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Setting the scale

The balance between complement factor H and its related proteins in health and disease

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General introduction and scope of this thesis

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The human body is constantly exposed to pathogens such as bacteria, fungi and viruses. The first lines of defense to protect ourselves from infection are our skin and the epithelial layer of our lungs and intestines, which form a physical barrier between the outside world and the human body. When this barrier is breached due to an injury, bacteria enter the body and come into contact with our blood. Within our body and for a great part within our circulatory system, several additional defense systems are present, collectively called the immune system. It is specialized in detecting and eliminating any (dangerous) foreign cell or particle that enters our body.

The immune system can be subdivided into two major branches: the adaptive immune system and the innate immune system. The adaptive immune system is initially slow but develops a memory during infections to be able to mount a stronger, highly specific and faster response upon a second encounter with the pathogen. In contrast, the other branch, the innate immune system, operates much faster as it is already present and fully equipped to eliminate foreign cells upon the first encounter, and does not require any additional development during an infection. However, the innate immune system is only able to recognize pathogens in a restricted way through the detection of conserved pathogen-associated molecular patterns, and therefore can be limited in its effectiveness or is easily evaded by pathogens. There is much cross-talk between the two branches of the immune system and they augment each other in the defense of the human body.

The complement system

An essential part of the innate immune system is called the complement system. Upon its discovery in the late 19th century, the system was identified as a heat-labile component in plasma, thought to only "complement" and support the antibacterial activities of the heat-stable component (reviewed in Le Friec & Kemper, 2009[1] and Nesargikar *et al.* 2012[2]). The heat-stable component was later identified as antibodies originating from the adaptive immune system, while the heat-labile component was found to be actually composed of many different proteins, which are nowadays collectively called the complement system. Furthermore, the complement system was found to be a highly conserved and evolutionary ancient system that is not only present in vertebrates but also in phylogenetically more distant species (the most distant species with complement proteins from humans being the Cnidaria *Hydra*, a small, fresh-water, polyp-shaped animal), preceding the adaptive immune system and antibodies in evolution[1]. In addition, while it does support the antibacterial role of antibodies from the adaptive immune system, the complement system can rely entirely on its own components to recognize, attack and kill invading pathogens. In a sense, one could even argue that the system was given a wrong name and it is actually the antibodies that complement the complement system. Especially in (very) young children in which the (adaptive) immune system is not yet fully developed, the complement system is crucial in the defense against pathogens.

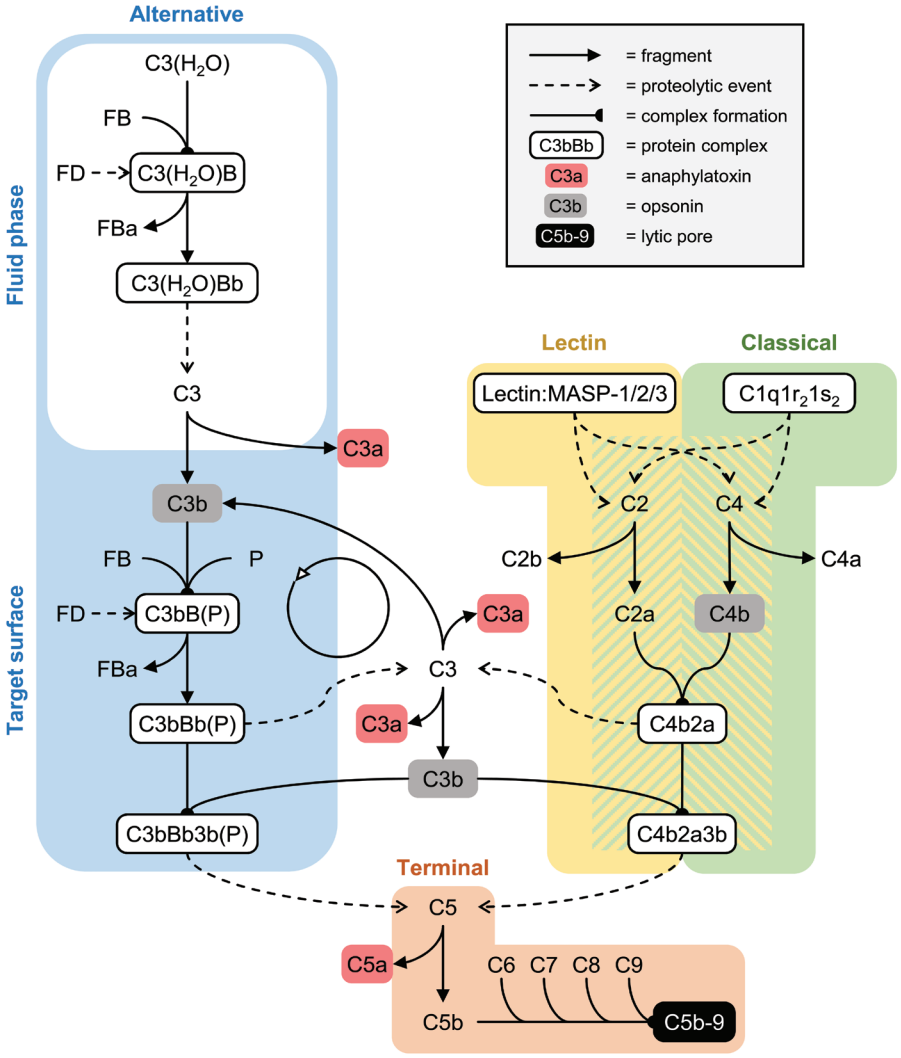

Figure 1 Schematic overview of the activation pathways of the complement system. The complement system can be activated via three distinct pathways: the alternative pathway (blue), the lectin pathway (yellow), or the classical pathway (green). All pathways lead to the formation of C3-convertases that cleave C3 into C3a, which is an anaphylatoxin (red), and C3b, which deposits on the target, opsonizing it (grey). C3b deposition will lead to the formation of C5-convertases, which cleave C5 into C5a, another, more potent anaphylatoxin, and C5b. C5b initiates the terminal pathway (orange) which will lead to the formation of the C5b-9 complex, also known as the membrane attack complex (black). The white box within the alternative pathway indicates the events that occur in fluid phase. While the complexes of the alternative pathway can form without properdin, indicated with (P), properdin is thought to stabilize formed surface complexes. Abbreviations used: C (followed by a number): complement component, FB: complement factor B, FD: complement factor D, P: Properdin, MASP-1/2/3: mannan-binding lectin associated serine proteases 1, 2 and/or 3.

Unlike other parts of the immune system, the complement system is solely composed of proteins, which, through a series of well-orchestrated proteolytic events, form an enzymatic cascade (**Fig. 1**). It is driven by three activation pathways which feed into one final cascade and comprises over 30 plasma and cell surface proteins[3]. Most proteins are named either complement components (C), followed by a number referring back to the order of their discovery, or complement factors (F), followed by a letter sometimes referring to their former name. The smallest cleavage fragments are indicated with a, while the larger fragments are indicated with b, with the exception to the rule for C2a and C2b (the latter being the smallest). The three pathways are termed the classical pathway (CP, the first one to be discovered), the lectin pathway (LP, the most recently discovered), and the alternative pathway.

The CP and LP are activated by pathogens via the binding of pattern recognition proteins to antibody–antigen complexes or distinct carbohydrate moieties on the surface of the pathogen, respectively. The pattern recognition protein of the CP is C1q, which is complexed with two of each C1r and C1s zymogens, together referred to as the C1 complex. The LP can be activated by several pattern recognition proteins, including mannan-binding lectin (MBL) and ficolins, each recognizing specific carbohydrate moieties associated with pathogens. MBL and ficolins also carry various zymogens, known as MBL-associated serine proteases (MASPs) of which humans possess three variants (MASP-1 to MASP-3). The exact composition of the LP recognition complexes is still unclear. Upon binding to the target, the zymogens of the CP and LP become activated and cleave C2 and C4. The subsequent fragments C4b and C2a form a C3-convertase on the targeted surface that in turn cleaves the central protein of the complement system, C3, into C3b and C3a. C3a and the later formed (and much more potent) C5a are anaphylatoxins, which function as immune modulators. C3a and C5a act as chemoattractants that activate not only immune cells of the innate and adaptive immunity but also endothelial cells (*i.e.* increasing vasodilatation and permeability) to induce inflammatory responses. Both C4b and C3b expose a highly reactive thioester moiety that covalently binds to hydroxy or amino groups on any surface in close proximity, thereby opsonizing the target. The AP is activated via the spontaneous, low rate turn-over of C3 in fluid phase, forming the active hydrolyzed C3 (C3(H_2O)). C3(H_2O) will bind factor B (FB) which in turn is cleaved and activated by factor D (FD), forming the fluid phase AP C3-convertase C3(H_2O)Bb. C3(H_2O)Bb cleaves C3, leading to the deposition of C3b on any surface. Similar to C3(H_2O), deposited C3b forms the C3-convertase C3bBb on the surface. This complex can be stabilized by properdin, extending its half-life (reviewed by Lesher *et al.* 2013[4]). Activation of the complement system via the AP will only proceed on surfaces that lack complement regulators. The formed C3-convertases on the targeted surface will in turn cleave more C3, resulting in more C3b deposition which will again initiate the AP and thus amplification of complement activation is achieved. This amplification loop is regarded to account for up to 80% of total complement activation, irrespective of the initial activation pathway that resulted

in C3b deposition, giving the AP a pivotal role within the complement system[5].

