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CFHR Gene Variations Provide Insights in the Pathogenesis of the Kidney Diseases Atypical Hemolytic Uremic Syndrome and C3 Glomerulopathy

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ABSTRACT

Sequence and copy number variations in the human *CFHR–Factor H* gene cluster comprising the complement genes *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4*, *CFHR5*, and *Factor H* are linked to the human kidney diseases atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy. Distinct genetic and chromosomal alterations, deletions, or duplications generate hybrid or mutant *CFHR* genes, as well as hybrid *CFHR–Factor H* genes, and alter the FHR and Factor H plasma repertoire. A clear association between the genetic modifications and the pathologic outcome is emerging: *CFHR1*, *CFHR3*, and *Factor H* gene alterations combined with intact *CFHR2*, *CFHR4*, and *CFHR5* genes are reported in atypical hemolytic uremic syndrome. But alterations in each of the five *CFHR* genes in the context of an intact *Factor H* gene are described in C3 glomerulopathy. These genetic modifications influence complement function and the interplay of the five FHR proteins with each other and with Factor H. Understanding how mutant or hybrid FHR proteins, Factor H::FHR hybrid proteins, and altered Factor H, FHR plasma profiles cause pathology is of high interest for diagnosis and therapy.

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Sequence and copy number variations in the human *CFHR* gene cluster are linked to the kidney disorders atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy,[1–6] and are furthermore associated with IgA nephropathy (IgAN), with a retinal disease, and with infections.[5–8] These copy number variations are caused by deletions, duplications, and insertions of gene or chromosomal segments and generate *CFHR::CFHR*, *CFHR::Factor H*, and *Factor H::CFHR* hybrid genes or *CFHR* genes with duplicated elements and result in FHR hybrid or mutant proteins[1] and alter the FHR and Factor H plasma repertoire. One clear difference is emerging: in aHUS, ultimately the protective complement action of Factor H on the endothelial surface is altered. In C3 glomerulopathy, the FHR mutants and a modified FHR plasma repertoire apparently affect local complement regulation, inducing cell proliferation and chronic inflammation. To evaluate how alterations in the five *CFHR* genes and the *Factor H* gene cause different renal pathologies, we here link the genetic scenarios, the specific FHR variants expressed in plasma, with the glomerular changes in these kidney diseases. We summarize the role of FHR proteins as emerging complement modulators, amplifiers, and inflammatory modifiers. In aHUS FHR mutants and in autoimmune aHUS pathogenic Factor H–binding autoantibodies alter Factor H regulation on endothelial surfaces and fluid-phase regulation remains intact. In C3 glomerulopathy, FHR mutants in the context of intact Factor H and FHL1 (the Factor H–like protein) affect complement regulation in the fluid phase and on the glomerular surface, and FHR mutants with duplicated interaction segments form large oligomers, which deregulate complement and compete with Factor H for surface binding. Thus, C3 glomerulopathy develops due to unique *CFHR* gene variations in the context of an intact *Factor H* gene.

Here, we summarize how modifications in the *CFHR* gene cluster described for aHUS and C3 glomerulopathy cause specific FHR mutants and how they affect the FHR plasma repertoire. Thereby, this review will focus on (*1*) providing an overview of complement initiation, with the two central enzymatic levels and the various effector actions; (*2*) the characteristic

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morphologic features of the human kidney disorders aHUS and C3 glomerulopathy; (*3*) giving a brief summary on the organization of the human *CFHR–Factor H* gene cluster with the Factor H and the five *CFHR* genes and on the structure of the encoded FHR proteins; (*4*) explaining how, in aHUS, *CFHR* gene variations generate Factor H::FHR3, Factor H::FHR1, and FHR1::Factor H hybrid proteins and how they alter FHR plasma levels; (*5*) describing which genetic *CFHR* changes occur in C3 glomerulopathy; (*6*) describing which FHR hybrid or FHR mutant proteins are expressed in this disease; (*7*) describing how altered FHR plasma levels result in C3 glomerulopathy; (*8*) summarizing the new insights into the role of the FHR proteins in IgAN, in complement control and disease pathology; and (*9*) providing concluding remarks and an outlook for diagnosis, biomarker profiling, and therapy.

COMPLEMENT: INITIATION, ENZYMATIC LEVELS, AND EFFECTOR PATHWAYS

Complement is a central homeotic system and defective complement causes many human diseases, in particular the kidney diseases aHUS and C3 glomerulopathy.[9,10] In order to link complement with *CFHR* gene variations a brief overview of complement, with the three activation pathways and with two central enzymatic checkpoints, is presented (Figure 1A). Activation of the alternative pathway occurs spontaneously in the fluid phase and propels on target surfaces; the lectin and the classic pathways are initiated on target surfaces by carbohydrates and antibodies bound to antigens.[9–11] Upon initiation, two subsequently acting enzymatic effector systems are formed. The first enzymatic level is mediated by two C3 convertases, the AP (C3bBb) and the LP/CP (C2bC4b) convertases, which cleave the same substrate C3 and generate C3a and C3b.[12] This enzymatic level includes a self-amplifying amplification loop that enhances activation. The second enzyme level generates C5 convertases. Again, two enzymes are generated; both the convertase (C3bBbC3b) of the AP and the convertase (C2bC4bC3b) of the LP/CP-pathway use C5 as substrate and generate C5a and C5b.

Complement action occurs in the fluid phase and on surfaces and multiple regulators control activation and the transition from fluid phase to the surface.[9] Both C3 convertases form the inflammatory anaphylatoxin C3a and the opsonin C3b. C3b is handled differently on self and nonself surfaces. C3b deposited on intact self surfaces is inactivated by complement proteases which act together with cofactors, and inactivated iC3b is further processed to C3dg and C3d. In addition, C3b when deposited to foreign surfaces initiates the amplification loop that amplifies C3b deposition. The second enzymatic level with the C5 convertase generates the potent anaphylatoxin C5a and deposits C5b, which initiates the pore-forming terminal complement complex (TCC) (Figure 1A).[13,14] Thus, two enzymatic steps generate different effector compounds (*1*) in the form of anaphylatoxins C3a and C5a that induce inflammation and attract host cells; (*2*) by opsonization of target surfaces with C3b; and (*3*) by forming the TCC, which generates a pore and damages the target membrane.[15,16]

An important challenge is to understand complement regulation and the actions of existing regulators and new modulators. Given that FHR proteins are emerging complement modulators and amplifiers, it is of interest to define at which specific step of a pathway and at which checkpoints each FHR protein acts. The different pathologies caused by the FHR mutants and hybrids, *i.e.*, endothelial damage in aHUS and in DEAP-HUS (homozygous *CFHR1-CFHR3* Deficiency and Autoantibody to Factor H Positive), and inflammatory and proliferative action in C3 glomerulopathy already indicate different regulatory and modulatory and amplifiers roles of FHR proteins in the complement cascade.

RENAL HISTOLOGIC CHANGES IN AHUS AND IN C3 GLOMERULOPATHY

Chromosomal changes in the *CFHR–Factor H* gene cluster result in genetic modifications that are causative for the two kidney diseases, genetic aHUS and C3 glomerulopathy. The morphologic and cellular alterations, which are caused by deregulated complement, are summarized in recent excellent reviews.[17,18] The morphologic appearance of aHUS in the glomeruli and the preglomerular arterioles can be characterized as thrombotic microangiopathy. Best understood is primary complement-mediated endothelial and mesangial cell damage due to dysregulation of the alternative complement cascade on the cell surface.[19] Defective complement control on endothelial surfaces results in cell lysis, followed by thrombus formation, loss of mesangial cells, and mesangiolysis as seen in histology (Figure 1C). In the chronic or repair phase, the newly formed endothelial cells produce new extracellular matrix, leading to double contours of the glomerular basement membrane (GBM) thickening.[17,18] In contrast, in C3 glomerulopathy, chronic complement activation in the fluid phase and/or on the surface leads to deposition of complement components in the mesangium and in the subendothelial space (membranoproliferative GN [MPGN] pattern), or within the GBM (intramembranous GN, dense deposit disease (DDD) pattern), and sometimes also at the outer aspects of the GBM (subepithelial deposits) (Figure 1D).[20] Depot formation is followed by mesangial and endothelial cell activation as well

Significance Statement

The human *CFHR–Factor H* gene cluster encodes the five FHR proteins that are emerging complement and immune modulators and the two complement regulators Factor H and FHL1. Genetic and chromosomal alterations in this cluster are associated with the human kidney diseases atypical hemolytic uremic syndrome and C3 glomerulopathy. Various genetic alterations result in the expression of mutant and altered FHR proteins, or FHR::Factor H and Factor H::FHR hybrid proteins. The modified FHR proteins together with an altered FHR and Factor H plasma repertoire, which often modify complement action in the fluid phase and cause morphologic alteration in the glomerulus, provide important views on FHR protein function in the kidney.

