

# Hereditary and acquired complement dysregulation in membranoproliferative glomerulonephritis

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## Summary

Membranoproliferative glomerulonephritis (MPGN) is a chronic progressive renal disease that is diagnosed on the basis of renal histological features. Several MPGN subtypes have been defined by the localization and composition of glomerular deposits (electron dense, Ig and C3). MPGN II or dense deposit disease (DDD) which is defined by the occurrence of electron dense deposits within the lamina densa of the glomerular basement membrane (GBM) is strongly associated with dysregulation of the alternative complement pathway (AP). However, C3 Nephritic Factor (C3NeF), an autoantibody against the alternative C3 convertase C3bBb, and mutations in regulatory proteins of the AP have also been identified in other subtypes of

MPGN and even in glomerulonephritis with mesangial C3 deposits. Clinically, MPGN is characterized by proteinuria (up to nephrotic range) and hypertension, frequent progression to end-stage kidney disease and disease recurrence after renal transplantation. The age of onset varies from childhood to adulthood. In the following we will review our current knowledge of pathogenesis of MPGN and will present a novel classification system of the disease based on pathogenesis rather than on morphology. A better understanding of the pathogenesis of MPGN is crucial for the development of novel, specific treatment strategies.

## Keywords

Membranoproliferative glomerulonephritis, haemolytic uremic syndrome, complement system, C3 deposition, glomerulonephritis C3 (GNC3), C3 deposition glomerulopathy (C3DG), Factor H (CFH)

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## Introduction

Membranoproliferative glomerulonephritis (MPGN) comprises a morphological spectrum of related but likely pathogenetically distinct disorders which are characterized by glomerular hypercellularity, increased mesangial matrix, and thickening of the peripheral capillary walls. The hallmark of MPGN is functional impairment of the glomerular basement membrane (GBM) with progressive loss of renal function eventually resulting in end stage kidney disease (ESKD). Clinical features at first manifestation are haematuria, (nephrotic range) proteinuria, impaired renal function, and hypertension (1).

A classification of the histological pattern of MPGN based on glomerular findings by light microscopy (LM) with further

specification by electron microscopy (EM) and staining for immunoglobulins (Ig) and complement components has been classically used during the last 30 years. All subtypes are characterized by mesangial cell proliferation and capillary wall thickening. MPGN I or MPGN with isolated C3 deposits are distinguished by subendothelial deposits of IgG and C3 or isolated C3 deposits, respectively. By contrast, MPGN II or dense deposit disease (DDD) is characterized by electron dense deposits within the lamina densa of the glomerular basement membrane (GBM). MPGN III with subendothelial, mesangial and subepithelial IgG and C3 deposits is very rare.

Recent progress in the understanding of the pathogenesis of MPGN, however, suggests that reclassification of MPGN based on pathogenesis rather than on morphology may be appropriate.

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A “membranoproliferative pattern of injury” is idiopathic or is found in a spectrum of diseases from immunocomplex mediated disorders (e.g. MPGN I and III) and paraprotein deposition diseases (e.g. cryoglobulinaemia type I). MPGN II/DDD seems to frequently result from impaired complement control, specifically from unrestricted activity of the alternative complement pathway (AP) C3 convertase C3bBb and has been suggested representing an independent disease entity (1, 2). Walker et al. highlight that the essential diagnostic feature of DDD is not the membranoproliferative pattern but the presence of electron dense transformation of GBM. Identification of acquired or hereditary defects in the regulation of the AP in all different MPGN subtypes suggests replacing the traditional, morphology based MPGN classification by a novel, pathophysiology based classification system.

Information about the prevalence of MPGN is rare and differs depending on age and country. In general, the overall incidence of MPGN is assumed to be 3-5 per million and seems to decrease in developed countries but to remain high or even increase in developing countries for which the pathogenetic role of hepatitis C for MPGN I may be contributory (3). The age of onset is variable between childhood and adulthood. Clinically, MPGN is characterized by proteinuria (up to nephrotic range) and hypertension, and frequently progression to ESKD and disease recurrence after renal transplantation. In patients with pediatric onset the median age at disease onset is about 10 years. Half of the patients present with nephrotic syndrome, the others with mild proteinuria, about 20% with macrohaematuria. About one third of the patients present with hypertension. The prognosis of MPGN – irrespective of the subtype – is unfavourable, and ESKD may develop during late childhood or early adolescence (4).