Eventually, the accumulation of C3b on the targeted surface leads to the formation of C5-convertases (by forming either C4b2a3b or C3bBb3b) that cleave C5 into C5a and C5b, of which the latter initiates the terminal pathway. The terminal pathway forms a membrane attack complex (MAC) through subsequent binding and incorporation of C5b, C6, C7, C8 and multiple C9 proteins into the membrane, forming a lytic pore. Lysis through the MAC, opsonization with C3b and C4b, and induction of inflammation via the anaphylatoxins C3a and C5a are the three main effector functions of the complement system and aid in the clearance of the target.

The genetic organization of complement regulation

As (part of) the complement system is rather a-specific, complement regulators are crucial in protecting host cells. In addition to fluid phase complement regulators, which are mostly secreted by the liver, human cells express various regulators on their surface. Together, these regulators target various steps in the complement cascade. The majority of complement regulator genes are located in a gene cluster referred to as the regulation of complement activation (RCA) cluster. All proteins encoded within the RCA cluster show a remarkable similarity in structure and overall composition. They predominantly consist of domains referred to as short consensus repeat (SCR) or complement control protein (CCP) domains, each of approximately 60 amino acids in length. These domains have a globular 3D structure that is highly conserved[6]. Distinct mechanisms of action, such as the inhibitory activities and binding to cell surfaces and/or complement components, can be attributed to different domains within each regulator. The RCA cluster is located on chromosome 1 and is evolutionary well-conserved[7]. It can be divided into two regions, one containing the genes encoding for C4-binding protein (*C4BP*), decay-accelerating factor (*DAF*), complement receptor 2 (*CR2*), complement receptor 1 (*CR1*) and membrane cofactor protein (*MCP*), while the other region encompasses the genes for factor H (*CFH*) and all the factor H-related proteins (*CFHR1-5*)[8,9].

Five *CFHR* genes have been reported in humans and they are termed *CFHR1* to *CFHR5*[20]. The genes are arranged in tandem on 1q31.3 and are located next to *CFH* in the following order; *CFH*, *CFHR3*, *CFHR1*, *CFHR4*, *CFHR2*, and *CFHR5* (**Fig. 2a**). The *CFHR* genes are likely to have arisen as a result of duplication events, originating from the *CFH* gene[7]. This is reflected in the high degree of sequence identity shared between *CFH* and the *CFHR* genes throughout the locus[21]. Furthermore, non-allelic homologous recombination events have occurred, resulting in copy number variation within the *CFH* locus. Two deletions have been reported, one spanning *CFHR3* to *CFHR1*, the other spanning *CFHR1* to *CFHR4* (**Fig. 2b**). The *CFHR3*/*CFHR1* deletion is the most common, with an average allele frequency of 20% in Europeans, about 4% being homozygous, *i.e.* carrying no gene copies of *CFHR3* and *CFHR1*[10–

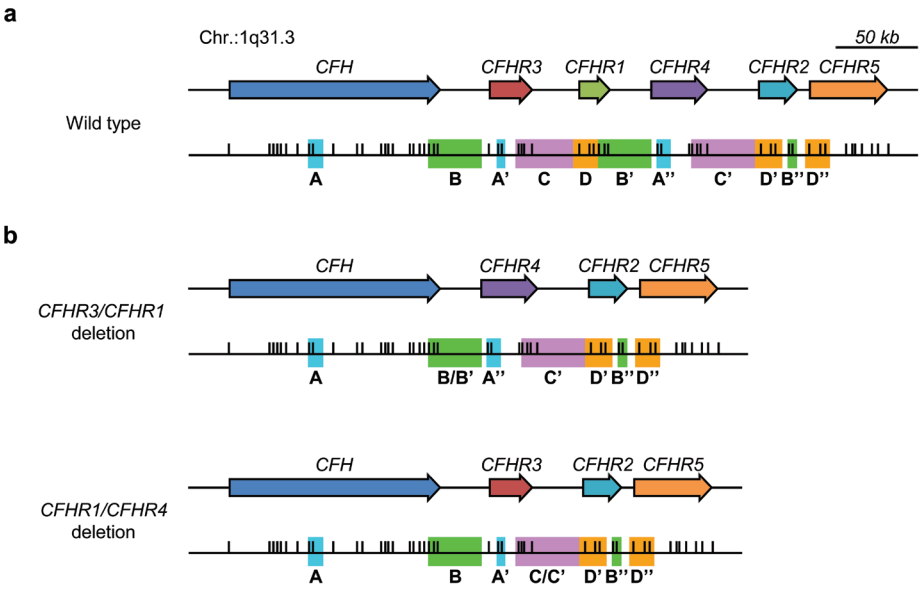

Figure 2 Schematic overview of the genetic organization of the FH protein family. (**a**) The FH protein family is encoded in tandem on 1q31.3. The colored arrows indicate the encoded genes in order. The vertical lines on the second strand indicate the locations of the introns, with the color boxes with letters indicating regions with high sequence similarity. Adapted from Rodriguez de Cordoba **et al.** (2012)[9]. (**b**) Representation of the two known copy number variations in the *CFH* locus. Due to non-allelic homologous recombination events, either *CFHR3/CFHR1* or *CFHR1/CFHR4* is deleted.

[15,22,23]. However, globally the allele frequency of the *CFHR3/CFHR1* deletion varies greatly (**Table 1**). The deletion is extremely rare or even absent in Mexican and Asian populations, while deletion of *CFHR3/CFHR1* is very common in people with African ancestry[11,15,18]. The highest allele frequency (55%) is found in the Nigerian population, where three in ten people are deficient for *CFHR3* and *CFHR1*.

The *CFHR1/CFHR4* deletion is far less common, with allele frequencies varying from 0.3% up to 3.8% (average = 1.5%), and only two reported individuals being homozygous for the *CFHR1/CFHR4* deletion[13,15] (**Table 1**). Next to the deletion of *CFHR3/CFHR1* and *CFHR1/CFHR4*, other variations in the *CFH* locus have been reported, albeit familial or single cases. These include duplication events[24–26] and recombination events leading to hybrid proteins[27–33]. While these variations associate with rare diseases it is still largely unknown if and how variations in the *CFHR* genes may cause disease.

The factor H protein family

Together, the six genes (*CFH* and *CFHR1-5*) in the *CFH* locus encode a total of eight glycoproteins (including two splice variants) referred to as the factor H protein family (**Fig.**

Table 1 Allele frequency across different populations of the two known CNV in the CFH locus

	Continent	Population[a]	*n*	Allele frequency deletion (% homozygous) *CFHR3/CFHR1*	*CFHR1/CFHR4*	ref.[b]
Healthy	Europe					
		British	505	16.4% (3.0%)	0.9% (0.0%)	10
			238	18.3% (3.4%)	n.d.	11
			119	6.3% (1.7%)	n.d.	12
		Spanish	269	24.5% (1.7%)	0.5% (0.0%)	13
			92	23.0% (3.0%)	n.d.	14
		Italian	90	24.4% (5.6%)	0.6% (0.0%)	15
			49	22.4% (6.1%)	n.d.	11
		Russian	41	25.6% (7.3%)	n.d.	11
	North America					
		Caucasian	352	22.1% (4.9%)	n.d.	16
			203[c]	27.6% (8.9%)	n.d.	17
			100	20.0% (4.0%)	n.d.	15
		European	275	19.8% (4.4%)	n.d.	18
			119	22.7% (5.0%)	n.d.	15
		Hispanic	196	17.9% (2.6%)	n.d.	18
		African	153	35.9% (14.4%)	2.9% (0.0%)	15
			106	42.0% (16.0%)	n.d.	18
		Mexican	155	12.7% (0.6%)	n.d.	15
			34	1.5% (0.0%)	n.d.	11
		Native	55	11.8% (1.8%)	0.9% (0.0%)	15
	South America					
		Columbian	333	25.5% (6.7%)	n.d.	16
			7	0.0% (0.0%)	n.d.	11
		Brazilian	22	0.0% (0.0%)	n.d.	11
	Africa					
		Kenyan[d]	149	23.8% (3.4%)	0.3% (0.0%)	15
			52	40.4% (19.2%)	2.9% (0.0%)	15
		Nigerian	120	54.2% (27.5%)	3.8% (0.8%)	15
			21	54.8% (33.3%)	n.d.	11
		sub-Saharan[e]	83	33.7% (8.4%)	n.d.	11
		Algerian	29	22.4% (6.9%)	n.d.	11
	Asia					
		Chinese	282	5.7% (0.7%)	n.d.	18
			240	6.3% (0.0%)	3.1% (0.0%)	15
			50	6.0% (0.0%)	n.d.	11
		Japanese	91	4.9% (0.0%)	1.6% (0.0%)	15
			29	0.0% (0.0%)	n.d.	11
		Pakistani	50	15.0% (0.0%)	n.d.	11
		Siberians	24	2.1% (0.0%)	n.d.	11
	Australia					
		Caucasian	327	19.3% (5.8%)	0.3% (0.0%)	19
aHUS	Europe					
		Spanish	326	22.0% (9.0%)	n.d.	14
			137	21.1% (10.2%)	1.4% (0.7%)	13
		British	196	25.5% (12.8%)	1.4% (0.0%)	10
			66	28.0% (10.6%)	n.d.	12
AMD	Europe					
		Spanish	141	9.6 (1.4%)	n.d.	13
	North America					
		Caucasian	576	11.0% (1.2%)	n.d.	16
			393	12.3% (1.8%)	n.d.	17
	South America					
		Columbian	629	10.4% (0.9%)	n.d.	16
	Australia					
		Caucasian	387	6.8% (0.5%)	0.8% (0.0%)	19

CNV: copy number variation, n.d.: not determined, aHUS: atypical hemolytic uremic syndrome, AMD: age-related macular degeneration.

