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Report

Factor H Mutations in Hemolytic Uremic Syndrome Cluster in Exons 18–20, a Domain Important for Host Cell Recognition

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Several recent studies have established an association between abnormalities of complement factor H (FH) and the development of hemolytic uremic syndrome (HUS). To identify the relative importance of mutations in FH as a cause of HUS, we have undertaken mutation screening of the FH gene in 19 familial and 31 sporadic patients with FH. Mutations were found in two familial and three sporadic patients, and these clustered in exons 18–20, a domain important for host recognition. Moreover, this study demonstrates that familial HUS is likely to be a heterogeneous condition.

Hemolytic uremic syndrome (HUS) (MIM 134370) is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. It can be classified as diarrheal (d+ HUS) or nondiarrheal (d− HUS), which in turn are classified as either sporadic or familial. Since Thompson and Winterborn's observation (1981) of two brothers with HUS and low levels of factor H (FH), there have been a number of reports of low FH levels in patients with HUS (Roodhooft et al. 1990; Pichette et al. 1994; Ohali et al. 1998; Rougier et al. 1998; Warwicker et al. 1998; Noris et al. 1999).

The alternative complement pathway distinguishes host from foreign tissue, and FH is both a key recognition protein in the system and a down-regulator of alternative pathway activity (Pangburn et al. 2000). C3b is continually deposited on all cell surfaces in contact with the blood. If it binds factor B, the alternative pathway is activated, leading to formation of the membrane attack complex and cell lysis. FH binds to host cell surfaces and competes with factor B to bind C3b. FH is also a cofactor for factor I–mediated breakdown of the C3 convertase C3bBb and has independent decay-accelerating properties (Zipfel et al. 1999).

FH is located in the "regulators of complement activation" cluster on chromosome 1. This gene cluster includes decay-accelerating factor, complement receptors type 1 and 2, membrane cofactor protein, C4-binding protein, clotting factor XIIIb, FH, and the FH-related protein genes (FHR1, FHR2, FHR3, and FHR4) (Zipfel and Skerka 1994; Pardo-Manuel de Villena et al. 1996; DiazGuillen et al. 1999; Zipfel et al. 1999). The gene family has arisen by multiple duplication events. Each of these genes encodes proteins, which consist of multiple (contiguous) homologous modules called "complement control protein modules" (CCPs). CCPs were previously known as short consensus repeats (SCRs). Each CCP is ~60 amino acids in length and has four cysteine residues that form two disulphide bridges, Cys1–Cys3 and Cys2–Cys4 (Schmidt et al. 1999).

FH comprises 20 CCPs, each encoded by a single exon, with the exception of the second CCP encoded by exon 2a and 2b. There is, in addition, an alternatively spliced product of the FH gene called "FHL1" or "reconectin," which consists of the first seven CCPs. FHR1, FHR3, and FHR4 each have five CCPs, and FHR2 has four CCPs. There is a high level of identity between FH

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exons 18, 19, and 20 and FHR1 exons 3, 4, and 5. The sequence identity is 100% between FH exon 18 and FHR1 exon 3, 97% between FH exon 19 and FHR1 exon 4, and 97% between FH exon 20 and FHR1 exon 5. The respective intronic sequences also show 99% identity (Male et al. 2000).

The molecular basis of the FH abnormality has been reported in only two families with HUS and in a single sporadic patient. HUS segregated as a dominant disorder in the first family, and affected individuals had normal FH levels. A single-base substitution leading to R1215G was identified (Warwicker et al. 1998). In the second family, the disorder was recessive, and FH levels were low in affected individuals. In this family, a base pair substitution and a 24–base pair deletion led to loss of the last seven amino acids of CCP 20, including a cysteine. This resulted in aberrant folding and decreased FH secretion (Ying et al. 1999; Buddles et al. 2000). The sporadic patient with HUS had an exon 1–truncating mutation on one allele and resultant half-normal FH levels (Warwicker et al. 1998).