In the following, we will review our current knowledge of MPGN associated with acquired or hereditary complement abnormalities and will present a novel classification system.

## Pathogenesis of MPGN associated with complement deficiencies – lessons from animal models

A link between dysregulation of the alternative complement pathway and the pathogenesis of MPGN has been appreciated for some 30 years with the finding of complement activation with low serum C3 levels and the identification of the C3 nephritic factor (C3NeF), an autoantibody of the alternative C3 convertase C3bBb (5). In further support of a complement-based pathogenesis of MPGN is information derived from animal models as summarized in the following (Table 1).

### Naturally occurring CFH-deficient piglets

In 1995 Jansen et al. and Hogasen et al. reported a Norwegian Yorkshire piglet breed which died within 11 weeks after birth from renal failure. Histomorphological examination of the kidneys by LM and EM revealed a phenotype identical with human MPGN II/DDD including glomerular hypercellularity, expansion of mesangial matrix, and linear deposition of electron-dense material within the lamina densa of the GBM which was preceded by deposits in the subendothelial space (6–8). Animals showed strong activation of the complement system resulting in

severely decreased plasma C3 levels, and massive glomerular C3 deposition was found by immuno-fluorescence (IF). In addition, immuno-electron microscopy (IEM) identified both C3 and terminal complement complex (TCC: C5b-9) within the lamina densa of the GBM (8).

Investigation of the cause of complement activation revealed deficiency of the 150 kDa Factor H (CFH) in plasma Western blotting and enzyme immunoassay (7). Genetic analyses identified two single nucleotide exchanges in position C1590G and T3610G of the coding region of CFH resulting in amino acid exchanges of non-framework residues L493V and I1166R within short consensus repeats (SCRs) 9 and 20 of CFH, respectively. While these mutations cause single amino acid exchanges they do not alter the overall protein structure and allow for expression of the normal size CFH within hepatocytes. However, protein release to plasma is prevented, and loss of the highly conserved Isoleucine in position 1166 within SCR20 was considered responsible (9).

In further support of a causative role of complement dysregulation caused by CFH deficiency for the pathogenesis of MPGN II/DDD, infusion of normal porcine plasma (20–30 ml/kg weekly) to replace for the deficient protein resulted in a decline of circulating TCC, an increase of plasma C3, and an increased animal survival (7).

### Genetically engineered mice

#### Mice deficient in CFH

Different from piglets, CFH knock-out mice were genetically engineered by disrupting the gene encoding CFH (*Cfh*<sup>-/-</sup>) in embryonic stem cells by gene targeting (10). As in naturally occurring CFH-deficient piglets, plasma C3 levels were markedly reduced, and the majority of C3 was converted to C3b in both *Cfh*<sup>-/-</sup> and +/- mice (10). However, compared to wild-type and heterozygous animals, only *Cfh*<sup>-/-</sup> mice developed haematuria and proteinuria and died significantly earlier. Renal histology showed typical findings of MPGN including mesangial hypercellularity, expansion of mesangial matrix, and thickening of the peripheral capillary loops. Ultrastructural analysis revealed a double-contour appearance of the GBM caused by mesangial cell interposition and electron-dense deposits in the subendothelial space (by IEM identified as C3 and C9) with the formation of a new basement membrane on the endothelial side of the deposits. Different from *Cfh*<sup>-/-</sup> piglets, however, no deposits were found within the lamina densa of the GBM (10). Thus, morphological findings in *Cfh*<sup>-/-</sup> mice were different from naturally occurring CFH-deficient piglets, however, showed similarity with human MPGN II/DDD since glomerular C3 deposition occurred in an IgG independent fashion.