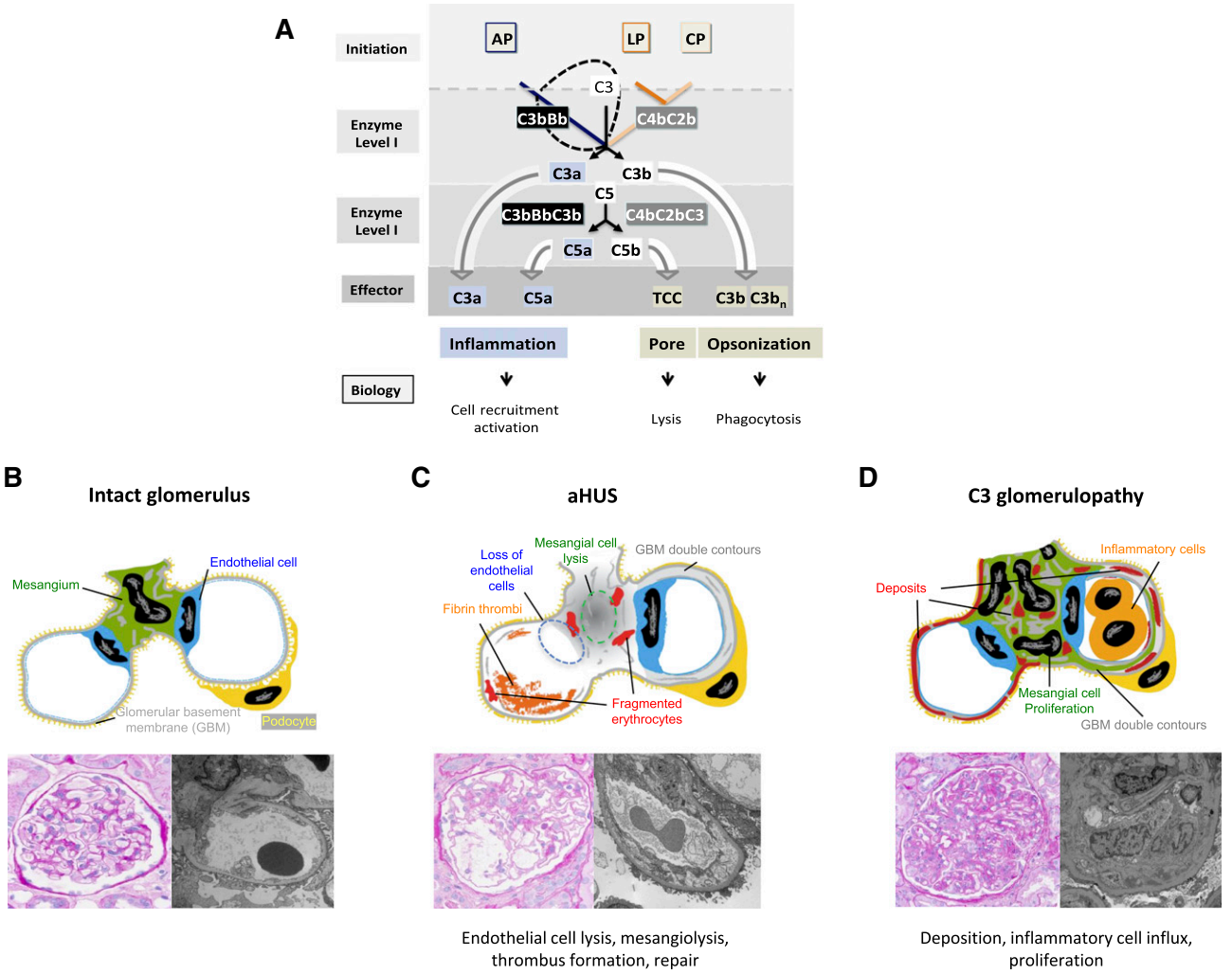

Figure 1. Overview of complement activation and effector pathways and morphologic changes in aHUS and C3 glomerulopathy. (A) Complement activation occurs *via* three pathways, the alternative pathway (AP), the lectin pathway (LP), and the classic pathway (CP), which are initiated on surfaces. The type of surface influences activation and the regulator repertoire decides on cascade progression or inhibition. The LP and CP are activated on surfaces by specific carbohydrate moieties (LP), by surface-deposited components (*e.g.*, C reactive protein, Pentraxin), and by IgGs (CP). A repertoire of regulators controls cascade progression in the fluid phase and on surfaces. The three pathways form specific surface-bound convertases; the AP results in the generation of AP-C3 convertase and the LP/CP trigger formation of the CP C3 convertase. The general role of both C3 convertases is to cleave the abundant plasma protein C3 (concentration 1000–1500 μg/ml) into the anaphylatoxin C3a and the opsonic C3b. The enzymatic response on the first enzymatic levels is frequently enhanced by the potent self-amplifying amplification loop. When activation proceeds the C3 convertases attach an additional C3b fragment, form the C3bBbC3b complex, changes substrate specificity and form a C5 convertase. C5 convertases of the AP and of the LP/CP pathways exist. The major role of the C5 convertase is to cleave C5 (plasma concentration 350 μg/ml) into the powerful anaphylatoxin C5a and to generate a surface-binding C5b. C5b subsequently initiates the TCC, which forms lytic pores. (B) Structure of an intact glomerulus. Top: Schematic view of mesangial area (green color) in the filtration unit with endothelial cells (blue), GBM (gray), and podocytes (yellow color). Bottom left: PAS staining of an intact pretransplant kidney. Right: Electron microscopic analysis of a pretransplant kidney with intact glomerular structure. (C) Glomerular changes in aHUS. Top: Schematic presentation of hypocellularity due to cell lysis and loss of endothelial and mesangial cells. Bottom left: PAS staining. Right: EM imaging reveals a pattern of thrombotic microangiopathy. (D) C3 glomerulopathy showing a proliferative pattern with hypercellularity and influx of infiltrating cells. Top: Schematic view of mesangial proliferation and thickening of GBM in MPGN (right glomerulus) and in DDD (left glomerulus). Bottom left: Glomerular changes revealed by PAS staining. Right: EM image showing thickening of the GBM and double contour formation.

as proliferation, leading to influx of macrophages and to endocapillary and mesangial hypercellularity. Constantly activated endothelial and mesangial cells form new matrixes leading to thickening of the GBM and double contours or multilayering, as well as mesangial matrix increase. This glomerular remodeling leads to the typical lobular appearance

that is summarized under the umbrella term MPGN.[5]

THE *FACTOR H–CFHR* GENE CLUSTER, FHR PROTEINS, AND CONFORMATION

The Human Factor H–CFHR Gene Cluster

The human *Factor H–CFHR* gene cluster is located on human chromosome 1q32 in the regulators of complement activation region.[19,21] The five *CFHR* genes are positioned downstream of the *Factor H* gene and are arranged in the order *CFHR3*, *CFHR1*, *CFHR4*, *CFHR2*, and *CFHR5*.[19] This cluster presents an instable, dynamic chromosomal region and a hotspot for structural rearrangements. The human *CFHR* gene cluster includes interspersed duplicated regions with strong nucleotide identity[22–24] (H. Richter, C. Skerka, and P. Zipfel, unpublished observations) (Figure 2, left panel). The segmental duplicated regions can include coding regions, *e.g.*, the repeat regions B and B' include exons that encode the C-terminal SCRs of Factor H and FHR1, respectively (see below).[25]

CFHR genes are conserved in evolution indicating essential biologic functions.[24,26] The four mouse genes *mCFHR-A*, *mCFHR-B*, *mCFHR-C*, and *mCFHR-D* differ in structure, domain composition, and sequence from the

