Heterozygous and Homozygous Factor H Deficiencies Associated with Hemolytic Uremic Syndrome or Membranoproliferative Glomerulonephritis: Report and Genetic Analysis of 16 Cases

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Abstract. Factor H (FH) is the major regulatory protein of the complement alternative pathway, with a structure consisting of a tandem array of 20 homologous units, called short consensus repeats (SCR). Reported are 16 FH-deficient patients. Among six patients with homozygous deficiency, four presented with membranoproliferative glomerulonephritis, and two with atypical hemolytic uremic syndrome (HUS). The ten other patients had heterozygous FH deficiency and developed atypical HUS. HUS onset occurred from birth to midadulthood, and disease progression was variable. Four children with homozygous or heterozygous FH deficiency and HUS underwent renal transplantation, which was successful in three but failed as a result of recurrence of HUS in one patient. All but one patient exhibited alternative pathway-mediated complement consumption, with no detectable FH antigenic levels or with 50% immunochemical or functional FH levels in the case of complete or partial deficiency, respectively. The molecular mechanisms of the deficiency were documented in all cases by exon-specific sequencing analysis. These mechanisms included nucleotide substitutions, insertion, or deletion located in SCR 2, 7, 11, 13, 15, and 20, leading to an amino acid substitution or to a stop codon. This report emphasizes the variability in the clinical progression of kidney diseases associated with FH deficiencies. Genetic analysis reveals the molecular abnormalities associated with FH deficiencies to be polymorphous.

The human complement system can be activated by three different pathways: the classical pathway, the lectin pathway, and the alternative pathway. Complement protein factor H (FH) is a regulatory protein of the alternative pathway. It inhibits the formation of the alternative C3-convertase and accelerates its decay. It also serves as a cofactor for the C3b-cleaving enzyme, factor I.

FH 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 (1,2), called either short consensus repeats (SCR) or complement control protein. Three C3-binding sites have been identified, one in SCR 1–4, which binds intact C3b, and two in SCR 6–10 and SCR 16–20, which bind C3c and C3d fragments, respectively (3,4). Three heparin-binding sites have also been identified in SCR 7, 13, and 20 (5–7).

The gene encoding FH is localized on the long arm of chromosome 1 at 1q32, a locus called RCA (regulators of complement activation) (8,9), which contains genes encoding different regulatory proteins of complement activation (10). A truncated protein, FHL-1, comprising the seven first SCR and four individual amino acids, is also generated by an alternative splicing mechanism (11,12).

FH deficiencies have been reported in both homozygous and heterozygous forms and were associated with atypical forms (nondiarrhea associated) of hemolytic uremic syndrome (HUS), type II or III membranoproliferative glomerulonephritis (MPGN), and other kidney diseases (reviewed in (13)). Some cases of systemic lupus erythematosus have also been reported in patients exhibiting combined FH and C2 deficiency

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(14,15). Some FH-deficient patients presented with increased susceptibility to meningococcal infection, probably secondary to acquired C3 or terminal components C5 to C9 deficiencies (16–18).

In 1998, Warwicker *et al.* (19) reported linkage of atypical HUS to a locus within the RCA gene cluster by genetic study of three large kindreds exhibiting no evidence of quantitative FH deficiency. They also found in one of the families a heterozygous nucleotide substitution, leading to the change of an amino acid in SCR 20. Additional genetic studies have also found several different heterozygous missense mutations between SCR 16 and 20, suggesting a particular role of the C-terminal domains of the protein in the pathophysiology of HUS (20–24). However, the molecular mechanism of FH deficiencies has only been demonstrated in five patients (15,19,21,22,25) and involved SCR 1, 2, 3, 9, 16, and 20 .

In the study presented here, we report on a series of 16 homozygous or heterozygous FH-deficient patients presenting with either HUS or MPGN. The deficiency was demonstrated at the protein level and characterized at the genetic level.

Materials and Methods

Patients

Patients were recruited from the departments of pediatric and adult nephrology of French university hospitals from 1997 to 2002. Informed consent of patients (or parents of children) was obtained before DNA analysis. Patients were selected on the basis of blood samples exhibiting low or undetectable antigenic or functional FH levels consistent with partial or complete FH deficiencies.

