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ORIGINAL ARTICLE

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Atypical haemolytic uraemic syndrome and mutations in complement regulator genes

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Abstract Haemolytic uraemic syndrome (HUS) is a thrombotic microangiopathy (TMA) disorder characterised by the association of haemolytic anaemia, thrombocytopenia and acute renal failure. Atypical forms (non-related to shigatoxin) may be familial or sporadic, with frequent recurrences and most of them lead to end stage renal failure. During the last years, different groups have demonstrated genetic predisposition of atypical HUS involving complement components factor H (FH), CD46 [or membrane co-factor protein (MCP)] and factor I. These three proteins are involved in the regulation of the alternative pathway of the complement system. Several series have reported mutations in the FH gene (called *HF1*) in between 10 and 22% of atypical HUS patients. At this time, four pedigrees corresponding to 13 cases have been reported with an MCP mutation and four cases with a sporadic disease presented factor I mutation. Whereas FH mutations were reported in both familial and sporadic forms of HUS, CD46 mutations were restricted to familial HUS, and factor I mutations were only observed in cases of sporadic HUS. We speculate that the penetrance of the disease may be variable regarding the identified susceptibility factors. Recently, the analysis of single nucleotide polymorphisms in both *HF1* and *MCP* in three large cohorts of HUS patients identified significant association between atypical HUS and HF1 and MCP particular alleles. All these results, together with the finding of anti-FH antibodies in some atypical HUS patients, strongly suggest that an abnormality in the regulation of the alternative pathway participates in the patho-physiological mechanisms of atypical HUS. The recent progress made in the determination of susceptibility factors for atypical HUS has

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permitted the development of new diagnostic tests and may eventually lead to new specific treatments to block the pathological process.

Introduction

Thrombotic microangiopathy (TMA) disorders include two main syndromes, the haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), and two related syndromes that occur during pregnancy, i.e. eclampsia and the haemolysis, elevated liver enzyme, low platelets syndrome (HELLP). The pathophysiology of these disorders involves an initial endothelial cell injury followed by occlusion of small arterioles and capillaries by platelet plugs. The common clinical features of TMA associate microangiopathic haemolytic anaemia, thrombocytopenia and variable organ damage. Although specific clinical and biological features allow to differentiate between them (Table 1), the distinction between HUS and TTP often remains uncertain. HUS is characterised by TMA lesions localised predominantly in the kidney leading to renal failure, which is classically acute, whereas neurological symptoms are predominant in TTP. Typical HUS usually occurs in relation to shigatoxin (Stx), also called verotoxin, produced by some strains of bacteria such as 0157/H7 *Escherichia coli*, principally in children. The infectious diarrhoea precedes the episode of HUS by 2 weeks. The history of diarrhoea is essential to define the diagnosis of "typical" post-infectious HUS (reviewed in [39]). Some drugs known to be toxic for endothelial cells also induce HUS (e.g. cyclosporine). It is when diarrhoea is absent or minimal that it is most difficult to differentiate typical HUS from atypical forms, which are not related to Stx, and may be sporadic (only one flare of the disease), relapsing (at least two episodes) or familial (with either the recessive or dominant mode of inheritance). These atypical forms have a much poorer outcome, and end stage renal failure or death is not uncommon. During the last 5 years, a clear link has been demonstrated between atypical HUS and genetic abnormality in complement regulator genes. Several studies have confirmed genetic predisposition both in familial and sporadic cases of atypical HUS involving complement component factor H (FH) [4, 6, 22, 26, 31]. Recently, two groups provided evidence that mutations in CD46 [or membrane co-factor protein (MCP)] may also predispose to HUS [23, 32]; more recently, our group reported on heterozygous factor I deficiency in a few individuals presenting sporadic HUS [10]. These three proteins are involved in regulating the alternative pathway of the complement system. FH inhibits the formation of the alternative C3-convertase and accelerates its decay. FH and CD46 serve as co-factors for the C3b-cleaving serine protease, factor

Table 1 Clinical and biological spectrum of thrombotic microangiopathic syndromes

	Haemolytic uraemic syndrome	Thrombotic thrombocytopenic purpura
Renal impairment	+++	+/−
Haemolysis	++	+++
Thrombocytopenia	++	+++
Hypertension	+	−/+
Fever	+	+
Central neurological signs	+/−	+++
Liver impairment	+/−	+/−

