JMG Online First, published on November 18, 2005 as 10.1136/jmg.2005.038315

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

Variations in the Complement Regulatory Genes Factor H (*CFH*) and Factor H Related 5 (*CFHR5*) are Associated with Membranoproliferative Glomerulonephritis Type II (Dense Deposit Disease)

Maria Asuncion Abrera-Abeleda[1,2], Carla Nishimura[1], Jenna L.H. Smith[1], Sanjeev Sethi[3], Jennifer L. McRae[4], Brendan F. Murphy[4], Giuliana Silvestri[5], Christine Skerka[6], Mihàly Józsi[6], Peter F. Zipfel[6], Gregory S. Hageman[7], Richard J.H. Smith[1,2,8]

[1]Department of Otolaryngology, [2]Interdepartmental PhD Program in Genetics, [7]Department of Ophthalmology and Visual Sciences, and [8]Department of Internal Medicine, Division of Nephrology, Carver College of Medicine, The University of Iowa, Iowa City, IA, USA; [3]Department of Laboratory Medicine and Pathology, The Mayo Clinic, Rochester, MN; , USA; [4]Immunology Research Centre, St Vincent's Hospital, Melbourne, Australia; [5]Division of Surgery and Perioperative Care, Department of Ophthalmology, Queen's University, Belfast, United Kingdom; [6]Department of Infection Biology, Leibniz-Institute for Natural Products Research and Infection Biology - Hans Knoell Institute, Beutenbergstrasse 11a, D-07745 Jena, Germany.

Corresponding author:
Richard JH Smith, Professor of Internal Medicine Division of Nephrology, Sterba Hearing Research Professor of Otolaryngology, Director - Molecular Otolaryngology Research Laboratories, 200 Hawkins Drive, The University of Iowa, Iowa City, IA 52242, USA. Telephone 319-356-3612; Fax 319-356-4108; richard-smith@uiowa.edu

Key words: membranoproliferative glomerulonephritis type II, dense deposit disease, complement factor H, complement factor H-related 5, end-stage renal failure

Copyright Article author (or their employer) 2005. Produced by BMJ Publishing Group Ltd under licence.

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

ABSTRACT
Introduction: Membranoproliferative glomerulonephritis type II or Dense Deposit Disease (MPGN II/DDD) causes chronic renal dysfunction that progresses to end-stage renal disease in about half of patients within 10 years of diagnosis. Deficiency of and mutations in complement Factor H (*CFH*) are associated with the development of MPGN II/DDD, suggesting that dysregulation of the alternative pathway of the complement cascade is important in disease pathophysiology.
Subjects: Patients with MPGN II/DDD were studied to determine whether specific allele variants of *CFH* and *CFHR5* segregate preferentially with the MPGN II/DDD disease phenotype. The control group was compromised of 131 persons in whom age-related macular degeneration had been excluded.
Results: Allele frequencies of four single nucleotide polymorphisms (SNPs) in *CFH* and three SNPs in *CFHR5* were significantly different between MPGN II/DDD patients and controls.
Conclusion: We have identified specific allele variants of *CFH* and *CFHR5* associated with the MPGN II/DDD disease phenotype. While our data can be interpreted to further implicate complement in the pathogenesis of MPGN II/DDD, these associations could also be unrelated to disease pathophysiology. Functional studies are required to resolve this question.

INTRODUCTION

The membranoproliferative glomerulonephritides are diseases of diverse and often obscure etiology that account for 4% and 7% of primary renal causes of nephrotic syndrome in children and adults, respectively[1]. Based on renal immunopathology and ultrastructural studies, three subtypes are recognized. Membranoproliferative glomerulonephritis (MPGN) types I and III are variants of immune complex-mediated disease; MPGN II, in contrast, has no known association with immune complexes[2].

MPGN II accounts for less than 20% of cases of MPGN in children and only a fractional percentage of cases in adults[1,3,4]. Both sexes are affected equally, with the diagnosis usually made in children between the ages of 5-15 years who present with non-specific findings like hematuria, proteinuria, acute nephritic syndrome or nephrotic syndrome[2]. More than 80% of patients with MPGN II are also positive for serum C3 nephritic factor (C3NeF), an autoantibody directed against C3bBb, the convertase of the alternative pathway of the complement cascade[5]. C3NeF is found in up to one-half of persons with MPGN types I and III and also in healthy individuals, making the electron microscopic demonstration of dense deposits in the glomerular basement membrane (GBM) necessary for a definitive diagnosis of MPGN II[2]. This morphological hallmark is so characteristic of MPGN II that the disease is more accurately referred to as Dense Deposit Disease (MPGN II/DDD) (Fig 1).

Spontaneous remissions of MPGN II/DDD are uncommon[3,4,6,7]. The more common outcome is chronic deterioration of renal function leading to end-stage renal disease (ESRD) in about half of patients within 10 years of diagnosis[7-10]. In some patients, rapid fluctuations in proteinuria occur with episodes of acute renal deterioration in the absence of obvious triggering events; in other patients, the disease remains stable for years despite persistent proteinuria.

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

C3NeF persists throughout the disease course in more than 50% of patients with MPGN II/DDD[5]. Its presence is typically associated with evidence of complement activation, such as a reduction in CH50, decrease in C3, increase in C3dg/C3d and persistently high levels of activation of the alternative pathway of the complement cascade. C3, the most abundant complement protein in serum (~1.2 mg/ml), normally undergoes low levels of continuous autoactivation by hydrolysis of its thioester in a process known as tick-over. C3 hydrolysis induces a large conformational protein change, making C3(H_20) similar to C3b, a cleavage product of C3. C3(H_20) associates with factor B to form C3(H_20)Bb, which cleaves C3 to C3b in an amplification loop that consumes C3 and produces C3bBb[2] (Fig 2).

In MPGN II/DDD, C3NeF binds to C3bBb (or to the assembled convertase) to prolong the half-life of this enzyme, resulting in persistent C3 consumption that overwhelms the normal regulatory mechanisms to control levels of C3bBb and complement activation[2]. Normal control involves at least seven proteins, four of which are present in serum (complement factor H (CFH), complement factor H-like protein 1 (CFHL1), complement factor I (CFI) and C4 binding protein (C4BP)) and three of which are cell membrane-associated (membrane co-factor protein (MCP, CD46), decay accelerating factor (DAF, CD55) and complement receptor 1 (CR1, CD35)) [2,11,12].

Of particular relevance to MPGN II/DDD is CFH, one of 7 proteins in the CFH family. In pigs and mice, its deficiency is associated with the development of renal disease that is similar at the light and electron microscopic level to MPGN II/DDD, and in humans its deficiency as well as mutations in the *CFH* gene have been reported in patients with MPGN II/DDD[11,13-16] (Fig 3).

The other 6 members of the CFH family include *CFHL1*, which is a splice isoform of *CFH*, and five CFH-related proteins encoded by distinct genes (*CFHR1-5*). There is little known about the latter five proteins, although they do show varying degrees of structural similarity to CFH[2]. Most interesting in this group with respect to MPGN II/DDD is CFHR5, because it shows the highest similarity to CFH and has been demonstrated in renal biopsies of patients with other types of glomerulonephritis [2,17]. In vitro studies have also shown that CFHR5 is present on surfaces exposed to complement attack, suggesting a possible role in the complement cascade [17].