We have now systematically looked for FH mutations in 19 affected individuals with one or more affected relatives and in 31 affected individuals with no family history of the disorder. The Newcastle and North Tyneside Area Health Authority Ethics Committee approved the study. We designed intronic primers to amplify specifically each exon of FH without amplifying the homologous exons of FHR1. Specificity of the primers for FH exons 18, 19, and 20 was verified by sequencing PCR products amplified from PAC 15d12, which contains the genomic sequence of FH exons 8–20, FHR1, and FHR3. Primers and conditions are shown in table 1. Mutational screening of each exon was performed on a panel of 50 patients (19 familial and 31 sporadic cases) affected with HUS. Bidirectional dideoxyfingerprinting, a technique with a high reported sensitivity and specificity, was used (Liu et al. 1996). Samples showing a change on the nondenaturing MDE gel were further analyzed by direct fluorescent sequencing of a purified PCR product. We now describe a further five patients with HUS and mutations in the FH gene (table 2) and one new polymorphism. All the changes occurred in exons 18–20 (CCPs18–20). The polymorphism was in exon 18 (N1050Y) and was found in two patients with HUS, and with an allele frequency of .04 in controls.

Two changes were observed in patient 1, a sporadic patient. The first, in exon 18, is a heterozygous C→G transition. This results in the substitution of the uncharged polar amino acid glutamine (Q) by an acidic species glutamic acid (E). The second, in exon 19, is a heterozygous single–base pair deletion (delA3559) and causes a frameshift that changes the terminal three amino acids of CCP 19. This results in a lysine to asparagine change (K1162N), a cysteine to alanine change (C1163A), and a leucine to tyrosine change (L1164Y). If normal splicing of the exon 19/20 boundary occurs, an alteration to the reading frame of exon 20 will result leading to a premature stop codon in exon 20 after abnormal isoleucine (I), arginine (R), and valine (V) residues. This is shown in figure 1.

Patient 2 is a member of an affected sibling pair with normal C3 and FH levels. A single heterozygous base pair substitution causes the replacement of aspartic acid (D) by the nonpolar glycine (G) in exon 19. This residue is conserved in human FH, FHR1, and FHR3 and FH in mouse and sand bass (*Parablax nebulifer*), suggesting a functional role for this site.

Patient 3, a sporadic patient, has normal C3 and FH levels and demonstrates the same change in exon 20 (R1215G) described by our group elsewhere (Warwicker et al. 1998). The patient is from a different region of the United Kingdom, with no known antecedents from the region of the family described by Warwicker et al. This change causes the substitution of a nonpolar amino acid in place of a highly conserved basic amino acid. The change is inherited from her father, who is unaffected.

Patient 4 is a sporadic patient in whom a single heterozygous base pair substitution causes the replacement of threonine (T) by arginine (R) in exon 20. FH levels were normal but C3 was low. His unaffected mother also shows the same change at this position. The change results in the substitution of an uncharged polar for a charged polar basic residue.

Patient 5 is a familial patient with an affected mother who died from HUS. The two changes seen are identical to the two amino acid differences between FH CCP 20 and FHR1 CCP5 (S1191L and V1197A). The changes were shown, by sequencing of cloned products, to lie on the same allele.

Thus, we have identified FH mutations in two patients known to have a single affected relative (one a parent and the other a sibling) and in three sporadic patients with HUS, providing further evidence that FH abnormalities are a cause of HUS. The mutations are clustered in exons 18–20, as shown in figure 2.

Three of the affected individuals have normal FH levels, indicating a functional abnormality in the secreted protein. This is an important observation because it means that normal FH levels do not exclude FH as underlying HUS in a particular individual. These changes were D1119G (CCP19), T1184R (CCP20), R1215G (CCP20), and a probable gene-conversion event changing a serine to leucine and a valine to alanine in CCP20. These CCPs are now known to have a role both in C3b binding and in binding to cell surfaces. FH binds to anionic structures on cell surfaces, and thus the polyanion heparin has been used to identify which CCPs bind to cell surfaces. Blackmore and colleagues (1996, 1998)