#### Mice deficient in CFH and Factor B (*Cfh*<sup>-/-</sup>*Bf*<sup>-/-</sup>)

To further elucidate the pathomechanisms eventually resulting in MPGN mice deficient in both CFH and Factor B (*Cfh*<sup>-/-</sup>*Bf*<sup>-/-</sup>) were generated. Soluble Factor B (CFB) attaches to surface bound C3b, becomes activated by Factor D (CFD) resulting in Bb. Stabilized by properdin, C3b and Bb form the alternative pathway C3 convertase C3bBb. Gain of function mutations of CFB resulting in decreased C3bBb decay have recently been identified in familial haemolytic uraemic syndrome (HUS) (11).

**Table 1: Animal models of MPGN.** CFH: complement Factor H; CFI: complement Factor I; CFB: complement Factor B; GBM: glomerular basement membrane; IEM: immuno-electron microscopy.

Animal model	Renal impairment	Localization of C3 deposits	Plasma C3 levels
<b>CFH knock-out piglets</b>	Yes	Electron-dense material and C3 deposits within GBM	Severely decreased C3 levels and absence of CFH protein
<b>Mice deficient in CFH (<i>Cfh</i><sup>-/-</sup>)</b>	Yes	Electron-dense deposits in the subendothelial space (by IEM identified as C3 and C9) No deposits within the lamina densa of the GBM	Severely decreased C3 levels and absence of CFH protein
<b>Mice deficient in CFH and CFB (<i>Cfh</i><sup>-/-</sup>/<i>Bf</i><sup>-/-</sup>)</b>	No	No subendothelial nor GBM deposits	Normal C3 levels
<b>Mice deficient in CFH and C5 (<i>Cfh</i><sup>-/-</sup>/<i>C5</i><sup>-/-</sup>)</b>	Yes	C3 deposition along GBM *Reduction in glomerular hypercellularity as compared to <i>Cfh</i> <sup>-/-</sup> mice	Severely decreased C3 levels and absence of CFH protein
<b>Mice deficient in CFI (<i>Cfi</i><sup>-/-</sup>)</b>	No	Increased mesangial expansion and C3 staining No C3 deposition along GBM No renal impairment	Decreased C3, CFB and CFH levels
<b>Mice deficient in CFH and CFI (<i>Cfh</i><sup>-/-</sup>/<i>Cfi</i><sup>-/-</sup>)</b>	No	No C3 deposition along GBM	Decreased C3 and CFB levels

In further support of a complement-mediated pathogenesis of MPGN, *Cfh*<sup>-/-</sup>/*Bf*<sup>-/-</sup> mice showed normal plasma C3 levels, and neither glomerular C3 deposition nor subendothelial electron-dense deposits were found. In addition, in these animals both renal function and renal histology were normal and not different from wild-type mice (10).

#### Mice deficient in CFH and C5

While results obtained in *Cfh*<sup>-/-</sup>/*Bf*<sup>-/-</sup> mice support a crucial role for the alternative C3 convertase C3bBb and the excessive cleavage of C3 in the pathogenesis of MPGN, the importance of factors downstream of C3 in the complement cascade is less clear. In order to clarify the contribution of complement factor C5, mice deficient for CFH and C5 (*Cfh*<sup>-/-</sup>/*C5*<sup>-/-</sup>) were generated (12). These animals showed a reduction in glomerular hypercellularity and had improved renal function and survival (12). Absence of C5, however, did not prevent C3 deposits along the GBM (12). This finding supports that GBM changes in MPGN are secondary to the presence of activated C3 while complement factor C5 seems to be important for the glomerular inflammatory response (12).

#### Mice deficient in CFH and Factor I (*Cfh*<sup>-/-</sup>/*Cfi*<sup>-/-</sup>)

Crucial for the regulation of the conversion of the central complement factor C3 to its active form C3b – a step which is mediated by the alternative complement pathway C3 convertase C3bBb – are the two plasma proteins Factor I (CFI) and CFH with CFH being cofactor of CFI. However, even though loss of function of either protein results in uncontrolled progression of the activation cascade of the alternative complement pathway resulting in the consumption of the plasma C3 pool, only CFH deficiency is known to cause a MPGN phenotype (10, 13). *Cfi*<sup>-/-</sup> mice did not develop spontaneous glomerulonephritis (GN) but, at eight months, demonstrated increased mesangial expansion

and increased mesangial C3 staining without deposition of C3 along the GBM. However, there was no evidence of renal impairment or GN, including MPGN II/DDD. It is notable that mesangial deposits of C3 in the absence of mesangial proliferation have recently been reported in patients with heterozygous mutations affecting either CFH or CFI (14).