A possible relationship between CHF/CFHR5 and MPGN II/DDD is further strengthened by the observation that patients with MPGN II/DDD develop an ocular phenotype called drusen. Drusen result from the deposition of abnormal extracellular deposits in the retina within the ocular Bruch's membrane beneath the retinal pigment epithelium. The drusen of MPGN II/DDD are clinically and compositionally indistinguishable from drusen that form in age-related macular degeneration (AMD) [18-20], which is the most common form of visual impairment in the elderly[21,22]. The single feature that distinguishes these two types of drusen is age of onset - drusen in MPGN II/DDD develop early, often in the second decade of life, while drusen in AMD are found in the elderly.

Four recent studies have implicated specific allele variants of *CFH* with AMD, suggesting that subtle differences in CFH-mediated regulation of the alternative pathway of complement may play a role in a substantial proportion of AMD cases[23-26]. One of these studies also showed that MPGN II/DDD and AMD patients segregate several of the same *CFH* risk alleles[23]. In this study, we sought to refine the association of allele

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

variations of *CFH* and *CFHR5* with MPGN II/DDD.

MATERIALS AND METHODS

Patients and Controls

Patients with biopsy-proven MPGN II/DDD were ascertained in nephrology divisions and enrolled in this study under IRB-approved guidelines. The control group was ascertained from ethnically-matched but not age-matched unrelated persons in whom AMD had been excluded by ophthalmologic examination.

Mutation Screening and Analysis

Coding and adjacent intronic regions of *CFH* and *CFHR5* were PCR amplified for 35 cycles of 30 seconds each at 94°C denaturing, 61°C annealing and 70°C extension. Product generation was verified by agarose gel electrophoresis and amplicons were then bi-directionally sequenced in patients with MPGN II/DDD. All novel and reported SNPs identified through data mining (Ensemble database, dbSNP, Applied Biosystems) were typed in the control population by denaturing high performance liquid chromatography (DHPLC) (Tables 1, 2). In brief, DHPLC analysis of each amplicon was performed at three different temperatures. Amplicons were analyzed using Wavemaker software to estimate optimal temperature, run time and acetonitrile gradient. Predicted temperatures were bracketed by +/-2°C to optimize sensitivity and maximize the likelihood that novel mutations would be detected[27].

Table 1. Primers used to amplify *CFH* coding sequence

Exon	Forward	Reverse
1	TGGGAGTGCAGTGAGAATTG	GCTAATGATGCTTTTCACAGGA
2	CCTGTGACTGTCTAGGCATTTT	TATGCCTGAATTATATCACTATTGCC
3	GCTTTGCTATGTTTAATTTTCCTT	AACTATGATGGAAATAATTAAATCTGG
4	TGCATATGCTGTTCATTTTC	GTCTTACATTAAAATATCTTAAAGTCTC
5	TTTCCTCCAATCTTATCCTGAG	CGTTCATTCTAAGGAATATCAGCA
6	CCTGATGGAAACAACATTTCTG	AACAGGGCCAGAAAAGTTCA
7	TGTTCATTTTAATGCCATTTTG	AGTTTTCGAAGTTGCCGAAA
8	CCTAGAAACCCTAATGGAATGTG	TGTTCAAGCAAAGTGACCAAA
9	TGAGCAAATTTATGTTTCTCATTT	ATGTCACCTTGTTTTACCAATGG
10	TGAATGCTTATGGTTATCCAGGT	AAAACCTGCAGGAACAAAGC
11	TCTTAGAATGGGAAATACTCAGATTG	TGGTTTTTCAGAAATTCATTTTCA
12	ATGTAAAATTAACTTTGGCAATGA	TTGCTGAAATAAGAATTAGAACTTTG
13	TGAATAAAAGAAGAAAATCTTTCCA	ATCTAAAACACATACATCATGTTTTCA
14	AAAACACATACATCATGTTTTCACAA	GATATGCCTCAACATTTCCAGTC
15	GTTGGTTTGATTCCTATCATTTG	TTGGAAAAGTAATAGGTATGTGTGTC
16	CTATGAGAATACAAGCCAAAAGTTC	TCTCTTGTGCTTCGTGTAAACAA
17	AACCCTTTGATTTTCATTCTTCA	TCAAAGTGAGGGGAATAATTGA
18	AATTTATGAGTTAGTGAAACCTGAAT	TCTTCATTCAAAGTGTAAGTGGTACC
19	ACAAAATGGCTAATATATTTTCTCAAG	TAATGTGTGGCCCAGCC
20	CAAAATGAACACTAGGTGGAACC	ATTTTGGGGGAGTATAGCAGG
21	CTGTGTTTGCGTTTGCCTTA	TTCACGTGGCTGGAAAAATC
22	TTGAAAACCTGAAAGTCTATGAAGA	TCAATCATAAAGTGCACACCTTT

Table 2. Primers used to amplify *CFHR5* coding sequence

Exon	Forward	Reverse
1	CAGTCCCATTTCTGATTGTTCCA	GCTGAGGATAATTTGAAGGGG
2	GTGATTCATCGATGTAGCTCTTT	AATGACCAGAGGAGCCTGGAA
3	TGATGTCAGTTTTCAAAGTTTTCC	ACCACTCTCTCAGTTTTGCTAATTAT
4	CACATTAAATTTGTTTCTGCAATGA	AGAAGTGATGAAACAAGAATTTGA
5	CCATTTAAGCATTATTTATGGTTTC	AAACAGGACAGTTACTATTACTTTGCA
6	AAATATTTTCAGAGTAAGCACTCATTT	TTTATCATTTTGATTGGGATTGT
7	TGCAGATATTTTATTGACATAATTGTT	GTTGATCTTGTTGCTTCTTTACAAGA
8	CCATTTTCCTGAAACACTACCC	TCTGTTGCACTGTACCCCAA
9	AATTATTTGAATTTCCAGACACCTT	TTTTGGACTAATTTCATAGAATAACCC
10	CTTAAATGCAATTTCACTATTCTATGA	TAGCCATTATGTAGCC

Haplotype Analysis

Construction of block structures with distribution of haplotypes was completed using Haploview, a publicly available software program developed at the Whitehead Institute (http://www.broad.mit.edu/mpg/haploview/)[28]. Two datasets, one consisting of each control's sex and genotype, and the other describing marker information including SNP identification and chromosomal location, were assimilated in Excel files, which were up-loaded into the Haploview program. The output consisted of linkage disequilibrium (LD) plots and the corresponding population frequencies with crossover percentages.

Stastical Analysis

The chi-square test of independence was used to detect differences in SNP frequencies between patients with MPGNII/DDD and controls. P-values$\leq$ 0.05 were considered significant. The LD plots for *CFH* and *CFHR5* were created using the control population.

RESULTS

Patients and Controls

Twenty-two patients with biopsy-proven MPGN II/DDD and 131 persons without AMD participated in this study. Mean age of the control group was 78.4 years, reflecting our ascertainment criterion to exclude AMD.

CFH, CFHR5 and MPGN II/DDD

Allele frequencies of four of seven *CFH* SNPs genotyped in the MPGN II/DDD patient group and the control population showed a significant association with the MPGN II/DDD disease phenotype at $p<0.05$. These SNPs included exon 2 I62V, IVS 2-18insTT, exon 9 Y402H and exon 10 A473A. Allele frequencies for exon 7 A307A, exon 13 Q672Q and exon 18 D936E were not significantly different between groups (Tables 3-5).

