4º CONGRESO DE LA SOCIEDAD GALLEGA DE NEFROLOGÍA

Enfermedades renales asociadas al complemento

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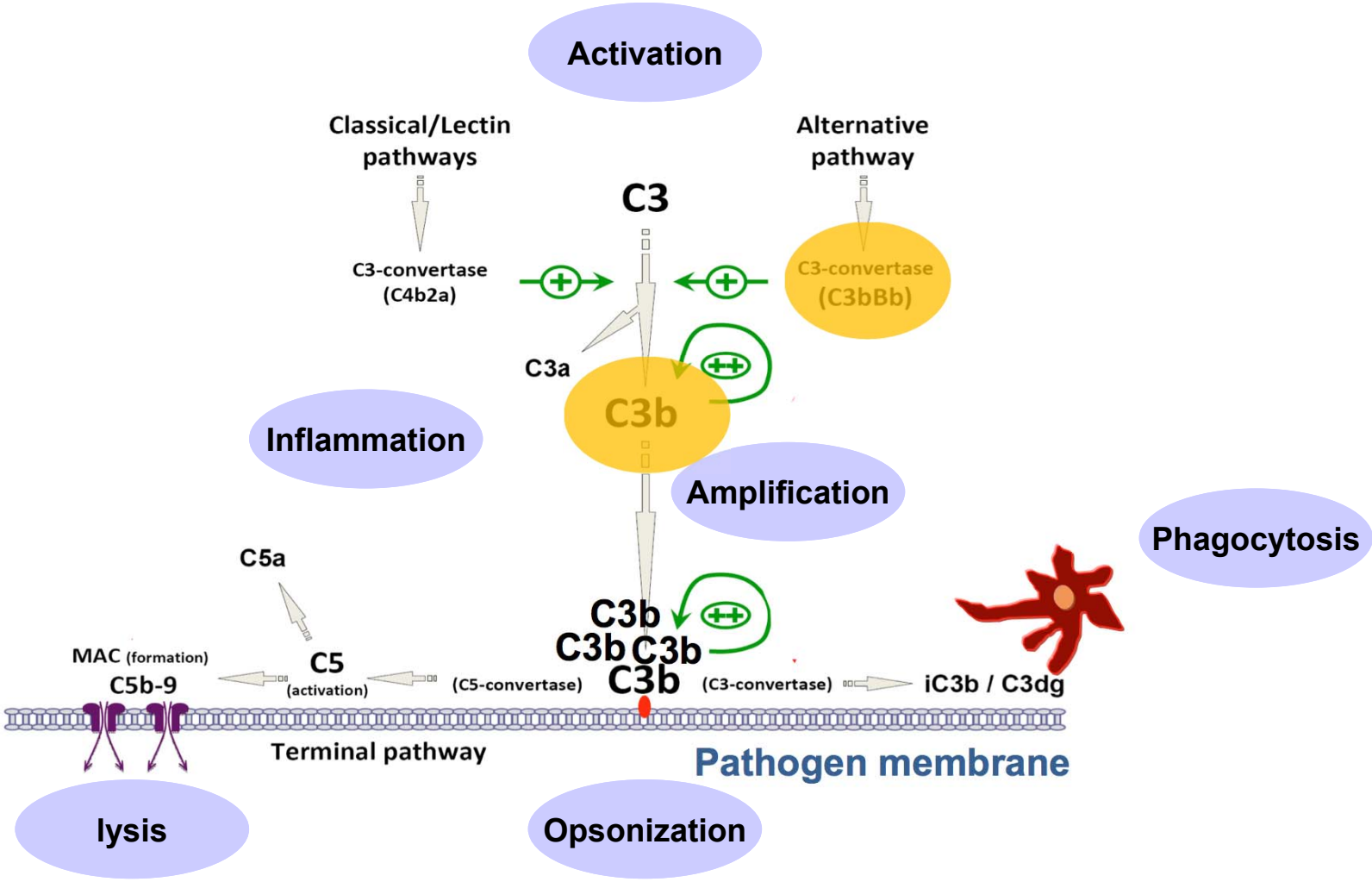
Madrid, Spain

Disclosures

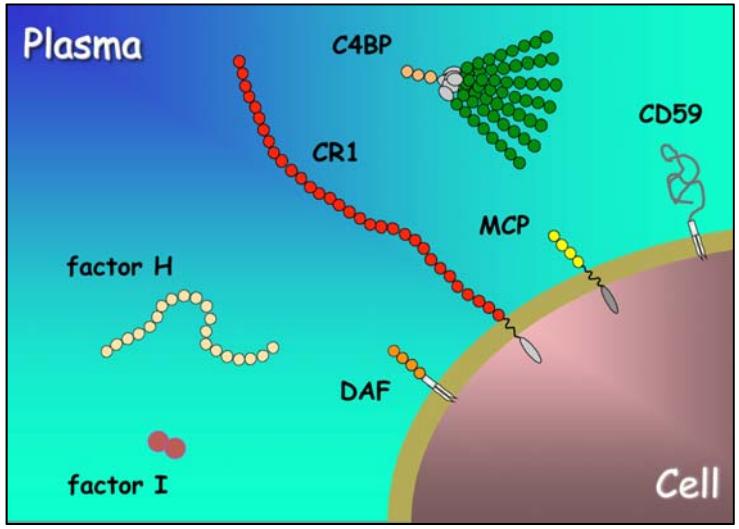
SRdeC has received fees from Alexion Pharmaceuticals for participation in advisory boards, experts meetings and teaching courses.