[a]Data on same ethnic populations from HapMap, Coriell Diversity Panel and HapMap phaseIII from Sivakumaran *et al.* (2011)[15] were grouped. [b]Only studies which confirmed the deletions at the

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3). The eponymous protein of this family was first described in 1965 as β1H globulin, a 155 kDa glycoprotein that was immunoprecipitated using antisera raised against β1C globulin[34]. β1C globulin is nowadays known as C3. In 1976, the function of β1H globulin as regulator of C3 was elucidated through a series of elegant experiments by two groups, of which one group introduced the name C3b-inactivator[35–37]. In 1981, a committee of the World Health Organization consisting of world leading experts on the complement system published a nomenclature list for proteins involved in the AP of complement activation, giving β1H globulin its definite and now well-known name: factor H (FH)[38]. A few years later, in 1988, the cDNA and amino acid sequences of FH were published, revealing that FH consists of 20 CCP domains that form a mature protein of 1213 amino acids[39], making its genetic linkage to other proteins in the RCA gene cluster apparent. In addition, a second transcript originating from the *CFH* gene was identified[40,41]. This splice variant encodes a 43 kDa protein named FH-like 1 (FHL-1) and comprises the first seven domains of FH followed by a unique C-terminal tail of four amino acids (SFTL). FH is a highly abundant plasma protein with an average concentration of 233–320 μg/mL or 1.5–2.1 μM[42–44] while FHL-1 is estimated to circulate at a concentration of 10-50 μg/mL or 0.2-1.2 μM[45] (**Table 2**). FH is highly glycosylated, with eight of the nine putative *N*-linked glycosylation sites found to be glycosylated *in vivo* (the first *N*-linked glycosylation site in domain 4 is unused)[46].

The FH-related (FHR) proteins were first discovered in the early 1990s with the identification of mRNA species encoding for FHR-1[51,52], FHR-2[53] and FHR-3[54]. The first protein product of *CFHR4* was described in 1997 as FHR-4[55] but with the discovery of FHR-4A in 2005, this protein was renamed to FHR-4B[56]. In 2001, FHR-5 was identified[57], completing the FH protein family. The domains of the FHR proteins show a striking yet variable degree of sequence similarity with domains of FH, ranging from 32% up to being completely, 100%, identical on amino acid level (**Fig. 3**). The FHRs differ in length, with FHR-2 being the smallest consisting of only four domains, followed by FHR-1, FHR-3 and FHR-4B, which all consist of five domains, and FHR-4A and FHR-5, consisting of nine domains each. The five domains of FHR-4B are completely identical to the first and the last four domains of FHR-4A[56]. FHR-5 is the only FHR that possesses domains that are highly similar to domains 10-14 of FH[57].

The FHR proteins can be subdivided into two sub-groups. The first group comprises FHR-1, FHR-2 and FHR-5, all having domains that are highly similar (42% up to 100%) on amino

genetic level, using either multiplex ligation-dependent probe amplification (MLPA) with multiple probes per gene or sequencing techniques, were included. [c]The Caucasian population described by Sawitzke *et al.* (2011)[17] is predominantly composed of Caucasians. [d]The two groups in the Kenyan population are the Maasai from Kinyawa, Kenya (n=149) and the Luhya from Webuye, Kenya (n=52). [e]The sub-Saharan population is composed of San from Namibia (n=6), Biaka pygmy from the Central Africa Republic (n=23), Mbuti pygmy from the Democratic Republic of Congo (n=13), Bantu from Kenya (n=11), Mandeka from Senegal (n=22) and Bantu from South Africa (n=8).

Table 2 Overview of the protein characteristics of the FH protein family

Name (abbreviation)	Sub-group	Gene	Domains	kDa	Mean conc. (µg/mL)	Notes
Factor H (FH)		*CFH*	20	155	233-320[42-44]	
Factor H-like 1 (FHL-1)		*CFH*	7	42	10-50[45] (estimation)	Splice variant
Factor H-related protein 1 (FHR-1)	1	*CFHR1*	5	37/43	70-100[47] (estimation)	2 glycosylation variants, dimerization motif
Factor H-related protein 2 (FHR-2)	1	*CFHR2*	4	24/29	50[20] (estimation)	2 glycosylation variants, dimerization motif
Factor H-related protein 3 (FHR-3)	2	*CFHR3*	5	36-50	50-80[48] (estimation)	Glycosylation variants
Factor H-related protein 4A (FHR-4A)	2	*CFHR4*	9	86	25.4[49] (incl.FHR-4B)	
Factor H-related protein 4B (FHR-4B)	2	*CFHR4*	5	42	25.4[49] (incl. FHR-4A)	Splice variant
Factor H-related protein 5 (FHR-5)	1	*CFHR5*	9	62	3.4-10.1[50]	Dimerization motif

acid level. A prominent feature of FHR-1, FHR-2 and FHR-5 is a dimerization motif, identified in domains 1 and 2 and not found in other members of the FH protein family[58]. Because of the dimerization motifs in FHR-1, FHR-2 and FHR-5, all possible species of homo- and hetero-dimers containing these FHR proteins have been detected *in vitro*. FHR-1 has two allelic variants, FHR-1*A and FHR-1*B, which differ in three amino acid residues (H157Y, L159V and E175Q) in domain 3[13]. Domain 3 of FHR-1*B is 100% identical to domain 18 of FH (FHR-1*A is depicted in **Fig. 3**). Furthermore, both FHR-1 and FHR-2 circulate in two glycosylation variants. FHR-1 is glycosylated at either one or two *N*-linked glycosylation sites resulting in two variants of 37 and 43 kDa, respectively, while FHR-2 carries either no *N*-linked glycans (24 kDa) or one *N*-linked glycan (29 kDa). The second group of the FHR proteins comprises FHR-3, FHR-4A and FHR-4B, which share a high degree of similarity in their first domains but do not dimerize nor seem to interact in another way with each other. FHR-3, FHR-4A and FHR-4B are heavily glycosylated, containing four (FHR-3 and FHR-4B) or even seven (FHR-4A) *N*-linked glycosylation sites.

Several reports have estimated or measured the levels of FHR proteins, claiming molar levels almost identical to FH (**Table 2**). FHR-1 was estimated to circulate at 70–100 µg/mL, while FHR-2 concentrations were estimated to be approximately 50 µg/mL[20,47], and FHR-3 has been estimated to circulate at 50–80 µg/mL[48]. Of note, neither the detail of the assays nor the possible cross-reactivity of the reagents used for these estimations were formally described. The total FHR-4 concentration, thus without distinguishing between FHR-4A and FHR-4B, was determined by ELISA and reported to be 6.5-53.9 µg/mL, with a mean concentration