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

Table 3. *CFH* SNPs in patients segregating MPGN II/DDD. Allele frequencies (f1 and f2) and number of patients by genotype are shown.

	EX2	I62V	IVS2	-18ins TT	EX7	A307A	IVS7	-53G>T	EX9	Y402H	EX10	A473A	EX13	Q672Q	IVS15	-30C>A	EX18	D936E	IVS18	-89T>C	Ex20	N1050Y
		rs800292				rs1061147				rs1061170		rs2274700		rs3753396				rs1065489				
		MPGN2		MPGN2		MPGN2		MPGN2		MPGN2		MPGN2		MPGN2		MPGN2		MPGN2		MPGN2		MPGN2
	GG	20	(T)9(T)9	20	CC	3	GG	8	CC	9	GG	18	AA	13	CC	8	GG	13	TT	19	AA	21
	GA	2	(T)11(T)9	2	CA	10	GT	10	CT	10	GA	4	AG	9	CA	10	GT	9	TC	3	AT	1
	AA	0	(T)11(T)11	0	AA	9	TT	4	TT	3	AA	0	GG	0	AA	4	TT	0	CC	0	TT	0
SUM		22		22		22		22		22		22		22		22		22		22		22
f1		.95G		.95 (T)9		.64A		.59G		.36Y		.90G		.80A		.59C		.80G		.93T		.98A
f2		.05A		.05 (T)11		.36C		.41T		.64H		.10A		.20G		.41A		.20T		.07C		.02T
At-Risk Haplotype		G		9		A		G		C		G		A		C		G		T		A
MPGN2-1		G,G		9,9		A,C		G,T		C,T		G,G		G,A		C,A		G,T		T,T		A,A
MPGN2-2		G,G		9,9		C,C		T,T		T,T		G,G		G,A		C,A		G,T		T,T		A,A
MPGN2-7		G,G		9,9		A,C		G,T		C,T		G,G		G,A		C,A		G,T		T,T		A,A
MPGN2-9		G,G		9,9		A,C		G,T		C,T		G,G		G,A		C,A		G,T		T,T		A,A
MPGN2-10		G,G		9,9		A,C		G,T		C,T		G,G		A,A		C,A		G,T		T,T		A,A
MPGN2-11		G,G		9,9		A,C		G,T		C,T		G,A		A,A		C,A		G,G		T,C		A,T
MPGN2-12		G,G		9,9		A,A		T,T		C,C		G,G		A,A		C,C		G,G		T,T		A,A
MPGN2-13		G,G		9,9		A,C		G,T		C,T		G,G		G,A		C,A		G,T		T,T		A,A
MPGN2-14		G,G		9,9		A,C		G,T		C,T		G,G		G,A		C,A		G,T		T,T		A,A
MPGN2-15		G,G		9,9		A,A		G,G		C,C		G,G		A,A		C,C		G,G		T,T		A,A
MPGN2-16		G,G		9,9		C,C		T,T		T,T		G,A		G,A		A,A		G,T		T,C		A,A
MPGN2-17		G,G		9,9		A,A		G,G		C,C		G,G		A,A		C,C		G,G		T,T		A,A
MPGN2-18		G,G		9,9		A,C		G,T		C,T		G,G		G,A		C,A		G,G		T,T		A,A
MPGN2-19		G,G		9,9		A,A		G,G		C,C		G,G		A,A		C,C		G,G		T,T		A,A
MPGN2-20		G,G		9,9		A,A		G,G		C,C		G,G		A,A		C,C		G,G		T,T		A,A
MPGN2-21		G,A		9,11		C,C		T,T		T,T		G,A		G,A		A,A		G,T		T,T		A,A
MPGN2-22		G,G		9,9		A,A		G,G		C,C		G,G		A,A		C,C		G,G		T,T		A,A
MPGN2-23		G,G		9,9		A,A		G,G		C,C		G,G		A,A		C,C		G,G		T,T		A,A
MPGN2-24		G,G		9,9		A,A		G,G		C,C		G,G		A,A		C,C		G,G		T,T		A,A
MPGN2-27-02		G,A		9,11		A,C		G,T		C,T		G,A		A,A		C,A		G,G		T,T		A,A
MPGN2-29		G,G		9,9		A,A		G,G		C,C		G,G		A,A		A,A		G,G		T,T		A,A
MPGN2-30		G,G		9,9		A,C		G,T		C,T		G,G		A,A		A,A		G,G		T,C		A,A

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

Table 4. Comparison of *CFH* SNP frequencies in MPGN II/DDD patients versus controls (allele frequencies given as f1 and f2)

SNP	f1 MPGN II/DDD	f2 MPGN II/DDD	f1 Controls	f2 Controls	P-value
Exon 2 I62V	42 (G)	2 (A)	202 (G)	60 (A)	0.0051
IVS2 -18insTT	42 (short)	2 (long)	194 (short)	68 (long)	0.0018
Exon 7 A307A	16 (C)	28 (A)	88 (A)	174 (C)	0.72
Exon 9 Y402H	28 (Y)	16 (H)	88 (Y)	174 (H)	0.00014
Exon 10 A473A	40 (G)	4 (A)	74 (G)	62 (A)	0.000013
Exon 13 Q672Q	35 (A)	9 (G)	217 (A)	41 (G)	0.45
Exon 18 D936E	35 (D)	9 (E)	115 (D)	19 (E)	0.32

Table 5. Coding SNPs associated with MPGN II/DDD and the related short consensus repeat (SCR) of *CFH*

SNP	SCR	Function of SCR
Exon 2 I62V	1	Interaction with C3b
Exon 9 Y402H	7	Heparin binding Interaction with C reactive protein
Exon 10 A473A	8	Interaction with C reactive protein

Five *CFHR5* SNPs were genotyped in the MPGN II/DDD patient group and control population, including one non-synonymous SNP (exon 2 P46S), two promoter SNPs (-249T>C , -20 T>C) and two intronic SNPs (IVS1+75T>A, IVS2+58C>T). Allele frequencies of three SNPs – exon 2 P46S, -249T>C and -20 T>C – were significantly different between groups at p<0.05 (Tables 6, 7).

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

Table 6. *CFHR5* SNPs in patients segregating MPGN II/DDD. Allele frequencies (f1 and f2) and number of patients by genotype are shown.

	Promoter	-249T>C	Promoter	-20T>C	IVS1	75T>A	Exon 2	P46S	IVS2	58C>T
		rs9427661		rs9427662		rs3748557		rs12097550		rs12097550
		MPGN2		MPGN2		MPGN2		MPGN2		MPGN2
	TT	21	TT	21	TT	16	CC	19	CC	16
	TC	1	TC	1	TA	5	CT	3	CT	5
	CC	0	CC	0	AA	1	TT	0	TT	1
SUM		22		22		22		19		22
f1		.98T		.98T		.84T		.93P		.84C
f2		.02C		.02C		.16A		.07S		.16T
At-Risk Haplotype		T		T		T		C		C
MPGN2-02		T,T		T,T		T,T		C,C		C,C
MPGN2-03		T,T		T,T		T,T		C,C		C,C
MPGN2-07		T,T		T,T		T,T		C,C		C,C
MPGN2-09		T,T		T,T		A,T		C,C		C,T
MPGN2-10		T,T		T,T		T,T		C,C		C,C
MPGN2-11		T,T		T,T		A,T		C,C		C,T
MPGN2-12		T,T		T,T		T,T		C,C		C,C
MPGN2-13		T,T		T,T		A,T		C,T		C,T
MPGN2-14		T,T		T,T		A,A		C,C		T,T
MPGN2-15		T,T		T,T		T,T		C,T		C,C
MPGN2-16		C,T		C,T		A,T		C,C		C,T
MPGn2-17		T,T		T,T		T,T		C,C		C,C
MPGN2-18		T,T		T,T		A,T		C,C		C,T
MPGN2-19		T,T		T,T		T,T		C,C		C,C
MPGN2-20		T,T		T,T		T,T		C,T		C,C
MPGN2-21		T,T		T,T		T,T		C,C		C,C
MPGN2-22		T,T		T,T		T,T		C,C		C,C
MPGN2-23		T,T		T,T		T,T		C,C		C,C
MPGN2-24		T,T		T,T		T,T		C,C		C,C
MPGN2-27-2		T,T		T,T		T,T		C,C		C,C
MPGN2-29		T,T		T,T		T,T		C,C		C,C
MPGN2-30		T,T		T,T		T,T		C,C		C,C