SRdeC is a founder member and shareholder of Secugen S.L.

Complement

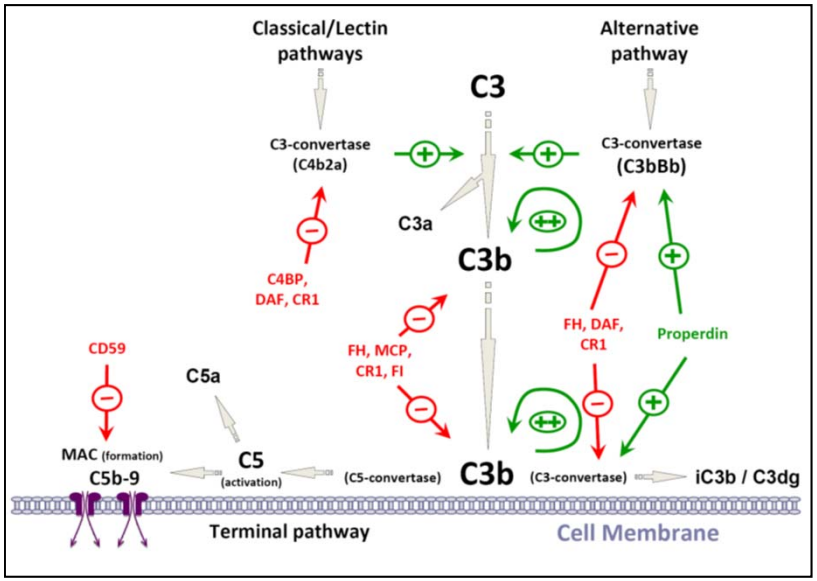

Activation
Classical/Lectin pathways
Alternative pathway
C3
C3-convertase (C4b2a)
C3-convertase (C3bBb)
C3a
C3b
Inflammation
Amplification
Phagocytosis
C5a
C3b
C3b C3b
C3b
MAC (formation)
C5b-9
C5
(activation)
(C5-convertase)
(C3-convertase)
iC3b / C3dg
Terminal pathway
Pathogen membrane
lysis
Opsonization

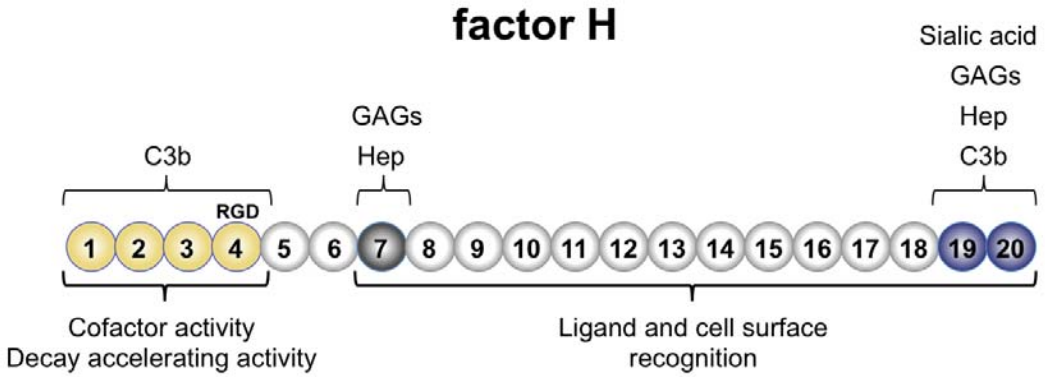
Complement Regulation

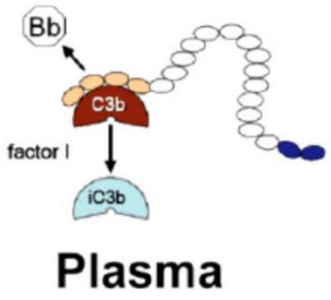


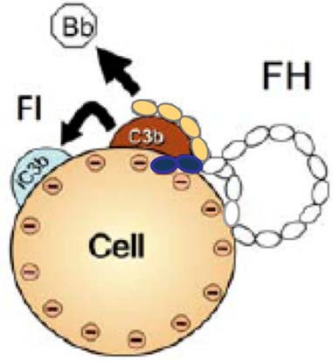
fH, factor H; CR1, complement recptor 1; DAF, decay accelerating factor; MCP, membrane cofactor protein; C4BP. C4b-binding protein