Complement Assays

Laboratory investigations to support the diagnosis were performed at the Laboratoire d'Immunologie Biologique of Hôpital Européen Georges Pompidou, Paris, a reference laboratory for the investigation of the complement system in France.

Freshly drawn EDTA plasma samples were obtained from all patients except patient 16. Measurement of CH50 activity was performed as described previously (26). Results were expressed as the percentage of mean values obtained with a reference plasma prepared from 100 healthy blood donors (normal range, 100% $\pm$ 30%). Plasma concentrations of C4, C3, and factor B (FB) antigens were measured by nephelometry (Dade Behring, Paris La Defense, France). Normal values established with pooled plasma from 100 healthy donors ranged 220 $\pm$ 120 mg/L, 960 $\pm$ 300 mg/L, and 200 $\pm$ 110 mg/L (mean $\pm$ 2 SD), respectively.

FH antigen concentration was measured by a sensitive ELISA method. Briefly, Nunc MaxiSorp ELISA plates (Nunc, Roskilde, Denmark) were coated with sheep polyclonal anti-human FH (The Binding Site, Birmingham, UK). After washing and blocking free reactive sites with PBS containing 1% BSA, the plasma to be tested was added at a dilution of 1:2000 for 1 h at 37°C. After washing, the plates were incubated with a mouse anti-human FH monoclonal antibody (Serotec, Fidlington, UK) for 1 h at 37°C. After additional washing, plates were incubated with an anti-mouse IgG labeled with horseradish peroxidase (Sanofi Pasteur, Marne-la-Coquette, France) and further incubated for 1 hr at 37°C. Enzymatic activity was revealed by using the orthophenyldiamine substrate. Results were expressed as the percentage of values obtained in the same experiment with a reference plasma pool prepared from 100 healthy blood donors (normal ranges were 100% $\pm$ 30%, as calculated by the results from 50 individual healthy donors). FH activity was measured by assessing the ability of test plasma to dissociate a preformed cell bound C3bBb convertase, as described previously (27).

Genomic FH DNA Sequencing

The DNA was extracted from whole blood using the proteinase K/phenol method (28). Uncloned genomic DNA was amplified by means of PCR using oligonucleotides flanking each exon. Primer sequences, length of the PCR amplified-fragments, and temperatures of hybridization used for each reaction are listed in Table 1. PCR products were then purified using the Multiscreen plates according to the manufacturer's instructions (Millipore, Molsheim, France). Direct DNA sequencing of the purified PCR products was then carried out by the Dye terminator cycle sequencing method (Applied Biosystems, Courtaboeuf, France) using a 96-capillary Sequencer 3700 (Applied Biosystems). Sequence analyses were performed using the Sequencher software. The number of nucleotide or codon referenced are indicated according to Warwicker *et al.* (19).

Results

Clinical features are summarized in Table 2.

Case Reports

Patients 1 and 2. In this family, two children who were first cousins had had relapsing HUS since age 7 and 11 mo, respectively. The family, which originated from Turquia, exhibited a high grade of consanguinity and has been previously reported (patients 4 and 5) (18). Patient 1 had been receiving fresh frozen plasma (FFP) treatment every week since age 4 (29). After 4 yr of follow-up, his renal function remained normal, and no relapse of hemolysis occurred. Kidney transplantation was performed at age 7 in patient 2. Twice-monthly plasma infusions were performed during the 2 yr after transplantation, and graft function is currently normal.

Patients 3 and 4. Two brothers of a Turkish family presented with MPGN. Data regarding the disease's onset and the clinical progression were not available.

Patient 5. This case has been reported by Levy *et al.* (30). This 6-yr-old boy presented with nephrotic syndrome with a normal renal function revealing type I MPGN. At age 23, renal function remained normal (creatinine clearance, 71 ml/min/1.73 m^2) with persistent nephrotic syndrome while receiving a treatment combining diuretics and angiotensin-converting enzyme inhibitors.

