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Lack of association between polymorphisms in C4b-binding protein and atypical haemolytic uraemic syndrome in the Spanish population

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Summary

Dysregulation of the alternative pathway of complement activation, caused by mutations or polymorphisms in the genes encoding factor H, membrane co-factor protein, factor I or factor B, is associated strongly with predisposition to atypical haemolytic uraemic syndrome (aHUS). C4b-binding protein (C4BP), a major regulator of the classical pathway of complement activation, also has capacity to regulate the alternative pathway. Interestingly, the C4BP polymorphism p.Arg240His has been associated recently with predisposition to aHUS and the risk allele His240 showed decreased capacity to regulate the alternative pathway. Identification of novel aHUS predisposition factors has important implications for diagnosis and treatment in a significant number of aHUS patients; thus, we sought to replicate these association studies in an independent cohort of aHUS patients. In this study we show that the C4BP His240 allele corresponds to the *C4BP*2* allele identified previously by isoelectric focusing in heterozygosis in 1·9–3·7% of unrelated Caucasians. Crucially, we found no differences between 102 unrelated Spanish aHUS patients and 128 healthy age-matched Spanish controls for the frequency of carriers of the His240 C4BP allele. This did not support an association between the p.Arg240His C4BP polymorphism and predisposition to aHUS in the Spanish population. In a similar study, we also failed to sustain an association between C4BP polymorphisms and predisposition to age-related macular degeneration, another disorder which is associated strongly with polymorphisms in factor H, and is thought to involve alternative pathway dysregulation.

Keywords: age-related macular degeneration, atypical haemolytic uraemic syndrome, C4b-binding protein, complement, polymorphism

Introduction

Human C4b-binding protein (C4BP) is an abundant plasma protein, synthesized primarily in the liver, that presents three isoforms, α7β1, α7β0 and α6β1 [1,2]. In normal plasma C4BPα7β1 is the major isoform. It is composed of seven identical α-chains (70 kDa) and one β-chain (45 kDa), linked covalently by their C-terminal regions [3,4]. The *C4BPA* and *C4BPB* genes are located within the regulator of complement activation gene cluster in chromosome 1q32 [5,6]. They are arranged in tandem with the 3′ end of the *C4BPB* gene located 4172 base pairs upstream of the 5′ end of the *C4BPA* gene [6]. C4BP is the principal fluid phase regulator of the classical pathway of complement activation [7–9]. C4BP also acts as a co-factor of factor I in the proteolytic inactivation of C3b and therefore also has potential to regulate the alternative pathway of complement activation [10]. C4BP is, however, a much weaker regulator than factor H when C3b is degraded on surfaces. Each α-chain of C4BP contains a C4b/C3b binding site, but only up to four molecules of C4b or C3b can bind one C4BP molecule [11]. The binding site for C4b spans Short Consensus Repeats (SCRs) 1–3 [12,13] and that for C3b also extends to SCR4 [10]. The α-chain of C4BP is a polymorphic protein in humans with three alleles identified by isoelectric focusing (IEF), one common (*C4BP*1*) and two relatively uncommon (*C4BP*2* and *C4BP*3*) [14,15].

Haemolytic uraemic syndrome (HUS) is characterized by thrombocytopenia, Coomb's test negative microangiopathic haemolytic anaemia and acute renal failure. Endothelial cell injury appears to be the primary event in the pathogenesis of HUS. Endothelial damage triggers a cascade of events that results in the formation of platelet-fibrin hyaline microthrombi that occlude arterioles and capillaries. A hallmark of HUS is the presence of schistocytes (fragmented cells) that generate as the red blood cells traverse these partially occluded microvessels [16]. The typical form of HUS follows a diarrhoeal prodrome and is associated with 0157:H7 *Escherichia coli* infections. However, 5–10% of HUS patients lack an association with this type of *E. coli* infections. This atypical form of HUS (aHUS) occurs in both adults and in very young children and has the poorest long-term prognosis. aHUS is associated with mutations or polymorphisms in the genes encoding the complement regulatory proteins factor H (*CFH*) [17–23], membrane co-factor protein (*MCP*) [24–27] and with mutations in the complement activating components factor B (*CFB*) [28] and C3 genes [29]. Importantly, mutations in the complement regulators factor H, MCP and factor I are loss-of-function mutations [27,30,31], while mutations in the complement activator factor B are gain-of-function mutations [28]. aHUS has also been associated with the deletion of the *CFHR1* and *CFHR3* genes [32] and the presence of antibodies against factor H [33,34].