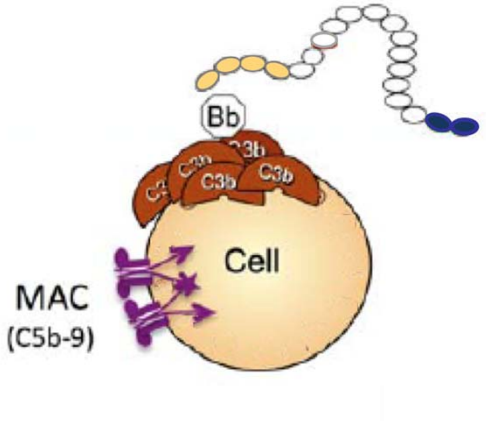
Factor H discriminates host surfaces from pathogens





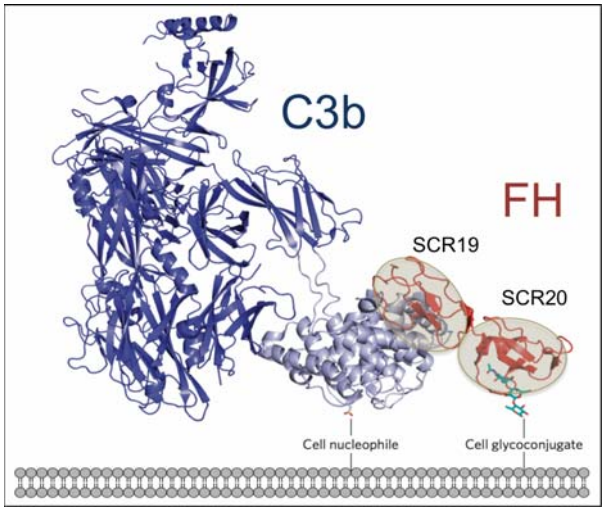


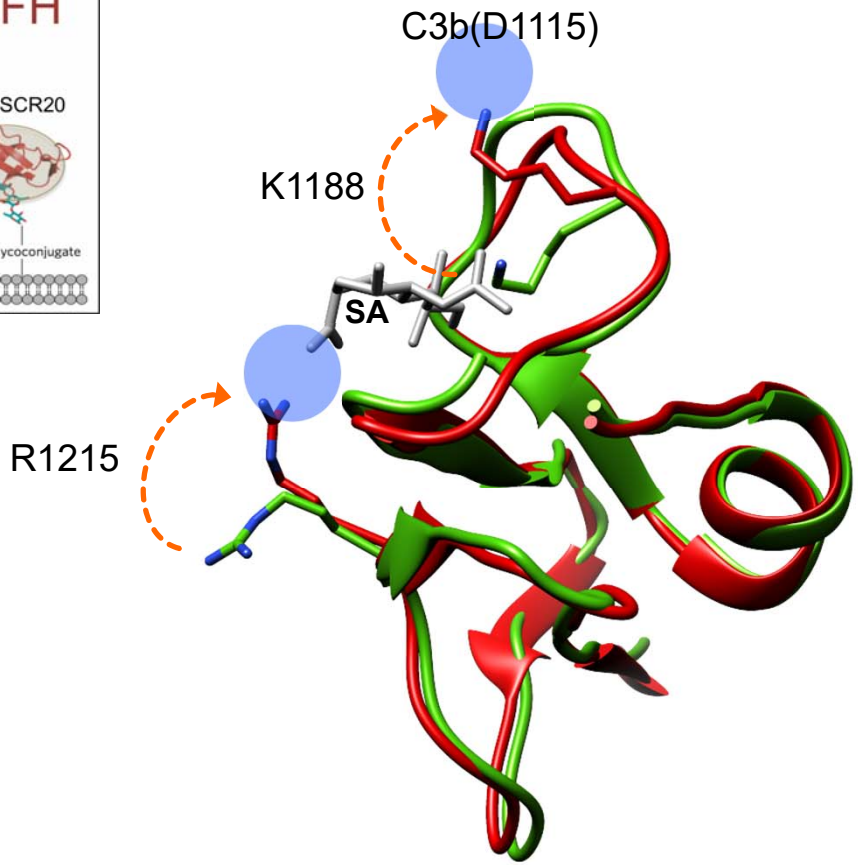


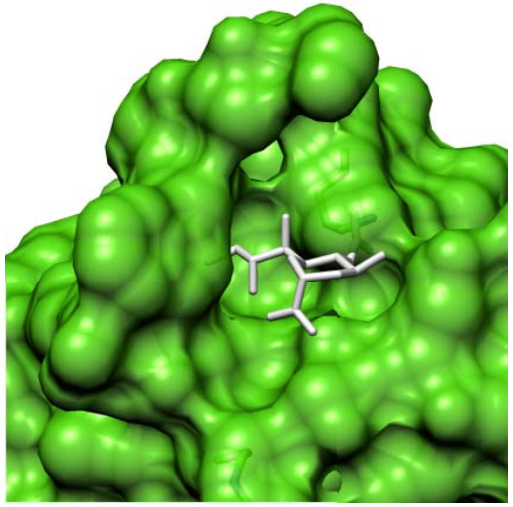


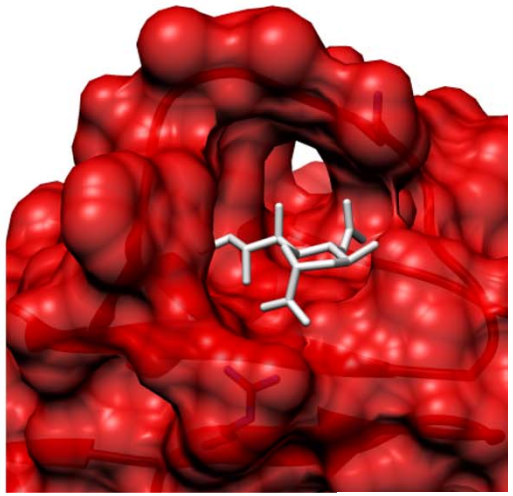
Rodriguez de Cordoba et al., MIMM (2004)