Patient 6. This white girl presented at age 8 with nephrotic syndrome secondary to type I MPGN. Two years after diagnosis, the patient exhibited hypertension and renal insufficiency (creatinine clearance: 38 ml/min/1.73 m^2) while receiving diuretics and angiotensin-converting enzyme inhibitors.

Patient 7. This case has already been reported (patient 3 in (18)). He presented with atypical HUS at the age of 6 mo with rapidly progressive renal failure that required dialysis. He remained on chronic hemodialysis without recurrence of hemolysis and received a kidney transplant at age 6. No recurrence of HUS has occurred during the 10 yr follow-up after transplantation.

Patient 8. This case has been previously reported (patient 1 in (18)). He presented with HUS at the age of 16 mo with

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Table 1. FH intronic primers and PCR conditions

Name	Primer Sequence	Annealing Temperature	Size of Amplicon (pb)
FH20-A	aac cgt tag ttt tcc agg att taa t	56°C	557
FH20-B	gtt tat tca aat caa tat gat gtt tc		
H-SCR19-A	ccc agc cct aaa gag aaa aa	55°C	473
H-SCR19-B	tca cca gaa atc aca aaa ct		
H-SCR18-A	ccc tat tac ttg tgttct g	60°C	406
H-SCR18-B	tca gtt gat ttg cta ctc a		
H-SCR17-4	cgg cta cca ata ttt ctt cag	56°C	460
H-SCR17-3	ctg ggc cca cac att ata taa		
H-SCR16-A	acc act tac act ttg aat ga	55°C	408
H-SCR16-B	tag aca gac aga cac cag aa		
H-SCR15-A	atg cca gaa tac aaa gtg ac	60°C	693
H-SCR15-B	aac aaa ata cag cca aaa tc		
H-SCR14-5	gtg ata att tat gaa aca gtt att g	57°C	404
H-SCR14-6	ctc tct tgt tta cac gaa gca c		
H-SCR13-A	aaa tga gaa ata gaa tac at	52°C	361
H-SCR13-B	aat aac ttg gtt ggt gaa at		
H-SCR12-A	gaa gac tgg aaa tgt tga gg	62°C	441
H-SCR12-B	tgt tga tgg aga gtg gac aa		
H-SCR11-A	aat aag gag ggg aag aaa gc	52°C	455
H-SCR11-B	atg aat aaa aga aga aaa tc		
H-SCR10-A	aca tcg ttc aag aaa cag t	56°C	463
H-SCR10-B	caa cct cac ttt att gtg g		
H-SCR9-A	cag att tat ttt cat ttt ga	50°C	364
H-SCR9-B	cca gtc ata gat tat ttt tg		
H-SCR8-A	tga tgt ctg ctt tgt tcc tg	62°C	505
H-SCR8-B	gtc ttg atg taa tgt ctt tg		
H-SCR7-A	gaa aaa cca aaa act aaa ta	52°C	598
H-SCR7-B	gaa aat cac agg aga aat aa		
H-SCR6-3	gag tgt tta tta cag taa aat ttc	57°C	315
H-SCR6-4	gtg ctc tcc ttt ctt cga tc		
H-SCR5-A	ttt tat tga gtc cct att tt	52°C	598
H-SCR5-B	atc ctt atg aga acc aca ga		
H-SCR4-A	tta gca ctc tac ttt tga tt	60°C	370
H-SCR4-B	tac ctg atg gaa aca aca tt		
H-SCR3-A	cct tag aat gaa cga tgt tt	50°C	469
H-SCR3-B	aga aaa gaa tca gga ata aa		
H-SCR2C-A	ttt gac tgg caa tag tga ta	52°C	402
H-SCR2C-B	ttt aga tag acc tgt gac tg		
H-SCR2B-A	ttt tcc cac tct ccc ata at	62°C	576
H-SCR2B-B	ccc agc caa tac atc atc at		
H-SCR2A-A	aaa agg aaa taa aaa tca gtt	52°C	500
H-SCR2A-B	agg aga agg agg aag gaa aaa		
H-SCR1-A	ctg tga aaa gca tca tta gc	54°C	390
H-SCR1-B	ata aga aaa agt cca aga gc		

rapidly progressive renal failure that required dialysis. He remained on chronic hemodialysis without recurrence of hemolysis and received a kidney transplant at age 4. No recurrence of HUS has occurred during the 4 yr follow-up after transplantation.