The data available support that aHUS involves alternative complement pathway dysregulation and probably develops as a consequence of defective protection of cellular surfaces from complement activation due to an improper function of different complement proteins [35]. In addition, it is generally accepted that multiple hits, involving plasma and membrane-associated complement regulatory proteins, as well as complement activators, are probably required to cause dysregulation and significantly impair protection to host tissues [24,28,36].

The C4BP has been implicated in the regulation of the alternative pathway [10]. Recently, a case–control study has tested the possibility that genetic variations in the *C4BPA* and *C4BPB* genes were associated with predisposition to aHUS and identified a relatively uncommon *C4BPA* polymorphism, p.Arg240His, that was present in 6/166 of aHUS patients *versus* 5/542 normal controls ($\chi^2 = 6{\cdot}021$; $P = 0{\cdot}014$) [37]. Interestingly, the aHUS-associated C4BP allele His240 showed decreased binding to C3b compared with the C4BP Arg240 allele.

In this study, we have established that the p.Arg240His is the nucleotide substitution that originates the *C4BP*2* protein polymorphic variant described previously by IEF and found to be present in heterozygosis in 1·9–3·7% of unrelated Caucasians. Most importantly, we show that this polymorphism is not associated with aHUS or age-related macular degeneration (AMD) in the Spanish population.

Materials and methods

Patients and controls

Our series of aHUS patients include 125 unrelated individuals. aHUS was diagnosed because of the presence of one or more episodes of microangiopathic haemolytic anaemia and thrombocytopenia defined on the basis of haematocrit < 30%, haemoglobin < 10 mg/dl, serum lactate dehydrogenase > 460 U/l, undetectable haptoglobin, fragmented erythrocytes in the peripheral blood smear and platelet count < 150 000/μl, associated with acute renal failure. Patients with Stx-HUS, defined as the presence of Shiga toxin in the stools (by the Vero cell assay) and/or of serum antibodies against Shiga toxin [by enzyme-linked immunosorbent assay (ELISA)] and/or lipopolysaccharide (O157, O26, O103, O111 and O145, by ELISA) were excluded. None of the aHUS patients had thrombotic thrombocytopenic purpura-like manifestations. Age at onset ranges from 1 month to 70 years, with 57 patients developing the disease aged under 13 years and 68 patients aged over 21 years. All patients were analysed routinely for mutations in the *CFH, MCP, CFI, C3* and *CFB* genes, tested for the presence of anti-factor H autoantibodies, genotyped for high-risk polymorphisms in the *CFH* and *MCP* genes and analysed for copy number variation of *CFHR1* and *CFHR3* and rearrangements in the *CFH-CFHR1-5* gene region. The overall number of patients who carry mutations or rearrangements in the complement genes or have detectable anti-factor H autoantibodies represent an estimable 50% and 6% of all the atypical HUS patients in our series, respectivelly.

For the studies reported here, the 125 unrelated aHUS patients in our cohort were divided into two groups, one including 102 unrelated Spaniards living in Spain and another group of 23 aHUS patients who were referred to us from outside Spain. This last group includes seven patients from other European countries, six from the USA, six from South America and four from Tunisia. Our control population for the aHUS cohort includes 128 healthy age-matched unrelated Spanish individuals from similar geographical regions to the aHUS patients.

The C4BP studies presented here also included the analysis of 143 patients > 60 years old with AMD, who presented with advanced choroidal neovascularization and drusen in both eyes, and the corresponding control cohort of 117 eye-examined, age-matched healthy Spanish controls with no family history of AMD.