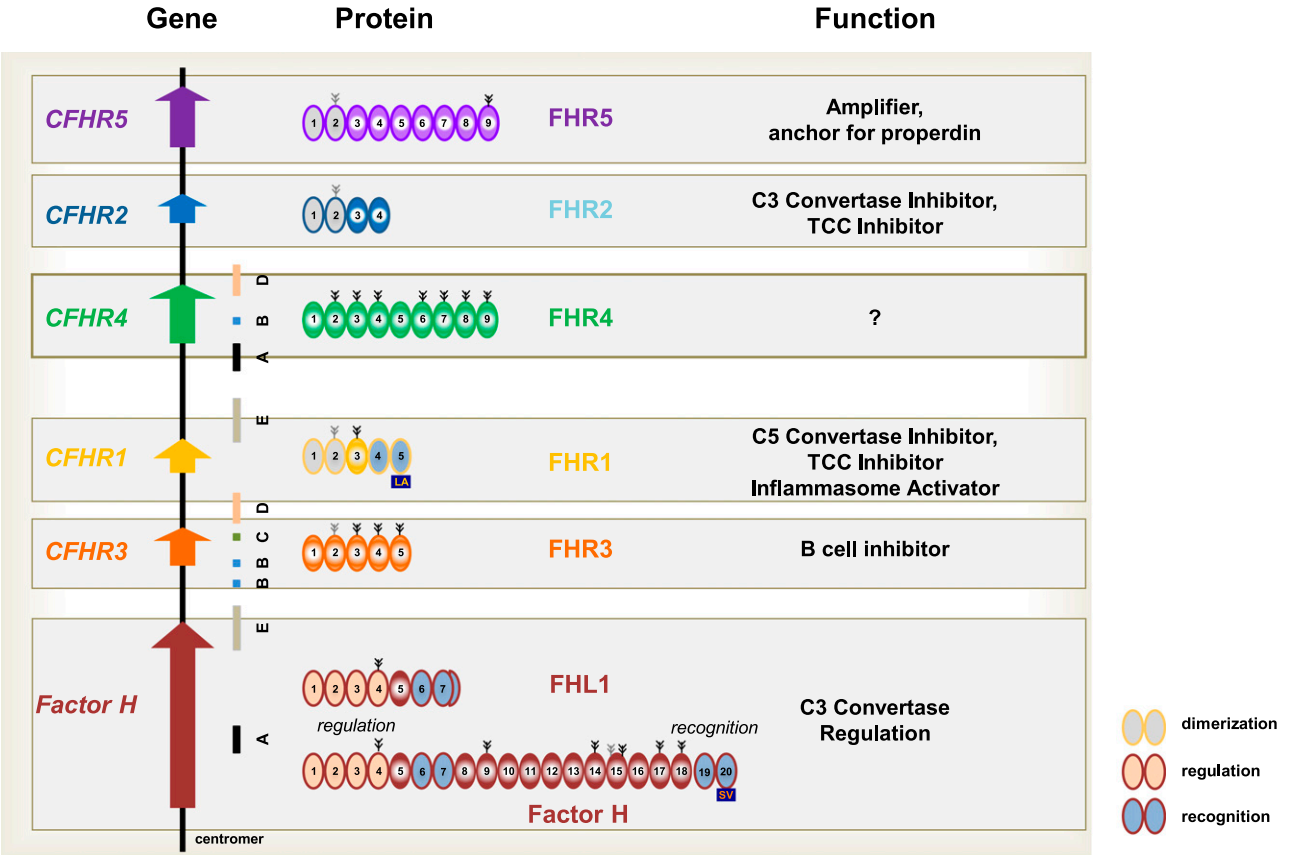

Figure 2. The human *Factor H–CFHR* gene cluster and the FHR, Factor H protein family. The human *Factor H–CFHR* gene cluster includes the *Factor H* gene and the five *CFHR* genes, which are located on chromosome 1q32. Left side: The *CFHR* genes are positioned downstream of the *Factor H* gene (bottom) and are arranged in the order *CFHR3*, *CFHR1*, *CFHR4*, *CFHR2*, and *CFHR5*. The human *CFHR* gene cluster includes interspersed duplicated regions, or segmental duplication elements, which have a high sequence identity and are shown as colored bars below the gene structure. Middle segment: Proteins. Domain organization of the secreted FHR proteins and of Factor H gene products FHL1 and Factor H. Each SCR domain is indicated and the domains of each protein are consecutively numbered. Attached carbohydrate side chains are shown above by the tree-like structure. Constitutively attached carbohydrate side chains have black lines, and facultatively attached carbohydrates have gray lines. The N-terminal multimerization domains of FHR1, FHR2, and FHR5 represented by two SCRs are filled with dashed lines. The C-terminal surface-binding regions of FHR1 and the surface-binding regions of Factor H and FHL1 are shown with a stippled pattern. FHR1: $L_{290}A_{296}$ indicates the two FHR1-specific residues, which differ from that of Factor H. Bottom: The human *Factor H* gene encodes two fluid-phase C3 convertase inhibitors, FHL1 and Factor H. The complement regulatory region is located in SCRs 1–4, is shown by the orange patterns, and is shared by FHL1 and Factor H. The C-terminal recognition regions, *i.e.*, SCRs 6–7 (FHL1/Factor H) and SCRs 19–20 (Factor H), are shown by blue patterns. The Factor H–specific two amino acids $S_{1191}V_{1196}$ in the most C-terminal recognition region are shown in the box below SCR 20. FHL1 has a unique six–amino-acid extension that follows SCR 7. Right side: Identified major functions of each FHR protein and of Factor H and FHL1.

human genes.[25–27] These substantial differences make it difficult to predict the mouse homologs of the human FHR protein.[26,28] Similar to the *MCP/CD46* (human) versus Crry (mouse) situation, mouse FHR homologs need to be identified by functional studies.

FHR Proteins Are Emerging Complement Modulators and Represent Immune and Inflammatory Regulators with Common and Unique Features

CFHR genes are mainly transcribed in the liver and the proteins are distributed in plasma. The five FHR proteins share structural homology with each other and with Factor H and the homology of single domains ranges from 35% to >90% (Figure 2, middle panel).[25–27] Domains with high homology can share overlapping ligand-binding profiles and functions. However, domains with low sequence homology and especially the different numbers of SCR domains of the FHR proteins indicate unique functions.

FHR proteins are emerging complement modulators, activators, and immune regulators. Each FHR protein binds to C3b, iC3b, C3dg, and C3d. Some, but not all, FHR proteins interact with ligands such as glycosaminoglycans, monomeric CRP, pentraxin-3, the lipid peroxidation product MDA (malondialdehyde), and laminins of the kidney, and bind to the surfaces of apoptotic and necrotic cells.[29–32] Often, two or more SCR domains form functional units or ligand-binding segments, including dimerization, multimerization, or cell binding. Extensive domain mapping for all five FHR proteins is necessary to localize such binding and interaction domains.

FHR proteins show sequence homology and share domains with the central fluid-phase regulator Factor H and also with FHL1. Factor H acts as a cofactor for the complement protease Factor I and regulates the stability of the C3 convertase.[33,34,35] FHL1 is also encoded by *Factor H* gene and the FHL1 mRNA is generated by alternative splicing.[36] FHL1 shares the regulatory N-terminal region with Factor H and has a surface-binding site in SCRs6–7 (Figure 2).

CFHR GENE VARIATIONS IN AHUS

aHUS is defined by the triad hemolytic anemia, thrombocytopenia, and renal damage. The disease develops in pediatric and in adult patients, and the clinical symptoms that are associated with the various subforms and patient management are summarized in excellent reviews.[3,37] aHUS is a rare disease and the frequency is one patient in a population of 500,000 (https://ghr.nlm.nih.gov/condition/atypical-hemolytic-uremic-syndrome).

HUS has many causes, and includes STEC-HUS caused by infections with Shiga toxin–producing *Escherichia coli*, pneumococcal HUS due to infections with *Streptococcus pneumoniae,* and aHUS.[9,38,39] The latter is also termed genetic aHUS and accounts for about 10% of total HUS cases. About 50%–60% of patients with genetic aHUS have alterations in one or several complement genes,[40–43] as well as in the *DGKe* (diacyl glycerine Kinase epsilon) gene, which encodes a cytoplasmic signaling protein, and in the *INF2* (Inverted Formin 2) gene, an intracellular actin binding protein.[44,45] Thus, the affected genes code for complement proteins that (*1*) form the alternative pathway C3 convertase (C3, Factor B), (*2*) regulate this central complement enzyme (Factor H, MCP/CD46, Factor I), (*3*) encode modulators (FHR1, FHR3, FHR4, thrombomodulin), and (*4*) code for TCC inhibitor (vitronectin) or for cytoplasmic proteins. For more detailed information on the genetic causes of aHUS see the excellent reviews that summarize these complex issues.[3,37]

CFHR–Factor H gene alterations are identified in aHUS.[3,37,46–58] Here, we describe how the various *CFHR1*, *CFHR3*, and *Factor H* gene alterations affect protein structure, protein function, and the FHR and Factor H plasma repertoire.