Patient 9. This 18-mo-old white girl was referred for HUS. She died 6 mo later despite weekly administration of FFP. There was no family history of renal disease.

Patient 10. This white girl presented with HUS at the age of 9 mo with rapidly progressive renal failure that required dialysis. She remained on chronic hemodialysis without recurrence of hemolysis and received a kidney transplant at age 5. Recurrence of HUS occurred 25 d later, with hemolysis and lesions of thrombotic microangiopathy (TMA) on the transplant biopsy, leading to transplantectomy. Presently aged 11, she remains on chronic hemodialysis. There is no family history of renal disease.

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Table 2. Clinical features associated with FH deficiencies

Family	Case	Disease	Onset of disease	FH Ag Level	Evolution[e]
			Homozygous deficiencies		
A: 2 first cousins	1[ab]	HUS	7 mo	<1%	Stable with weekly FFP administration
	2[a]	HUS	11 mo	<1%	No recurrence 18 mo after renal transplantation and FFP administration
B: 2 brothers	3	MPGN	<12 yr[d]	<1%	—
	4	MPGN	<14 yr[d]	<1%	—
C	5[c]	MPGN	6 yr	<1%	Stable renal function 12 yr after diagnosis
D	6	MPGN	10 yr	<1%	CRI 2 yr after diagnosis
			Heterozygous deficiencies		
E	7[a]	HUS	6 mo	50%	No recurrence 10 yr after renal transplantation
F	8[a]	HUS	16 mo	35%	No recurrence 4 yr after renal transplantation
G	9	HUS	18 mo	45%	Death 6 mo after onset
H	10	HUS	9 mo	30%	Recurrence of HUS 25 d after renal transplantation
I	11	HUS	26 yr	45%	No recurrence 5 yr after onset
J	12	HUS	34 yr	41%	Recurrence of haemolysis, progressive renal failure
K	13	HUS	29 yr	37%	Three recurrences
L	14	HUS	4 mo	94%	Chronic haemodialysis
M	15	Chronic renal failure	41 yr	55%	Recurrence of TMA lesions on renal transplant
			Undetermined deficiency		
N	16	HUS	At birth	Not determined Parents: 52%	Death 15 d after onset

[a] Case report in Rougier *et al.* ((18)).
[b] Case report in Nathanson *et al.* ((28)).
[c] Case report in Levy *et al.* ((29)).
[d] The disease's onset was not available, ages at the time of investigation are done.
[e] FFP, fresh frozen plasma; CRI, chronic renal insufficiency.

Patient 11. This 26-yr-old white woman was referred for anemia, thrombocytopenia, and acute renal failure 3 mo after her first childbirth and while receiving oral contraceptives. Renal biopsy showed TMA lesions. At the acute phase, she received plasma exchanges and hemodialysis treatment. She regained independent renal function (creatinine clearance: 50 ml/min/1.73 m^2), but she presented with chronic hemolysis requiring weekly administration of FFP during 4 mo. A second pregnancy occurred 5 yr after the initial episode, without recurrence of the disease. There was no family history of renal disease.

Patient 12. This 34-yr-old white woman presented with hemolytic anemia, thrombocytopenia, and acute renal failure that required hemodialysis. Analysis of renal biopsy specimens revealed the presence of severe TMA lesions. After the initial episode, she received weekly FFP during 3 mo and long-term oral prednisolone. She regained independent renal function (creatinine clearance: 44 ml/min/1.73 m^2). There was no family history of renal disease.

Patient 13. This white woman was studied at age 37 after she experienced three episodes of HUS since the age of 29. Analysis of renal biopsy material revealed TMA lesions. Renal function remained normal between episodes of HUS. There was no family history of renal disease.

Patient 14. This white girl presented with HUS few weeks after birth, leading to end-stage renal failure. Presently aged 20, she remains on chronic hemodialysis. No relapse of hemolysis has occurred. Four years before her initial presentation, her unique brother had severe HUS at age 4 d and died a few weeks later.