Genomic DNA was generated from a buccal swab (BuccalAmp DNA Extraction kit; Epicentre Biotechnologies, Madison, WI, USA) or peripheral blood leucocytes using standard procedures. All protocols included in these studies have been approved by national and/or local institutional review boards, and all subjects gave their informed consent.

In addition, we had DNA samples available for these studies from all members of five of the original pedigrees

that were used to demonstrate the IEF polymorphism in the α-chain of C4BP [15]. None of these individuals were included in the case–control association studies presented here.

The IEF analysis of C4BP

The IEF analysis of C4BP was performed as described [14] by using fresh ethylenediamine tetraacetic acid-containing serum or serum samples stored at –80°C. Samples were treated with neuraminidase before immunoprecipitation with a rabbit polyclonal antibody against human C4BP. Immunoprecipitates were analysed by IEF under completely denaturing conditions on vertical 4·5% polyacrylamide slab gels and stained with Coomassie blue for analysis.

C4BPA sequencing and genotyping analysis

All *C4BPA* exons were amplified from genomic DNA using the primers shown in Table 1. These amplifications were performed with the Certamp Kit for Complex Amplifications (purchased from Biotools, Madrid, Spain). The thermal cycle started with 3 min at 94°C followed by 35 cycles of 30 s at 94°C for denaturation, 40 s at the annealing temperature shown in Table 1 and another 40 s at 72°C. These 35 cycles were followed by a final elongation step of 6 min at 72°C. Automatic DNA sequencing was performed in an ABI 3730 sequencer using a dye terminator cycle sequencing kit (Applied Biosystems, NJ, USA) with one of the two primers used in the polymerase chain reaction (PCR) amplification of the exons. Genotyping of the polymorphism c.719G>A (Arg240His) in exon 7 and c.1615G>C (Glu539Gln) in exon 11 of *C4BP* was also performed by automatic DNA sequencing of PCR-derived amplicons.

Table 1. Primers for the amplification and sequencing of the *C4BPA* exons.

Name	Primers 5′-3′	Temp °C
C4BPA-E2F	CAGAGGCCAATCCTTACTG	58
C4BPA-E2R	AGTGAATGCACAGATGATCTTC	
C4BPA-E3F	ATCACTCACTAGGTGTATTGAACAG	58
C4BPA-E3R	ATGTACATTGAAATCAGTAATTGAC	
C4BPA-E4F	TGCTGCAATTATTTAACTGGTC	58
C4BPA-E4R	AAATGAGAATTCTCAACGTCTATC	
C4BPA-E5/6F	TACTGAATGGAGTGCTTAATGG	58
C4BPA-E5/6R	ATTGCATCAGAGCGATAGTTAG	
C4BPA-E7F	CCTTGTATCTACTTGACATGCAC	60
C4BPA-E7R	CTGTAATTCATAGTAGGCCTAT	
C4BPA-E8F	GCACATTTGAGCGAATGTC	58
C4BPA-E8R	TTCTGGCACTAACAGTTTACTAATC	
C4BPA-E9F	AGCAGACTTGGAGTGTCTTG	58
C4BPA-E9R	CAAGAATCATGCAAGGTATATG	
C4BPA-E10F	TTGGCTACGTGCTCTTACAG	56
C4BPA-E10R	GAGCCTATATTCATACTAGAGCAAG	
C4BPA-E11F	GTGTCTTAATGTATCTCAGCTGTC	60
C4BPA-E11R	AATCCTGACAAGTCAGATTTCAC	
C4BPA-E12F	GGTCTTGGTTCAATCCTGTAG	56
C4BPA-E12R	CCAAGTACCTATTATTTGTGGC	