Table 7. Comparison of *CFHR5* SNP frequencies in MPGN II/DDD patients versus controls (allele frequencies given as f1 and f2)

SNP	F1 MPGNII/DDD	f2 MPGNII/DDD	f1 Controls	f2 Controls	P-value
Promoter -249T>C	43 (T)	1 (C)	178 (G)	28(A)	0.033
Promoter -20T>C	43 (T)	1 (C)	178 (G)	28(A)	0.033
IVS1 +75T>A	37(T)	7 (A)	161 (A)	41 (C)	0.38
Exon 2 P46S	41 (P)	3(S)	205 (P)	1 (S)	0.00023
IVS2 +58C>T	37 (C)	7 (T)	158 (C)	28 (T)	0.28

Haploblocks

Haplotype blocks showed that A307A and Y402H are in linkage disequilibrium in *CFH* while -249T>C and -20T>C are in linkage disequilibrium in *CFHR5* (Fig 4).

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

DISCUSSION

The alternative pathway of complement represents an elegant system to protect humans from pathogens. Its central component, C3, circulates at a high concentration in plasma and is distributed throughout body fluids[29]. Its activation creates a toxic local environment that damages foreign surfaces and results in the elimination of microbes. To prevent unrestricted complement activation, host cells and tissue surfaces down-regulate the amplification loop using a combination of surface-attached and membrane-bound regulators of complement. Some host cells express a single membrane-bound regulator of complement in high copy number, while other cells express several membrane-bound regulators and also attach soluble fluid-phase regulators. A few tissues lack membrane-bound regulators and depend exclusively on the attachment of soluble regulators[2].

In the kidney, endothelial and mesangial cells express two membrane-bound regulators of complement, MCP and DAF[30,31]. Podocytes express four: MCP, DAF, CR1 and CD59. Both mesangial cells and podocytes also secrete the soluble regulator, CFH, which is up-regulated in membranous nephropathy in response to complement activation and inflammation[32,33]. CFH acts in an autocrine fashion by binding directly to the secreting mesangial cells and podocytes.

The GBM, in contrast, is unique. It lacks endogenous membrane-bound regulators to protect it from complement-mediated injury, however its highly negatively charged surface binds and absorbs CFH[16]. The dependency of the GBM on CFH for local complement control is consistent with the finding of pathologic mutations in *CFH* in a few persons with MPGN II/DDD[13,34].

Our data identifying several allele variants of *CFH* and *CFHR5* associated with MPGN II/DDD is consistent with the hypothesis that complement control plays a role in the pathogenesis of this disease. A comparison of our data with reported AMD data adds additional support, as the allele frequency for each of the identified at-risk SNP variants we observed in *CFH* is higher in the MPGN II/DDD patient cohort than in the AMD patient cohort, and strong evidence implicates *CFH* in AMD[23-26]. Although it is not known whether the amino acid changes in exons 2 and 9 of CFH impact function, these changes are found in domains that interact with C3b and heparin, and differences in C3b/C3d and heparin binding have been demonstrated with several amino acid changes in CFH that are associated with another renal disease, atypical hemolytic uremic syndrome[38] (Tables 4 and 5).

With the exception of CFH, the function of other members of the Factor H-related family is largely unknown and their expression patterns have not been explored, however studies of CFHR5 have shown that it has properties similar to CFH, including heparin, CRP and C3b binding[17] (Fig 3). This similarity suggests that like CFH, CFHR5 could play a role in MPGN II/DDD. Consistent with this possibility is our finding of CFHR5 expression in renal biopsies from two patients with MPGN II/DDD (data not shown).

Our genotyping data show that some allele variants of *CFHR5* preferentially associate with the MPGN II/DDD disease phenotype. Included are two SNPs in the promoter region of *CFHR5* which could affect transcription, one by removing a binding site for C/EBPbeta and the other by adding a GATA-1 binding site. The other significant association changes a proline to serine in exon 2. Since exons 1and 2 of CFHR5 encode a domain homologous to short consensus repeat 6 (SCR6) of CFH, which is integral to heparin and CRP binding, this change could affect complement activation and control.

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

SUMMARY

Humans, pigs and mice deficient in CFH develop MPGN II/DDD[11,13-15], implicating local dysfunction of the alternative pathway of the complement cascade in this disease. We have identified specific allele variants of *CFH* and *CFHR5* associated with the MPGN II/DDD disease phenotype. While our data can be interpreted to further implicate complement in the pathogenesis of MPGN II/DDD, these associations could also be unrelated to disease pathophysiology. Functional studies are required to resolve this question.

ACKNOWLEDGMENTS

We are indebted to the persons with MPGNII/DDD whose participation has made this research possible. We are also grateful for the participation of persons who served as control subjects for this study.

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in JMG and any other BMJPGL products and sublicences such use and exploit all subsidiary rights, as set out in our licence (http://jmg.bmjjournals.com/misc/ifora/licenceform.shtml).

LEGENDS

Figure 1. Light micrograph showing marked glomerular hypercellularity with dense intramembranous deposits that cause capillary wall thickening in a person with MPGN II/DDD. The deposits can form a segmental, discontinuous or diffuse pattern in the lamina densa of the glomerular basement membrane (GBM). By light microscopy, they are eosinophilic and refractile, stain brightly with periodic acid-Schiff and are highly osmophilic, which explains their electron-dense appearance (A). Even by electron microscopy the deposits lack substructure and appear as very dark homogeneous smudges (B). The exact composition of dense deposits remains unknown (bar, 5 um).

Figure 2. The alternative pathway of the complement cascade is systematically activated at a high level in patients with MPGN II/DDD. Normally, continuous low-level activation of C3 occurs by spontaneous hydrolysis in a process known as tick-over. C3 hydrolysis is associated with a large conformational protein change shown at the top of the diagram. The conformational change makes C3(H_20) similar to C3b, a C3 cleavage product. The initial convertase, C3(H_2O)Bb, activates C3 to form C3b. Although C3b has a fleeting half-life, if it binds to IgG, cells or basement membranes, it is protected from immediate inactivation. $(C3b)_2$-IgG complexes form in the fluid phase and bind properdin (P), which facilitates factor B binding and the generation of C3bBb, the convertase of the alternative pathway, shown here as a Bb$(C3b)_2$-IgG-properdin complex. The amplification loop is depicted by the red arrows. C3NeF prolongs the half-life of C3 convertase and is shown in the inset. One mechanism to degrade C3 convertase is through its interaction with complement factor H, shown at the bottom right. Deficiency of and mutations in complement factor H are associated with MPGN

II/DDD.