Table 1

FH Intronic Primers and PCR Conditions

Intronic Primer Position	Primer Sequence	Annealing Temperature (°C)	Magnesium Concentration (mM)
Leader Sequence	F: ggagtgcagtgagaattggg	53	2.5
	R: caacaatgtcaaaagccactc		
1	F: ctccatagatatggggttagg	55	1.5
	R: cagagccagactccatctc		
2a	F: ccttacattcaatctgtcttc	53	1.5
	R: ccatcatagttaaactttcagg		
2b	F: gacactcagaatggcatcgag	55	1.5
	R: gatcaggctgcattcgtttttg		
3	F: cctccaatcttatcctgaggatg	55	1.5
	R: gctgatattccttagaatgaacg		
4	F: cctgatggaaacaacatttctg	45	2.5
	R: cataaattagcactctacttttg		
5	F: gccattttgtattatgctaagg	55	1.5
	R: cttactttgtatatacaataagac		
6	F: gagtgtttattacagtaaaatttc	53	3.0
	R: gtgctctcctttcttcgatc		
7	F: ttagtaactttagttcgtcttcag	55	3.0
	R: ggtccattggtaaaacaaggtg		
8	F: ggttttcagttacaaatgactc	53	1.5
	R: gaaattatatcagcccccac		
9	F: gctttttcttcttagaattgggg	53	3.0
	R: caactatccttaacaattcctc		
10	F: tctgatgcccctctgtatgacc	45	3.0
	R: cttatttcagcaattgtaagataag		
11	F: gaagaaaatctttccatttttactg	43	3.5
	R: caaaatacaaaagttttgacaag		
12	F: acttgtcaaaactttttgtattttg	55	1.5
	R: ggaaatgttgaggcttatctg		
13	F: gattaagtcataattttaccatgc	55	1.5
	R: cacacatacctattacttttcc		
14	F: gtgataatttatgaaacagttattg	45.6	3.5
	R: ctctcttctttacacgaagcac		
15	F: caaattatactcactttaaaatccg	55	3.0
	R: cccctcactttgataacaagag		
16	F: gtgatgtcatagtagctcctg	55	1.5
	R: ctagagtccctgtttactttc		
17	F: cgctattttagaatccattacatg	55	1.5
	R: ggcctcccaaagtgctggg		
18	F: gtaactgttatcagttgatttgc	60	1.5
	R: tagccctgctatactccccc		
19	F: ttcttccaggactcatttctttc	54	1.5
	R: gtgagtattttgttacaaacagtg		
20	F: tttattcaaatcaatatgatgtttc	53	2.5
	R: agttctgaataaaggtgtgcac		

NOTE.—Conditions were as follows: 94°C for 4 min, then 30 cycles of 94°C for 1 min, annealing temperature for 1 min, and 72°C for 1 min.

cloned a series of FH mutants in a eukaryotic expression vector and then tested the heparin-binding capacity of the mutant proteins. They concluded that the heparin binding sites are CCP7 and CCP20. Further evidence comes from the observation that a monoclonal antibody with an epitope within CCP19 or CCP20 inhibits heparin binding by FH (Prodinger et al. 1998). The basic amino acids arginine and lysine are primarily responsible for heparin binding, and thus the arginine to glycine change R1215G is likely to disrupt heparin binding and in vivo binding of FH to host cells. We have cloned each of these mutations so that we can assess their affect on heparin binding.

FH deficiency has also been reported in patients with glomerulonephritis (Levy et al. 1986), systemic lupus erythematosis (SLE) (Lopez-Larrea et al. 1987), and recurrent meningococcal meningitis (Nielsen et al. 1989). The molecular basis has been studied in one child with

Table 2

Mutations Detected by Bidirectional Dideoxyfingerprinting

Patient	Mutation	Effect	CCP	Family History	Comments
1	C3299G	Q1076E	18	Sporadic	…
	delA3559	Frameshift	19	Sporadic	Same patient as above
2	A3429G	D1119G	19	Sibling pair	(D) Conserved residue
3	C3716G	R1215G	20	Sporadic	Previously reported in AD kindred. (R) Conserved residue
4	C3624G	T1184R	20	Sporadic	…
5	C3645T	S1191L	20	Mother affected	Probable gene-conversion event
	T3663C	V1197A			

NOTE.—Q = glutamine, E= glutamic acid, D = aspartic acid, R =arginine, G = glycine, T = threonine, S = serine, L = leucine, V = valine, and A= alanine. The system of base pair/amino acid numbering is adapted from Ripoche et al. (1988). The amino acid number includes the 18–amino acid leader.