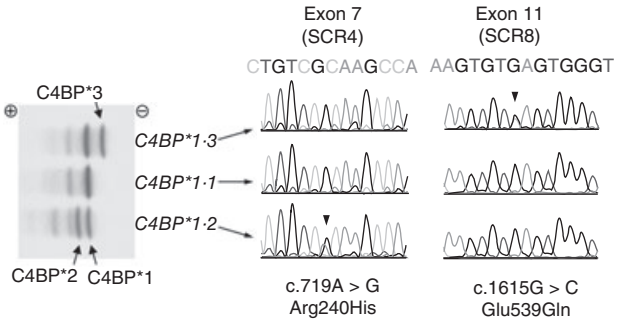

Fig. 1. Sequence analysis of the *C4BPA* isoelectric focusing (IEF) alleles. IEF on polyacrylamide gels under completely denaturing conditions of neuraminidase-treated affinity-purified C4b-binding protein (C4BP) proteins from *C4BP*1/C4BP*3*, *C4BP*1/C4BP*1* and *C4BP*1/C4BP*2* individuals is shown on the left side of the figure. Each C4BPα polypeptide chain discloses a banding pattern composed of three bands, the cathodal band being the more heavily stained. The cathodal bands corresponding to the three *C4BPA* alleles are indicated by arrows. On the right side fragments of the electropherograms corresponding to the regions of exon 7 (SCR4) and exon 11 (SCR8) of *C4BPA* gene from the same *C4BP*1/C4BP*3*, *C4BP*1/C4BP*1* and *C4BP*1/C4BP*2* individuals are shown to illustrate the nucleotide substitutions that identify the *C4BP*2* (c.719G>A; Arg240His) and *C4BP*3* (c.1615G>C; Glu539Gln) alleles.

Statistical analysis

To acquire statistical data comparable with that generated by Blom *et al.* [34], we analysed the differences in the *C4BP* allelic frequencies between the aHUS, AMD and control groups by the Pearson's χ^2 test. A *P*-value of 0·05 or less was considered to be statistically significant.

Results

Identification of the amino acid substitutions that characterize the three IEF *C4BPA* alleles

Genetic polymorphism of *C4BPA* was first described at the protein level using IEF [15]. Subsequently, in a sample of 516 normal unrelated Caucasians, three *C4BPA* alleles, denominated *C4BP*1, C4BP*2* and *C4BP*3*, were identified with estimated allele frequencies of 0·986, 0·010 and 0·004 respectively [15]. *C4BP*2* and *C4BP*3* have been found only in heterozygosis with *C4BP*1*.

To identify the amino acid substitutions that characterize the *C4BPA* IEF alleles we selected one *C4BP*1/C4BP*1* homozygote, one *C4BP*1/C4BP*2* heterozygote and one *C4BP*1/C4BP*3* heterozygote and determined the nucleotide sequences of all the coding exons of *C4BPA* in their genomic DNA. Figure 1 summarizes these analyses.

c.719G>A was the only nucleotide change resulting in an amino acid sustitution (Arg240His) observed between the *C4BP*1/C4BP*1* homozygote and the *C4BP*1/C4BP*2* heterozygote. Similarly, c.1615G>C underlies the single amino acid difference (Glu539Gln) between the *C4BP*1/C4BP*1* homozygote and the *C4BP*1/C4BP*3* heterozygote.

To confirm that the Arg240His and the Glu539Gln amino acid changes identified the *C4BP*2* and *C4BP*3 C4BPA* allelic variants, we genotyped all members of five available pedigrees in which we have previously shown segregation of the *C4BP*2* and *C4BP*3* alleles (Fig. 2). In agreement with the sequencing results, data from these families showed co-segregation of His240 with *C4BP*2* alleles and Gln539 with *C4BP*3*, demonstrating that the amino acid substitutions Arg240His in SCR4 and Glu539Gln in SCR8 identify the *C4BP*2* and *C4BP*3* allelic variants respectively.