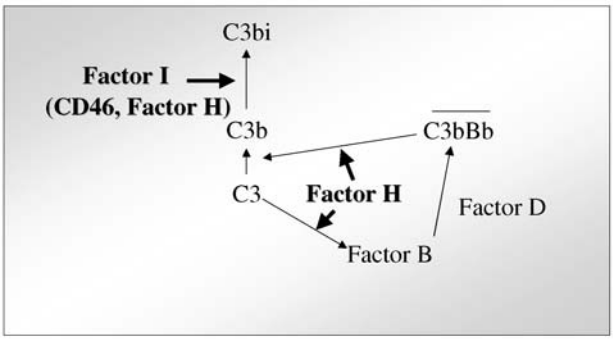

Fig. 1 Representation of the alternative pathway regulation

I (Fig. 1). The purpose of this article is to review and analyse the known genetic mutations presently reported in these three genes.

Factor H

Factor H 1 gene (*HF1*) is localised on the long arm of chromosome 1 at 1q32, a locus called regulators of complement activation (RCA) [34, 44] which contains genes encoding different regulatory proteins of complement activation, including MCP [12]. All these proteins are characterised by the presence of a modular structure consisting of a tandem array of homologous units of about 60 amino acid residues called short consensus repeats (SCR) or complement control protein (CCP) and by their capacity to bind C3b or C3 cleavage fragments.

Mainly synthesised by the liver, FH is a single-chain serum glycoprotein of 150 kD comprising 20 SCR. Several observations of FH deficiencies in both homozygous and heterozygous forms have been reported with atypical forms of atypical HUS after the first description by Thompson and Winterborn in 1981 [40]. In 1998, Warwicker et al. [42] reported linkage of atypical HUS to a locus within the RCA gene cluster containing FH gene, by genetic study of three large kindred 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 the SCR20. Additional genetic studies have also found several different heterozygous missense mutations between SCR16–20. To date, about 200 cases of patients presenting with atypical HUS have been analysed for FH genetic abnormalities in four main series [8, 22, 26, 31]. In these series, the frequency of FH mutation reported varied between 10 to 22%. Moreover, additional reports have also described patients presenting with atypical HUS [9, 30] (reviewed in Table 2). Until now, about 50 different genetic abnormalities have been reported in the FH gene. As previously noted, most of them are located at the C-terminal domains of the protein: 63% are located in the exons encoding the SCR18 to 20, and 84% of them are located in the SCR20 representing more than 50% of the total mutations described. However, there may be some biases in this analysis. Indeed, in some of the first reports, only exons encoding the C-terminal domains of the protein were

Table 2 Summary of factor H mutations reported in association with aHUS

Reference	No. of studied cases	No. of mutated cases	First clinical description age at onset	HUS form	FH antigenic levels	C3 antigenic levels	FH genetic study
Warwicker [42]	4 pedigrees, 6 cases	2					
		1	11 years	Familial	Normal	Moderately low	SCR20: Arg1197Gly
		1		Sporadic	50%	Low	SCR1: Del 4 bp
Caprioli [4]	5 pedigrees, 7 cases	7 cases					
		Patient 1	31 years	Sporadic	Normal	Moderately low	SCR20: Arg1210Cys
		Family 1	0.5 years, 8 years, 1 years	Familial	Normal	Moderately low	SCR20: Arg1215Glu
		Family 3	22, 25 years (1 post-partum)	Familial	Normal	Moderately low	SCR8: DelA 1494–1496, codon stop
		Family 24	6, 3, 3.5 years	Familial	Normal	Moderately low	SCR20: Arg1210Cys
		Family 29	10 neonatal cases	Familial	<10%	Very low	SCR20: A/T+24 bp del
Richards [31]	50 cases, 19 familial and 31 sporadic	5 (10%)					
		Patient 1		SHU	ND	ND	SCR18: Gln1076Glu+SCR19: Del A 3559 Stop codon SCR20
		Patient 2		Familial	Normal	Normal	SCR19: Asp1119Gly
		Patient 3		Sporadic	Normal	Normal	SCR20: Arg1215Gly
		Patient 4		Sporadic	Normal	Moderately low	SCR20: Thr1183Arg
		Patient 5		Familial	Normal	Normal	SCR20: Ser1191Leu+Val1197Ala (FHR1 CCP5)
Remuzzi [30]	1	1	Neonatal	Familial	Normal	Normal	SCR20: Trp1183Arg

Table 2 (continued)