type II mesangiocapillary (membranoproliferative) glomerulonephritis who had normal levels of FHL1 but undetectable levels of FH by radioimmunoassay. He was found to have a maternally inherited change in CCP9 (C518R) and a paternally inherited change in CCP16 (C991Y). Both of these mutations led to loss of a cysteine residue, such that disulphide bridges essential for protein folding could not form. Abnormal secretion of the protein was confirmed by pulse chase experiments (Ault et al. 1997). FH deficiency inherited as an autosomal recessive trait in the Norwegian Yorkshire pig is also associated with mesangiocapillary glomerulonephritis (Hogasen et al. 1995), but the molecular mechanism responsible has not been described. There has been a recent report of FH deficiency in a consanguineous Italian family (Sanchez-Corral et al. 2000) in which the proband had SLE and chronic renal failure and her two brothers had had one and three episodes of meningococcal meningitis. They had a homozygous truncating mutation in exon 3 and, hence, absence of both FH and FHL1.

Thus FH abnormalities have been reported in association with type II mesangiocapillary glomerulonephritis, SLE, recurrent meningococcal meningitis, and HUS. Affected individuals in the two families with SLE and meningococcal meningitis (Fijen et al. 1996; Sanchez-Corral et al. 2000) have had absent FH and, in one, concurrent absence of FHL1, and the disorder has been autosomal recessive. In the one case of mesangiocapillary glomerulonephritis that has been studied extensively, FH has been absent, but FHL1 levels were normal, and, again, the disorder was autosomal recessive. For HUS, both dominant and recessive pedigrees have been reported. In some families, the FH level has been very low, whereas in other cases it has been normal. From the changes reported here, we hypothesize that mutations in SCR 19/20 that affect the host recognition by FH predispose to HUS. However, this does not explain why the previously reported sporadic patient with half-normal FH levels and one normal copy of the gene developed HUS, whereas adults with reduced FH levels in the autosomal recessive pedigrees have not had a clinical phenotype. Nor does it explain why, in the two reports of low FH levels due to aberrant protein folding and decreased secretion, the phenotype in one family is mesangiocapillary glomerulonephritis and in the other family is HUS. However, hemizygous mutations in CCPs 19 and 20 do appear to be a cause of HUS. In the large dominant pedigree with R1215G we reported elsewhere, there was incomplete penetrance and, in the individual in whom we have identified this change in this series, it was inherited from an unaffected parent. Similarly, in the other sporadic patient in this series for whom we had parental samples, the change had been inherited from an unaffected parent. Thus, in a proportion of patients who present with typical sporadic HUS, there will be an underlying genetic predisposition, and this may have implications for other family members. It

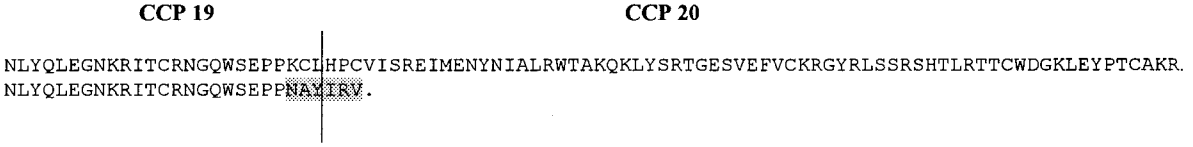

Figure 1 Comparison of CCP19 and 20 wild-type amino acid sequence with the predicted sequence in patient 1 with delA3559. The abnormal amino acids are highlighted. The vertical line indicates the boundary between CCP 19 and 20.