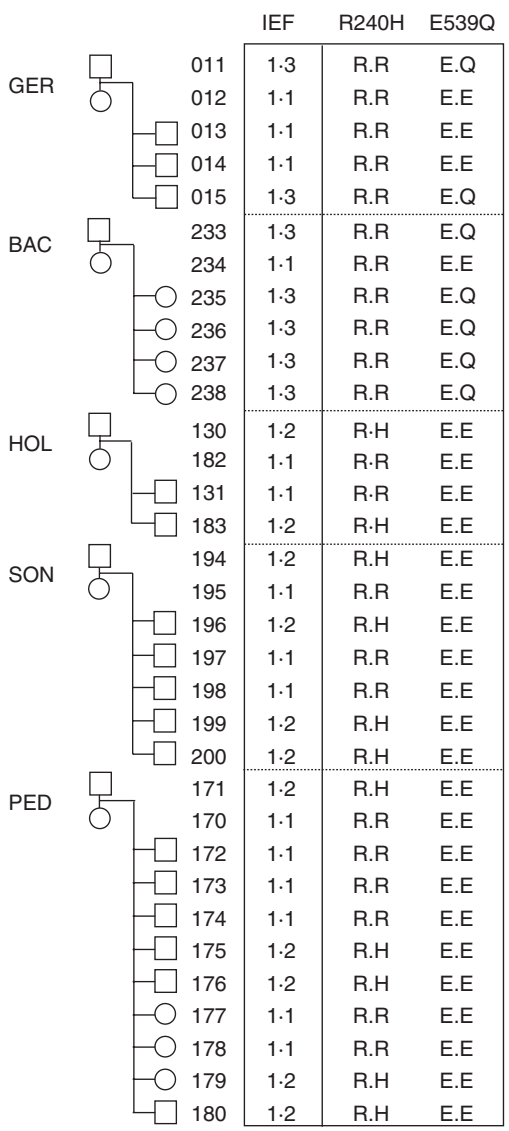

Fig. 2. Segregation analysis of the *C4BPA* alleles. Five pedigrees carrying the *C4BP*2* or the *C4BP*3* isoelectric focusing (IEF) protein variants are shown to illustrate the perfect segregation of these alleles with the c.719G>A (His240; *C4BP*2*) and c.1615G>C (Gln539; *C4BP*3*) nucleotide substitutions. The numbers on the right of the pedigrees are the codes for the samples.

C4BPA genotyping analysis in the aHUS and AMD Spanish cohorts

Recently, Blom *et al.* [34] reported that the p.Arg240His polymorphism is associated with aHUS and performed functional analyses showing that a recombinant C4BP molecule composed of His240 C4BPα polypeptide chains had impaired capacity to regulate the alternative pathway when compared with a similar molecule composed of Arg240 C4BPα polypeptide chains. Both binding of C4BP to C3b on surfaces and in the fluid phase was decreased in the His240 C4BP molecules, resulting in impaired co-factor capacity for degradation of C3b by factor I. In contrast, C4b-binding and degradation of C4b were not affected by this *C4BPA* polymorphism. These are important data that fit with current hypotheses regarding the pathogenesis of aHUS and provide a novel candidate gene contributing to aHUS predisposition. However, the aHUS cohort used by Blom *et al.* [34] to calculate the frequency of the His240 *C4BPA* allele included related patients carrying the Arg240 *C4BPA* allele, leading to an overestimation of the frequency of the His240 *C4BPA* allele. In addition, the replication study performed by the same authors in an independent cohort of aHUS patients was at the limit of statistical significance ($\chi^2 = 3{\cdot}808$; $P = 0{\cdot}051$). To resolve these uncertainties and clarify the association of this polymorphism with disease, we set out to test the association of the p.Arg240His C4BP polymorphism with aHUS in our independent cohort of aHUS patients. In addition, we extended these association studies to our cohort of AMD patients as AMD also involves dysregulation of the alternative pathway [38–40]. Our cohorts of patients consist of 102 unrelated aHUS Spanish patients and 143 unrelated AMD Spanish patients (see Materials and methods). The results of these studies are summarized in Table 2 and illustrate that there are no differences in the frequencies of the *C4BPA* His240 carriers between aHUS patients or AMD patients and their respective control populations. Therefore, we concluded that the p.Arg240His *C4BPA* polymorphism is not associated with increased risk to aHUS or to AMD in the Spanish population. None of the 23 non-Spanish patients in our cohort of aHUS patients carried the His240 *C4BPA* allele. Similarly, none of the 17 patients in the Spanish membraneproliferative glomerulonephritis type II cohort carried the His240 *C4BPA* allele, but this number of patients is too small to draw any conclusion.