Reference	No. of studied cases	No. of mutated cases	First clinical description age at onset	HUS form	FH antigenic levels	C3 antigenic levels	FH genetic study
Neumann [22]	111 cases, 8 familial	16 (14.4%)					
		1, 2, 3	31, 25, 24 years	Sporadic	Normal	Normal	SCR11:Cys630Trp/12 :Ser714X/14 :Glu850Lys
		4	22 years	Familial	Normal	Normal	SCR16: Trp978Cys
		5, 6, 7, 8, 9, 10	33, 26, 2, 37, 28, 18 years	Sporadic	Normal	Normal	SCR17: Cys1043Arg/18: Gln1076Glu, 19: Val1134Gly/Tyr1142Asp/Trp1157Arg/splice defect
		11	1 year	Familial	Normal	Normal	SCR20: Trp1183Arg
		12, 13, 14, 15,	13, 40, 33, 59 years	Sporadic	Normal	Normal	SCR20: Arg1210Cys/1216 DelThr/ Pro1226Ser/3768 delAGAA
		16	39 years	Sporadic	Normal	Normal	SCR17 Lys1021Phe+20 Arg1210Cys
Dragon-Durey [6]	11 pedigrees, 12 cases	12					
		1, 2	7, 11 months	Familial	<1%	Very low	SCR15: Tyr 899 X homozygous
		7	6 months	Sporadic	50%	Moderately low	SCR15: Gln924X
		8	16 months	Sporadic	<50%	Low	SCR13: +A 2303→774X
		9	18 months	Sporadic	<50%	Low	SCR2: −25 bp at 371 bp→136X
		10	9 months	Sporadic	<50%	Low	SCR15: Cys915Ser
		11	26 years	Sporadic	<50%	Low	SCR15: His893Arg
		12	34 years	Sporadic	<50%	Low	SCR11: Cys673Tyr
		13	29 years	Sporadic	<50%	Low	SCR20: Phe1199Ser
		14	4 months	Familial	Normal	Low	SCR20: Trp1183Leu
		15	41 years	Sporadic	<50%	Normal	SCR15: His893Arg
		16	At birth	Sporadic	ND	ND	SCR7: Gln400Lys
Filler [9]	1	1	1 year	Familial	Normal	Normal	SCR20: c.3546_3581dup36

Table 2 (continued)

Reference	No. of studied cases	No. of mutated cases	First clinical description age at onset	HUS form	FH antigenic levels	C3 antigenic levels	FH genetic study
Esparza Gordillo [8]	41 cases	9 (22%)					
		Cas 2	23 years	Sporadic	Normal	Normal	SCR20: Trp1183Leu
		Cas 3	53 years	Sporadic	50%	ND	SCR20: Val1197Ala/Del exon2
		Cas 12	12 years	Familial	Normal	Normal	SCR20: Leu1189Ar
			13 years	Sporadic	Normal	Normal	SCR16: Thr956Met
				ND	Normal	ND	SCR20: Val1197Ala
					Normal	ND	SCR20: Arg1210Cys
					Normal	ND	SCR20: Ser1191Trp
					Normal		SCR20: Leu1189Phe
					Low	ND	C 3221A>T

ND not determined

analysed. Moreover, different genetic molecular techniques were used in the various studies: either screening by single strand conformation polymorphism followed by sequencing analysis, or direct sequencing analysis. This difference may lead to variability in the sensitivity of mutation detection in the various studies.

Only a few reported mutations have been associated to an FH deficiency as defined by antigenic plasma levels below the normal ranges of the technique—antigenic levels of approximately half the normal value were consistent with partial FH deficiency, undetectable levels consistent with complete FH deficiency. However, the antigenic FH level was not determined in every case, and one could suppose that the mutations leading to a premature stop codon, or involving one of the four cysteines of the SCR involved in S-S bonds [14], were responsible for the absence of circulating protein as previously demonstrated [1, 37]. This point is of particular importance because the functional consequences in a non-producing allele are very different from those in a mutated allele. In the first case, the consequence is a haplo-insufficiency of FH, in the other case, the production of an abnormal circulating protein. This abnormal circulating FH might influence the normal protein's function or disturb other proteins.