Structural basis for sialic acid-mediated self-recognition by FH



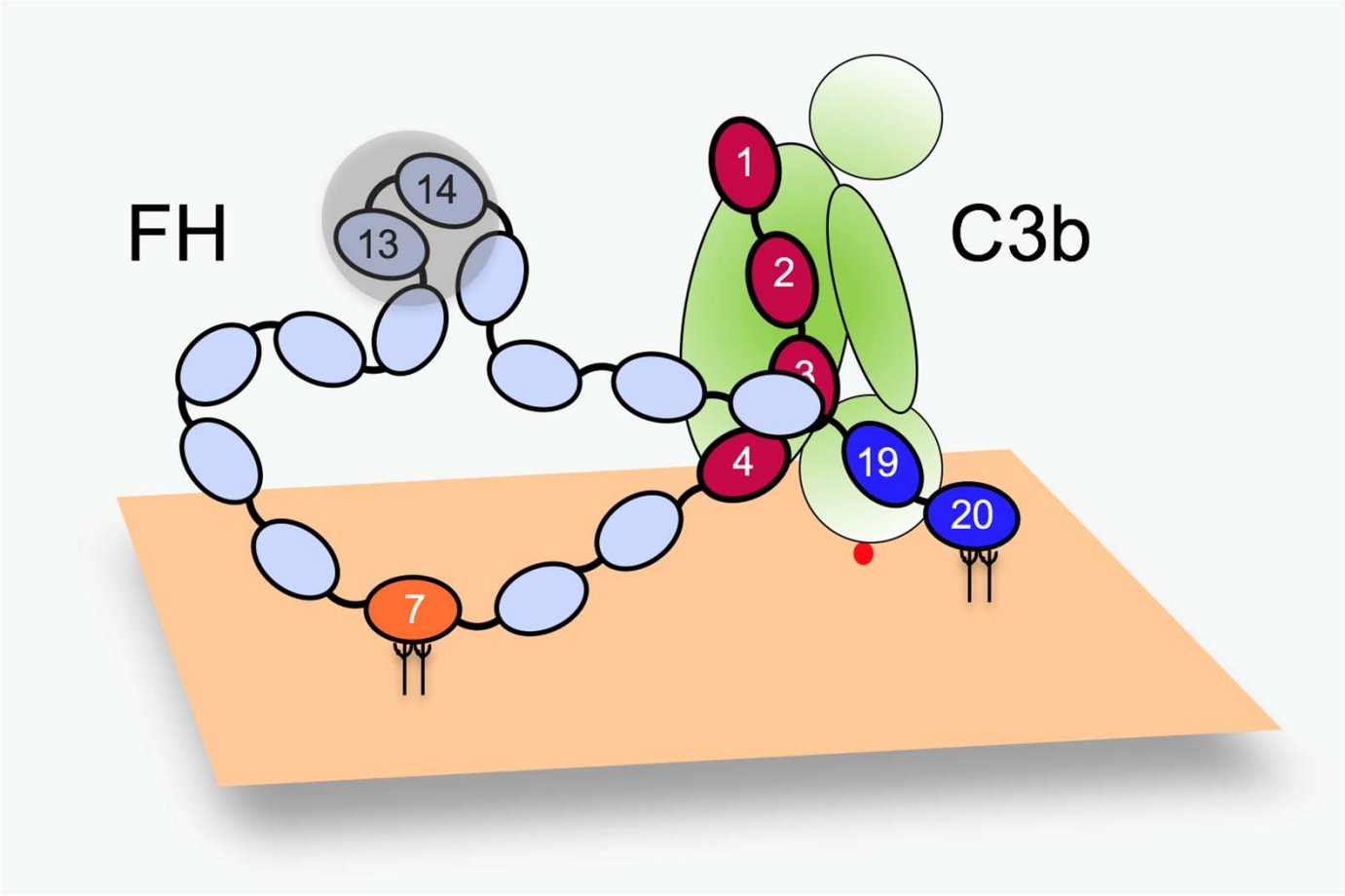






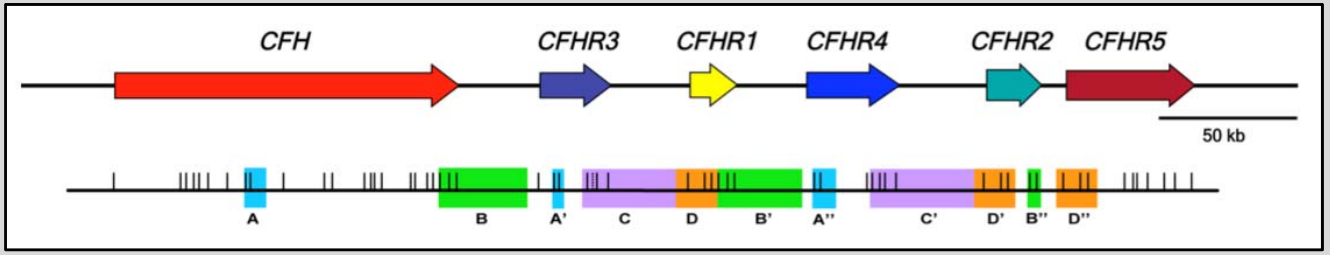
Blaum et al. Nat Chem Biol. 2015
Goicoechea de Jorge et al., Semin. Immunopathol. 2017

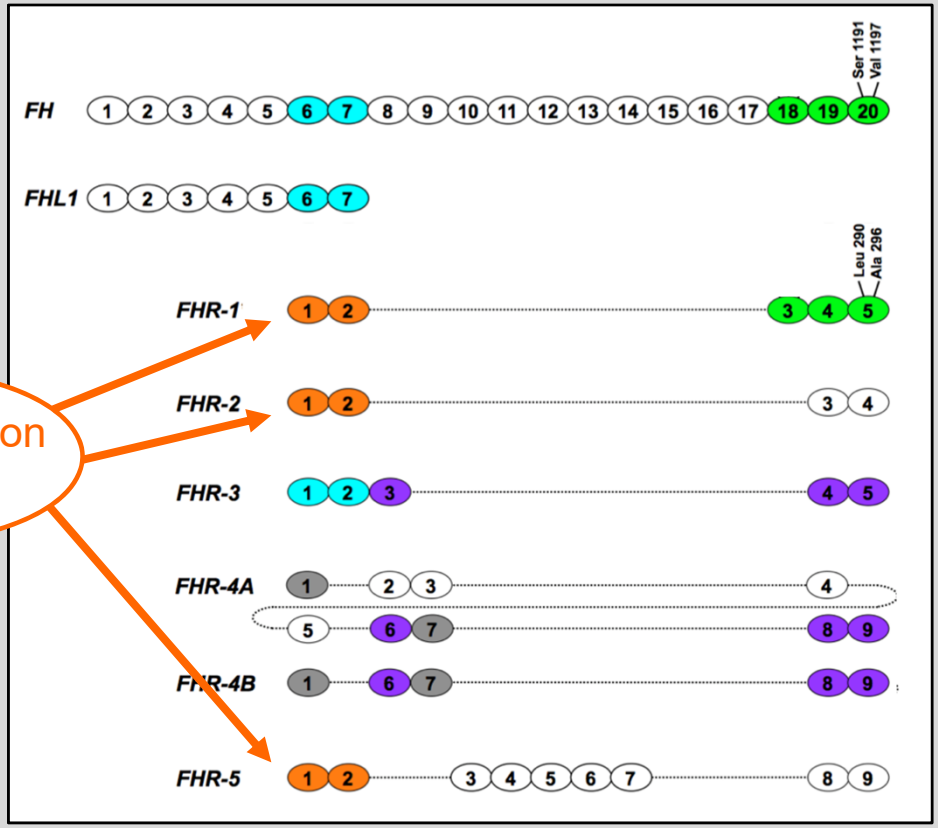