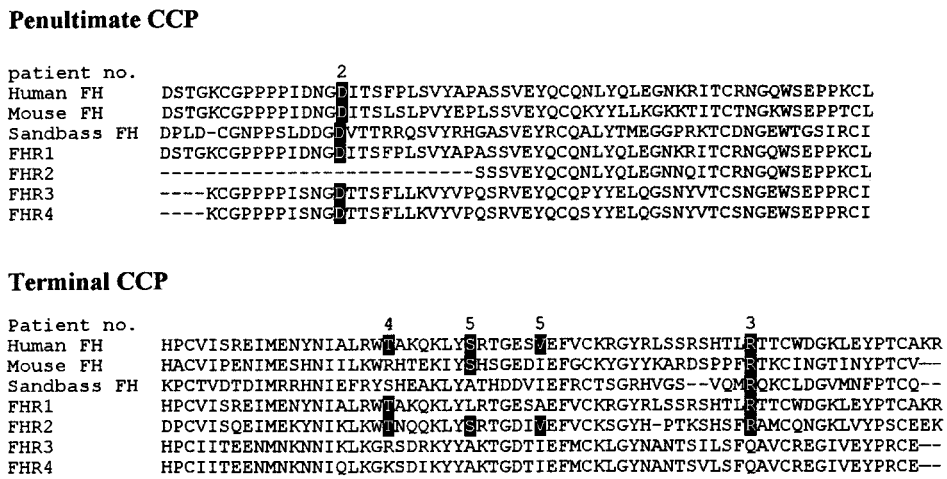

Figure 2 Amino acid sequence of the last two carboxy terminal CCPs for human, mouse, and sand bass FH and for human FHR1, 2, 3, and 4. The degree of homology at the mutation sites for patients 2, 3, 4, and 5 are shown by highlighting.

seems likely that the genetic change is a predisposing factor and that an environmental insult then precipitates the disorder, but it is possible that polymorphisms or mutations in other genes modify the phenotype.

Finally, we have failed to identify mutations in 17 families and 28 individuals with HUS, despite use of a method that is extremely sensitive in detection of single base changes. Although this method does not detect large deletions or genomic rearrangements, the low mutation-detection rate in the familial cases suggests this may be a heterogeneous condition. The family samples that we have are not suitable for linkage studies, but microsatellite markers in some of the families are not consistent with a locus on chromosome 1q32 (unpublished data), again suggesting involvement of additional genes.

In conclusion, mutations in FH exons 19/20 that affect host recognition by FH predispose to HUS, but changes in FH do not account for all cases of familial HUS. C3 and FH levels are not reliable as a screening test for FH-associated HUS.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for HUS [MIM 134370])

SWISS-PROT, http://www.ebi.ac.uk/swissprot/ (for human FH, CAA68704; FHR1,CAA39666; FHR2, I37388; FHR3, CAB53064; and FHR4, NP_006675; for mouse FH, M12660; and for sand bass FH, AAA92556)

References

Ault BH, Schmidt BZ, Fowler NL, Kashtan CE, Ahmed AE, Vogt BA, Colten HR (1997) Human factor H deficiency—mutations in framework cysteine residues and block in H protein secretion and intracellular catabolism. J Biol Chem 272:25168–25175

Blackmore TK, Sadlon TA, Ward HM, Lublin DM, Gordon DL (1996) Identification of a heparin-binding domain in the 7th short consensus repeat of complement factor H-1. J Immunol 157:5422–5427

Blackmore TK, Hellwage J, Sadlon TA, Higgs N, Zipfel PF, Ward HM, Gordon DL (1998) Identification of the second heparin-binding domain in human complement factor H. J Immunol 160:3342–3348

Buddles MR, Donne RL, Richards A, Goodship J, Goodship THJ (2000) Complement factor H gene mutation associated with autosomal recessive atypical hemolytic uremic syndrome. Am J Hum Genet 66:1721–1722

DiazGuillen MA, Rodriguez de Cordoba S, HeineSuner D (1999) A radiation hybrid map of complement factor H and factor H–related genes. Immunogenetics 49:549–552

Fijen CAP, Kuijper EJ, Bulte MTT, Vandeheuvel MN, Holdrinet ACJM, Sim RB, Daha MR, Dankert J (1996) Heterozygous and homozygous factor-H deficiency states in a Dutch family. Clin Exp Immunol 105:511–516

Hogasen K, Jansen JH, Mollnes TE, Hovdenes J, Harboe M (1995) Hereditary porcine membranoproliferative glomerulonephritis type-II is caused by factor H deficiency. J Clin Invest 95:1054–1061

Levy M, Halbwachs-Mecarelli L, Gubler MC, Kohout G, Bensenouci A, Adet P, Hauptmann G, Fesavre P (1986) Factor

H deficiency in two brothers with atypical dense intramembranous disease. Kidney Int 30:949–956

Liu Q, Feng J, Sommer SS (1996) Bi-directional dideoxy fingerprinting (Bi-ddF): a rapid method for quantitative detection of mutations in genomic regions of 300-600 bp. Hum Mol Genet 5:107–114

Lopez-Larrea C, Dieguez MA, Enguix A, Dominguez O, Marin B, Gomez E (1987) A familial deficiency of complement factor H. Biochem Soc Trans 15:648–649