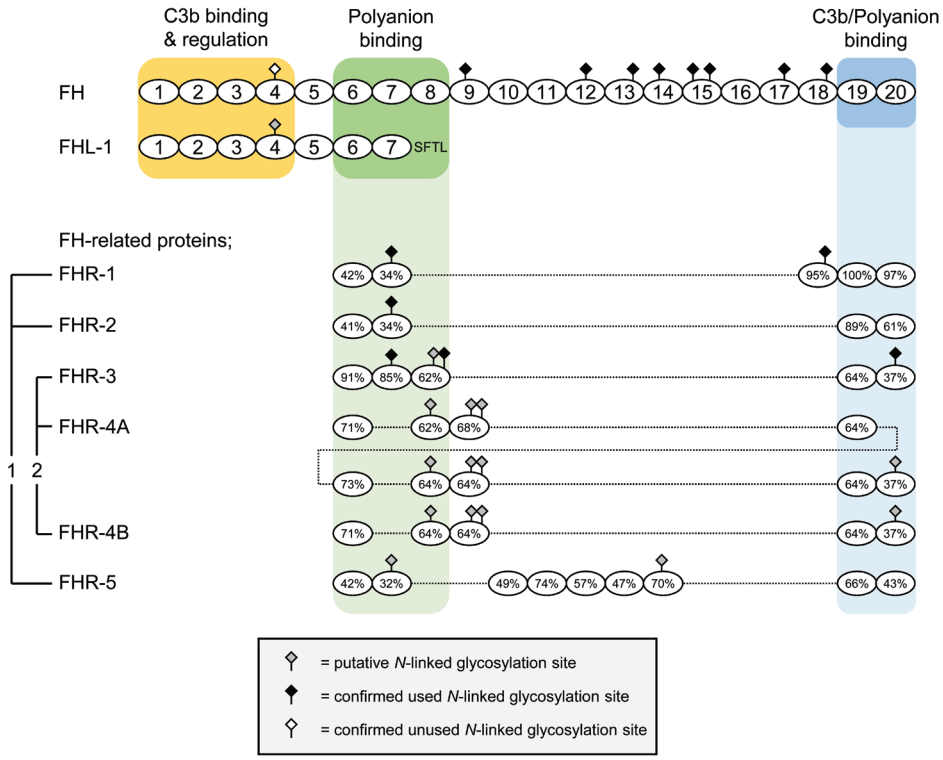

Figure 3 Schematic overview of the FH protein family. The FH protein family comprises eight proteins. Known *N*-linked glycosylation sites as well as putative or confirmed unused sites are indicated. Numbers within each domain of the FHR proteins represent the similarity in percentage as compared to the same domain as in FH. The two FHR protein sub-groups, FHR-1/FHR-2/FHR-5 (sub-group 1) and FHR-3/FHR-4A/FHR-4B (sub-group 2), are indicated by lines. Regions identified to have similar functional properties are indicated with colored boxes. Percentage similarity was calculated based on the domains as indicated by Uniprot (www.uniprot.org, accession numbers FH/FHL-1: P08603, FHR-1: Q03591, FHR-2: P36980, FHR-3: Q02985, FHR-4A/FHR-4B: Q92496, and FHR-5: Q9BXR6) and with the use of ClustalW (www.ebi.ac.uk/Tools/msa/clustalw2/).

of 25.4 µg/mL[49]. FHR-5 is reported to circulate at 3.4-10.1 µg/mL[50]. It is, however, unclear whether monomers of FHR-5, homodimers of FHR-5, or heterodimers of FHR-5 with FHR-1 and/or FHR-2 were detected. Due to the high similarity between the FHRs and with FH, it has proved to be challenging to accurately distinguish between the different members of the FH protein family. Many antibodies raised against one protein of the FH family are shown to be reactive against other FH protein family members.

Complement regulation by the FH protein family

Today, FH is widely recognized as one of the most crucial complement regulators for the protection of human cells and surfaces from complement and is still of great interest to many

research groups. FH regulates the AP by inhibiting the formation of the C3-convertase C3bBb in three ways (**Fig. 4**). First, the decay of the formed C3-convertase C3bBb is accelerated via a process known as decay-accelerating activity. Secondly, the formation of new C3bB complexes is prevented by competition of FH with FB for the binding of C3b. Lastly, and arguably the most important mechanism, FH functions as a cofactor for the FI-mediated cleavage of C3b into inactivated C3b (iC3b) (reviewed by Nilsson *et al.*, 2011[59]), which will no longer propagate the complement system. Deposited iC3b still functions as an opsonin and is further degraded on human cell surfaces via other complement regulators. Regulation by FH occurs both in fluid phase as well as on the host cell surface. While fluid phase complement regulation by FH only requires binding to fluid phase C3b or C3(H_2O), complement regulation on host surfaces requires additional pattern recognition by FH. FH recognizes and binds polyanionic residues, including glycosaminoglycans (GAGs), heparan sulfates and sialic acid moieties, and its affinity for membrane-bound C3b increases in the presence of such ligands[60–62]. The requirement of polyanionic residues for FH to bind to C3b on a surface leads to the discrimination of human from non-human by FH. In principle, pathogens lack the appropriate polyanionic residues that are recognized by FH, and thus are successfully targeted by the complement system.

Following the discovery that FH consist of 20 domains, researchers have been able to attribute the different functions of FH to specific domains or regions within FH (**Fig. 3**). Domains 1-4 are indispensable for the cofactor and decay-accelerating activity[63,64]. In addition, these domains, together with domains 19-20, mediate C3b binding with domains 1-4 preferentially binding intact C3b and domains 19-20 binding intact C3b, iC3b and the C3d region of C3b[65–67]. Domains 6-8 and domain 20 have been found to be important for binding polyanionic residues, with a crucial role for domain 7 in the recognition of different human surfaces and consequently the surface complement regulatory properties of FH[68–71]. The common FH polymorphism Y402H resides within domain 7 (and is thus affecting both FH and FHL-1) and was demonstrated to be decisive for the proper recognition of the polyanionic ligands as well as oxidized lipids found in Bruch's membrane in the eye[72–74].

The Y402H polymorphism is strongly associated with age-related macular degeneration (AMD)[75–77]. AMD is one of the leading causes of blindness worldwide, accounting for 8.7% of all blindness[78,79]. AMD is a multifactorial disease and, next to common risk factors such as aging and environmental factors, associated with a wide range of different genetic variations (reviewed by Fritsche *et al.* 2014[80]). Late-stage AMD can be subdivided in dry AMD, marked by geographic atrophy, and wet AMD, characterized by choroidal neovascularization. In dry AMD, deposits of cell debris, called 'drusen', form within the retinal pigment endothelium layer and Bruch's membrane, disturbing the flow of nutrients, metabolites and oxygen from the choroidal vasculature to the photoreceptors, which results in atrophy. In wet AMD,

the formation of leaky new vasculature within the retinal pigment endothelium results in fluid accumulation and hemorrhages in the retina. In both wet and dry AMD, complement activation and regulation (via FH and/or FHL-1) are thought to play a role.

Mutations in domain 19-20 of FH have been reported to affect the polyanionic binding capacity of FH and are associated with atypical hemolytic uremic syndrome (aHUS)[62]. aHUS is a severe kidney disease, presenting with hemolytic anemia, thrombocytopenia and acute renal failure (reviewed by Noris & Remuzzi, 2009[81]). aHUS is commonly associated with genetic mutations and about 60% of these mutations are found within genes that encode for proteins of the complement system, the most frequently (about 30%) mutated gene being *CFH*. Furthermore, auto-antibodies against FH are found in about 10% of the aHUS patients[82]. These auto-antibodies are commonly associated with homozygosity for the *CFHR3/CFHR1* deletion. Although the overall allele frequency does not differ from the healthy population, homozygosity for the *CFHR3/CFHR1* deletion is increased in aHUS patients[10,12–14] (**Table 1**). Possibly, the lack of FHR-1 is involved in the formation of auto-antibodies against FH in most of these patients. The last three domains of FHR-1 are almost completely identical to the last three domains of FH and most auto-antibodies formed are found to be reactive against both FH and FHR-1[83].

The current models regarding FH surface regulation envisage domains 1-4 and 19-20 binding to human cell membranes via deposited C3b while domains 6-8 and 20 contribute via interactions with polyanions expressed on the cell surface. Upon binding, FH is hypothesized to form a loop which crosses at domain 5 and 18, bringing the domains 1-4 and 19-20 closely together on the C3b molecule[84–86] (**Fig. 4a**). There seems to be no difference between human FH and FHL-1 regarding complement regulating capabilities[64]. However, as FHL-1 lacks domains 8-20, which contain important surface-binding regions, it has been suggested to play a different role in complement regulation on human surfaces. A clear functional and distinct role for FHL-1 in the complement system remained elusive until recently. In contrast to the larger molecule FH, FHL-1 was found to be capable of diffusing through and bind to the Bruch's membrane, which is located in the human eye, and is therefore likely to act locally as the main soluble complement regulator[87].