Figure 3. The Regulators of Complement Activation is a gene cluster on chromosome 1. It includes genes that encode the seven proteins in the complement factor H family. Structurally, these proteins are similar, each being built on a motif of distinct functional domains called short consensus repeats (SCRs). CFH has 20 SCRs. The interacting partners with some of these SCRs has been determined and is shown on the top right (CRP, C reactive protein; Hep, heparin). Complement factor H-like 1 (CFHL1) is a splice isoform of *CFH*, while complement factor H-related proteins 1-5 (CFHR1-5) are each encoded by a unique gene (*CFHR1-5*). The SCRs of CFHR1-5 are similar to some of the SCRs in CFH, as denoted by the numbers in the ovals. For example, CFHR5 has 9 SCRs, with the first two being similar to SCRs 6 and 7 of CFH and therefore having CRP and heparin binding properties. SCRs5-7 of CFHR5 have the numbers 12-14 within the corresponding ovals because these SCRs are similar to SCRs 12-14 of CFH and have C3b and heparin binding properties.

Figure 4. Linkage disequilibrium plots show that A307A and Y402H are in linkage disequilibrium in *CFH* and that -249T>C and -20T>C are in linkage disequilibrium in *CFHR5* (n=103). Haplotype frequencies and cross-over frequencies between blocks are shown to the right.

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

REFERENCES

1. Orth SR, Ritz E. The nephrotic syndrome. *New Engl J Med* 1998;**338**:1202-1211.
2. Appel GB, Cook HT, Hageman G, Jennette JC, Kashgarian M, Kirschfink M, Lambris JD, Lanning L, Lutz HU, Meri, S, Rose NR, Salant DJ, Sethi S, Smith RJH, Smoyer W, Tully HF, Tully SP, Walker P, Welsh M, Würzner R, Zipfel PF. Membranoproliferative glomerulonephritis type II (Dense Deposit Disease): an update. *J am Soc Nephrol* 2005;**16:**1392-1403.
3. Habib R, Gubler MC, Loriat C, Maiz HB, Levy M. Dense deposit disease. A variant of membranoproliferative glomerulonephrtitis. *Kidney Int* 1975;**7:**204-215.
4. Habib R, Antignac C, Hinglais N, Gagnadoux MF, Broyer M. Glomerular lesions in the transplanted kidney in children. *Am J Kidney Diseas* 1987;**10:**198-207.
5. Schwertz R, Rother U, Anders D, Gretz N, Scharer K,. Kirschfink M. Complement analysis in children with idiopathic membranoproliferative glomerulonephritis: a long-term follow-up. *Pediatr Allergy Immunol* 2001;**12:**166-172.
6. Cameron JS, Turner DR, Heaton J, Williams DG, Ogg CS, Chantler C, Haycock GB. Idiopathic mesangiocapillary glomerulonephritis. Comparison of types I and II in children and adults and long-term prognosis. *Am J Med* 1983;**74:**175-192.
7. Barbiano di Belgiojoso G, Tarantino A, Colasanti G, Bazzi C, Guerra L, Durante A. The prognostic value of some clinical and histological parameters in membranoproliferative glomerulonephritis. *Nephron* 1977;**19**:250-258.
8. Droz D, Noel LH, Barbanel C, Grunfeld JP. Évolution a long terme des glomérulonéphrites membranoproliferative de l'adulte: remissionspontanée durable chez 13 malades avec étude de biopsies rénales itératives dans 5 cas. *Neprhrologie* 1982;**3**:6-11.
9. McEnery, PT. Membranoproliferative glomerulonephritis: The Cincinnati experience cumulative renal survival from 1957 to 1989. *J Pediatr* 1990;**116:**S109-S114.
10. Swainson, CP, Robson JS, Thomson D, MacDonald MK. Mesangiocapillary glomerulonephritis: A long-term study of 40 cases. *J Pathol* 1983;**141:**449-468.
11. Meri S, Pangburn MK. Regulation of alternative pathway complement activation by glycosaminoglycans: specificity of the polyanion binding site on factor H. *Biochem Biophys Res Commun* 1994;**198:**52-59.
12. Pascual M, Steiger G, Sadallah S, Paccaud JP, Carpentier JL, James R, Schifferli, JA. Identification of membrane-bound CR1 (CD35) in human urine: evidence for its release by glomerular podocytes. *J Exp Med* 1994;**79:**889-899.
13. Dragen-Durey MA, Fremeaux-Bacchi V, Loirat C, Blouin J, Niaudet P, Deschenes G, Coppo P, Herman Fridman W, Weiss L. Heterozygous and homozygous factor H deficiencies associated with hemolytic uremic syndrome or membranoproliferative glomerulonephritis: report and genetic analysis of 16 cases. *J Am Soc Nephrol* 2004;**15:**787-795.
14. Jansen JH, Hogasen K, Harboe M, Hovig T. In situ complement activation in porcine membranoproliferative glomerulonephritis type II. *Kidney Int* 1998;**53:** 331-349.
15. Pickering MC, Cook HT, Warren J, Bygrave AE, Moss J, Walport MJ, Botto M. Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. *Nat Genet* 2002;**31:** 424-428.
16. Zipfel PF, Smith RJH, Heinen S. The role of complement in membranoproliferative glomerulonephritis. In Complement and Kidney Disease 2005; In press.
17. Murphy B, Georgiou T, Machet D, Hill P, McRae J. Factor H-related protein-5: a

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

novel component of human glomerular immune deposits. *Am J Kid Dis* 2002;**39:**24-27.