All the described mutations are heterozygous except the rare cases of homozygous FH deficiencies and four cases of compound heterozygous patients [8, 22, 31]. It is interesting to note that certain mutations have been reported in different studies. All of these (for example, the Arg1210Cys mutation) are located in the SCR20 [4, 8, 22]. The Trp1183 has been reported changed to an arginine [22, 30] or to a leucine residue [6, 8]. The Ser1191 has been reported mutated to a leucine [31] or to a tryptophan [8]. The arginine1215 has been found changed to a glycine in one case [31] and to a glutamine in another study [4]. The elevated frequency of mutations located in the same residue suggests that these amino acids are extremely important for the protein function involved in the disease process. The functional consequences of some of these mutations have been studied using different approaches: purification of plasma FH from FH mutated patients [36], generation and recombinant expression of different mutated regions of FH in the baculovirus system [18, 24] or molecular modelling of the FH C-terminal domains [27]. These different works demonstrated that these mutations impaired the binding of FH to surface-bound C3b as well as to heparin and endothelial cells. However, none of the studies was allowed to determine whether any one binding was predominantly impaired in atypical HUS. Different authors put forward the hypothesis that patients exhibiting FH mutation essentially carry a dysfunction in the protection of cellular surfaces—in particular of endothelial cell surfaces—against complement activation, leading to a specific susceptibility for the development of atypical HUS.

Determining whether particular FH genotypes/phenotypes are associated with specific clinical features would help to understand the role of factor H in HUS. This can be analysed for instance for FH deficient patients, in whom genetic abnormalities led to the same phenotype, for instance FH haplo-insufficiency (50% normal FH in blood, no abnormal form of FH), irrespective of the precise mutation. However, the clinical course of these patients is heterogeneous [6]. Some have one episode of HUS leading in one shot to complete renal failure, others have a complete recovery and still others suffer from several recurrences with increasing renal damage. Furthermore, onset of the disease may occur in early childhood or later in life. Some FH-deficient patients have presented unique features of TMA. One deficient patient developed chronic renal failure without precise diagnosis; the FH deficiency was suspected because of a renal graft failure and TMA lesions seen on the kidney biopsy. Even the renal graft outcome may vary among FH deficient patients. Among patients ex-

hibiting FH mutated protein, clinical features may vary according to the identified mutation. Unfortunately, the clinical descriptions of these patients were frequently insufficient for such analysis. However, among patients exhibiting the same mutation, the HUS mode of inheritance may differ, suggesting an incomplete penetrance of the disease transmission. For example, the Arg1210Cys mutation was associated with a familial form in one case and with the sporadic form in three cases [4, 22]. Moreover, in one of the sporadic cases, familial genetic analysis identified three carriers, healthy at the time of the investigation [4]. The Trp1183 to arginine or to leucine mutations were also found to be associated with a familial form in three cases and a sporadic form in two cases [6, 8, 22, 30]. However, the mutation Trp1183 to arginine has been reported by us and by two other groups in association with a familial form of aHUS with early onset [6, 30, 22].

Anti-factor H autoantibodies

As described in TTP patients, in whom a von Willebrand factor protease (ADAMST-13) deficiency may be inherited or acquired secondary to IgG antibodies, we have recently reported three atypical HUS children exhibiting persistent anti-FH antibodies leading to an acquired FH deficiency [7]. The presence of anti-factor H IgG was detected using an enzyme-linked immunosorbent assay (ELISA) method with coated purified human factor H. One patient presented with a sporadic form leading to end stage renal disease (ESRD) at the first flare, and the others presented with a recurrent form of HUS. Two of them exhibited persistent low C3 levels consistent with alternative pathway activation, whereas C3 levels were normal in the last case. The plasma factor H decay accelerating activity, investigated using an FH functional test, was found to be decreased in the three patients, and we demonstrated for two patients that this perturbation correlated with the anti-FH antibody titers. Plasma factor H antigenic levels and factor H gene analysis were normal, indicating that the presence of anti-FH antibodies leads to an acquired functional FH deficiency. As reported in many patients presenting with an acquired ADAMST-13 deficiency [5], all three patients exhibited anti-nuclear antibodies (ANA) which may be related to this particular form of disease. The association of ANA with organ-specific auto-antibodies is frequently reported in various autoimmune disorders pointing to the importance of immune dysregulation in the induction of these diseases. To date, none of the three patients has presented any symptom or any other biological marker of systemic autoimmune disease such as systemic lupus erythematosus.

The high frequency of "auto-antibody mediated" ADAMST-13 deficiency among the TTP patients contrasts with the apparent low frequency of anti-FH antibody in HUS patients in whom genetic factors seem to be predominant. However, such auto-antibodies might be responsible for more atypical HUS than reported so far, particularly in the still large group of patients with atypical HUS not explained by a mutation. Indeed, anti FH auto-antibodies have not yet been looked for systematically, and it is quite possible that they are present only transiently. The main conclusion, however, is that HUS may occur in the context of an auto-immune disease with the development of anti-FH-specific antibodies leading to acquired FH deficiency and HUS. This new mechanism of FH deficiency/HUS should lead to new diagnostic approaches, i.e. rapid measurement of anti-FH autoantibodies, and when positive, to adequate treatment approaches such as plasmapheresis and/or immunosuppression.