The physiological role of the FHR proteins is far less understood and still a matter of debate. All FHRs lack domains similar to the first four domains of FH (and FHL-1), which were identified to contain all regulatory activities of FH, suggesting that none of the FHRs play a role in directly inhibiting complement activation. An initial study indeed showed no clear evidence of any regulatory activity for plasma-derived FHR-1 and FHR-2 on C3b[88]. Contradictory, later studies did report complement regulatory activities, showing that recombinant (r) FHR-2 inhibited the formation of C3-convertases similar to FH, although

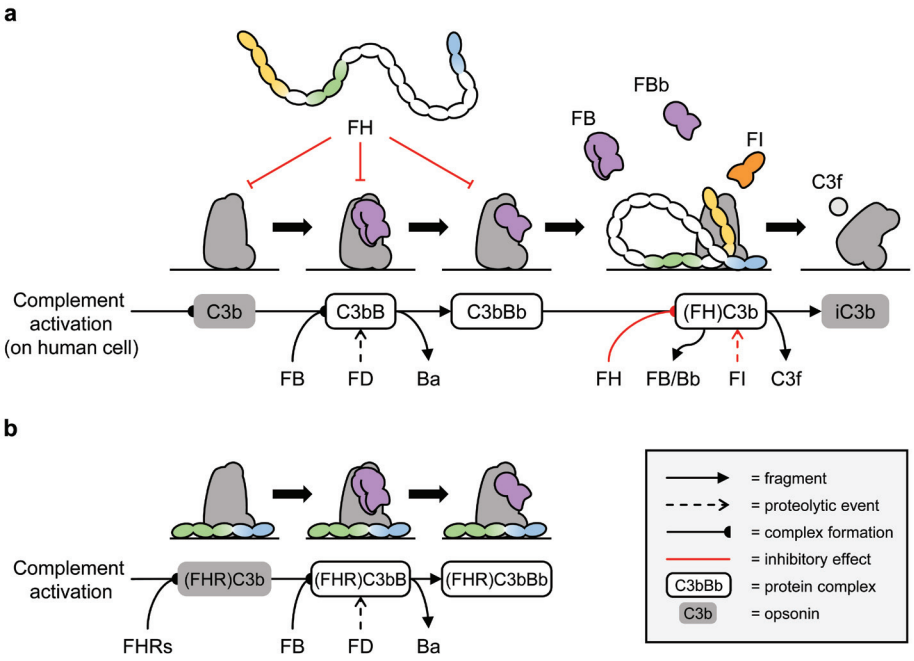

Figure 4 Schematic overview of the regulation of C3b by FH on the human cell surface. (**a**) Upon complement activation on a human cell surface, FH binds to deposited C3b, formed C3bB and/or C3bBb complexes. This will result in dissociation of FB and Bb from the complex, known as decay accelerating activity. Bound to C3b, FH prevents association of FB to C3b through competition for the same binding site on C3b. Furthermore, FH acts as a cofactor for complement factor I (FI), resulting in degradation of C3b and the release of C3f. The remaining iC3b, while still covalently bound to the surface and acting as an opsonin, can no longer further activate the complement system. Of note FH also inhibits the activation of the alternative pathway in fluid phase by binding to C3(H2O) and inhibiting complex formation in a similar way. (**b**) Schematic representation of the hypothesized role of the FH-related proteins (FHRs). The FHRs are hypothesized to compete with FH for the binding to deposited C3b, C3bB and/or C3bBb complexes. Depending on the type of human cell surface targeted, different FHR proteins might compete with FH. By preventing the binding of FH, the FHRs allow the formation of C3bBb complexes, and thus complement activation to progress.

not in a competitive manner[89]. rFHR-1 was reported to inhibit the complement system by binding to C5 and C5b6[47], thus preventing insertion of the MAC, although other studies did not find such activities[58,90]. Cofactor activity was reported for rFHR-3, rFHR-4B and rFHR-5, with rFHR-3 and rFHR-4B even having a synergistic effect on FH cofactor activity[48,91,92]. However, the high protein concentrations required to achieve these effects question the physiological relevance[93]. In addition, a recent study did not find any indication of cofactor activity for FHR-5[58].

While the proposed direct complement regulatory activities for FHR proteins are still under

debate, it is clear that all FHR proteins are capable of binding the same ligands as FH. Binding to C3b, and/or fragments thereof, was reported for all FHR proteins[26,48,49,58,88,89,91,92]. Binding to heparin (as a surrogate for polyanionic residues) was reported for FHR-1[47], FHR-2[89], FHR-3[91] and FHR-5[92], with FHR-4A and FHR-4B seemingly devoid of any affinity for heparin[91]. Interactions with pentraxin 3 and C-reactive protein were reported for FHR-1[94], FHR-4A[95], FHR-4B[95] and FHR-5[92,96]. Furthermore, next to interacting with the same ligands as FH, binding to lipoproteins (HDL or LDL) was reported for FHR-1[97], FHR-2[97], FHR-4B[55] and FHR5[92].

FHRs as antagonists of FH

The observations outlined above have led to a new hypothesis regarding the physiological role of the FHR proteins within the complement system and more particularly with regard to FH. As all FHRs, although to different extents, bind the same ligands as FH and seemingly lack physiological relevant complement regulatory activity, the FHR proteins are currently hypothesized to be antagonists of FH. Thus, they act as de-regulators of the complement system by competing with FH for the binding to deposited C3b on cell surfaces[93]. Consequently, the deposited C3b is not negatively regulated and complement activation can continue (**Fig. 4b**). However, the FHR proteins seem to have different binding affinities to the ligands compared to FH, which is likely caused by differences in the involved domains. With regard to this, the reported homo- and hetero-dimerization of FHR-1, FHR-2 and FHR-5 likely plays a crucial role[58]. Lower affinities of monomeric FHR proteins for ligands, compared to FH, would favor binding of FH while homo- and hetero-dimerization of FHR-1, FHR-2 and FHR-5 might provide a necessary increase in avidity to compete with FH for ligand binding. The composition of the hetero-dimers might even confer a certain level of tissue or surface specificity to the FHR proteins as there seem to be differences in ligand specificity.

The physiological relevance of the FHR proteins is supported by association of variations in the FHR proteins with various diseases. For instance, mutations and duplication events in *CFHR1* and *CFHR5* have been associated with renal diseases such as C3 glomurelopathy[24,26,98]. Furthermore, an indication for the proposed antagonistic role of FHRs is the protective association of the *CFHR3/CFHR1* deletion with AMD, reflected in a decreased allele frequency of the *CFHR3/CFHR1* deletion in AMD patients[13,16,17,19,99] (**Table 1**). Although the underlying mechanism of this protective association is still unknown, it is possible that, in line with the hypothesized antagonistic role of FHRs, the lack of FHR-1 and/or FHR-3 favors complement regulation by FH/FHL-1 in the eye.

An important piece of information that is lacking with regard to the FHR proteins is their physiological concentration. Determining the physiological concentration will aid in clarifying whether the FHRs can truly inhibit complement activation on their own and

whether they indeed would be able to act as antagonists of FH. The challenge in measuring the FHR proteins is the difficulty in specifically distinguishing one FHR from another. As the domains that comprise the FHRs are not only very similar to FH but also to other FHR proteins, lack of specific antibodies has thus far limited the specific measurement of FHR proteins, resulting in rough estimations for most. Furthermore, the discovery of hetero- and homo-dimerization makes proper quantification even more difficult and questions previously reported concentrations using methods that did not account for the effects of dimerization on the detection of the FHRs.

The FH protein family and meningococcal disease

Variations in the complement system, including the FH protein family, are commonly associated with infectious diseases. Many pathogens have evolved mechanisms to evade recognition and destruction by the complement system, including the excretion of complement inhibitors, but more importantly and relevant for this thesis, through the recruitment of human complement regulators to their own surface (reviewed by Lambris *et al.* 2008[100]). As FH is an abundant, freely circulating, potent human complement inhibitor, many pathogens have evolved mechanisms to hijack FH from our circulation. These include mimicry and expression of human cell surface polyanionic residues, which are recognized by FH as a "human" surface, while other pathogens such as the bacteria *Borrelia burgdorferi*[101], *Streptococcus pneumoniae*[102], *Staphylococcus aureus*[103], and *Salmonella enterica*[104] express highly specialized proteins that actively bind human FH to recruit it to their surface. One of the most infamous bacteria that relies on this mechanism is the Gram-negative human commensal *Neisseria meningitidis*. It is carried asymptomatically in the nasopharynx of 10% of the general population, but carriage varies from 5% in infants up to 27% in young adults, going down again to 8% in older adults[105–107]. Upon infection, *N. meningitidis* can cause meningococcal disease (MD), a severe, life-threatening, infectious disease which commonly presents with sepsis and/or meningitis, and has a high mortality (10-15%) and morbidity, with 12-20% of the cases resulting in severe clinical sequelae (*e.g.* paralysis, amputations)[108]. MD mostly affects young children, with the highest incidence in the first year of life[109].

N. meningitidis expresses factor H-binding protein (fHbp), a specialized protein that binds FH at domain 6 and 7 with high affinity, resulting in C3b inhibition and consequently prolonging the survival of the meningococci in the human circulation[110,111]. In 2010, a genome-wide association study revealed that not only variations within the *CFH* gene, but also variations within *CFHR3* are associated with altered susceptibility for MD[112]. This led to the hypothesis that FHR-3, of which domains 1 and 2 have the highest similarity of the FHR proteins with domains 6 and 7 of FH, competes with FH for the binding with meningococcal fHbp. Binding of FHR-3 would, in line with the previously discussed antagonist hypothesis, allow complement activation and increase the clearance of *N. meningitidis*. Indeed, a few reports

already indicate that FHR proteins are also bound by bacterial proteins that were initially identified to bind FH. For instance, FHR-1, FHR-2 and FHR-5 were reported to be bound by borrelial complement regulator-acquiring surface proteins (CRASPs)[113,114], while FHR-3 is, next to FH, also bound by streptococcal M protein[45]. However, the functional consequences of these interactions are not yet understood. Furthermore, the physiological relevance of these interactions is again highly dependent on the physiological concentration of the FHR proteins in relation to the FH concentration. Therefore, next to clarifying their function, there is an increasing need to adequately and specifically determine the concentration of each of the FHR proteins.

