19] Several short deletions of one nucleotide in the factor H gene have been associated with heterozygous factor H deficiency in a patient with atypical HUS. Recently, Caprioli *et al*[21] reported mutation A304V in one patient with a sporadic form of atypical HUS and perhaps an inefficient insert into the lipid bilayer in normal cells. Functional studies were not available for the three new heterozygous mutations. These mutations were not found in over 100 healthy controls, which excludes the fact that the mutations may represent polymorphisms. Most likely these change may also impair the capacity of the regulation of the AP.

Our data show that HUS and glomerulonephritis C3 share common genetic susceptibility factors and that acquired or constitutional uncontrolled activation of the CAP leads to various diseases, ranging from HUS to glomerulonephritis C3 (fig 3).

Our results suggest that C3NeF leads to systemic uncontrolled CAP activation, as represented by the C3 consumption noted in patients with glomerulonephritis C3 with MPGN. Conversely, patients in the glomerulonephritis C3 without MPGN group with factor H or factor I mutations had normal C3 (except for patient No 7 with a factor H heterozygous deficiency) which suggests that mutations observed in CAP regulatory genes lead to tissue restricted uncontrolled CAP activation causing C3 deposition, usually in the absence of systemic activation. The difference in CAP activation patterns may explain the wide spectrum of renal diseases associated with CAP dysregulation. Interestingly, in three patients, C3 levels were decreased in the absence of detectable C3Nef or factor H, factor I and MCP mutations. Thus other yet unidentified genetic or acquired factors may lead to abnormal CAP regulation and renal disease. For example, complement component deficiencies were found with significantly higher frequency among patients with MPGN type I and III in the study of Coleman *et al*.[30]

In summary, glomerulonephritis C3 should be added to the expanding spectrum of diseases associated with factor H, factor I and MCP genes mutations. Thus genetic screening is required in patients with glomerulonephritis C3, regardless of the level of circulating C3. The evolution of glomerulonephritis C3 is highly unpredictable, with 15% of our patients reaching end stage renal disease. To date, there is no treatment that has proven efficacy in these patients. The detection of C3NeF and factor H, factor I or MCP mutations in some of our patients suggests that new therapies specifically aimed at controlling CAP activation may represent a possible treatment option for glomerulonephritis C3.

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