Structural basis for FH recognition of opsonized surfaces

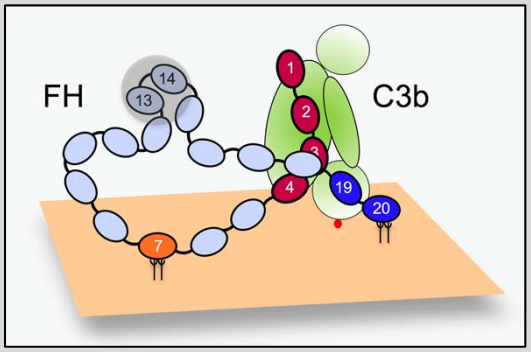


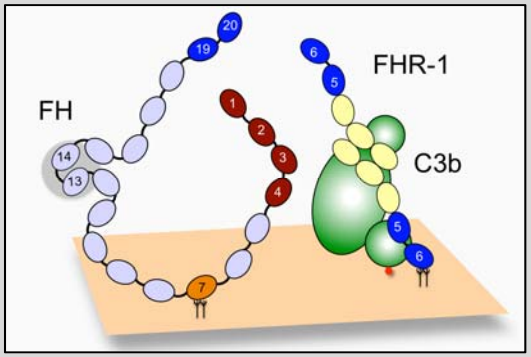
Prosser et al. JEM (2007); Schmidt et al. JI (2008); Harder et al. JI (2016); Martin-Merinero et al. KI (2017)