Scope of this thesis

The goal of the project described in this thesis was to determine the role of the FHR proteins in the complement system, with special interest in the balance between FH and the FHR proteins in meningococcal disease. We aimed to establish the physiological concentration of all FHR proteins in human blood in order to further elucidate the physiological relevance of the proposed antagonistic properties of the FHRs proteins. This was approached by developing highly specific reagents for both FH and the FHR proteins in order to measure the FH and FHR protein levels in healthy and patient samples, and study possible interactions of the FH protein family with human cells and bacteria. During the project, it became apparent that developing assays and studying the complete FH protein family would not be feasible. Therefore, the current project has focused on FH and the monomeric FHR proteins FHR-3, FHR-4A and FHR-4B. The dimeric FHR proteins (FHR-1, FHR-2 and FHR-5) are part of another, still ongoing study.

In **Chapter 2**, we describe the development and characterization of anti-FH monoclonal antibodies to be used for further characterization of FH. We tested the functional consequences of blocking domains of FH using these newly developed antibodies, demonstrating that most antibodies were inhibiting specific functions of FH, depending on the targeted domain. This work led directly to the discovery of one particular monoclonal antibody, which we describe in **Chapter 3**. This monoclonal antibody, directed against FH, demonstrated opposite effects compared with the other antibodies, as it was found to be potentiating the function of FH. We explored the possible therapeutic applications of this antibody *in vitro* and demonstrated that upon complement activation, potentiating FH with this monoclonal antibody will aid in the protection of human cells from complement-mediated damage. This was demonstrated *in vitro* using aHUS patient material. Importantly, this FH potentiation does not seem to affect the complement-mediated clearance of bacteria, which offers great advantages over many of the currently available or clinically studied complement-targeting drugs. In **Chapter 4**, we describe the development and characterization of antibodies directed against FHR-3. Using these antibodies, we were able to, for the first time, quantify FHR-3 levels in human plasma.

We revealed that the gene copy number variation of *CFHR3* carried in the human population is of major influence on the concentration of FHR-3 in human plasma. Furthermore, FHR-3 levels were found to be much lower compared to FH levels, challenging the physiological relevance of FHR-3 as described in the "antagonist" hypothesis. In **Chapter 5**, we describe the development and characterization of FHR-4A antibodies. With the use of specific FHR-4A antibodies and cross-reactive FHR-4A/FHR-4B antibodies, we demonstrated a large difference in circulating FHR-A and FHR-4B levels, the latter being undetectable. This led us to develop the first specific FHR-4A ELISA, which revealed a high degree of variation in FHR-4A levels in humans. In **Chapter 6**, we investigated a previously described genotype in *CFHR3* that was linked to aHUS and describe a clear genotype-phenotype relationship between variations in *CFHR3* and FHR-3 levels. Allotype *CFHR3*B*, which was associated with aHUS, also associated with higher FHR-3 levels in serum, suggesting that FHR-3 might be involved in aHUS. In **Chapter 7**, we describe FH and FHR-3 levels in children that recovered from meningococcal disease in order to elucidate the occurrence of the proposed competition between FH and FHR-3 *in vivo* as an underlying cause for meningococcal disease susceptibility. We demonstrated that while FHR-3 levels were normal, FH levels were increased in these patients. This increase in FH levels correlated with a SNP found in the *CFHR3* gene which associated with increased susceptibility for meningococcal disease. By employing CRISPR/Cas9 technology, we, for the first time, revealed that disturbed genetic regulation of FH levels by the *CFHR3* gene, rather than altered FHR-3 protein levels, is the underlying mechanism for increased susceptibility for meningococcal disease. In **Chapter 8**, we describe the levels of FH and FHR-3 in samples drawn during meningococcal disease, which were measured to further understand the role of FH and FHR-3 during the acute stage of the disease. We show that FH and FHR-3 are decreased during acute meningococcal infection and recover quickly over time. No indications of an apparent competition between FH and FHR-3 were found. Acute FH levels were found to associate with clinical parameters of disease severity, highlighting the pivotal role of FH in meningococcal disease. Lastly, in **Chapter 9**, the results described in this thesis are summarized and further discussed.

References

1. Le Friec, G. & Kemper, C. Complement: Coming full circle. *Arch. Immunol. Ther. Exp.* **57,** 393–407 (2009).
2. Nesargikar, P. N., Spiller, B. & Chavez, R. The complement system: history, pathways, cascade and inhibitors. *Eur. J. Microbiol. Immunol.* **2,** 103–111 (2012).
3. Walport, M. J. Complement; First of Two Parts. *N. Engl. J. Med.* **344,** 1058–1066 (2001).
4. Lesher, A. M., Nilsson, B. & Song, W. C. Properdin in complement activation and tissue injury. *Mol. Immunol.* **56,** 191–198 (2013).
5. Harboe, M., Ulvund, G., Vien, L., Fung, M. & Mollnes, T. E. The quantitative role of alternative pathway amplification in classical pathway. *Clin. Exp. Immunol.* **138,** 439–446 (2004).
6. Krych-Goldberg, M. & Atkinson, J. P. Structure-function relationships of complement receptor type 1. *Immunol. Rev.* **180,** 112–122 (2001).
7. Rodríguez de Córdoba, S., Díaz-Guillén, M. a. & Heine-Suñer, D. An integrated map of the human regulator of complement activation (RCA) gene cluster on 1q32. *Mol. Immunol.* **36,** 803–808 (1999).
8. Heine-Suñer, D. *et al.* A high-resolution map of the regulator of the complement activation gene cluster on 1q32 that integrates new genes and markers. *Immunogenetics* **45,** 422 – 427 (1997).
9. Rodríguez de Córdoba, S., Tortajada, A., Harris, C. L. & Morgan, B. P. Complement dysregulation and disease: From genes and proteins to diagnostics and drugs. *Immunobiology* **217,** 1034–1046 (2012).
10. Moore, I. *et al.* Association of factor H autoantibodies with deletions of CFHR1, CFHR3, CFHR4, and with mutations in CFH, CFI, CD46, and C3 in patients with atypical hemolytic uremic syndrome. *Blood* **115,** 379–387 (2010).
11. Holmes, L. V *et al.* Determining the population frequency of the CFHR3/CFHR1 deletion at 1q32. *PLoS ONE* **8,** 1–7 (2013).
12. Zipfel, P. F. *et al.* Deletion of complement factor H-related genes CFHR1 and CFHR3 is associated with atypical hemolytic uremic syndrome. *PLoS Genet.* **3,** 0387–0392 (2007).
13. Abarrategui-Garrido, C., Martínez-Barricarte, R., López-Trascasa, M., Rodríguez de Córdoba, S. & Sánchez-Corral, P. Characterization of complement factor H-related (CFHR) proteins in plasma reveals novel genetic variations of CFHR1 associated with atypical hemolytic uremic syndrome. *Blood* **114,** 4261–4271 (2009).
14. Bernabéu-Herrero, M. E. *et al.* Complement factor H , FHR-3 and FHR-1 variants associate in an extended haplotype conferring increased risk of atypical hemolytic uremic syndrome. *Mol. Immunol.* **67,** 276–286 (2015).
15. Sivakumaran, T. A. *et al.* A 32 kb critical region excluding Y402H in CFH mediates risk for age-related macular degeneration. *PLoS ONE* **6,** e25598 (2011).
16. Hageman, G. S. *et al.* Extended haplotypes in the complement factor H (CFH) and CFH-related (CFHR) family of genes protect against age-related macular degeneration: characterization, ethnic distribution and evolutionary implications. *Ann. Med.* **38,** 592–604 (2006).
17. Sawitzke, J., Im, K. M., Kostiha, B., Dean, M. & Gold, B. Association assessment of copy number polymorphism and risk of age-related macular degeneration. *Ophthalmology* **118,** 2442–2446 (2011).
18. Zhao, J. *et al.* Association of genetic variants in complement factor H and factor H-related genes with systemic lupus erythematosus susceptibility. *PLoS Genet.* **7,** 1–9 (2011).
19. Cantsilieris, S., White, S. J., Richardson, A. J., Guymer, R. H. & Baird, P. N. Comprehensive analysis of Copy Number Variation of genes at chromosome 1 and 10 loci associated with late age related macular degeneration. *PLoS ONE* **7,** e35255 (2012).
20. Skerka, C., Chen, Q., Frémeaux-Bacchi, V. & Roumenina, L. T. Complement factor H related proteins (CFHRs). *Mol. Immunol.* **56,** 170–180 (2013).
21. Male, D. A., Ormsby, R. J., Ranganathan, S., Giannakis, E. & Gordon, D. L. 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 (2000).
22. Feifel, E. *et al.* Polymorphism and deficincy of human factor H-related proteins p39 and p37. *Immunogenetics* **36,** 104–109 (1992).
23. Hughes, A. E. *et al.* A common CFH haplotype, with deletion of CFHR1 and CFHR3, is associated with lower risk of age-related macular degeneration. *Nat. Genet.* **38,** 1173–1177 (2006).
24. Gale, D. P. *et al.* Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. *Lancet* **376,** 794–801 (2010).
25. Vernon, K. A. *et al.* Recurrence of complement factor H-related protein 5 nephropathy in a renal transplant.