Membrane co-factor protein (or CD46)

Membrane co-factor protein (MCP) is a widely expressed type 1 transmembrane glycoprotein that inhibits complement activation on host cells. MCP is the target for several pathogens. It is a receptor for measles virus (MV), group A *Streptococcus pyogenes*, pathogenic *Neisseria* species and human herpesvirus 6. The amino terminus of MCP consists of four complement control protein (CCP) repeats. Following this is an alternatively spliced region for extensive O glycosylation (termed the STP domain B and C). The extracellular portion of MCP is followed by a hydrophobic transmembrane domain and a cytoplasmic anchor. Through alternative splicing, MCP is expressed on most cells with two distinct cytoplasmic tails and mediates signalling events. Structurally, MCP consists of four alternatively spliced isoforms that co-exist on most cells. MCP is widely distributed, being present on leukocytes, platelets, endothelial cells, epithelial cells and fibroblasts, and regulates complement activation by serving as a membrane-bound co-factor for the plasma serine protease factor I to cleave C3b and C4b. Sites for C3b and C4b interactions have been mapped by CCP deletions primarily to modules 2, 3 and 4 [17]. In addition, the MV binding site has been localised to CCPs 1 and 2.

The *MCP* gene is located within the RCA cluster on chromosome 1 with 14 exons spanning ~43 kb [33]. MCP belongs to the RCA family of structurally, functionally and genetically related receptor and inhibitory complement proteins. Other members are CR1 (CD35) and CR2 (CD21), decay accelerating factor (DAF; CD55) and the plasma proteins, C4b-binding protein (C4BP) and FH. Because MCP lies within the RCA cluster on chromosome 1, it was considered as a candidate gene in the two families from the original linkage study in whom an FH mutation had not been found. Richards et al. [32] proceeded to sequence MCP in 30 HUS families and identified for the first time functionally significant mutations in seven patients (three pedigrees) presenting a family history of the disease with a recessive form of inheritance. In one family, a heterozygous 6 bp deletion (GACAGT) in the exon 6, resulting in the loss of two amino acids (ΔD237/S238), was detected in affected individuals and associated with a reduced expression of MCP at the surface of peripheral blood mononuclear cells (PBMCs). In this family, three male siblings were affected after the second decade of life (at 27, 31 and 35 years of age). The clinical course was similar in all three, and the clinical episode of HUS was accompanied by an irreversible loss of renal function. C3 levels were normal at presentation. Subsequently, all three received a cadaver renal transplant with no recurrence of HUS in the allografts, which is of interest considering that the allograft expressed normal MCP. In two families, a transition (T822C) resulting in a serine to proline change, S206P, in the exon 6 encoding CCP4 was found. In one of these families, two male siblings were affected at the ages of 8 and 15 years, respectively. Renal function recovered in both cases. The two affected individuals were heterozygous for the S206P substitution. In the other family, one male and one female sibling were affected, both of whom also made a complete recovery. The affected individuals in this family were homozygous for the substitution, their parents being first-degree relatives. In the affected individuals from both families, fluorescence-activated cell sorter (FACS) analysis revealed that MCP expression in PBMCs was normal. However, the binding of C3b to MCP in PBMC lysates was markedly reduced in affected heterozygotes and undetectable in affected homozygotes. The functional consequences of the S206P substitution were confirmed in CHO cells expressing this mutant. These studies indicated that the mutant protein's ability to interact with C3b was severely impaired (Table 3).