FH "de-regulation" by the FH-related proteins





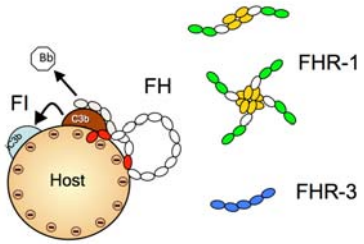




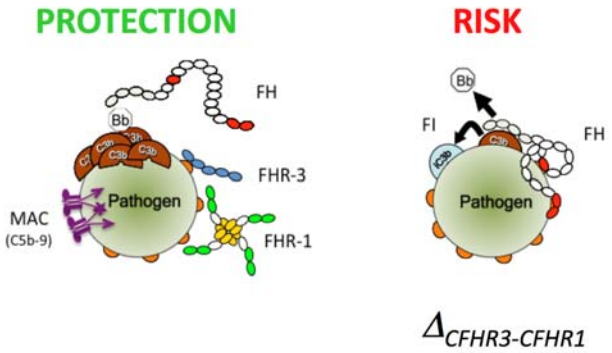
Józsi et al. Trends Immunol (2015)

FHRs in health and disease

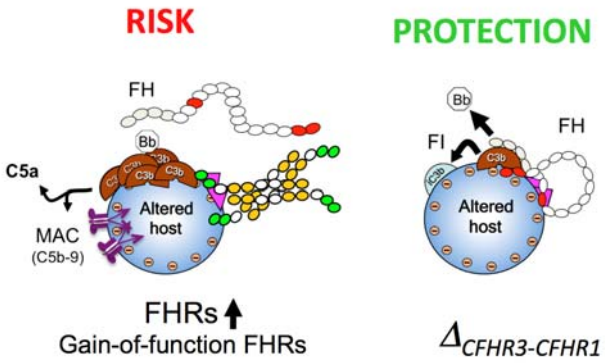
Host surfaces



Pathogens



Altered surfaces



Goicoechea de Jorge et al. PNAS 2013; 110: 4685-90.
Tortajada et al. JCI 2013; 123: 2434–2446.
Józsi et al. Trends Immunol (2015)
Tortajada el at KI 2017;

Altered host surfaces refer, for example, to ECM and other cell surface components modified by aging, microbial and chemical agents, or by deposition of immunecomplexes (like those containing galactose deficient-IgA), or even to iC3b, C3dg opsonised surfaces.