CFHR Copy Number Variations

In aHUS, chromosomal changes affect one or several *CFHR* genes and may also involve the *Factor H* gene (Figure 3, Supplemental Table 1). FHR variants, as well as FHR1::Factor H and Factor H::FHR hybrids, are expressed and the FHR, as well as the Factor H plasma inventories, are altered, but FHL1 mRNA is transcribed from the defective Factor H allele. Four types of modifications exist: (*1*) deletions of chromosomal segments resulting in *Factor H::CFHR3* or (*2*) *Factor H::CFHR1* hybrid genes, (*3*) insertions of chromosomal segments generating extra *CFHR1::Factor H* hybrid genes, or (*4*) homozygous deletions of the *CFHR3-CFHR1* or *CFHR1-CFHR4* gene segments. Similar, but not identical, scenarios were identified in unrelated families.

ad (i) Factor H::CFHR3 hybrids. Factor H::CFHR3 hybrid genes result from chromosomal deletions. One scenario is a *Factor H::CFHR3* hybrid gene that has *Factor H* exons i-xxii linked to the complete *CFHR3* gene[46] (Figure 3A, upper line). The encoded 186-kDa, 24-domain protein has Factor $H_{1–19}$ attached to FHR3. A related hybrid gene, with a different chromosomal breakpoint and generated by *de novo* deletion, codes for a Factor $H_{1–17}$::FHR3 protein composed of 22 domains[47] (Supplemental Table 1). Both Factor H::FHR3 hybrids harbor the N-terminal regulatory region, lack the C-terminal recognition region of Factor H, and have instead full-length FHR3 added at the C terminus. The hybrid proteins are expressed in plasma. The Factor $H_{1–19}$FHR3 hybrid has cofactor activity, and binds with high intensity to immobilized C3b and heparin resulting in defective surface binding and regulation.[46] Also, the highly related Factor $H_{1–17}$::FHR3 hybrid shows impaired surface binding and defective surface regulation.[47] Both hybrid genes allow transcription of FHL1 mRNA, and FHL1 is present in plasma. These genomic settings alter the FHR, Factor H plasma inventory in the same manner. FHR3 and Factor H plasma levels are reduced and intact proteins are encoded by the second, intact allele. *CFHR1*, *CFHR2*, *CFHR4*, and *CFHR5* genes are

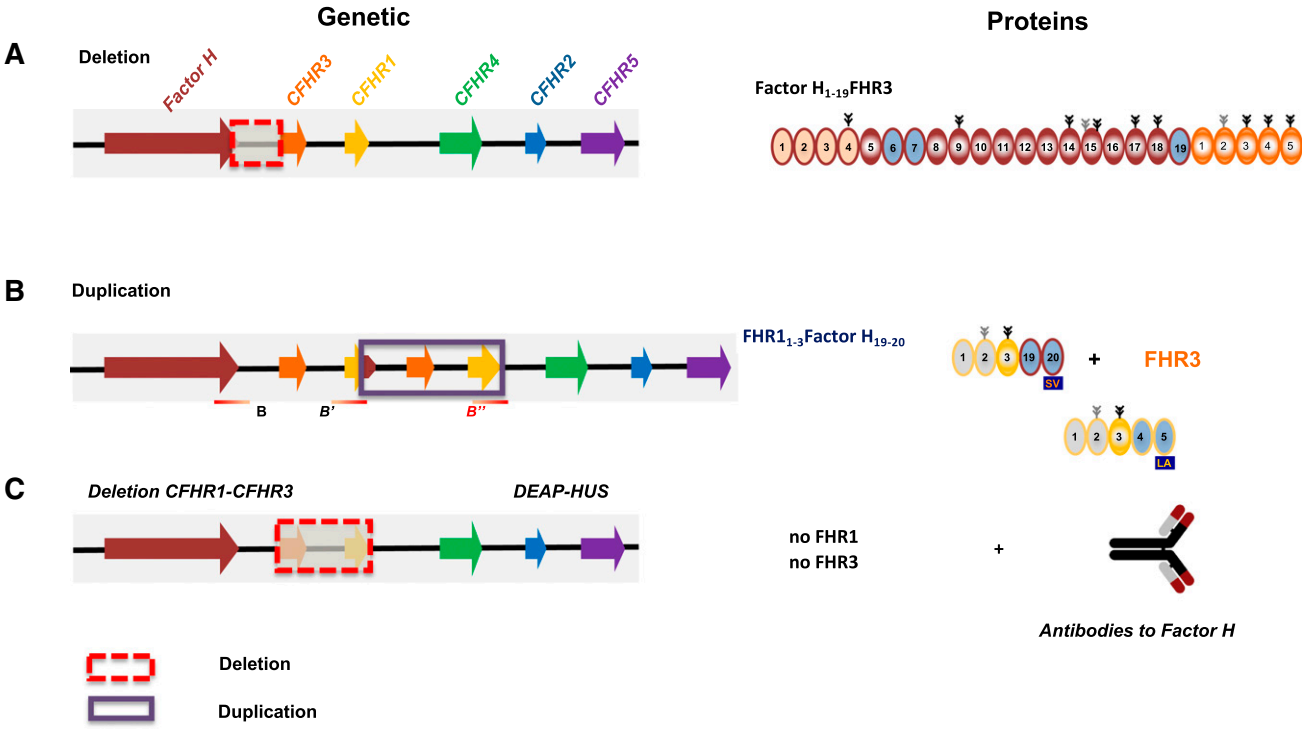

Figure 3. *CFHR* gene variations in aHUS and aHUS-associated FHR protein variants. (A) Deletion of chromosomal segments: *Factor H::CFHR3* hybrid gene. Genetic deletion generating a *Factor H::FHR3* hybrid gene (left side) and the corresponding Factor H::FHR3 hybrid variant are shown using the color code of Figure 2. The deleted chromosomal segment is shown with the box with red, stippled lines. (B) Insertional mutagenesis (duplications) of chromosomal segments generating a *CFHR1::Factor H* hybrid gene. Genetic duplication (boxes with blue lines) generates an extra *CFHR1::Factor H* hybrid gene together with an extra CFHR3 allele (left side), and the corresponding FHR1::Factor H hybrid protein is shown. The changes affect the FHR plasma repertoire (see Table 1). (C) In patients with DEAP-HUS, the deletion of *CFHR3-CFHR1* is often associated with the formation of autoantibodies that bind to the C terminus of Factor H.

present in two copies and the corresponding proteins are expressed at normal levels (Figure 3).

ad (ii) Factor H::FHR1 hybrids. A Factor *H::CFHR1* hybrid gene that has *Factor H* exons *i–xxi* (SP, Factor $H_{1–18}$) linked to *CFHR1* exons *v-vi* ($FHR1_{4–5}$) was reported in a UK family with eight affected individuals[48] (Supplemental Table 1). A related hybrid gene, caused by *de novo* deletion, has a different breakpoint and has Factor H exons *i–xxii* linked to *CFHR1* exon *vi*[49] (Supplemental Table 1). Again, FHL1 mRNA is transcribed from the hybrid genes. Both hybrid proteins, *i.e.*, Factor $H_{1–18}$::$FHR1_{4–5}$ and Factor $H_{1–19}$::$FHR1_{5}$, have the FHR1-specific C-terminal residues and represent Factor H_{LA} (Factor $H_{L1191A1197}$).[48,49] The patients have reduced Factor H, FHR3, and FHR1 plasma levels and normal FHR4, FHR2, and FHR5 levels (Figure 3, Table 1).

ad (iii) Extra FHR1::Factor H hybrids. Chromosomal duplications generating an extra *CFHR1::Factor H* hybrid gene and a third *CFHR3* allele were

Table 1. CFHR Gene Variations in aHUS Affect FHR and Factor H but not FHL1 Plasma Levels

Variable	Intact FHL1	Affected				Intact		
		Factor H		FHR3	FHR1	FHR 4	FHR2	FHR5
Factor HFHR3	100	50	Factor HFHR3	50	100	100	100	100
Factor HFHR3	100	50	Factor HFHR3	50	100	100	100	100
Factor HFHR1	100	50	Factor HFHR1	100	50	100	100	100
Factor HFHR1	100	50	Factor HFHR1	100	50	100	100	100
FHR1::Factor H	100	100	FHR1::Factor H	150	100	100	100	100
FHR1::Factor H	100	100	FHR1::Factor H	150	100	100	100	100
ΔΔFHR3FHR1	100		100	0	0	100	100	100
ΔFHR3FHR1	100		100	50	50	50	100	100
ΔΔFHR1FHR4	100		100	100	0	0	100	100

Numerical data presented as % plasma levels.

reported as *de novo*[50] and as familial cases.[51] The de novo case had an extra chromosomal segment, consisting of *Factor H* exons *xxii-xxiii*, the *CFHR3* gene, and *CFHR*1 exons *i–iv*[50] (Figure 3B). In the familial scenario, the 49-year-old patient and the affected 20-year-old daughter had a chromosomal segment duplicated that includes Factor H exon *xxiii*, the *CFHR3* gene, and *CFHR1* exons *i–v*[51] (Supplemental Table 1). Thus, duplicated segments are inserted within either the B or B' segmented duplicated elements of the *CFHR* gene cluster. The *CFHR1::Factor H* hybrid genes encode an extra $FHR1_{1–3}$::Factor $H_{19–20}$ (or $FHR1_{1–4}$::Factor H_{20}) termed $FHR1_{SV}$ and the extra *CFHR3* allele results in elevated FHR3 plasma levels (150%) (Figure 3B). Plasma levels of FHR1, FHR2, FHR4, FHR5, Factor H, and FHL1 are normal. The hybrid protein with the Factor H–specific C-terminal residues has a Janus-faced character. $FHR1_{SV}$ forms multimers with itself and with FHR1 and FHR2, and does compete with Factor H for surface binding.[50–52]

Frequency of CFHR Gene Mutations in aHUS

In the Spanish cohort of 513 patients with aHUS, six patients (1.2%) expressed $FHR1_{SV}$ variants.[53] In an Italian aHUS cohort (154 patients), seven patients (4.5%) had heterozygous *CFHR* gene rearrangements.[54] Five patients presented with *Factor H::FHR1* hybrids (2.8%); two patients showed the extra $FHR1_{SV}$ hybrid and a third the *CFHR3* allele (1.3%) and altered FHR1/FHR3 plasma levels.[54]