Even more remarkable, mice with the combined deficiency of CFH and CFI (*Cfh*<sup>-/-</sup>/*Cfi*<sup>-/-</sup>) do not present with C3 deposition along the GBM (13). However, when supplemented with CFI containing serum, C3 plasma levels of *Cfh*<sup>-/-</sup>/*Cfi*<sup>-/-</sup> mice dropped, C3  $\alpha$ -chain fragments appeared in the circulation and subsequently (within 72 hours) glomerular C3 deposition occurred similar in pattern as obtained in *Cfh*<sup>-/-</sup> mice (13).

These observations support the hypothesis that in the pathogenesis of MPGN the C3b inactivating activity of CFI is inevitably required and only the presence of C3 metabolites in the circulation results in glomerular C3 deposition eventually creating the MPGN phenotype. Since this was found even in the absence of CFH the question about an additional cofactor for CFI – different from CFH – remains still open.

Taken together, over the past years animal models tremendously contributed to advance our understanding of MPGN pathology. Especially the elegant studies in genetically engineered mice by Pickering et al. clarified the key role for fluid phase complement control mainly via CFH and CFI and guided our understanding of the role of plasma C3 and C3-split products for the development of glomerular deposits. While minor phenotypical differences between murine and human glomerular immunohistopathology seem to be acceptable, observations of the appearance of GBM dense deposits prior to detectable C3 deposition are not in keeping with the established causal order of events leading up to glomerular pathology in MPGN indicating that our understanding of the details of the disease pathomechanism is still incomplete and requires further elucidation.

## The role of the alternative complement pathway (AP) for the pathogenesis of MPGN

### Hypocomplementaemia and C3 in patients with MPGN

The AP is in a continuous state of activation with several control mechanisms. Normal control involves at least seven proteins, three of which are present in serum (CFH, CFI, and C4 binding protein [C4BP]), and three of which are cell membrane-associated (membrane cofactor protein [MCP, CD46], decay accelerating factor [DAF, CD55]) and complement receptor 1 [CR1, CD35]). MPGN was originally designated as a form of chronic GN with persistent hypocomplementaemia. However, while C3 is always seen by immunofluorescence microscopy (IF), systemic AP activation as reflected by low C3 and normal C4 levels is a frequent but not an obligatory marker. MPGN II/DDD is a rare disease characterized by the deposition of abnormal electron-dense material within the GBM of the kidney and – interestingly – also often within Bruch's membrane in the retina of the eye (16). The AP is frequently but not permanently activated at high level during the follow up of the disease. In a retrospective study of 75 patients with MPGN II/DDD the serum C3 level was moderately decrease at the time of diagnosis in 95% of patients, and severe depression of C3 levels (<20 mg/dl) was present in 20% of patients (17). At the opposite in the series of Little et al. only 29% of patients with MPGN II/DDD showed at the time of diagnosis a low C3 level (18, 19). Several papers described fluctuation of C3, but there is no clear information on the persistence of hypocomplementaemia during the follow-up. In the series of Schwartz et al. during follow-up, C3 levels were persistently normal in 18% of the patients with MPGN II/DDD, and continuously low C3 levels did not have any prognostic value for the clinical outcome.

MPGN I is rare and its morphologic hallmark is the presence of deposits mainly situated at subendothelial sites in the glomerular capillaries. Two main sub-types of MPGN I are recognized based on deposit composition via IF (20). The most common variant, MPGN I, is characterized by the predominant presence of subendothelial immune complexes with deposits containing immunoglobulins and classical pathway components. MPGN I can be idiopathic or more frequently secondary to known causes, i.e. infections, systemic immune or chronic liver diseases. Conversely, the term MPC3 refers to a MPGN subtype with isolated C3 deposits at subendothelial sites without evidence of IgG deposits. MPC3 has no known association with immunocomplexes and is most frequently idiopathic. Deposits exclusively containing complement C3 without immunoglobulins are highly suggestive of AP activation. Thus, except for the location of the deposits, marked differences in etiology and pathogenesis between MPGN I and MPGN C3 exist.