Discussion

The search for novel polymorphisms conferring increased risk for aHUS is important, as current genetic analysis of the

Table 2. Frequencies of *C4BPA* polymorphisms in patients and controls.

	n	His240	Gln539	Statistics	References
Controls					
Caucasians 1	70	3 (3·7%)	n.d.	–	[14]
Caucasians 2	516	10 (1·9%)	4 (0·77%)	–	[15]
Caucasians 3	542	5 (0·9%)	n.d.	–	[34]
Spaniards 1	128	1 (0·78%)	n.d.	–	This report
Spaniards 2	117	1 (0·85%)	n.d.	–	This report
Patients					
aHUS (UK)	166	6* (3·6%)	n.d.	$\chi^2 = 6{\cdot}021$; $P = 0{\cdot}014^{\dagger}$	[34]
aHUS (France)	170	5 (2·9%)	n.d.	$\chi^2 = 3{\cdot}808$; $P = 0{\cdot}051^{\dagger}$	[34]
aHUS (Spain)	102	1 (0·98%)	0	$\chi^2 = 0{\cdot}026$; $P = 0{\cdot}872^{\ddagger}$	This report
AMD (Spain)	143	1 (0·7%)	n.d.	$\chi^2 = 0{\cdot}020$; $P = 0{\cdot}886^{\ddagger}$	This report

*Includes two related individuals; †Caucasians 3 is the control population for these cohorts; ‡Spaniards 1 and Spaniards 2 are the controls for the atypical haemolytic uraemic syndrome and age-related macular degeneration Spanish cohorts respectively; n.d., Not done.

CFH, *MCP*, *CFI*, *CFB* and *C3* genes identifies only mutations in approximately 50% of unrelated patients. Novel genes harbouring aHUS-associated polymorphisms that are supported fully by statistical and biological significance are excellent candidate genes for also carrying mutations associated with aHUS; these genes should be added to those analysed routinely in aHUS patients. Based on the studies reported by [37], *C4BPA* may be one of these novel genes. However, the association studies implicating *C4BPA* with aHUS were not supported fully by statistical significance in a replication study by the same authors. To clarify these data, we sought to replicate the association of the p.Arg240His *C4BPA* polymorphism with aHUS in our cohort of patients. Unfortunately, however, our results found no differences between patients and controls for the frequency of carriers of the *C4BPA* His240 allele. One possible explanation for the differences between our results and those reported by Blom *et al.* [34] is that the frequency of this relatively uncommon *C4BPA* polymorphism varies between different populations (Table 2). This may be particularly critical in the case of aHUS, with limited numbers of patients from many different geographical origins. Since 1983 we have analysed three different Caucasians populations, two from the New York area in the United States and one from Spain, with frequencies of His240 carriers ranging from 0·78 to 3·7. Notably, the frequencies of His240 carriers in the two aHUS cohorts and the control populations described by Blom *et al.* [34] are included within this range (Table 2). In the studies presented in this report we selected our patient and control groups to include only Spaniards from similar geographical areas and found identical numbers of carriers in two independent control populations and two cohorts of patients, one with aHUS and the other with AMD (Table 2).

In summary, our data do not support an association between the p.Arg240His *C4BPA* polymorphism and aHUS. Therefore, despite functional analyses which potentially give biological significance for the association of this polymorphism with aHUS, the statistics supporting the association are insufficient to consider the inclusion of the *C4BPA* gene in the routine mutational analysis of complement genes in aHUS patients.

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References

1 Hillarp A, Hessing M, Dahlback B. Protein s binding in relation to the subunit composition of human c4b-binding protein. FEBS Lett 1989; **259**:53–6.

2 Sanchez-Corral P, Garcia OC, Rodriguez de Cordoba S. Isoforms of human C4b-binding protein: I. Molecular basis for the C4BP isoform pattern and its variations in human plasma. J Immunol 1995; **155**:4030–6.

3 Hillarp A, Dahlback B. Novel subunit in C4b-binding protein required for protein S binding. J Biol Chem 1988; **263**:12759–64.