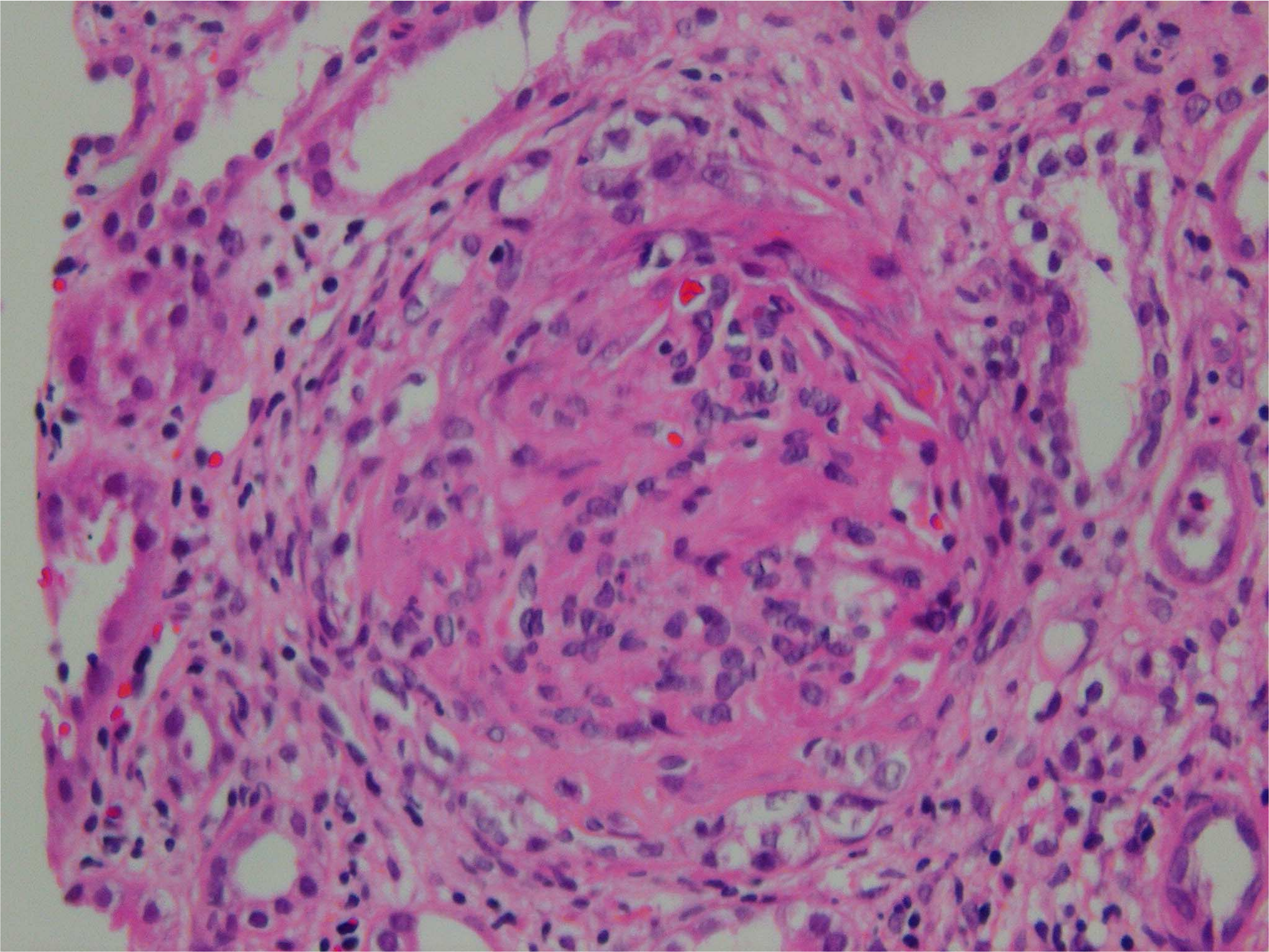
18. Mullins R F, Aptsiauri N, Hageman G S. Structure and composition of drusen associated with glomerulonephritis: implications for the role of complement. *Eye* 2001;**15:**390-5.
19. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res* 2001;**20:**705-32.
20. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol* 2002;**134:**411-31.
21. Klein R, Peto T, Bird A, Vannewkirk MR. The epidemiology of age-related macular degeneration. *Am J Ophthalmol* 2004;**137:**486-95.
22. van Leeuwen R, Klaver CC, Vingerling JR, Hofman A, de Jong PT. Epidemiology of age-related maculopathy: a review. *Eur J Epidemiol* 2003;**18**:845-54.
23. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM, Smith RJ, Silvestri G, Russell SR, Klaver CC, Barbazetto I, Chang S, Yannuzzi LA, Barile GR, Merriam JC, Smith RT, Olsh AK, Bergeron J, Zernant J, Merriam JE, Gold B, Dean M. Allikmets R.Common haplotype in the complement regulatory gene, factor H (*HF1/CFH*), predisposes individuals to age-related macular degeneration. *Proc Nat Acad Sci* 2005;**102:** 7227-32.
24. Edwards AO, Ritter III R, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H Polymorphism and age-related macular degeneration. *Science* 2005;**308:**421-424.
25. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel EA, Pericak-Vance MA. Complement factor H variant increases the risk of age-related macular degeneration. *Science* 2005;**308:**419-421.
26. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, Sangiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005;**308:**385-389.
27. Prasad S, Kölln KA, Cucci RA, Trembath RC, Van Camp G, Smith RJH. Pendred syndrome and DFNB4 - Mutation screening of *SLC26A4* by denaturing high-performance liquid chromatography and the identification of seven novel mutations. *Am J Med Genet* 2004;**124A:**1-9.
28. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2004;**21:**263-5.
29. Walport MJ. Complement. First of two parts. *N Engl J Med* 2001;**344:**1058-1066.
30. van den Dobbelsteen ME, Verhasselt V, Kaashoek JG, Timmerman JJ, Schroeijers WE, Verweij CL, van der Woude FJ, van Es LA, Daha MR. Regulation of C3 and factor H synthesis of human glomerular mesangial cells by IL-1 and interferon-gamma. *Clin Exp Immunol* 1994;**95:**173-180.
31. Timmerman JJ, van der Woude FJ, van Gijlswijk-Janssen DJ, Verweigj CL, van Es LA, Daha MR. Differential expression of complement components in human fetal and adult kidneys. *Kidney Int* 1996;**49:**730-740.
32. Angaku M. Complement regulatory proteins in glomerular diseases. *Kidney Int* 1998;**54:**1419-1428.