MPGN III is considered a variant of MPGN I in which there are changes in the capillary wall similar to those of membranous nephropathy, that is, epimembranous deposits with interspersed projections of basement membrane material. However, secondary forms of MPGN III are not observed. As most of the studies were performed before the etiological importance of hepatitis C virus (HCV) was appreciated, the frequency of hypocomplemen-

taemia in patients with MPGN I is unclear but thought to be less than 50%.

Recently, Servais et al. reported 19 cases of GN with isolated C3 deposits characterized by exclusive or predominant mesangial C3 deposits in the kidney and impairment of kidney filtration referred to as glomerulonephritis C3 (GNC3) or – as also suggested – C3 deposition glomerulopathy (C3DG) (21). Patients were divided into two groups based on renal pathology findings. In group I (n=13), renal biopsies disclosed typical features of MPGN I with isolated C3 deposits (MPC3). In group II renal biopsies disclosed a peculiar pattern of mesangial and epimembranous deposits (MESC3) without mesangial proliferation. Five out of 13 patients in the MPC3 group had low C3 levels compared to 2/6 patients in the MESC3 group. In one case of MPC3, a very low C3 level was noted at the time of diagnosis, while C3 was normal 10 years later. Taken together, these findings suggest that MESC3 is a type of GN which is associated with AP dysregulation.

### Acquired and genetic abnormalities in patients with MPGN

So far four distinct scenarios, all of them leading to complement dysregulation have been linked to the three forms of MPGN and to GNC3 without MPGN: the presence of the autoantibody C3 Nephritic Factor (C3NeF), complete CFH deficiency due to mutations in the CFH gene, an anti-CFH autoantibody in the context of lymphoproliferative disease or, recently, heterozygous mutations in complement regulatory genes leading to quantitative or functional deficiency in AP control (22).

### C3 Nephritic Factor

First reported in MPGN patients, but not specific for this disease, C3 Nephritic Factor (C3NeF) is an IgG autoantibody which binds the AP C3 convertase C3bBb thus preventing the normal decay of this enzyme. C3NeF stabilizes both the fluid phase and membrane bound C3 convertase. C3NeF has been shown to be responsible for chronic C3 consumption secondary to an increase in the half-life of C3bBb and can, thus, bypass all the other mechanisms involved in the regulation of C3bBb (5, 23).

As C3NeF was also found in MPGN patients without hypocomplementaemia and in healthy subjects, it was suggested that not all C3NeF are identical and that they may have different target epitopes, although functional studies are scanty (24). For example, C3 consumption in MPGN I patients has been found to be much smaller than expected when caused by C3NeF. However, in the same patients decreased C5-levels suggested also stabilization of the properdin-dependent C3/C5 convertase (C3Bb2BbP) (25). In contrast, an *atypical* C3NeF which stabilizes cell-bound C3 convertase but has only a weak effect on the fluid phase C3 convertase has been suggested in patients who presented with normal C3 levels (26).

After the initial description of one patient with a case of acute GN subsequent to a skin infection, a nephritic factor of the classical pathway (C4NeF), which stabilizes the classic pathway C3 convertase C4b2a was found in 20% of patients with hypocomplementemic MPGN alone or in combination with C3NeF (27, 28). However besides these reports, no further information is available on the prevalence of this autoantibody.

The reason for genesis of nephritic antibodies is unknown. Typical dense deposits have been reported in a consanguineous Turkish family in whom both the presence of C3NeF and a homozygous deletion of a single lysine residue within SCR4 associated with impaired C3 regulatory capacity was identified (29). Interestingly, C3NeF has also been found in three siblings presenting with different (or none) degrees of renal disease suggesting an additional genetic background for the generation of this antibody (30). The functional impact of genetic variants may be to alter the kinetics of complement regulation or to expose novel epitopes that facilitate formation of nephritic antibodies, however no study has addressed this point so far.