Patient 15. This 41-yr-old white man presented with hypertension and severe renal failure. Analysis of renal biopsy material revealed ischemic glomeruli and fibrotic lesions in arteria and interstitium; no deposition of C3 was detected. Hemodialysis was started 3 mo later. Uncontrolled hyperten-

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sion led to a bilateral nephrectomy. The patient received a kidney transplant 2 yr later. Eighteen months after the transplantation, recurrence of progressive renal failure associated with hemolytic anemia occurred, and he required again hemodialysis. Analysis of graft biopsy material revealed TMA. There was no family history of renal disease.

Patient 16. A white newborn girl presented with severe HUS, leading to death at day 15. Investigations were secondarily performed on her parents, who were first cousins.

Complement Component Assessment

Plasma complement profiles at the time of diagnosis are listed in Table 3. Results of complement investigation were confirmed at least twice at two different occasions. Patients 1 to 6 exhibited severe complement consumption through the alternative pathway, as indicated by very low plasma levels of CH50, C3, and FB. All six patients presented with a lack of detectable FH antigen at 1:2000 and 1:40 plasma dilutions, indicative of plasma concentration of FH protein below 1%. Functional FH activity was undetectable. These results are in agreement with homozygous FH deficiency.

Patients 7 to 13 exhibited mildly decreased CH50, low C3, and FB plasma levels suggestive of mild alternative pathway–mediated complement activation. Antigenic plasma levels of FH were between 35% and 50% of normal values, suggesting heterozygous FH deficiency. In patient 12, a first blood sample, obtained during plasma therapy, showed normal levels of CH50, C3, FB, and FH. A second blood sample was analyzed a few months later; it showed evidence of alternative pathway complement activation and half normal levels of plasma FH.

Patient 14 also presented with alternative pathway activation with mildly decreased plasma levels of C3 and FB. However, in this case, the plasma concentration of FH protein was normal, and functional activity was found to be decreased (50% of normal value), suggesting a heterozygous functional FH deficiency.

Patient 15 exhibited half levels of antigenic FH associated with normal plasma levels of CH50, C3, and FB. No complement exploration could be performed for patient 16. Investigations were, however, performed in both healthy parents, who exhibited half of normal value of FH antigenic level associated with normal plasma levels of C3 and FB.

Characterization of the Genetic Defect

For all patients, all 22 FH exons were sequenced and analyzed. Results of the genetic analysis are illustrated in Figure 1 and listed in Table 4. They were confirmed on two different samples. Each mutation described was the only genetic abnormality found in each patient. The genetic abnormalities described were not found in a sample of at least 100 individuals (>200 chromosomes investigated).

In five cases, the molecular abnormalities that were found created a nonsense mutation. Patients 1 and 2 (family A) and patient 7 presented with a nucleotide substitution, leading to the direct creation of a stop codon in SCR 15 at positions 899 and 924, respectively. Patient 8 presented with a single nucleotide +A insertion in SCR 13 at position 2303, leading to a

Table 3. Results of complement exploration at the time of diagnosis

Case	Factor H Antigenic Levels[a] (70 to 130%)	C3[a] (660 to 1250 mg/L)	Factor B[a] (90 to 320 mg/L)	CH50[a] (70 to 130%)	C4[a] (93 to 320 mg/L)
1	<1	50	17	24	328
2	<1	70	15	<10	384
3	<1	170	70	<10	600
4	<1	100	50	<10	250
5	<1	<40	12	<10	263
6	<1	<40	17	<10	133
7	50	580	190	98	320
8	35	310	200	68	250
9	45	430	79	71	220
10	30	240	250	59	370
11	45	270	80	62	140
12[b]	41/*94*	453/*783*	60/*139*	83/*103*	215/*151*
13	37	382	55	61	237
14	94[c]	470	170	72	230
15	55	834	103	124	256
16 (Father)	52	812	150	93	160
16 (Mother)	46	799	182	136	205

[a] Normal values are indicated in parenthesis.
[b] In italics are indicated results obtained under FFP therapy.
[c] FH functional activity = 50%.