Functional Consequences of *CFHR* Gene Alterations

Familial or *de novo* modifications generating heterozygous *CFHR* gene variants cause aHUS and identify a role of FHR variant proteins in disease pathology. The *CFHR1*, *CFHR3*, and *Factor H* genes are affected; FHR variants are expressed; and the FHR, Factor H plasma repertoire is altered (Table 1). Three *CFHR* scenarios cause aHUS: (*1*) chromosomal deletions, (*2*) chromosomal duplications, and (*3*) homozygous FHR1-FHR3 deficiency. ad (i) chromosomal deletions generate *Factor H::CFHR3* or *Factor H::CFHR1* hybrid genes and the encoded hybrid proteins have the N-terminal regulatory region together with additional Factor H domains linked to either FHR3 or the recognition region of FHR1. The hybrid proteins show normal Factor H regulation in the fluid phase and lack Factor H surface binding, but use FHR3 or FHR1 domains for surface binding. Intact FHL1 mRNA is transcribed from the mutant allele. ad (ii) duplications result in extra *CFHR1::Factor H* hybrid genes. The encoded $FHR1_{SV}$ protein has dual character. This Janus-faced protein combines the N-terminal dimerization region of FHR1 with the C-terminal recognition region of Factor H. The protein lacks the discriminatory part of the FHR1, specifically the LA residues. The chromosomal duplications also generate a third *CFHR3* allele, resulting in higher FHR3 plasma levels. ad (iii) homozygous deletion of an approximately 24-kb chromosomal segment that encompasses the *CFHR3-CFHR1* genes is observed in about 15% of predominantly young patients with aHUS (Figure 3C).[22,55–58] For these patients with DEAP-HUS, this genetic scenario is frequently associated with the presence of autoantibodies that bind to the C-terminal region of Factor H (SCRs 19–20) and block surface binding.[23,56–59] The exact mechanisms whereby in HUS the homozygous deficiency of CFHR1 and CFHR3 breaks tolerance and induces antibody production are not clear. However, one link is that FHR3 binds to the C3d fragment and blocks the adjuvant effect of C3d in B cell activation.[60]

GLOMERULOPATHY DUE TO *CFHR* GENE VARIATIONS

C3 glomerulopathy is a very rare disorder affecting 1–2 people per million people worldwide and is equally common in men and women (https://ghr.nlm.nih.gov/condition/c3-glomerulopathy). C3 glomerulopathy is frequently associated with proteinuria, hematuria, high creatinine plasma levels, and altered complement plasma levels. Kidney function decreases with time and within 10 years about 50% of patients progress to ESKD. Two major forms are separated, DDD and C3 GN, which form related kidney problems.[61,62] Precise diagnosis requires kidney biopsy specimens. This heterogeneous disease is induced by various triggering events that lead to complement activation in the fluid phase and on glomerular surfaces, thus explaining a link of defective alternative complement and proteinuria and hematuria. The genetic and autoimmune causes are carefully summarized in recent outstanding reviews[63–65]. Here, we focus on *CFHR* copy number variations in the context of an intact Factor H gene, which represent a specific genetic form of C3 glomerulopathy. All five *CFHR* genes can be affected and in this case the *Factor H* gene remains intact. Mutant FHR proteins are expressed and the FHR plasma repertoire is disturbed, whereas Factor H and FHL1 levels are normal.

CFHR Gene Variations in C3 Glomerulopathy

Chromosomal Deletions

A 26-year-old Italian patient diagnosed with DDD showed variations in several *CFHR* genes.[63] A deletion on one allele generates a *CFHR3i-iv::CFHR4ix,x* hybrid gene that encodes an $FHR3_{1–3}$::$FHR4_{8,9}$ hybrid protein (Supplemental Table 1). The second allele has the common *CFHR3::CFHR1* deletion. In addition, the patient has two heterozygous mutations (p.Q211X and p.R254×) in the *CFHR2* gene that terminate transcription. This results in a unique plasma repertoire with an FHR3::FHR4 hybrid protein; lack of FHR1, FHR2, and FHR3; reduced FHR4 levels; and normal FHR5, Factor H, and FHL1 plasma levels.

In an Indian family, the 8-year-old index patient diagnosed with glomerulopathy histologically classified as an overlap of C3 GN and DDD has a chromosomal deletion that spans from *CFHR1v* to the *CFHR5*-gene promoter.[64] The $CFHR1_{i-iv}$::*CFHR5* hybrid gene encodes a 90-kDa $FHR1_{1–3}$::FHR5 protein, and is associated with low FHR1, FHR2, FHR4, and FHR5 plasma levels

(Supplemental Table 1). The father and a 3-year-old sister have this deletion on one allele, combined with the common *CFHR1-CFHR3* deletion on the second allele. Both patients lack FHR1, and have reduced FHR3, FHR2, FHR4, and FHR5 plasma levels. The hybrid protein, with two interacting regions, forms large oligomers, which include the hybrid, FHR1, FHR2, and FHR5.

Two related German patients diagnosed with MPGN-II had a heterozygous deletion of a 24-kbp chromosomal segment, resulting in a *CFHR2*$_{i\text{-}iii}$*::CFHR5* hybrid gene that encodes an FHR2$_{12}$::FHR5 hybrid protein.[65] FHR2 and FHR5 plasma levels are reduced, whereas FHR1, FHR3, FHR4, FHL1, and Factor H levels are normal (Figure 4A). The kidney biopsy specimens from both patients showed C3b, C5b-9 deposition, and FHR5, as well as properdin staining.[65] This FHR2$_{12}$FHR5 hybrid protein with two multimerization domains forms large oligomers, consisting of FHR2$_{1-2}$FHR5, FHR1, FHR2, and FHR5, and forms a hyperactive fluid-phase C3 convertase that consumes C3. In addition, the surface-bound hybrid anchors properdin and serves as a hyper-amplifier of complement.

Insertional Mutagenesis of Intragenic Segments

Two scenarios are reported for Spanish patients with C3 glomerulopathy, which add new genetic material in the *CFHR1* gene. One patient diagnosed with C3 glomerulopathy has in one *CFHR1* allele a duplication, which includes the *CFHR1* promoter and exons *i-iii,* resulting in a mutant gene with two transcription start sites (Figure 4B).[66] The upstream promoter generates a longer transcript, which codes FHR1$_{1-2}$FHR1. The mutant proteins with two multimerization segments form large oligomeric complexes with other FHR proteins, which enhance hemolysis (Supplemental Table 1).

A 12-year-old boy with an upper respiratory tract infection by *Haemophilus influenza* from another Spanish family diagnosed with C3 glomerulopathy had a heterozygous intragenic duplication in one *CFHR1* allele combined with the *CFHR3-CFHR1* deletion on the second allele.[67] Duplication of exons *i-v* results in an FHR1 mutant protein composed of nine SCRs (Figure 4B). The patients have normal FHR2, FHR3, FHR4, FHR5, FHL1, and Factor H levels. The affected