4 Dahlback B, Smith CA, Muller Eberhard HJ. Visualization of human C4b-binding protein and its complexes with vitamin K-dependent protein S and complement protein C4b. Proc Natl Acad Sci USA 1983; **80**:3461–5.

5 Rodriguez de Cordoba S, Sanchez-Corral P, Rey-Campos J. Structure of the gene coding for the alpha polypeptide chain of the human complement component C4b-binding protein. J Exp Med 1991; **173**:1073–82.

6 Pardo-Manuel F, Rey-Campos J, Hillarp A, Dahlback B, Rodriguez De Cordoba S. Human genes for the a and b chains of complement C4b-binding protein are closely linked in a head-to-tail arrangement. Proc Natl Acad Sci USA 1990; **87**:4529–32.

7 Scharfstein J, Ferreira A, Gigli I, Nussenzweig V. Human C4-binding protein. I. Isolation and characterization. J Exp Med 1978; **148**:207–22.

8 Fujita T, Gigli I, Nussenzweig V. Human C4-binding protein. II. Role in proteolysis of C4b by C3b-inactivator. J Exp Med 1978; **148**:1044–51.

9 Gigli I, Fujita T, Nussenzweig V. Modulation of the classical pathway C3 convertase by plasma proteins C4 binding protein and C3b inactivator. Proc Natl Acad Sci USA 1979; **76**:6596–600.

10 Blom AM, Kask L, Dahlback B. CCP1–4 of the C4b-binding protein a-chain are required for factor I mediated cleavage of complement factor C3b. Mol Immunol 2003; **39**:547–56.

11 Ziccardi RJ, Dahlback B, Muller-Eberhard HJ. Characterization of the interaction of human C4b-binding protein with physiological ligands. J Biol Chem 1984; **259**:13674–9.

12 Accardo P, Sanchez-Corral P, Criado O, Garcia E, Rodriguez De Cordoba S. Binding of human complement component C4b-binding protein (C4BP) to *Streptococcus pyogenes* involves the C4b-binding site. J Immunol 1996; **157**:4935–9.

13 Blom AM, Kask L, Dahlback B. Structural requirements for the complement regulatory activities of C4BP. J Biol Chem 2001; **276**:27136–44.

14 Rodriguez de Cordoba S, Ferreira A, Nussenzweig V, Rubinstein P. Genetic polymorphism of human C4-binding protein. J Immunol 1983; **131**:1565–9.

15 Rodriguez de Cordoba S, Rubinstein P. New alleles of C4-binding protein and factor H and further linkage data in the regulator of complement activation (RCA) gene cluster in man. Immunogenetics 1987; **25**:267–8.

16 Noris M, Remuzzi G. Hemolytic uremic syndrome. J Am Soc Nephrol 2005; **16**:1035–50.

17 Caprioli J, Bettinaglio P, Zipfel PF *et al.* The molecular basis of familial hemolytic uremic syndrome: mutation analysis of factor H gene reveals a hot spot in short consensus repeat 20. J Am Soc Nephrol 2001; **12**:297–307.

18 Caprioli J, Castelletti F, Bucchioni S *et al.* Complement factor H mutations and gene polymorphisms in haemolytic uraemic syndrome: the C-257T, the A2089G and the G2881T polymorphisms are strongly associated with the disease. Hum Mol Genet 2003; **12**:3385–95.

19 Manuelian T, Hellwage J, Meri S *et al.* Mutations in factor H reduce binding affinity to C3b and heparin and surface attachment to endothelial cells in hemolytic uremic syndrome. J Clin Invest 2003; **111**:1181–90.

20 Perez-Caballero D, Gonzalez-Rubio C, Esther Gallardo M *et al.* Clustering of missense mutations in the C-terminal region of factor H in atypical hemolytic uremic syndrome. Am J Hum Genet 2001; **68**:478–84.

21 Richards A, Buddles MR, Donne RL *et al.* Factor H mutations in hemolytic uremic syndrome cluster in exons 18–20, a domain important for host cell recognition. Am J Hum Genet 2001; **68**:485–90.