Male DA, Ormsby RJ, Ranganathan S, Giannakis E, Gordon DL (2000) Complement factor H: sequence analysis of 221 kb of human genomic DNA containing the entire fH, fHR-1 and fHR-3 genes. Mol Immunol 37:41–52

Nielsen HE, Christensen KC, Koch C, Thomsen BS, Heegard NHH, Tranum-Jensen J (1989) Hereditary, complete deficiency of complement factor H associated with recurrent meningococcal disease. Scand J Immunol 30:711–718

Noris M, Ruggenenti P, Perna A, Orisio S, Caprioli J, Skerka C, Vasile B, Zipfel PF, Remuzzi G (1999) Hypocomplementemia discloses genetic predisposition to hemolytic uremic syndrome and thrombotic thrombocytopenic purpura: role of factor H abnormalities. Italian Registry of Familial and Recurrent Hemolytic Uremic Syndrome/Thrombotic Thrombocytopenic Purpura. J Am Soc Nephrol 10:281–293

Ohali M, Shalev H, Schlesinger M, Katz Y, Kachko L, Carmi R, Sofer S, Landau D (1998) Hypocomplementemic autosomal recessive hemolytic uremic syndrome with decreased factor H. Pediatr Nephrol 12:619–624

Pangburn MK, Pangburn KL, Koistinen V, Meri S, Sharma AK (2000) Molecular mechanisms of target recognition in an innate immune system: interactions among factor H, C3b, and target in the alternative pathway of human complement. J Immunol 164:4742–4751

Pardo-Manuel de Villena F, HeineSuner D, Rodriguez de Cordoba S (1996) Ordering of the human regulator of complement activation gene cluster on 1q32 by two-colour FISH. Cytogenet Cell Genet 72:339–341

Pichette V, Querin S, Schurch W, Brun G, Lehnernetsch G, Delage JM (1994) Familial hemolytic-uremic syndrome and homozygous factor-H deficiency. Am J Kidney Dis 24: 936–941

Prodinger WM, Hellwage J, Spruth M, Dierich MP, Zipfel PF (1998) The c-terminus of factor H: monoclonal antibodies inhibit heparin binding and identify epitopes common to factor H and factor H-related proteins. Biochem J 331: 41–47

Ripoche J, Day AJ, Harris JR, Sim RB (1988) The complete amino acid sequence of human complement factor H. Biochem J 249:593–602

Roodhooft AM, McLean RH, Elst E, Van Acker KJ, Vanacker KJ (1990) Recurrent hemolytic uremic syndrome and acquired hypomorphic variant of the third component of complement. Pediatr Nephrol 4:597–599

Rougier N, Kazatchkine MD, Rougier J-P, Fremeaux-Bacchi V, Blouin J, Deschenes G, Soto B, Baudouin V, Pautard B, Proesmans W, Weiss E, Weiss L (1998) Human complement factor H deficiency associated with hemolytic uremic syndrome. J Am Soc Nephrol 9:2318–2326

Sanchez-Corral P, Bellavia D, Amico L, Brai M, Rodriguez de Cordoba S (2000) Molecular basis for factor H and FHL-1 deficiency in an Italian family. Immunogenetics 51: 366–369

Schmidt BZ, Fowler NL, Hidvegi T, Perlmutter DH, Colten HR (1999) Disruption of disulfide bonds is responsible for impaired secretion in human complement factor H deficiency. J Biol Chem 274:11782–11788

Thompson RA, Winterborn MH (1981) Hypocomplementaemia due to a genetic deficiency of β-1H globulin. Clin Exp Immunol 46:110–119

Warwicker P, Goodship THJ, Donne RL, Pirson Y, Nicholls A, Ward RM, Goodship JA (1998) Genetic studies into inherited and sporadic haemolytic uraemic syndrome. Kidney Int 53:836–844

Ying L, Katz Y, Schlesinger M, Carmi R, Shalev H, Haider N, Beck G, Sheffield VC, Landau D (1999) Complement factor H gene mutation associated with autosomal recessive atypical hemolytic uremic syndrome. Am J Hum Genet 65: 1538–1546

Zipfel PF, Jokiranta TS, Hellwage J, Koistinen V, Meri S (1999) The factor H protein family. Immunopharmacology 42: 53–60

Zipfel PF, Skerka C (1994) Complement factor H and related proteins: an expanding family of complement- regulatory proteins? Immunol Today 15:121–126