Table 3 Summary of CD46 mutations reported in association with aHUS

Mutation	Age at onset of the disease	Clinical presentation	Reference
6 bp deletion (HE)	27	Caucasian male ESRD after the first flare No recurrence after renal transplantation	[32]
6 bp deletion (HE)	31	Caucasian male ESRD after the first flare No recurrence after renal transplantation	[32]
6 bp deletion (HE)	35	Caucasian male ESRD after the first flare No recurrence after renal transplantation	[32]
S206P (HE)	8	Caucasian male HUS with complete recovery of renal function without dialysis (one flare)	[32]
S206P (HE)	15	Caucasian male HUS with complete recovery of renal function (one flare)	[32]
S206P (HO)	17	Turkish male HUS with complete recovery of renal function	[32]
S206P (HO)	9	Turkish female HUS with complete recovery of renal function Recurrence 2 years later	[32]
2 bp deletion in exon 6 (HE)	16 months	Caucasian female Haemolytic anaemia and thrombocytopenia with normal renal function Six recurrences of TMA with deteriorating renal function ESRD at 20 years	[23]
2 bp deletion in exon 6 (HE)	9	Caucasian male HUS with complete recovery of renal function (two recurrences)	[23]
C 903-907 del	ND	ND	[8]
IVS2+1 G>C	ND	ND	[8]
P131S	ND	ND	[8]
R69T	ND	ND	[8]

HE heterozygous, *HO* homozygous, *ND* not determined

Noris et al. [23] have also described a family in which two siblings had HUS associated to a MCP mutation. The female sibling was first affected at the age of 16 months and had six recurrences of HUS, all associated with deteriorating renal function. She was in complete renal failure after a last episode of HUS at the age of 20. Her male sibling had two episodes of HUS at the age of 9, but made a complete recovery with no renal sequelae. The female sibling exhibited a mildly decreased C3 plasma level suggestive of mild alternative pathway-mediated complement activation. In her brother, the antigenic plasma levels of C3 were in the normal range. Mutation screening in both siblings revealed a heterozygous two base-pair deletion in MCP exon 6 which encodes CCP4, causing a change in three amino acids and the creation of a premature stop codon. The mutation was associated with a reduced expression of MCP on PBMC. Recently, Esparza-Gordillo [8] reported four novel mutations in MCP in

patients with atypical HUS (c.903–907 del; IVS2+1 G>C, P131S, R69T). In all but one case (R69T), the expression of MCP was low at the surface of the cells (Table 3).

Atypical haemolytic uraemic syndrome and factor I

Because persistently low C3 levels with no evidence of mutation in all FH exons were observed in some HUS patients, and because the serine protease factor I plays an important role in inhibiting the alternative pathway amplification loop, factor I was considered as a candidate gene for atypical HUS. Factor I cleaves the alpha chains of C4b and C3b and is thereby involved in the regulation of both the classical and alternative complement pathways. However, factor I cleavage is efficient only in the presence of co-factors. C4 bp, FH and MCP first bind C4b and C3b, allowing factor I cleavage [25].

The factor I gene is located outside the RCA cluster on chromosome 4q25, spans 63 kb and comprises 13 exons. The protein is a heterodimer of about 88,000 MW consisting of a non-catalytic heavy chain of 50,000 MW linked to a catalytic light chain of 38,000 MW by a disulphide bond. The protein is synthesised as a single chain precursor of 565 amino acids, predominantly in the liver. Four basic amino acids are then excised from the precursor prior to secretion of the heterodimer. Like many complement proteins, factor I has a modular structure. The heavy chain contains two low-density lipoprotein receptor (LDLr) domains, a CD5 domain, a module found only in factor I and complement proteins C6 and C7 (FIM; also called FIMAC). The light chain of factor I, which is the serine proteinase region of the molecule, is encoded in the terminal five exons. Factor I deficiency has been described previously as a recessive disorder in ~39 kindred and is usually associated with a predisposition to pyogenic infection due to a secondary deficiency of C3 and factor B [16, 41].

To date, four atypical HUS patients exhibiting a factor I mutation have been reported by two different groups. Our group reported for the first time the clinical history of three patients presenting different genetic abnormalities in the factor I gene [10]. After pregnancy, patient 1, a 32-year-old Caucasian woman, developed acute renal failure, hypertension and haemolytic anaemia. Renal biopsy disclosed a pattern of TMA predominantly in the glomeruli, with few vascular lesions. HUS recurred 2 and 4 months later with partial renal function recovery but persistent hypertension. One year later she was started on maintenance haemodialysis. Patient 2, a 17-month-old Caucasian boy, suffered from HUS with severe anaemia and mild renal involvement (hypertension and proteinuria). He relapsed 6 months later. Three years after the onset, he requires anti-hypertensive treatment, but his renal function remains normal. Patient 3, a 26-year-old woman, developed chronic renal failure without precise diagnosis. She had lost her first kidney transplant as a result of HUS coinciding with acute rejection and was hospitalised for recurrence of HUS following a second renal transplant. Renal biopsies confirmed histological features of thrombotic micro-angiopathy.