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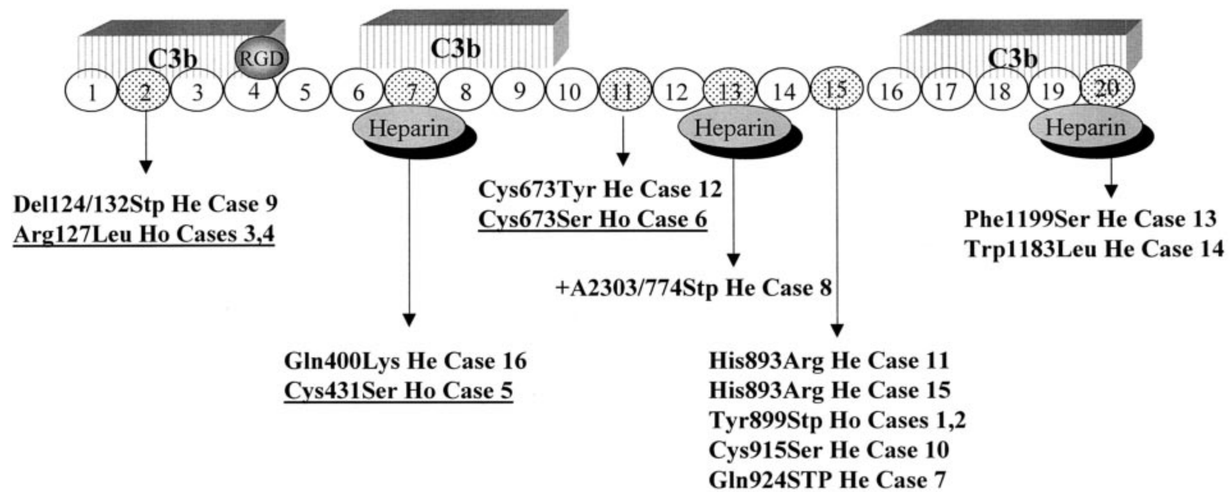

Figure 1. Location of the mutations found in the 16 factor H (FH)–deficient patients. Mutations are depicted on a schematic diagram of the structure of FH where the 20 short consensus repeat (SCR) and functional binding domains are illustrated. The mutations found in patients presenting with membranoproliferative glomerulonephritis (MPGN) are underlined. The codon and nucleotide numbers are indicated according to the mRNA FH sequence. Ho, homozygous mutations; He, heterozygous mutations.

Table 4. Molecular characterization of the genetics defects

Family	Case	Molecular defect	Codon change	No of mutated SCR
		Homozygous deficiencies		
A: 2 first cousins	1	899TAT → TAA	Tyr899Stop homozygous	15
	2	899TAT → TAA	Tyr899Stop homozygous	15
B: 2 brothers	3	127CGT → CTT	Arg127Leu homozygous	2
	4	127CGT → CTT	Arg127Leu homozygous	2
C	5	431TGT → AGT	Cys431Ser homozygous	7
D	6	673TGT → TCT	Cys673Ser homozygous	11
		Heterozygous deficiencies		
E	7	924CAG → TAG	Gln924Stop heterozygous	15
F	8	767AAC → AA(+A) C	+A at 2303pb → 774Stop heterozygous	13
G	9	124ATT → AT(−25bp)G	−25 bp at 371 bp → 136Stop heterozygous	2
H	10	915TGC → AGC	Cys915Ser heterozygous	15
I	11	893CAT → CGT	His893Arg heterozygous	15
J	12	673TGT → TAT	Cys673Tyr heterozygous	11
K	13	1199TTT → TCT	Phe1199Ser heterozygous	20
L	14	1183TGG → TTG	Trp1183Leu heterozygous	20
M	15	893CAT → CGT	His893Arg heterozygous	15
		Undetermined deficiency		
N	16	400CAA → AAA	Father and Mother: Gln400Lys heterozygous	7

change in the amino acids 767 to 773 and the creation of a stop codon at position 774. In patient 9, the deficiency was secondary to a 25-bp deletion in SCR 2 between nucleotides 371 and 397, leading to the change in the amino acids 124 to 135 and the creation of a stop codon in SCR 3 at position 136.