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

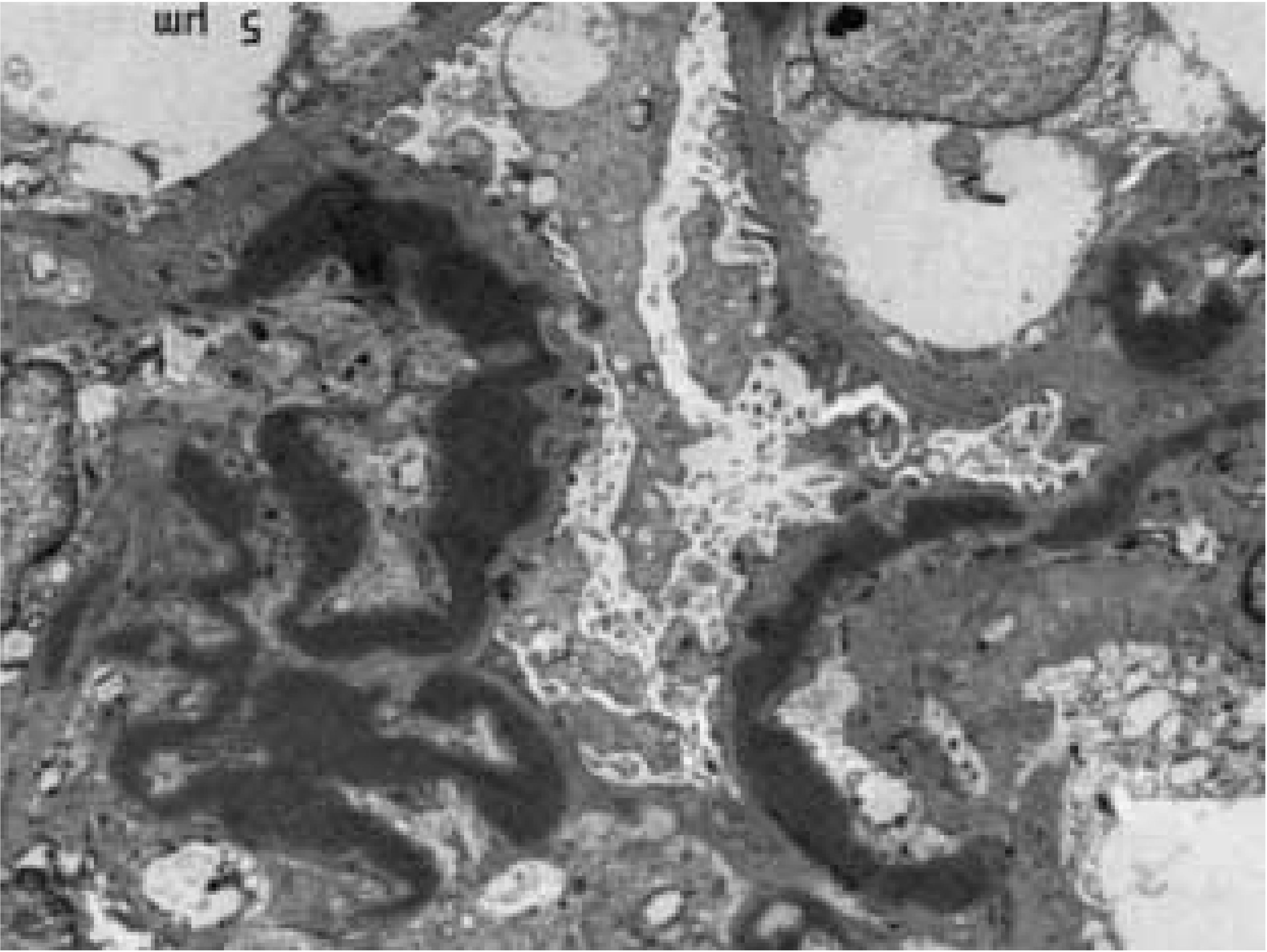
33. Bao L, Spiller OB, St. John PL, Haas M, Hack BK, Ren G, Cunningham PN, Doshi M, Abrahamson DR, Morgan BP, Quigg RJ. Decay-accelerating factor expression in the rat kidney is restricted to the apical surface of podocytes. *Kidney Int* 2002;**62:**2010-2021.
34. Ault BH, Schmidt BZ, Fowler NL, Kashtan CE, Ahmed AE, Vogt BA, Colten HR. Human factor H deficiency. Mutations in framework cysteine residues and block in H protein secretion and intracellular catabolism. *J Biol Chem* 1997;**272:** 25168-25175.
35. Colville D, Guymer R, Sinclair RA, Savige J. Visual impairment caused by retinal abnormalities in mesangiocapillary (membranoproliferative) glomerulonephritis type II (“dense deposit disease”). *Am J Kidney Dis* 2003;**42:** E2-E5.
36. Duvall-Young J, Short CD, Raines MF, Gokal R, Lawler W. Fundus changes in mesangiocapillary glomerulonephritis type II: clinical and fluorescein angiographic findings. *Br J Ophthalmol* 1989;**73:**900-906.
37. Holz FG, Pauleikhoff D, Klein R, Bird AC. Pathogenesis of lesions in late age-related macular disease. *Am J Ophthalmol* 2004;**137:**504-510.
38. Manuelian T, Hellwage J, Meri S, Caprioli J, Noris M, Heinen S, Jozsi M, Neumann HPH, Remuzzi G, Zipfel PF. Mutations in factor H reduce binding affinity to C3b and heparin and surface attachment to endothelial cells in hemolytic uremic syndrome. *J Clin Invest* 2003;**111:**1181-1190.

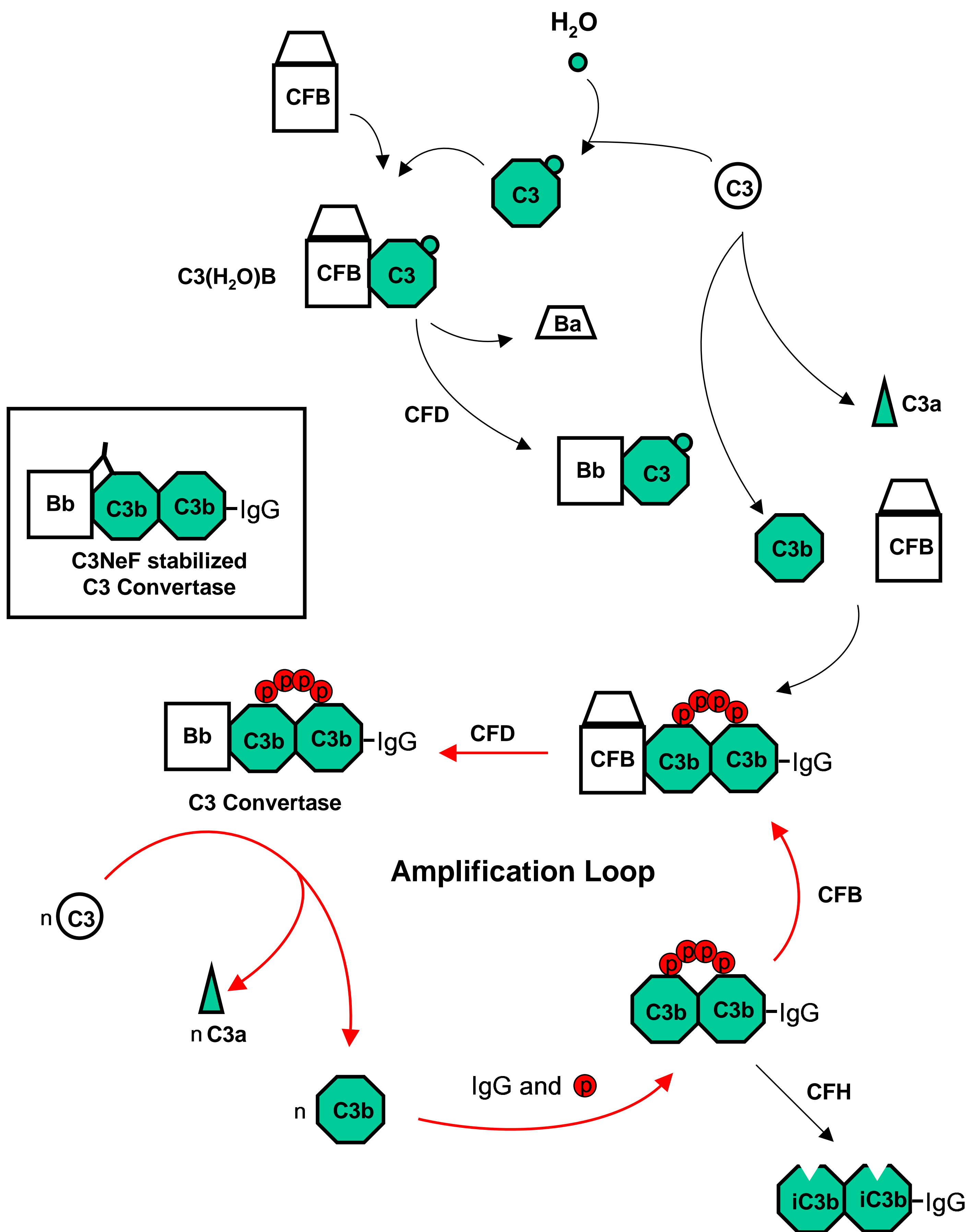
J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