At the time of diagnosis, patients 1 and 2 had low C3 and factor B levels, whereas patient 3 presented normal C3 levels. All patients had normal FH antigenic levels and normal FH gene sequence analysis. Patients 1 and 3 had decreased antigenic levels of factor I (50% of normal value), whereas patient 2 had normal factor I antigenic levels. Sequencing analysis of all 13 factor I gene exons revealed nonsense mutations in exons 11 (G456X) and 13 (W528X) in both cases, exhibiting heterozygous factor I deficiency. In the other case, a missense mutation in exon 13 was found, resulting in an aspartic acid to valine change at

position 506 (D506V) (Table 4). This residue belongs to five highly conserved amino acids GD*S*GG thought to be critical for protease activity. Recently, a new mutation, located in exon 13 of factor I gene was also reported by another group [8].

Haemolytic uraemic syndrome and the complement control proteins

These results strongly suggest that mutations in three complement genes implicated in the regulation of alternative pathway predispose to atypical HUS. However, the differential diagnosis of the different forms of HUS remains often difficult. Diarrhoea preceding HUS is considered a clear criterion for the diagnosis of typical Stx-related HUS, but this could be misleading because it is not rare for diarrhoea to be absent in typical HUS [3] and may be present in atypical HUS, secondary to microangiopathic lesions of the gut. In the literature, episodes of diarrhoea have already been reported among HUS patients presenting with FH deficiency [2, 28, 29, 35]. In fact there are no definite criteria to distinguish between typical and atypical sporadic HUS. The other forms of atypical HUS are easier to define after exclusion of secondary HUS (neoplasia, organ transplantation, toxic drugs, etc.) since they are indisputable in cases of recurrent and familial forms of HUS (although there are rare reports of patients having two episodes of typical HUS) [39]. Sometimes, the clinical features are incomplete, and an atypical HUS may be suspected solely because of the presence of TMA lesions in the kidney.

Among patients with atypical HUS, a subgroup of between 10 and 22% carry mutations in the gene encoding for FH. At this time, only four pedigrees corresponding to 13 cases have

Table 4 Summary of factor I mutations reported in association with aHUS

Mutation	Domain protein	Age at onset of the disease	Clinical presentation	Consequence	Reference
R456X	Serine protease	32 years	Caucasian woman ESRD after three recurrences during 1 year	Heterozygous deficiency	[10]
D506V	Serine protease (exon 13)	17 months	Caucasian boy Recurrent forms (one relapse after 6 months) 2 years after the onset, he requires anti-hypertensive treatment but his renal function is normal	Mutation of the aspartic acid at −1 of the active serine to valine may lead to functional factor I deficiency	[10]
Try 528 Stop	Serine protease (exon 13)	26 years	Caucasian woman Recurrence of HUS following renal graft. two renal failures	Heterozygous deficiency	[10]
c.1624 inAT	Serine protease (exon 13)	ND	ND	Heterozygous deficiency	[8]

ND not determined

been reported with an MCP mutation and four cases with a sporadic disease presented factor I mutation. The number of patients is small, and the analysis of genotype-associated phenotype may be still difficult. Whereas FH mutations were reported in both familial and sporadic forms of HUS, MCP mutations were restricted to familial HUS, and factor I mutations were only observed in cases of sporadic HUS. The relation between penetrance and external factors determining the acute attacks of HUS remains unresolved. A characteristic feature of atypical HUS is reduced penetrance and variable inheritance; one possible explanation for this is that polymorphic changes in complement proteins act as modifiers. Recently, the analysis of single nucleotide polymorphisms in both *HF1* and *MCP* genes in three large cohorts of HUS patients identified common variants of the proteins and a strong and significant association between atypical HUS and HF1 and MCP particular alleles [8, 11].

For the time being, renal transplantation in a patient with FH mutation apparently entails a high risk of graft loss (less than 50 cases reported), whereas no recurrence of the disease was reported in the three patients with MCP mutation. In one of the patients with factor I mutation, the kidney transplant failed twice following recurrence of the disease. Among FH mutated patients, the recurrence range is 30–75% according to different reports. FH is a plasma protein synthesised by the liver, whereas MCP is synthesised in part locally by the kidney. The presence of MCP in the transplant kidney may be sufficient to protect against recurrent HUS.