Loss of AP control seems to be crucial for the pathogenesis of MPGN lesions. However, neither plasma C3 levels nor presence or absence of C3NeF correlate with the clinical course of the disease or have predicative value in identifying a recurrence risk of the disease in renal transplants. It has been suggested that C3NeF should only be considered as marker for some forms of MPGN (31). The role of C3NeF in causing nephritis in these patients and its contribution to the chronic and progressive nature of the disease is unknown.

In most patients with MPGN II/DDD, loss of complement regulation is likely caused by C3NeF. Two independent series of patients with MPGN with only C3 deposits and idiopathic MPGN I showed the presence of C3NeF at the time of the investigation, in 5/13 patients (38%) and in 11/26 patients (42%), respectively (14, 32). On the other hand, it would not be of surprise if C3 consumption was secondary to the activation of the classical pathway in cases of MPGN associated with immune complexes. In this context the presence of C3NeF has been noted but both frequency and pathogenetic role remain unclear. In a study reported by West et al., subendothelial deposits in MPGN III were also found to be closely associated with nephritic factor; they were present in 14/26 (54%) patients with hypocomplementaemia but in none of the 18 patients with normocomplementaemia (33).

C3NeF is also found in approximately 83% of patients with acquired partial lipodystrophy (APLD), many of whom associated with low C3 levels. APLD is a rare disease with loss of fat tissue typically of the upper half of the body. The prognosis is mainly determined by renal insufficiency due to MPGN which develops after a median of approximately eight years in less than 20% of patients and presents with variable histological and electron microscopical findings. Glomerular dense deposits at both locations, intramembranous and subendothelial, have been reported in patients with APLD (review in [34]). Mathiesen provided an explanation for this association demonstrating that adipocytes are lysed in the presence of the nephritic factor. The precise mechanism, however, is not yet known.

### Homozygous CFH deficiency

Although rare, hereditary CFH deficiency is associated with a high risk to develop a renal disease, in particular MPGN. CFH is a single-chain serum glycoprotein of 150 kD with a modular structure consisting of a tandem array of 20 homologous units of about 60 amino acid residues each, called short consensus repeats (SCR). Three C3-binding sites have been identified; one in SCRs 1–4 which binds intact C3b, and two in SCRs 6–10 and

SCRs 16–20 which bind C3c and C3d fragments, respectively. In addition, three heparin-binding sites have been identified in SCRs 7, 13 and 20. The gene encoding for CFH is localized on the long arm of chromosome 1 at 1q32, a locus called RCA (regulators of complement activation) which contains genes encoding different regulatory proteins of complement activation. CFH is a plasma protein produced mostly by the liver that plays a central role in the regulation of the AP both in fluid phase and on cellular surfaces by binding to C3b thus destabilizing C3bBb. In fluid phase this interaction results in dissociation of C3bBb and in irreversible inactivation of C3b to iC3b by CFI. On cell surfaces CFH competes with CFB to bind to C3b targeted surfaces and also favors the degradation of bound C3b by cofactor activity.

Complete CFH deficiency is associated with severely decreased C3 and CFB. The diagnosis is made by the quantification of circulating CFH, which is undetectable. In the literature four families have been reported with complete CFH deficiency and MPGN (review in [22]). CFH deficiency was due to homozygous CFH mutations (three cases) or compound heterozygous mutation (one case). Levy et al reported the first case of CFH deficiency in two Algerian brothers who had early-onset glomerulonephritis with segmental MPGN II/DDD with an atypical pattern with abundant granular C3 deposits within the mesangium and along the capillary walls on IF (35). The genetic abnormality was a homozygous C431S located in SCR7 (36).

Glomerular deposition of fibrillar collagen a characteristic finding for collagen type III glomerulopathy has been reported in one individual with a complete CFH deficiency and two heterozygous mutation involving a cysteine in SCRs 9 (C518R) and 16 (C991Y) (37, 38). Schmidt et al. subsequently demonstrated that the 150 kDa protein was retained in the intracellular fraction (39). The cause and pathogenesis of this idiopathic glomerular disease and the implication of complement are entirely elusive. Most surprisingly, one patient who had a C673S homozygous mutation presented at eight years of age with nephritic syndrome secondary to MPGN I without immune complex disease (36). No histological information was available for the two brothers of a Turkish family who presented with a R127L homozygous mutation (36).