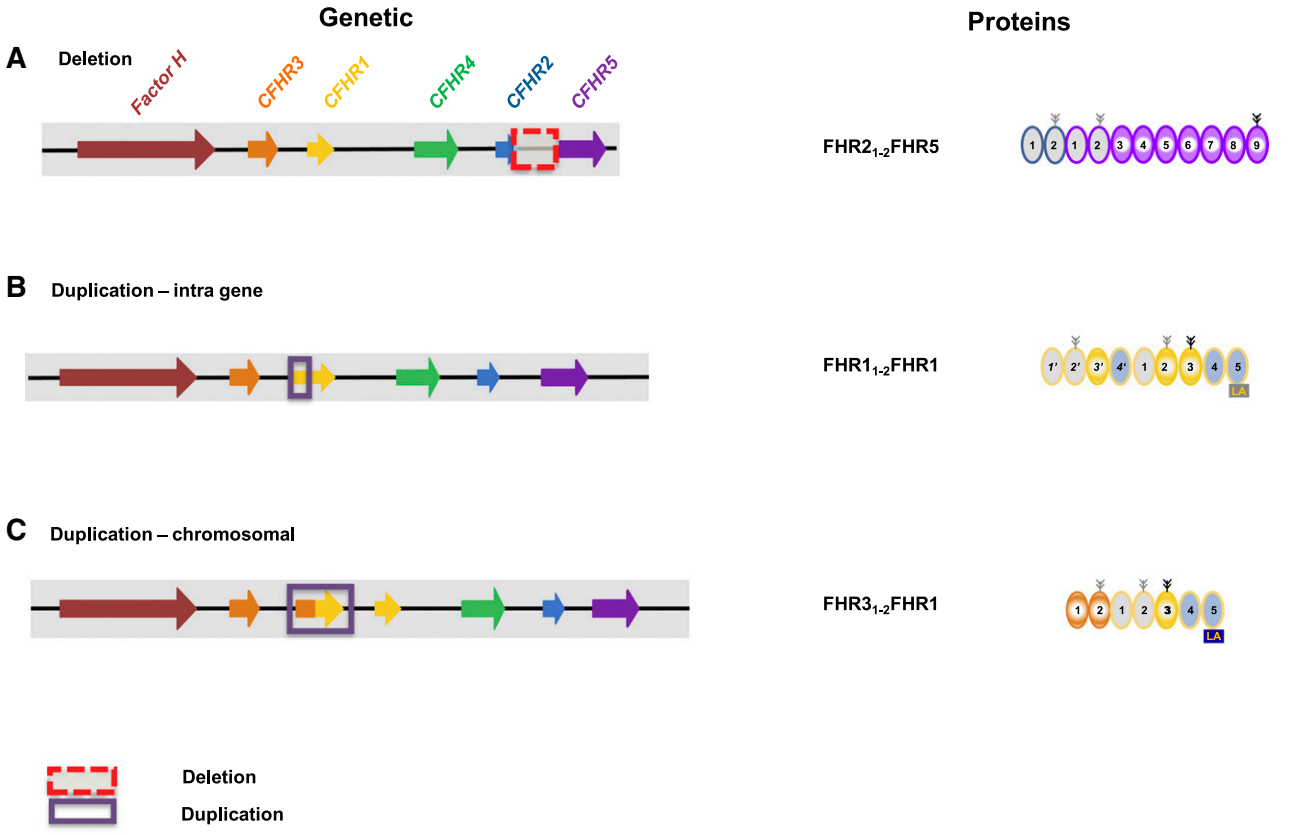

Figure 4. *CFHR* gene variations in C3 glomerulopathy and associated FHR protein variants. (A) Deletion of large chromosomal segments. Deletion of a large chromosomal segment, which includes exons of the *CFHR2* gene and the intragenic region spanning to the *CFHR5* gene, generates a *CFHR2::CFHR5* hybrid gene. This gene encodes an FHR2$_{1-2::}$FHR5 hybrid protein which is shown on the right. (B) Insertional mutation of intragenetic segments: Duplication of *CFHR1* gene segments results in an FHR1 mutant protein. Duplication of the first three exons results in a mutant *CFHR1* gene. The encoded protein FHR1$_{1-2}$FHR1 has duplicated interaction segments (right panel). (C) Insertional mutageneses of chromosomal segments results in extra hybrid genes. A duplicated extra gene has *CFHR3* exons i–iii linked to the last exons of the *CFHR1* gene and generates a new *CFHR3::CFHR1* hybrid gene that encodes an FHR3$_{1-2}$::FHR1 hybrid protein.

individuals have low C3 plasma levels and the kidneys showed intense C3 deposition, mesangial hypercellularity, and osmiophilic deposits along the GBM. The FHR1$_{1-4}$FHR1 mutant forms large oligomers, which include the mutant, FHR1, FHR2, and FHR5, and which bound with higher intensity to surface-attached C3b and acted as effective competitors for Factor H.

FHR5::FHR5 mutants with duplicated SCRs1–2 were reported in patients of a large Cypriote cohort, who presented with microscopic hematuria, synpharyngitic macroscopic hematuria, and C3 GN, and showed slightly reduced C3 plasma levels.[68–70] Kidney biopsy specimens revealed mesangial matrix expansion, capillary wall thickening, and glomerular C3, as well as C1q, IgA, and IgG staining combined with subendothelial electron dense deposits.[70] In the *CFHR5* gene, the patients have exons *i-iii* duplicated (Supplemental Table 1). The FHR5$_{1-2}$FHR5 mutant protein has two multimerization domains. An almost identical duplication of *CFHR5* exons *i-iii* was identified in UK family of non-Cypriote origin. The patients expressed the same FHR5$_{1-2}$FHR5 mutants in plasma.[71] Both the Cypriote and the UK patients have reduced FHR5 plasma levels. All other FHR proteins, FHL1, and Factor H are present at normal levels.

Duplication of Extra Gene Segments

Insertion of an extra *CFHR3$_{12}$::CFHR1* hybrid gene between the *FHR3* and *FHR1* genes was reported in a UK family.[72] The affected members had the biopsy-based diagnosis of MPGN. All five *CFHR* genes and the *Factor H* gene are intact; thus, the FHR3$_{1-2}$FHR1 hybrid is expressed together with the five FHR proteins, FHL1, and Factor H (Figure 4C). The renal biopsy specimens showed C3b staining, glomerular inflammation, and a membranoproliferative type III pattern.

A *CFHR5::CFHR2* hybrid gene was identified in a US patient with familial C3 glomerulopathy.[73] This extra hybrid gene has *CFHR5* exons *i-iii* (SP, SCRs1,2) attached to the *CFHR2* gene (Supplemental Table 1). The encoded FHR5$_{1-2}$::FHR2 protein forms large oligomers, which include the hybrid, FHR1, FHR2, and FHR5.

Glomerulopathy due to CFHR Gene Variations

The eight scenarios show heterozygous variations and are familial. Three scenarios result from deletion of large genomic segments. Two cases combine the deletion with heterozygous *CFHR3-CFHR1* deletion on the second allele. Two Indian patients, but not the index patient, combine the *CFHR4::CFHR2* and *CFHR3-CFHR1* deletions. The Italian patient combines the mutations with *CFHR2* gene mutations. Two case scenarios from Spain show duplications in the *CFHR1* gene, the Cypriot patients have a *CFHR5* gene duplication, and two other duplications result in extra *CFHR3-CFHR1* or *CFHR5-CFHR2* hybrid genes (Supplemental Table 1). *CFHR* gene variations cause C3 glomerulopathy and the disease develops in the context of an intact Factor H gene and presence of Factor H in plasma. One principle is emerging: *CFHR* gene variations in C3 glomerulopathy result in variant proteins that have two interaction segments, and for several proteins formation of large oligomeric complexes is shown. Thus, FHR mutant proteins and together with altered FHR plasma levels in the context of an intact *Factor H* gene cause glomerular pathology (Supplemental Table 1).

Distinct Genetic Scenarios Cause Related Glomerular Changes

C3 glomerulopathy is caused by distinct genetic scenarios; *e.g.*, by heterozygous *CFHR* gene mutations in the context of intact *Factor H* alleles and also by homozygous (or compound-heterozygous) *Factor H* gene mutations and intact *CFHR* genes. Thus, alterations in different genes cause the same or highly related glomerular alterations and the same pathologies,[20,74,75] and highlight the role of FHR proteins as complement modulators.

CFHR GENE CLUSTER VARIATIONS IN OTHER (KIDNEY) DISEASES

IgAN

CFHR1, *CFHR3*, and *CFHR5* gene modifications and variations of FHR plasma levels cause pathology in IgAN and these scenarios develop in the context of intact Factor H and FHL1.[76–79] In addition, Factor H genetic variations apparently influence complement regulation in IgAN.[80,81] Furthermore, mutations in another complement gene, such as the MBL gene, are reported in IgAN.[82] Genome-wide association studies identified the *CFHR* gene cluster as a susceptibility locus in IgAN and opposing effects are reported.[83] Homozygous *CFHR1/CFHR3* deletion resulting in the absence of FHR1 and FHR3 in plasma is protective.[84–86] In addition, *CFHR5* is an IgAN susceptibility gene and mutant FHR5 proteins show altered C3b binding.[87] Current work in IgAN is focusing on FHR1 and FHR5 plasma levels.[88–91] Elevated FHR1 and also FHR5 plasma levels and higher FHR1::Factor H ratios influence alternative pathway regulation, and correlate with glomerular filtration and disease severity.[91]

CFHR5 Gene Variations in Other Kidney and Retinal Diseases

Sequence variations in the *CFHR5* gene are also reported in aHUS,[91–93] in C3 glomerulopathy/DDD,[94] for a case with *S. pneumoniae* infection,[95] and for the retinal disease AMD.[96] For these scenarios it will be necessary to address whether all reported changes are pathogenic or whether the exchanges represent rare polymorphic variants.