According to the lack of identified susceptibility factors in more than 60% of atypical HUS patients, the actual challenge is to determine new genetic or acquired factors. At this time, several candidate genes in the complement regulation system have been investigated. There are five principal regulators: C4-binding protein (C4BP) and FH are plasma proteins

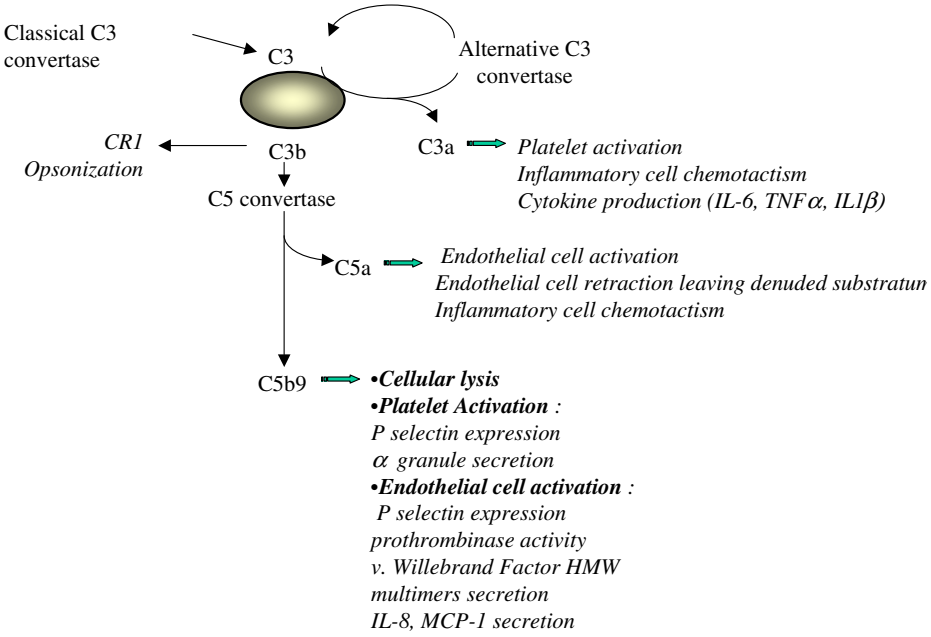

Fig. 2 The alternative pathway dysregulation in atypical HUS may lead to different signalising pathways capable of participating to the microangiopathic lesions in the kidney

that regulate the classical and alternative pathway, respectively, by both decay accelerating and co-factor activity; DAF (CD55), MCP and complement receptor 1 (CR1, CD35) are all membrane bound and regulate both the classical and the alternative pathways. No mutation in CR1 was identified in patients with familial, recurrent or sporadic HUS [23]. No mutation was found in the DAF gene in a cohort of 41 patients by Esparza-Gordillo et al. [8]. FH-related protein-5 (FHR-5) is a human plasma protein with structural similarities to human FH and the four other recently described FH-related proteins. Murphy et al. [19] showed FHR-5 to be strongly associated with glomerular complement deposition and suggested a role in complement activation or regulation. However, no FHR-5 mutation was found in a study performed in 25 HUS patients [23].

To date, three genes have been identified as susceptibility factors for atypical HUS. All three genes are crucially implicated in the regulation of the complement alternative pathway. These data allowed us to suggest that an alternative pathway dysregulation participates in the pathological mechanisms leading to HUS. The impairment of alternative pathway regulation leads to the excessive liberation of different cleavage fragments such as C3a and C5a and to the formation of the C5b9 complex. All three components mediate different signalling pathways capable of participating to the microangiopathic lesions in the kidney (Fig. 2). C3a and C5a are anaphylatoxins, which have the capacity to activate platelets and endothelial cells [38, 43], and chemotactic factors, which enhance inflammation, and finally have the property to induce pro-inflammatory cytokines [15]. C5b9 complexes are able to induce platelets activation with induction of P-selectin expression, release of dense granule adenosine triphosphate, secretion of α granules containing high-weight von Willebrandt factor multimers and prothrombinase activation [45]. Furthermore, C5b9 appears to play a central role in microangiopathy as demonstrated in an animal model [13, 20, 21].

Conclusions

The recent progress made in the determination of the susceptibility factors for atypical HUS suggest new diagnostic investigations in these patients. For the time being, prospective studies should include the genetic analysis of FH, factor I, MCP and possibly other complement regulators, as well as the determination of anti-FH antibodies. A clearer definition of the various forms of HUS will help to define new treatment strategies including immunosuppression when anti-factor H antibodies are present, or to determine whether transplantation should be performed (recurrences when FH mutations are present, none for MCP), and if a transplantation is desired, which family member might be a donor (avoidance of a member with a mutation).

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