Complement is a major player in several diseases

Infectious diseases, sepsis, anaphylaxis

I/R injury

Paroxismal nocturnal hemoglobinuria

Psoriasis

Systemic lupus erythematosus

Lipodistrofia

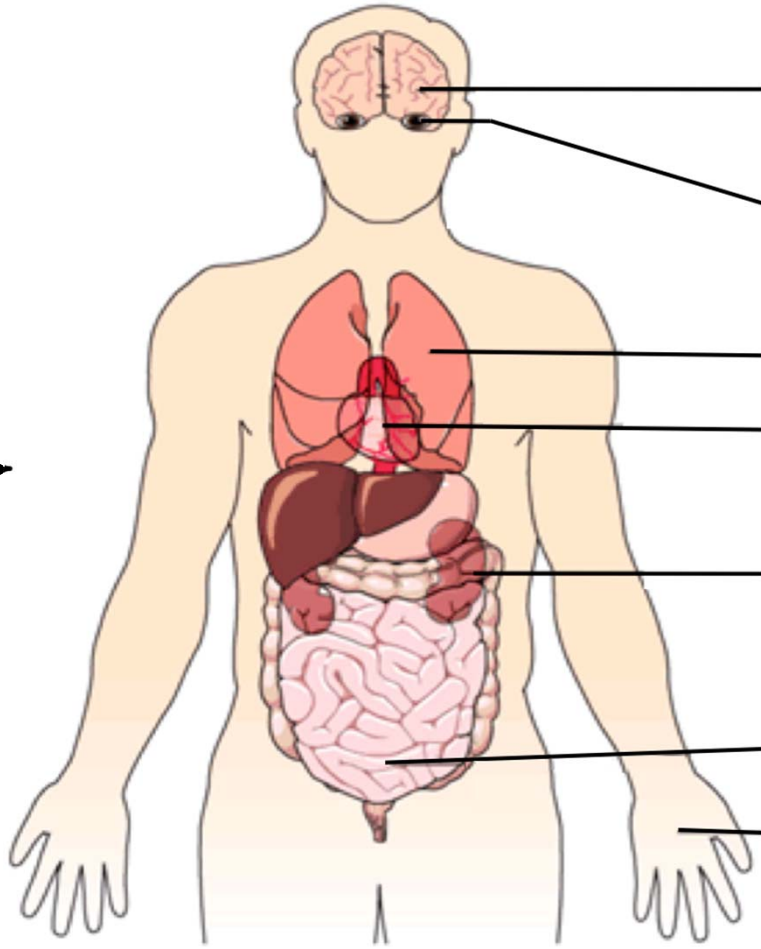
Transplant rejection

Multiple sclerosis

Allergic inflammation

Myasthenia gravis

Cancer



Stroke

Alzheimer's disease

Schizophrenia

Age-related macular degeneration

Asthma

Myocardial infarction

C3 Glomerulopathy

Atypical Hemolytic uremic syndrome

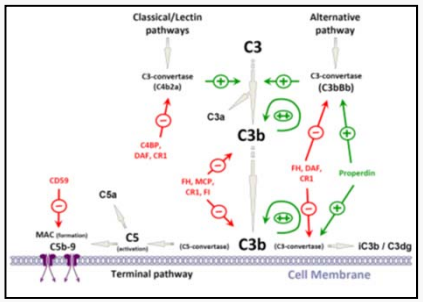
IgA Nephropathy

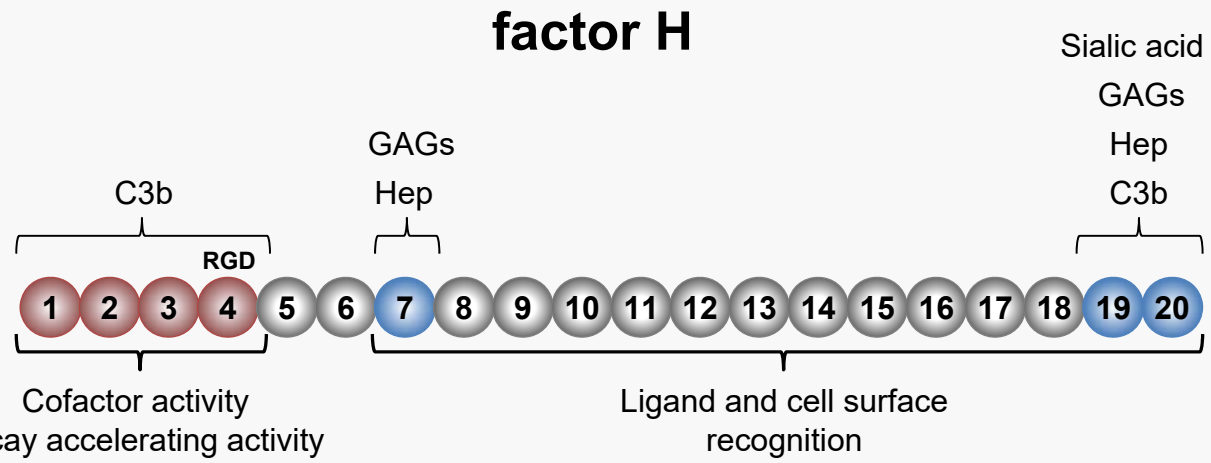
Crohn's disease

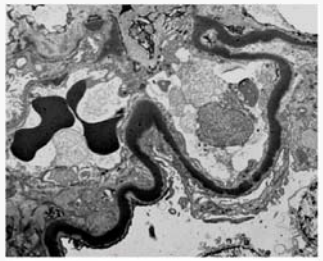
Rheumatoid arthritis

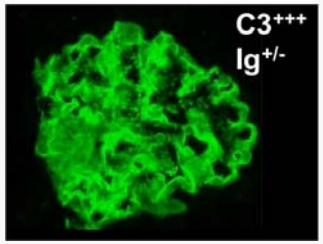
Adapted from: Ricklin D, Lambris JD. Nat Biotechnol. 2007 25:1265-75.

CFH genotype-phenotype correlations





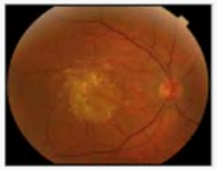


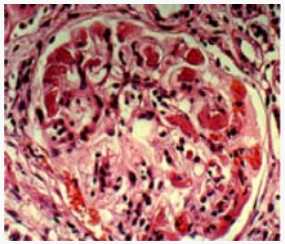


Plasma

Altered surface

surface





DDD
del K229 (hom)
FH deficiency (hom)