FHR Protein Deposition in Fibrillary GN and Pauci-Immune Kidneys

Proteome analyses identified FHR1, FHR2, and FHR5 deposition in glomeruli of patients with the rare kidney disease fibrillary GN along with strong deposition of DNA-JB9[97] and in pauci-immune necrotizing crescentic GN.[98]

The Challenge of Homozygous CFHR1-CFHR3 Deletion

Homozygous *CFHR1-CFHR3* deficiency has many faces: it confers risk in DEAP-HUS,[23]

in SLE,[99] and for infections with the Gram-negative bacterium *Neisseria meningitidis*,[100] but has protective effects in IgAN[78] and AMD.[101,102] Importantly, homozygous *CFHR1-CFHR3* deficiency is not a risk or protective marker on its own. Homozygous *CFHR1-CFHR3* deficiency is present in 5%–8% of healthy whites and the frequency is even higher in the healthy Asian (approximately 18%) and African populations (approximately 28%).[103,104]

FHR VARIANTS PROVIDE CLUES ON FHR PROTEIN FUNCTION AND IDENTIFY PATHOLOGIC ROLES OF FHR OLIGOMERS

CFHR gene alterations cause pathology, which can manifest in different tissues, and variations within the same *CFHR* gene can cause different pathologies in tissue compartments. Parameters affected include the following categories

The C Terminus of FHR1

FHR1 and Factor H share almost identical three C-terminal SCRs that include the recognition regions. Thus, FHR1 and Factor H bind the same or related targets and together fine-tune local complement action. However, the most C-terminal SCRs of FHR1 and Factor H differ; $FHR1_{LA}$ uses L_{290},A_{296} in SCR5 and Factor H_{SV}, S_{1191},V_{1197} in SCR20 (see Figure 2). Exchange of the two residues LA and SV has pathologic consequences; $FHR1_{SV}$, with the Factor H_{SV} residues, and similarly Factor H_{LA}, with the $FHR1_{LA}$ residues, cause aHUS.[105–107]

FHR Plasma Levels

CFHR cluster variations result in unique FHR–Factor H plasma profiles (Tables 1 and 2). FHR plasma concentration is a timely topic because plasma levels reported for healthy individuals vary by up to two orders of magnitude.[108–112] For example, FHR1 plasma levels range from 0.2 μg/ml to 120 μg/ml.[76,108–110,113] Complex formation of FHR proteins and association of FHR proteins in lipoprotein particles (see below) may explain such variations and also indicate assay-based detection aspects.

FHR Proteins Form Multimers that Show a Preference for Interaction Partners

The SCR backbone provides flexibility. FHR proteins apparently interact with themselves, with other FHR proteins, and potentially also with Factor H, and bind to C3b and to plasma and cell surface constituents. In physiologic settings, FHR proteins and also Factor H adapt various conformations and can form dimers, tetramers, or multimers (Figure 5A).[58,66,67,111–115] Such multimers enhance regulatory action; for example, Factor H mini-variants, which are linked *via* an FHR1 multimerization region, are more potent in complement control.[116,117] For both FHR1 mutants with duplicated interaction segments,[67,68] for the FHR1-FHR5,[65] and also for the FHR2::FHR5[66] hybrid formation of large oligomeric complexes is reported, which enhance complement activity.

Furthermore, FHR1, FHR2, and FHR4A are integrated into lipid particles, which also include ApoE, ApoAI, ApoB100, LPS-binding protein, fibrinogen, and other uncharacterized proteins.[118,119] Thus, unique interaction profiles and a preference for interacting partners indicate different regulatory roles of FHR oligomers (Figure 5B).

Payload Composition Influences Oligomer Function

The disease-associated FHR mutants (FHR1::FHR1, FHR1::FHR5, FHR5::FHR5) and hybrids (FHR1::FHR5-, FHR2::FHR5, FHR5::FHR2) form oligomers that contain the specific FHR variant, FHR1, FHR2, and FHR5. Apparently, payload composition and the combinations of functionally distinct FHR proteins influence oligomer function (Figures 5, C and D and 6).[65–68]

FHR Proteins as Biomarkers for Diagnosis and Therapy

CFHR gene variations define specific genetic forms that integrate in the overall disease spectrum of aHUS and C3 glomerulopathy. The current diagnosis for C3 glomerulopathy is on the basis of kidney biopsy specimens. However, for both disorders minimal invasive diagnostic approaches are advantageous for biomarker profiling.

CFHR gene variations are valuable genetic markers and, when combined with altered FHR protein mobility, variations

Table 2. CFHR Gene Variations in C3 Glomerulopathy affect FHR Plasma Levels, but not Factor H and FHL1 plasma levels

Variable	Intact		Affected									
	FHL1	Factor H	FHR3		FHR1		FHR 4		FHR2		FHR5	
FHR3::FHR4	100	100	0	FHR3::FHR4	0		50		100 *Mutated*		100	
FHR1::FHR5	100	100	100		50	FHR1::FHR5	50		50		50	
FHR1::FHR5	100	100	50		0	FHR1::FHR5	50		50		50	
FHR2::FHR5	100	100	100		100		100	50	FHR2::FHR5		50	
FHR1::FHR1	100	100	100		50	FHR1::FHR1	100		100		100	
FHR1::FHR1	100	100	100		50	FHR1::FHR1	100		100		100	
FHR5::FHR5	100	100	50		50		100		100		FHR5::FHR5	50
FHR3::FHR1	100	100	100		FHR3::FHR1	100	100		100		100	
FHR5::FHR2	100	100	100		100		100	100	FHR5::FHR2		100	

Numerical data presented as %.

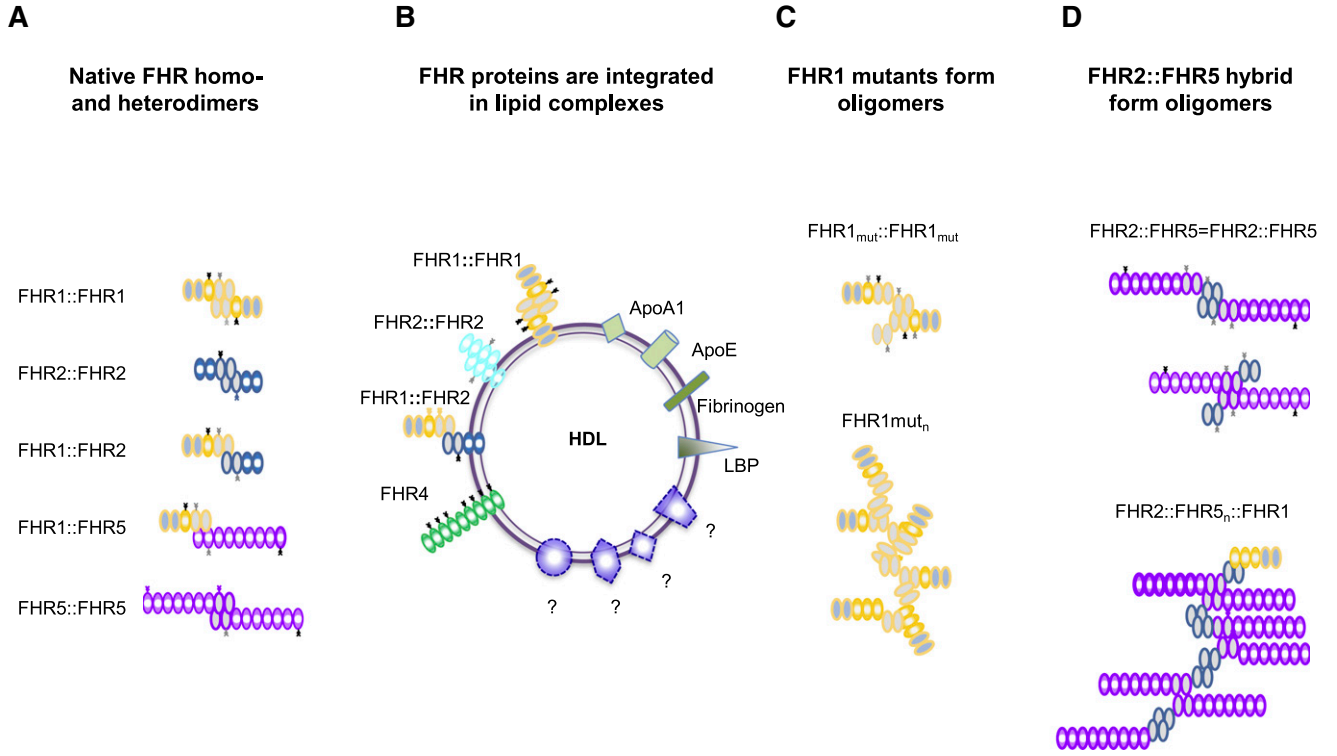

Figure 5. FHR proteins form homodimers and heterodimers; FHR hybrid proteins with two multimerization segments form oligomers. (A) The three proteins FHR1, FHR2, and FHR5 form dimers *via* their N-terminal domains (gray domains). The N-terminal multimerization segments of each FHR protein interact with a second protein and form dimers and even multimers. FHR1 and FHR2 can form heterodimers. (B) FHR proteins are contained in lipid complexes. FHR1, FHR2, and FHR4-A are associated with lipids HDL and LDLD. Lipids include FHR1 and FHR2 together with ApoAI, LPS-binding protein, fibrinogen, and additional so-far-uncharacterized proteins. FHR4-A is present in triglyceride-rich lipid particles, which also include ApoE, ApoAI, ApoE, ApoAIV, ApoB48, and ApoB100. (C) The FHR1 mutants with two interaction sites form larger complexes. Upper panel: A single FHR1::FHR1 mutant with two interaction segments that bind FHR1, FHR2, and FHR5 and allow formation of larger complexes. Oligomerization or branching is disrupted when FHR1, FHR2, or FHR5 with one interaction segment is integrated. (D) The FHR2::FHR5 hybrid forms larger complexes. Upper panel: A single FHR2::FHR5 hybrid has two interaction segments that allow formation of larger complexes. The FHR2::FHR5 hybrid protein can multimerize *via* the FHR2 (upper constellation) or *via* the FHR5 multimerization domain with FHR5 (bottom scenario). Oligomerization or branching is disrupted when FHR1 or FHR2 with one interaction segment is attached to the FHR2 domain, or when FHR5 is bound to the FHR5 domain.

of FHR plasma levels provide important diagnostic information. FHR plasma levels, the evaluation of complement split products, TCC levels, complement activity, and C3 levels allow monitoring of complement in a direct and timely manner. Such parameters can also be used evaluate the efficacy of approved complement therapeutics and new inhibitors that are developed and tested in clinical trials.[120,121] For renal biopsy specimens, a correlation of FHR protein deposition with morphologic alterations and immunohistologic staining patterns will provide important diagnostic information. In this regard, FHR-specific mABs are advantageous and should be developed.