Am. J. Transplant. **11,** 152–155 (2011).
26. Tortajada, A. *et al.* C3 glomerulopaty-associated CFHR1 mutation alters FHR oligomerization and complement regulation. *J. Clin. Invest.* **123,** 2434–2446 (2013).
27. Venables, J. P. *et al.* Atypical haemolytic uraemic syndrome associated with a hybrid complement gene. *PLoS Med.* **3,** e431 (2006).
28. Francis, N. J. *et al.* A novel hybrid CFH/CFHR3 gene generated by a microhomology-mediated deletion in familial atypical hemolytic uremic syndrome. *Blood* **119,** 591–601 (2012).
29. Malik, T. H. *et al.* A Hybrid CFHR3-1 Gene Causes Familial C3 Glomerulopathy. *J. Am. Soc. Nephrol.* **23,** 1155–1160 (2012).
30. Eyler, S. J. *et al.* A novel hybrid CFHR1/CFH gene causes atypical hemolytic uremic syndrome. *Pediatr. Nephrol.* **28,** 2221–2225 (2013).
31. Chen, Q. *et al.* Complement factor H-related hybrid protein deregulates complement in dense deposit disease. *J. Clin. Invest.* **124,** 145–155 (2014).
32. Valoti, E. *et al.* A novel atypical hemolytic uremic syndrome-associated hybrid CFHR1/CFH gene encoding a fusion protein that antagonizes factor H-dependent complement regulation. *J. Am. Soc. Nephrol.* **26,** 1–11 (2015).
33. Maga, T. K. *et al.* A novel deletion in the RCA gene cluster causes atypical hemolytic uremic syndrome. *Nephrol. Dial. Transplant.* **26,** 739–741 (2011).
34. Nilsson, U. R. & Müller-Eberhard, H. J. Isolation of beta 1F globulin from human serum and its characterization as the fith component of complement. *J. Exp. Med.* 277–298 (1965).
35. Weiler, J. M., Daha, M. R., Austen, K. F. & Fearon, D. T. Control of the amplification convertase of complement by the plasma protein beta1H. *Proc. Natl. Acad. Sci. U. S. A.* **73,** 3268–3272 (1976).
36. Whaley, K. & Ruddy, S. Modulation of the alternative complement pathway by beta 1H globulin. *J. Exp. Med.* **144,** 1147–1163 (1976).
37. Whaley, K. & Ruddy, S. Modulation of C3b hemolytic activity by a plasma protein distinct from C3b inactivator. *Science* **193,** 1011–1013 (1976).
38. Nomenclature of the alternative activating pathway of complement. *Bull. World Health Organ.* **59,** 489–491 (1981).
39. Ripoche, J., Day, A. J., Harris, T. J. & Sim, R. B. The complete amino acid sequence of human complement factor H. *Biochem. J.* **249,** 593–602 (1988).
40. Schwaeble, W. *et al.* Human complement factor H: expression of an additional truncated gene product of 43 kDa in human liver. *Eur. J. Immunol.* **17,** 1485–1489 (1987).
41. Misasi, R. *et al.* Human complement factor H: An additional gene product of 43kDa isolated from human plasma shows cofactor activity for the cleavage of the third component of complement. *Eur. J. Immunol.* **19,** 1765–1768 (1989).
42. Esparza-Gordillo, J. *et al.* Genetic and environmental factors influencing the human factor H plasma levels. *Immunogenetics* **56,** 77–82 (2004).
43. Hakobyan, S. *et al.* Measurement of factor H variants in plasma using variant-specific monoclonal antibodies: application to assessing risk of age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* **49,** 1983–1990 (2008).
44. Sofat, R. *et al.* Distribution and determinants of circulating complement factor H concentration determined by a high-throughput immunonephelometric assay. *J. Immunol. Methods* **390,** 63–73 (2013).
45. Kotarsky, H. *et al.* Identification of a domain in human factor H and factor H-like protein-1 required for the interaction with Streptococcal M proteins. *J. Immunol.* **160,** 3349–3354 (1998).
46. Fenaille, F. *et al.* Site-specific N-glycan characterization of human complement factor H. *Glycobiology* **17,** 932–944 (2007).
47. Heinen, S. *et al.* Factor H-related protein 1 (CFHR-1) inhibits complement C5 convertase activity and terminal complex formation. *Blood* **114,** 2439–2447 (2009).
48. Fritsche, L. G. *et al.* An imbalance of human complement regulatory proteins CFHR1, CFHR3 and factor H influences risk for age-related macular degeneration (AMD). *Hum. Mol. Genet.* **19,** 4694–4704 (2010).
49. Hebecker, M. & Józsi, M. Factor H-related protein 4 activates complement by serving as a platform for the assembly of alternative pathway C3 convertase via its interaction with C3b protein. *J. Biol. Chem.* **287,** 19528–19536 (2012).
50. Vernon, K. A. *et al.* Acute presentation and persistent glomerulonephritis following streptococcal infection in a patient with heterozygous complement factor H-related protein 5 deficiency. *Am. J. Kidney Dis.* **60,** 121–125 (2012).
51. Estaller, C., Koistinen, V., Schwaeble, W., Dierich, M. P. & Weiss, E. H. Cloning of the 1.4-kb mRNA species

of human complement factor H reveals a novel member of the short consensus repeat family related to the carboxy terminal of the classical 150-kDa molecule. *J. Immunol.* **146,** 3190–3196 (1991).
52. Skerka, C., Horstmann, R. D. & Zipfel, P. F. Molecular cloning of a human serum protein structurally related to complement factor H. *J. Biol. Chem.* **266,** 12015–12020 (1991).
53. Skerka, C., Timmann, C., Horstmann, R. D. & Zipfel, P. F. Two additional human serum proteins structurally related to complement factor H - Evidence of a family of factor H-related genes. *J. Immunol.* **148,** 3313–3318 (1992).
54. Skerka, C., Kuhn, S., Gunther, K., Lingelbach, K. & Zipfel, P. F. A novel short consensus repeat-containing molecule is related to human complement factor H. *J. Biol. Chem.* **268,** 2904–2908 (1993).
55. Skerka, C. *et al.* The human factor H-related protein 4 (FHR-4). *J. Biol. Chem.* **272,** 5627–5634 (1997).
56. Józsi, M. *et al.* FHR-4A: a new factor H-related protein is encoded by the human FHR-4 gene. *Eur. J. Hum. Genet.* **13,** 321–329 (2005).
57. McRae, J. L. *et al.* Human factor H-related protein 5 (FHR-5); A new complement-associated protein. *J. Biol. Chem.* **276,** 6747–6754 (2001).
58. Goicoechea De Jorge, E. *et al.* Dimerization of complement factor H-related proteins modulates complement activation in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 4685–4690 (2013).
59. Nilsson, S. C., Sim, R. B., Lea, S. M., Fremeaux-Bacchi, V. & Blom, A. M. Complement factor I in health and disease. *Mol. Immunol.* **48,** 1611–1620 (2011).
60. Rodríguez de Córdoba, S., Esparza-Gordillo, J., Goicoechea De Jorge, E., Lopez-Trascasa, M. & Sanchez-Corral, P. The human complement factor H: functional roles, genetic variations and disease associations. *Mol. Immunol.* **41,** 355–367 (2004).
61. Zipfel, P. F., Heinen, S., Józsi, M. & Skerka, C. Complement and diseases: Defective alternative pathway control results in kidney and eye diseases. *Mol. Immunol.* **43,** 97–106 (2006).
62. Blaum, B. S. *et al.* Structural basis for sialic acid-mediated self-recognition by complement factor H. *Nat. Chem. Biol.* **11,** 77–83 (2015).
63. Gordon, D. L., Kaufman, R. M., Blackmore, T. K., Kwong, J. & Lublin, D. M. Identification of complement regulatory domains in human factor H. *J. Immunol.* **155,** 348–356 (1995).
64. Kühn, S., Skerka, C. & Zipfel, P. F. Mapping the complement regulatory domains in the human factor H-like protein 1 and in factor H. *J. Immunol.* **155,** 5663–5670 (1995).
65. Jokiranta, T. S., Hellwage, J., Koistinen, V., Zipfel, P. F. & Meri, S. Each of the three binding sites on complement factor H interacts with a distinct site on C3b. *J. Biol. Chem.* **275,** 27657–27662 (2000).
66. Jokiranta, T. S. *et al.* Binding of complement factor H to endothelial cells is mediated by the carboxy-terminal glycosaminoglycan binding site. *Am. J. Pathol.* **167,** 1173–1181 (2005).
67. Józsi, M., Oppermann, M., Lambris, J. D. & Zipfel, P. F. The C-terminus of complement factor H is essential for host cell protection. *Mol. Immunol.* **44,** 2697–2706 (2007).
68. Blackmore, T. K., Sadlon, T. A., Ward, H. M., Lublin, D. M. & Gordon, D. L. Identification of a heparin binding domain in the seventh short consensus repeat of complement factor H. *J. Immunol.* **157,** 5422–5427 (1996).
69. Blackmore, T. K. *et al.* Identification of the second heparin-binding domain in human complement factor H. *J. Immunol.* **160,** 3342–3348 (1998).
70. Prosser, B. E. *et al.* Structural basis for complement factor H linked age-related macular degeneration. *J. Exp. Med.* **204,** 2277–2283 (2007).
71. Schmidt, C. Q. *et al.* A new map of glycosaminoglycan and C3b binding sites on factor H. *J. Immunol.* **181,** 2610–2619 (2008).
72. Clark, S. J. *et al.* Impaired binding of the age-related macular degeneration-associated complement factor H 402H allotype to Bruch's membrane in human retina. *J. Biol. Chem.* **285,** 30192–30202 (2010).
73. Weismann, D. *et al.* Complement factor H binds malondialdehyde epitopes and protects from oxidative stress. *Nature* **478,** 76–81 (2011).
74. Clark, S. J. *et al.* Tissue-specific host recognition by complement factor H is mediated by differential activities of its glycosaminoglycan-binding regions. *J. Immunol.* **190,** 2049–2057 (2013).
75. Edwards, A. O. *et al.* Complement factor H polymorphism and age-related macular degeneration. *Science* **308,** 421–424 (2005).
76. Haines, J. L. *et al.* Complement factor H variant increases the risk of age-related macular degeneration. *Science* **308,** 419–421 (2005).
77. Klein, R. J. *et al.* Complement factor H polymorphism in age-related macular degeneration. *Science* **308,** 385–389 (2005).
78. Bourne, R. R. A. *et al.* Causes of vision loss worldwide, 1990-2010 a systematic analysis. *Lancet Glob. Heal.*