Most of the genetic abnormalities found involved a cysteine residue (4/5). All four cysteines affected are involved in the formation of disulfide bonds, and mutations involving these residues would impede the formation of intrachain disulfide bonds leading to the change of the tertiary structure of CFH.

In contrast, to our knowledge, no case of MPGN has so far been reported in patients with uncontrolled AP activation on the background of complete CFI deficiency.

### Heterozygous CFH, CFI and MCP gene mutations

A connection between heterozygous mutations in complement regulatory proteins was recently made by Servais et al. (14). They reported two cases of MPGN I with only C3 deposition and a heterozygous mutation in the CFH (G650V in SCR11) or a double heterozygous mutation in MCP (V181M and A304V) gene. They have identified for the first time several cases of a peculiar primary GN characterized by isolated mesangial C3 deposits in the absence of mesangial proliferation on the back-

ground of heterozygous mutations in CFH (R1210C and P76X) and FI (A222G and G243D) genes. Three genetic abnormalities are clearly associated with complement dysregulation. The A304V mutation rendered MCP unable to control the AP on cell surface (40), the R1210C mutation resulted in impaired CFH protection of host surfaces against complement activation (41), and finally short deletion of one nucleotide in the CFH gene is associated with heterozygous CFH deficiency (36). These findings suggest that the disease is caused by a defect in local complement regulation with formation of complement deposits in the kidney. However, genetic studies demonstrated that heterozygous mutations of MCP, CFH or CFI predispose for atypical HUS (aHUS) (42). Thus, aHUS and C3GN (C3DG) share common genetic susceptibility factors and acquired or constitutional factors resulting in uncontrolled activation of the AP resulting in a phenotypical spectrum.

### Anti CFH autoantibodies

An unique monoclonal Ig lambda light chain dimer (protein LOI) was isolated from the serum and urine of a patient with hypocomplementemic MPGN (43). *In vitro* the lambda light-chain dimer efficiently activated the AP. The antibody was found to bind to the SCR3 of CFH and thereby inhibit the C3 convertase in fluid phase. While CFH antibodies recognizing the CFH C-terminus have been identified in several cases of aHUS patients (44, 45), there is no report of this type of antibodies in MPGN patients.

### Multiple genetic predispositions for MPGN?

MPGN accounts for less than 20% of patients with complete CFH deficiency. The majority have developed atypical forms HUS with an early onset of the disease. The reason why some individuals with homozygous CFH deficiency develop aHUS, MPGN, other glomerular diseases or remain free of any apparent disease, is still unclear. However, reports regarding healthy CFH-deficient subjects as well as the spectrum of clinical manifestation indicate that other genetic or environmental factors, including micro-organisms or drugs, probably play an additional role in the initiation and/or the expression of the disease. In this regard, an increased prevalence of the C3 haplotype and later of a common CFH variant, H402Y, that has been shown to be a susceptibility factor for age-related macular degeneration (AMD), or of variants of Factor H related protein 5 (CFHR5) have been found in association with MPGN II/DDD, although their functional implication is still unknown (46).

### The novel point of view: A disease spectrum defined by complement dysregulation

Complement regulation is essential for the integrity of kidney cells and particularly the GBM. There is evidence of a link between C3GN (C3DG) and defective complement control, particularly caused by fine defects in local complement regulation or by uncontrolled activation of the AP. According to Pickering et al. we propose to regroup the renal pathology associated with C3 deposition and evidence of complement dysregulation (22).

C3GN (C3DG) (21) may refer to the patients with MPGN with isolated C3 deposits (C3 MPC3) characterized by isolated granular C3 deposits in the mesangium and along the GBM; cases with C3 deposits in the lamina densa of the GBM may be referred to as MPGN II/DDD; and the rare cases of patients characterized by mesangial and epimembranous C3 deposits in the absence of mesangial proliferation may be referred to as GN with mesangial C3 deposits (MESC3). As no major differentiating features in the clinical presentation and in the frequency of AP abnormalities between MPGN I and the other subtypes have been reported, we propose to include the group of patients with MPGN I among the group of C3GN (C3DG).