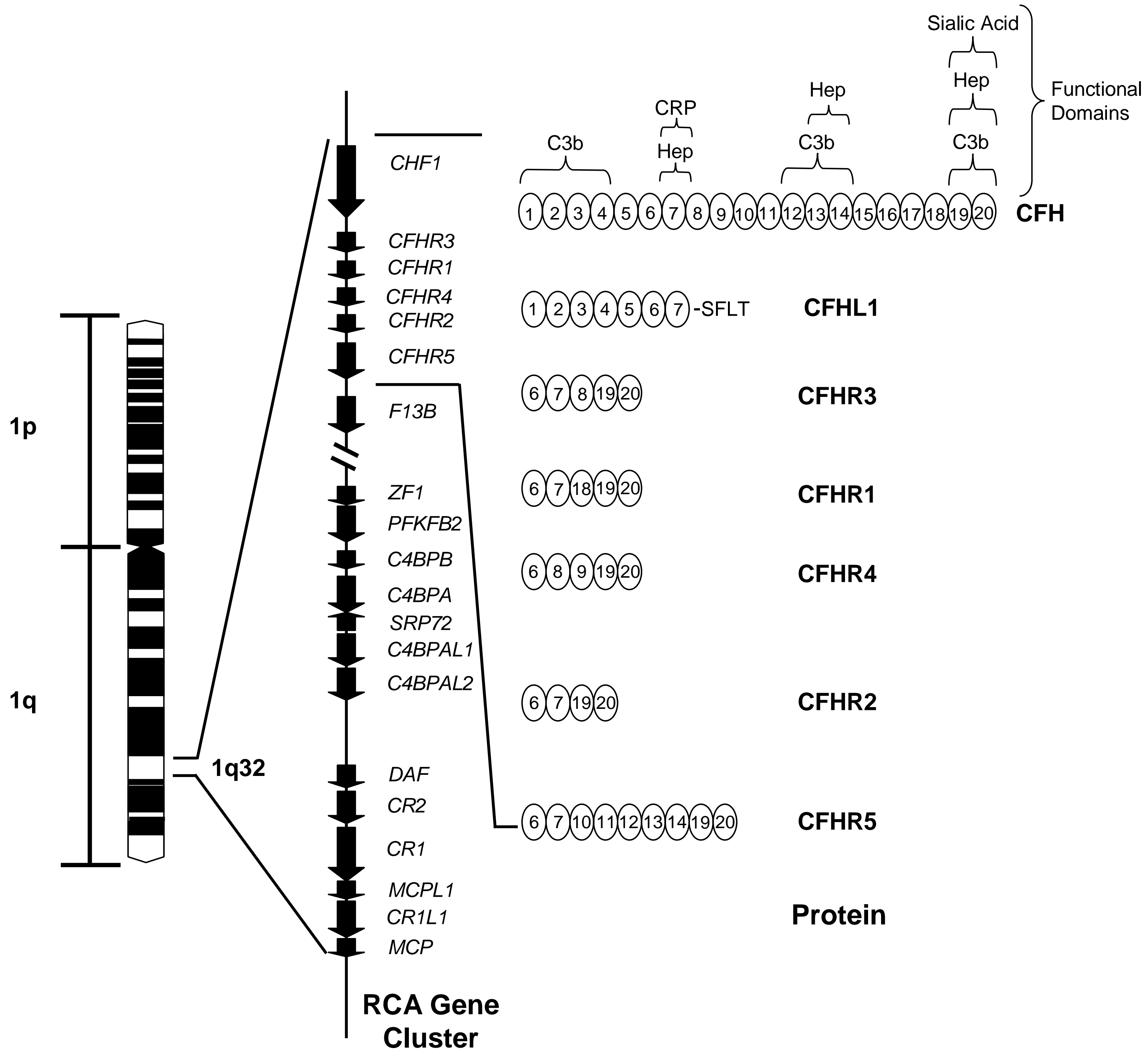


J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.

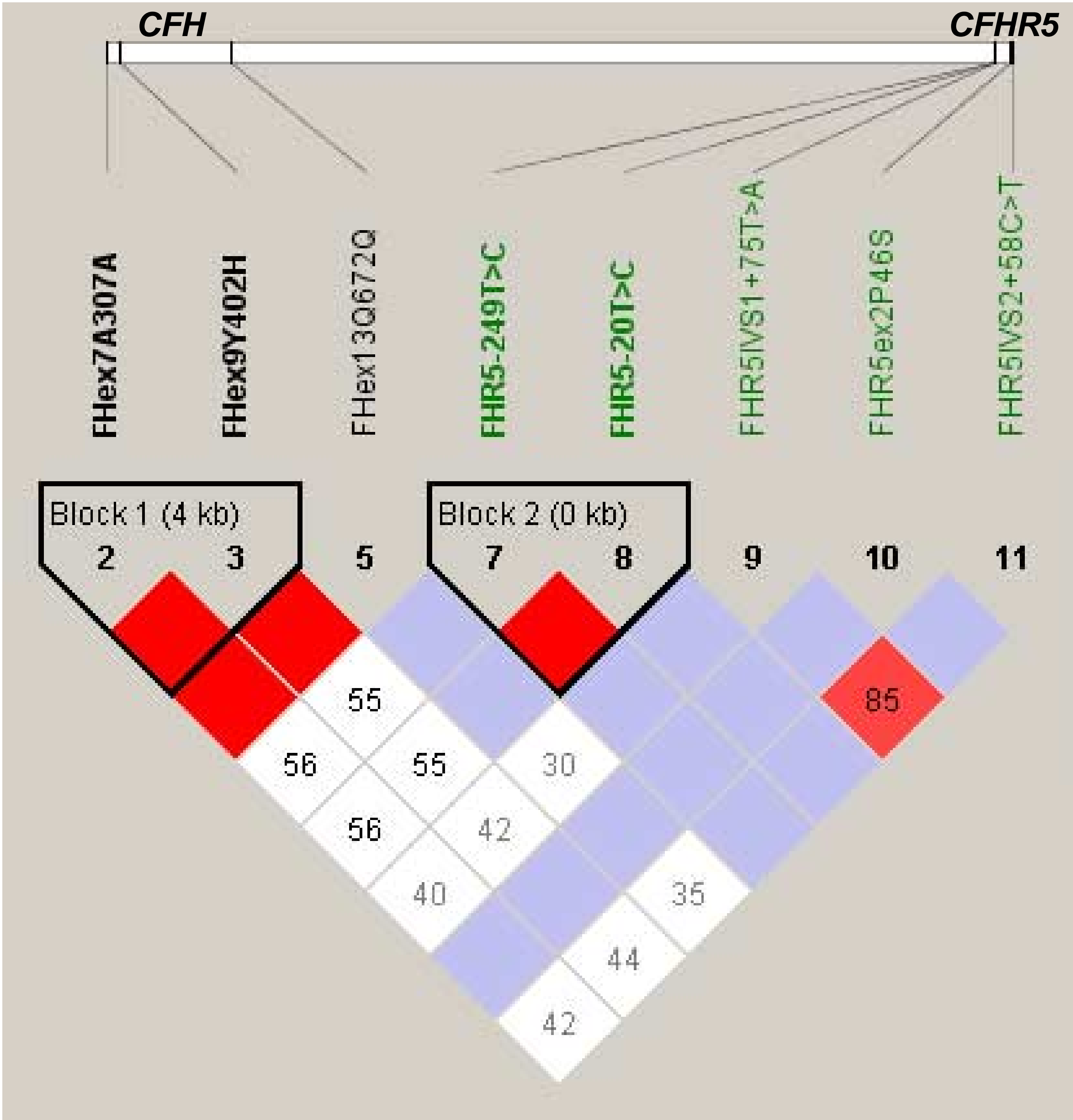




J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.



J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.



J Med Genet: first published as 10.1136/jmg.2005.038315 on 18 November 2005. Downloaded from http://jmg.bmj.com/ on December 6, 2021 by guest. Protected by copyright.