1, e339–349 (2012).
79. Wong, W. L. *et al.* Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: A systematic review and meta-analysis. *Lancet Glob. Heal.* **2,** e106–116 (2014).
80. Fritsche, L. G. *et al.* Age-related macular degeneration: genetics and biology coming together. *Annu. Rev. Genomics Hum. Genet.* **15,** 151–171 (2014).
81. Noris, M. & Remuzzi, G. Atypical hemolytic–uremic syndrome. *N. Engl. J. Med.* **361,** 1676–1687 (2009).
82. Nester, C. M. *et al.* Atypical aHUS: state of the art. *Mol. Immunol.* **67,** 31–42 (2015).
83. Józsi, M. *et al.* Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. *Blood* **111,** 1512–1514 (2008).
84. Morgan, H. P. *et al.* Structural analysis of the C-terminal region (modules 18-20) of complement regulator factor H (FH). *PLoS ONE* **7,** 19–24 (2012).
85. Harder, M. J. *et al.* Comparative analysis of novel complement-targeted inhibitors, miniFH, and the natural regulators factor H and factor H-like protein 1 reveal functional determinants of complement regulation. *J. Immunol.* **196,** 866–876 (2015).
86. Schmidt, C. Q. *et al.* Selectivity of C3-opsonin targeted complement inhibitors: a distinct advantage in the protection of erythrocytes from paroxysmal nocturnal hemoglobinuria patients. *Immunobiology* **221,** 503–511 (2016).
87. Clark, S. J. *et al.* Identification of factor H-like protein 1 as the predominant complement regulator in Bruch's membrane: implications for age-related macular degeneration. *J. Immunol.* **193,** 4962–4970 (2014).
88. Timmann, C., Leippe, M. & Horstmann, R. D. Two major serum components antigenically related to complement factor H are different glycosylation forms of a single protein with no factor H-like complement regulatory functions. *J. Immunol.* **146,** 1265–1270 (1991).
89. Eberhardt, H. U. *et al.* Human factor H-related protein 2 (CFHR2) regulates complement activation. *PLoS ONE* **8,** e78617 (2013).
90. Strobel, S. *et al.* Factor H-related protein 1 neutralizes anti-factor H autoantibodies in autoimmune hemolytic uremic syndrome. *Kidney Int.* **80,** 397–404 (2011).
91. Hellwage, J. *et al.* Functional properties of complement factor H-related proteins FHR-3 and FHR-4: binding to the C3d region of C3b and differential regulation by heparin. *FEBS* **462,** 345–352 (1999).
92. McRae, J. L. *et al.* Human factor H-related protein 5 has cofactor activity, inhibits C3 convertase activity, binds heparin and C-reactive protein, and associates with lipoprotein. *J. Immunol.* **174,** 6250–6256 (2005).
93. Józsi, M., Tortajada, A., Uzonyi, B., Goicoechea de Jorge, E. & Rodríguez de Córdoba, S. Factor H-related proteins determine complement-activating surfaces. *Trends Immunol.* **36,** 374–384 (2015).
94. Kopp, A. *et al.* Atypical hemolytic uremic syndrome-associated variants and autoantibodies impair binding of factor H and factor H-related protein 1 to pentraxin 3. *J. Immunol.* **189,** 1858–1867 (2012).
95. Hebecker, M. *et al.* Molecular basis of C-reactive protein binding and modulation of complement activation by factor H-related protein 4. *Mol. Immunol.* **47,** 1347–1355 (2010).
96. Csincsi, Á. I. *et al.* Factor H-related protein 5 interacts with pentraxin 3 and the extracellular matrix and modulates complement activation. *J. Immunol.* **194,** 4963–4973 (2015).
97. Park, C. T. & Wright, S. D. Plasma lipopolysaccharide-binding protein is found associated with a particle containing apolipoprotein A-I, phospholipid, and factor H-related proteins. *J. Biol. Chem.* **271,** 18054–18060 (1996).
98. Athanasiou, Y. *et al.* Familial C3 Glomerulopathy associated with CFHR5 mutations: clinical characteristics of 91 patients in 16 pedigrees. *Clin. J. Am. Soc. Nephrol.* **6,** 1436–1446 (2011).
99. Schmid-Kubista, K. E. *et al.* Contribution of copy number variation in the regulation of complement activation locus to development of age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* **50,** 5070–5079 (2009).
100. Lambris, J. D., Ricklin, D. & Geisbrecht, B. V. Complement evasion by human pathogens. *Nat. Rev. Microbiol.* **6,** 132–142 (2008).
101. Kraiczy, P., Skerka, C., Kirschfink, M., Brade, V. & Zipfel, P. F. Immune evasion of Borrelia burgdorferi by acquisition of human complement regulators FHL-1/reconectin and Factor H. *Eur. J. Immunol.* **31,** 1674–1684 (2001).
102. Dave, S., Brooks-Walter, A., Pangburn, M. K. & McDaniel, L. S. PspC, a pneumococcal surface protein, binds human factor H. *Infect. Immun.* **69,** 3435–3437 (2001).
103. Sharp, J. A. *et al.* Staphylococcus aureus surface protein SdrE binds complement regulator factor H as an immune evasion tactic. *PLoS ONE* **7,** e38407 (2012).
104. Ho, D. K., Jarva, H. & Meri, S. Human complement factor H binds to outer membrane protein Rck of Salmonella. *J. Immunol.* **185,** 1763–1769 (2010).

105. Yazdankhah, S. P. & Caugant, D. A. Neisseria meningitidis: an overview of the carriage state. *J. Med. Microbiol.* **53,** 821–832 (2004).
106. Trotter, C. L. & Greenwood, B. M. Meningococcal disease in the African meningitis belt. *Lancet Infect. Dis.* **7,** 797–803 (2007).
107. Christensen, H., May, M., Bowen, L., Hickman, M. & Trotter, C. L. Meningococcal carriage by age: a systematic review and meta-analysis. *Lancet Infect. Dis.* **10,** 853–861 (2010).
108. Center for Disease Control and Prevention. in *Epidemiology and Prevention of Vaccine-Preventable Diseases* (eds. Hamborsky, J., Kroger, A. & Wolfe, C.) 231–246 (Public Health Foundation, 2015).
109. Bijlsma, M. W. *et al.* Epidemiology of invasive meningococcal disease in the Netherlands, 1960-2012: an analysis of national surveillance data. *Lancet Infect. Dis.* **14,** 805–812 (2014).
110. Schneider, M. C. *et al.* Functional significance of factor H binding to Neisseria meningitidis. *J. Immunol.* **176,** 7566–7575 (2006).
111. Schneider, M. C. *et al.* Neisseria meningitidis recruits factor H using protein mimicry of host carbohydrates. *Nature* **458,** 890–893 (2009).
112. Davila, S. *et al.* Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. *Nat. Genet.* **42,** 772–776 (2010).
113. Haupt, K. *et al.* Binding of human factor H-related protein 1 to serum-resistant Borrelia burgdorferi is mediated by Borrelial complement regulator-acquiring surface proteins. *J. Infect. Dis.* **196,** 124–133 (2007).
114. Siegel, C. *et al.* Complement factor H-related proteins CFHR2 and CFHR5 represent novel ligands for the infection-associated CRASP proteins of Borrelia burgdorferi. *PLoS ONE* **5,** e13519 (2010).