After a period of publication quiescence, MPGN II/DDD has recently been the focus of several papers. One of these papers was a study of the histology of 81 cases of MPGN II/DDD around the world; the authors reported that only 25% of biopsies had a membranoproliferative pattern on LM, whereas the incidence of mesangial hypercellularity was much higher (2). Based on this observation it seems important to reconsider the histological description of the disease, and Walker et al. propose that cases with presence of electron dense deposits within the GBM should be reclassified as DDD in the overall scheme of glomerular disease (2). The role of complement in these several histological patterns remains unknown.

### Treatment perspectives

Treatment options for patients with all subtypes of MPGN are scarce. While in idiopathic cases of MPGN I immunosuppressive treatment (e.g. steroids) seems to be beneficial, in MPGN II(DDD – besides supportive measures addressing proteinuria and/or hypertension using angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor type 1 (ARB) blockers – treatment recommendations do not exist. Different from other GNs, steroids do not seem to be successful in MPGN II/DDD.

The understanding of MPGN II/DDD as complement-mediated disease, however, offers novel and promising treatment options. Patients with a mutation in one (or more) complement regulatory proteins resulting in the deficiency or a functional defect with subsequent over-activation of the complement system, especially the AP, can be treated with plasma infusions or the purified or recombinantly expressed pure missing or defective protein to substitute for its missing function (21, 47). Patients with an antibody (e.g. C3NeF) would benefit from plasma exchange or plasma pheresis or the use of immunosuppressants (e.g. the anti-CD20 antibody rituximab). Finally, antibodies directed against certain components of the complement cascade may be beneficial through their potential to prevent the activation cascade from progressing. Eculizumab, a humanized monoclonal anti-C5 IgG antibody is such an antibody which in the past has successfully been used in the treatment of paroxysmal nocturnal haemoglobinuria (PNH) in adults with minimal side effects only (48, 49).

In addition, sulodexide combination of a low-molecular-weight heparin (~80%) and a dermatan sulphate (~20%) with profibrinolytic and antithrombotic function is considered a therapeutic option in MPGN II/DDD. Sulodexide inhibits he-

paranases – glycosidases which cleave glucosaminoglycan side chains of GBM and podocyte membrane proteins and are increased in GBMs of MPGN II/DDD patients – thus improving both GBM filter function and CFH binding. A beneficial effect has been found in thrombotic conditions and in diabetic nephropathy (3).

Except for plasma infusion none of these treatment conditions has been used in prospective treatment trials in children, yet. Thus, no treatment recommendations for children with MPGN II/DDD other than supportive therapy exists to date, and prospective treatment studies are desperately needed.

In conclusion, the outcome of excessive AP activation is diverse, and our understanding of the exact mechanisms by which complement abnormalities lead to specific disease phenotypes is still poor. More in depth analyses are required for a better understanding of the pathogenesis and, thus, the development of specific therapies.

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## Abbreviations

AMD, age-related macular degeneration; AP, alternative complement pathway; APLD, acquired partial lipodystrophy; atypical HUS, atypical haemolytic uraemic syndrome; C3bBp, alternative pathway C3 convertase; C3Bb2BbP, C3/C5 convertase; C3DG, C3 deposition glomerulopathy; C3NeF, C3 nephritic factor; C4NeF, C4 nephritic factor; C4b2a, classical pathway C3 convertase; C4BP, C4 binding protein; Complement regulatory proteins: CFB, Factor B; CFH, Factor H; CFI, Factor I; CR1, Complement receptor 1; DAF, Decay accelerating factor; EM, electron microscopy; IEM, immuno-electron microscopy; IF, immunofluorescence microscopy; LM, light microscopy; MCP, Membrane cofactor protein; DDD, dense deposit disease; ESKD, end-stage kidney disease; GBM, glomerular basement membrane; GN, glomerulonephritis; GNC3, glomerulonephritis C3; MESC3, mesangial C3 deposits; MPC3, MPGN subtype with isolated subendothelial C3 deposits without IgG deposits; MPGN I-III, membranoproliferative glomerulonephritis subtypes I-III; RCA, regulators of complement activation; SCR, short consensus repeats; TCC, terminal complement complex.

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