In four patients, a nucleotide substitution leading to substitution of a cysteine for another amino acid was found in SCR 7 (patient 5), 11 (patients 6 and 12), and 15 (patient 10). In the seven remaining cases, the molecular abnormality was a nucleotide substitution, leading to the change of an amino acid localized in SCR 2, 7, 15, and 20 and involving an arginine in 4 cases (patients 3, 4, 11, and 15).

In families A (patient 1), B, E, F, and H, genetic analyses were also performed in the parents of the propositus, and the same heterozygous molecular abnormality was found in both parents in families A (patient 1) and B, and in one of the parents in the other families (data not shown). All carrier relatives exhibited half antigenic levels of FH.

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Discussion

The study presented here reports on a series of 16 patients presenting with atypical HUS or MPGN associated with homozygous or heterozygous inherited FH deficiency. As in previous reports (14–17,19,22), we defined a FH deficiency on the basis of antigenic or functional plasma levels. We have considered that undetectable levels of FH are consistent with complete FH deficiency, whereas half antigenic levels are consistent with partial FH deficiency. In all cases of complete or partial deficiency, we have identified the associated homozygous or heterozygous mutation in the FH gene.

All deficient patients, except patient 15, exhibited at the time of diagnosis evidence of alternative pathway–mediated complement consumption with low plasma levels of C3 and FB. In all relatives of patients who were found to be FH deficient, C3 and FB were within normal ranges. As previously reported (13,18), levels of C3 and FB may vary among FH-deficient individuals. Therefore, normal C3 and FB levels do not exclude the diagnosis of heterozygous FH deficiency. Of note, in one patient, treatment with plasma probably increased the levels of plasma complement components and thus masked partial FH deficiency.

Genetic analysis and exon-specific amplification of genomic DNA by PCR followed by direct sequence analysis revealed the molecular abnormality in all 16 patients. These abnormalities, except in patient 14, led to FH deficiency in which the mutated genes are unable to encode any circulating protein. None of the genetic abnormalities found here, except one, have been previously described. Various FH deficiency mechanisms were identified, including one insertion of a single nucleotide, one 25-bp deletion, and single base pair substitutions arising in several different exons. Interestingly, exon 15 was involved in 6 of 16 cases. The other mutated exons were exon 2a (three cases), 7 (two cases), 11 (two cases), 13 (one case), and 20 (two cases). Most of the genetic abnormalities found led to the creation of a nonsense mutation (5 of 16) or involved a cysteine (4 of 16).

In the FH, all four cysteines of each SCR are involved in disulfur bonds (31). Mutations involving these residues would impede the formation of intrachain disulfide bond, leading to the change of the secondary structure of the protein. Similar mutations involving a cysteine in SCR 9 and 16 were previously reported by Ault *et al.* (25); Schmidt *et al.* (32) then demonstrated that the 150-kD protein was retained in the intracellular fraction.

In accordance with these findings, in our patients, all amino acid substitutions implicating a cysteine were associated with undetectable antigenic levels of FH in the case of homozygous substitutions (patients 5 and 6) or low FH antigenic levels in the case of heterozygous substitutions (patients 10 and 12). Interestingly, patients 6 and 12 exhibited a mutation involving the same cysteine residue (673), which was changed to a serine in patient 6 (homozygous substitution) and to a tyrosine in patient 12 (heterozygous substitution).

Two other single heterozygous base pair substitutions involved an arginine that was changed to a leucine in family B or substituted a histidine in two unrelated patients (patients 11 and 15). The latter mutation was the only same mutation that we found in two different patients. Finally, another recurrent mutation was found in patient 14, who exhibited a previously described mutation in SCR 20 (22). In agreement with this report, we found this mutation associated with low C3 levels and normal quantitative levels of FH. Our data suggest that this mutation affects FH activity, as assessed by the ability of FH to dissociate a preformed C3bBb convertase.