CONCLUSIONS, PERSPECTIVE, AND FUTURE DIRECTIONS

CFHR–Factor H gene cluster variations are associated with several kidney disorders, including aHUS, C3 glomerulopathy, and also IgAN. These associations show important roles of the FHR proteins in maintaining glomerular integrity. FHR proteins are complement modulators and complement activators, and FHR1 regulates inflammasome activity. The proteins act alone, and also cooperate with each other and with Factor H. For example, FHR1 balances the local action of the complement inhibitor Factor H, and when attached to the necrotic surfaces activates the inflammasome. The unique roles of the individual FHR proteins in the complete cascade suggest that patients with CFHR gene defects may benefit from treatment with one of the new complement inhibitors that are currently in development.[122]

The major challenges ahead are (*1*) to link the genetic changes in the *CFHR* cluster with precise morphologic and immunohistochemical staining of renal biopsy specimens, with cellular and morphologic alterations, and with plasma biomarkers; (*2*) to understand the exact physiologic role of each FHR protein; and (*3*) to define the interplay of the FHR proteins as complement

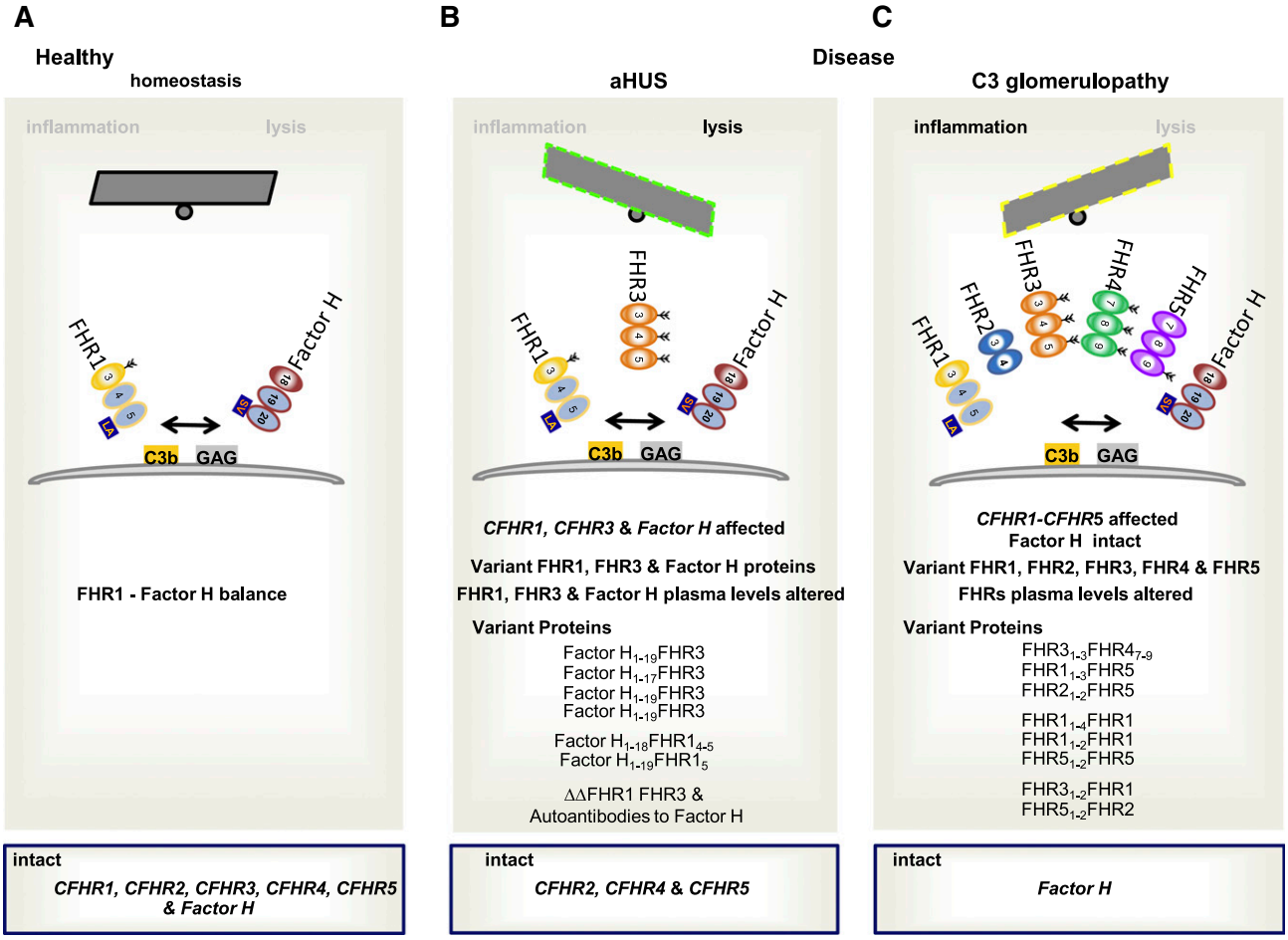

Figure 6. FHR mutant and hybrid proteins affect complement regulation at target sites. Gene mutations and copy number variations in the human *Factor H–CFHR* gene cluster in aHUS and C3 glomerulopathy affect protein structure and protein levels in plasma and influence the fate of complement control at target surfaces. (A) Normal homeotic scenario. FHR1 and Factor H with their homologous C-terminal regions bind to surface-deposited C3b at danger sites and to glycosaminoglycans (GAGs). The proteins are shown and the three C-terminal recognition region is presented, and the FHR1-specific LA and the Factor H–specific SV residues in the most C-terminal domains are shown. The balance of FHR1 and Factor H at modified target sites is influenced by the structure of the proteins; by Factor H, FHL1, and FHR plasma levels; by the binding intensities of the proteins to the ligands; and by the density of C3b and the type and composition of GAGs at sites of damage. FHR1 competes with Factor H for surface binding and, in a proper combination, the two regulators adjust and fine-tune local complement action. Thereby, FHR1 initiates inflammasome activation and Factor H dissociates the alternative pathway C3 convertase and inhibits complement progression. (B) Scenarios in aHUS and C3 glomerulopathy. aHUS scenario, left panel: Factor H::FHR3, Factor H::FHR1, and FHR1::Factor H hybrid proteins together with altered plasma levels influence the local FHR1, Factor H balance at a damage surface (left site). C3 glomerulopathy scenario, right panel: The various FHR hybrid and mutant proteins, many of which having two interacting segments and form large oligomeric complexes, influence the local FHR1, Factor H balance at damaged surfaces. Scenarios in aHUS, left panel: Various scenarios in the form of deletions; insertional mutations, including intragene; as well as chromosomal duplications generate *CFHR–Factor H* or *Factor H–CFHR* hybrid genes. In aHUS, *Factor H* and the *CFHR1* and *CFHR3* genes are affected, but *CFHR4*, *CFHR2*, and *CFHR5* genes remain intact. Scenarios in C3 glomerulopathy, right panel: Various scenarios in the form of deletions; insertional mutations, including intragene; as well as chromosomal duplications generate *CFHR-CFHR* hybrid genes. In C3 glomerulopathy all five CFHR genes can be affected, but the Factor H gene remains intact.

modulators or amplifiers with each other and with Factor H. A strong interdisciplinary approach including geneticists, complementologists, nephropathologists, and clinicians is required to establish precise diagnostic and prognostic parameters for the FHR-associated disorders. Advanced understanding of the disease mechanisms will guide physicians and patients through this complex disease spectrum.

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DISCLOSURES

Dr. Zipfel provided advice on complement to Vifor Fresenius Medical Care Renal Pharma, Generic Assays and received speaker honorarium from Alexion. All of the remaining authors have nothing to disclose.

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SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2019050515-/DCSupplemental.

Supplemental Table 1. Morphologic differences in atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy.

Supplemental Table 2. Alterations in the Factor H–CFHR gene cluster in aHUS/dEAP-HUS and C3 glomerulopathy.

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