22 Venables JP, Strain L, Routledge D *et al.* Atypical haemolytic uraemic syndrome associated with a hybrid complement gene. PLoS Med 2006; **3**:1957–67.

23 Warwicker P, Goodship THJ, Donne RL *et al.* Genetic studies into inherited and sporadic hemolytic uremic syndrome. Kidney Int 1998; **53**:836–44.

24 Esparza-Gordillo J, Goicoechea de Jorge E, Buil A *et al.* Predisposition to atypical hemolytic uremic syndrome involves the concurrence of different susceptibility alleles in the regulators of complement activation gene cluster in 1q32. Hum Mol Genet 2005; **14**:703–12.

25 Fremeaux-Bacchi V, Kemp EJ, Goodship JA *et al.* The development of atypical haemolytic-uraemic syndrome is influenced by susceptibility factors in factor H and membrane cofactor protein: evidence from two independent cohorts. J Med Genet 2005; **42**:852–6.

26 Noris M, Brioschi S, Caprioli J *et al.* Familial haemolytic uraemic syndrome and an MCP mutation. Lancet 2003; **362**:1542–7.

27 Richards A, Kemp EJ, Liszewski MK *et al.* Mutations in human complement regulator, membrane cofactor protein (CD46), predispose to development of familial hemolytic uremic syndrome. Proc Natl Acad Sci USA 2003; **100**:12966–71.

28 Goicoechea De Jorge E, Harris CL, Esparza-Gordillo J *et al.* Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. Proc Natl Acad Sci USA 2007; **104**:240–5.

29 Fremeaux-Bacchi V, Regnier C, Blouin J *et al.* Protective or aggressive: paradoxical role of C3 in atypical hemolytic uremic syndrome. Mol Immunol 2007; **44**:172.

30 Fremeaux-Bacchi V, Dragon-Durey MA, Blouin J *et al.* Complement factor I: a susceptibility gene for atypical haemolytic uraemic syndrome. J Med Genet 2004; **41**:e84.

31 Sanchez-Corral P, Perez-Caballero D, Huarte O *et al.* Structural and functional characterization of factor H mutations associated with atypical hemolytic uremic syndrome. Am J Hum Genet 2002; **71**:1285–95.

32 Zipfel PF, Edey M, Heinen S *et al.* Deletion of complement factor H-related genes CFHR1 and CFHR3 is associated with atypical hemolytic uremic syndrome. PLoS Genet 2007; **3**:e41.

33 Dragon-Durey MA, Loirat C, Cloarec S *et al.* Anti-factor H autoantibodies associated with atypical hemolytic uremic syndrome. J Am Soc Nephrol 2005; **16**:555–63.

34 Jozsi M, Strobel S, Dahse HM *et al.* H autoantibodies block C-terminal recognition function of factor H in hemolytic uremic syndrome. Blood 2007; **110**:1516–18.

35 Rodriguez de Cordoba S, Goicoechea de Jorge E. Translational mini-review series on complement factor H: genetics and disease associations of human complement factor H. Clin Exp Immunol 2008; **151**:1–13.

36 Esparza-Gordillo J, Goicoechea de Jorge E, Garrido CA *et al.* Insights into hemolytic uremic syndrome: segregation of three independent predisposition factors in a large, multiple affected pedigree. Mol Immunol 2006; **43**:1769–75.

37 Blom AM, Bergström F, Edey M *et al.* A novel non-synonymous polymorphism (p.Arg240His) in C4b-binding protein is associated with atypical hemolytic uremic syndrome and leads to impaired alternative pathway cofactor activity. J Immunol (Baltimore, MD: 1950) 2008; **180**:6385–91.

38 Hageman GS, Anderson DH, Johnson LV *et al.* A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA 2005; **102**:7227–32.

39 Lotery A, Trump D. Progress in defining the molecular biology of age related macular degeneration. Hum Genet 2007; **122**:219–36.

40 Gold B, Merriam JE, Zernant J *et al.* Variation in factor B (BF) and complement component 2 (2) genes is associated with age-related macular degeneration. Nat Genet 2006; **38**:458–62.