In our study population, a HUS was found to be associated with both homozygous or heterozygous deficiency, whereas only HUS or thrombomicroangiopathic lesions and no case of MPGN was found in patients with heterozygous deficiency. In six cases, the deficiency was complete and associated with HUS in two and with MPGN in four. Homozygous FH deficiency has already been reported to be associated with MPGN (30) or with other kidney diseases such as collagen type III glomerulopathy (25,33). Two animal models—pigs (34) and the more recently described FH knockout ($FH^{-/-}$) mice—spontaneously develop MPGN (35). Conversely, none of the partially deficient ($FH^{+/-}$) mice showed histologic evidence of MPGN at the age of 8 mo. Data from experiments introducing a second mutation in the gene encoding complement FB in $FH^{-/-}$ mice suggested that the MPGN lesions were secondary to uncontrolled C3 activation. In contrast, to our knowledge, no case of MPGN has so far been reported in factor I–deficient patients who also exhibit uncontrolled alternative pathway activation. This calls into question the potential role of C3b fragments, which could be generated in FH-deficient patients but not in factor I–deficient patients in the development of MPGN. Interestingly, none of the $FH^{+/-}$ mice or FH± pigs developed HUS. In humans, reports of HUS were rarely associated with complete FH deficiency (36,37) and more frequently reported in association with partial FH deficiency (18,19,22,38–40).

In our study population, the onset of HUS occurred from birth to midadulthood, and the disease progression was variable. In four cases (patients 11, 12, 13, and 15), HUS occurred in adulthood. It developed postpartum in one patient. In patient 15, FH deficiency was diagnosed as a result of TMA lesions on the kidney to be transplanted. HUS occurred in childhood in eight patients. Two children died quickly (patients 9 and 15), and five children presented with end-stage renal disease. Four of them received a kidney transplant, which was successful in three (patients 2, 7, and 8), with a follow-up of 18 mo to 10 yr. One of these children with complete FH deficiency, patient 2, also received FFP once every 2 wk. In only one of the four children (patient 10), the kidney transplant failed rapidly within 25 d as a result of disease recurrence. The success of a kidney transplantation in three of four patients is worth emphasizing, considering the poor results of kidney transplantation reported by others (13,41,42). A combined kidney and liver transplantation was thus discussed in cases of FH deficiency or a mutated FH gene (41,42). The remaining child exhibiting a complete FH deficiency (patient 1) presented with HUS and retained normal renal function with weekly FFP infusion, as previously reported (29).

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The reason why some individuals with homozygous or heterozygous FH deficiency develop HUS or MPGN, and why some heterozygous FH-deficient people remains free of apparent disease, is still unclear. The role of uncontrolled alternative pathway seems to be demonstrated in the pathophysiology of MPGN lesions. In HUS, FH heterozygous mutations were recently described in the C-terminal domains of the protein that binds to the C3d fragment within C3b and where heparin-binding residues are located (19–23). These mutations result in normal antigenic levels of FH and no alternative pathway activation.

Recent molecular modeling studies showed that these mutations are located in, or close to, conserved basic residues involved in polyanionic binding surface (24). Other experiments indicate that the heparin- and C3d-binding sites are overlapping in SCR 20 (43). Structural and functional characterization of three SCR 20–mutated FH demonstrated that these proteins showed very low binding to surface-bound C3b (44,45). Taken together, these data suggest that mutations within this region may lead to indiscriminate complement attack against self cells, in particular against endothelial cells, which express high surface density of heparin-like glycosaminoglycans. Endothelial cell activation may result in a procoagulant phenotype that may be perpetuated by complement activation. Haplo insufficiency of FH may result in uncontrolled alternative pathway activation and C3b deposition followed by microangiopathic injury. Furthermore, FH exhibits other functions, such as cofactor activity for factor I, which could be abnormal in FH-deficient individuals. However, reports regarding healthy FH-deficient subjects as well as healthy mutated FH carriers indicate that other genetic or environmental factors, including microorganisms or drugs, probably play an additional role in the initiation and/or the progression of the disease.

This report of a large number of FH-deficient patients emphasizes the variability of the progression of the kidney diseases associated with FH deficiencies. Genetic analysis shows that the molecular abnormalities of FH deficiencies are polymorphous and not restricted to the C-terminal domains of the protein. [Printer: Reference (7) is cited here for parsing. Please remove this parenthetical information.]

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