AMD
Y402H

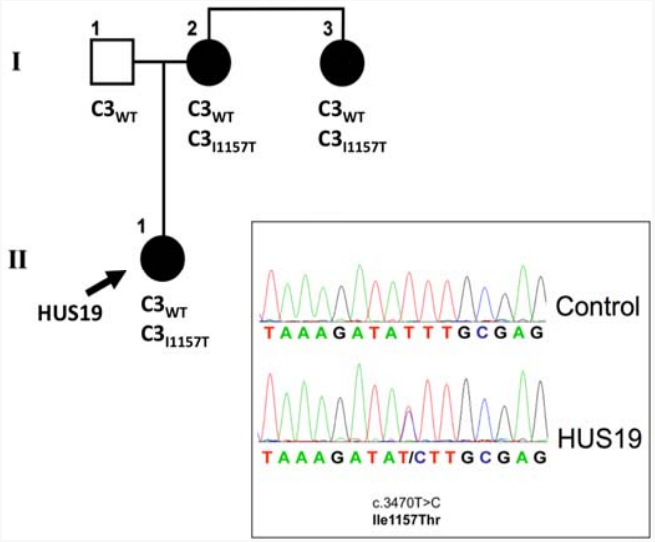
aHUS
C-ter mutations

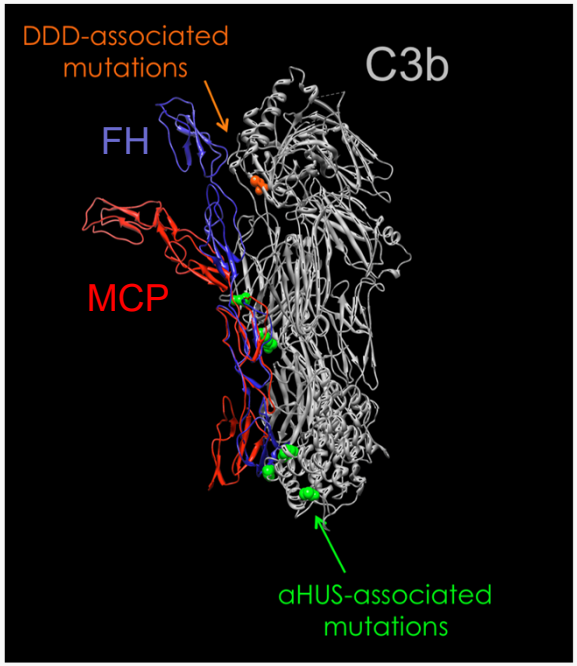
C3 genotype-phenotype correlations

Distinct C3 gain-of-function mutations associate with aHUS and DDD

aHUS

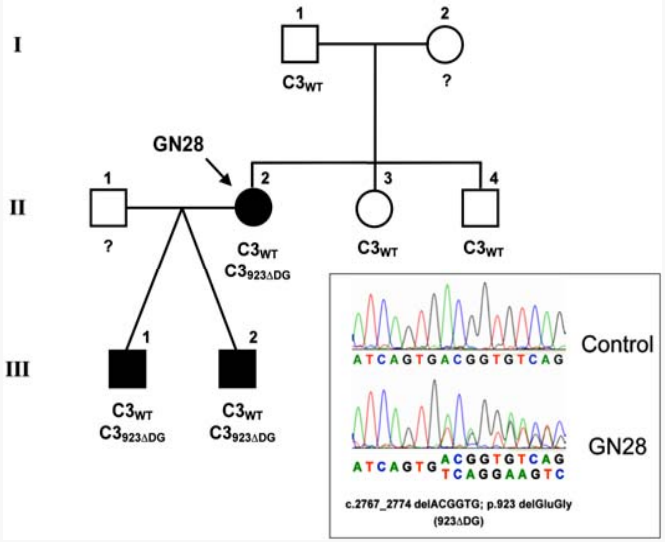
I1157T (R161W)





C3G

923 delDG (I756T)



Mutations in C3 associated with aHUS impair regulation by MCP

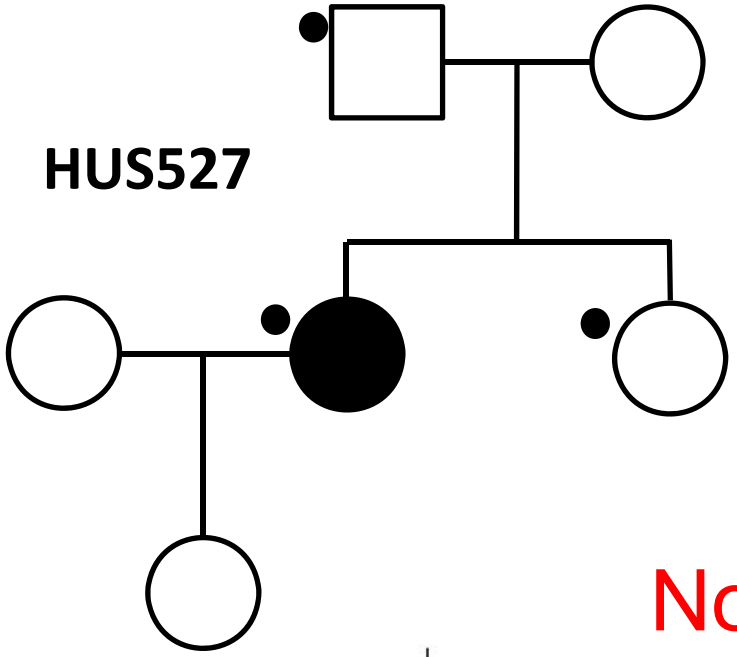
Mutations in C3 associated with C3G impair regulation by FH (and CR1)

Martinez-Barricarte et al. JCI 2010; Martinez-Barricarte et al. MIMM 2015; Chauvet et al. JASN 2016

CFHRs genetic variants

- Physiopathological consequences; Genotype-phenotype correlations
- Genomic mutations; Diagnostic implications

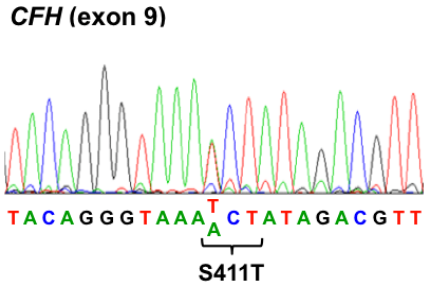
Novel aHUS pathogenic variants in *CFHR1*



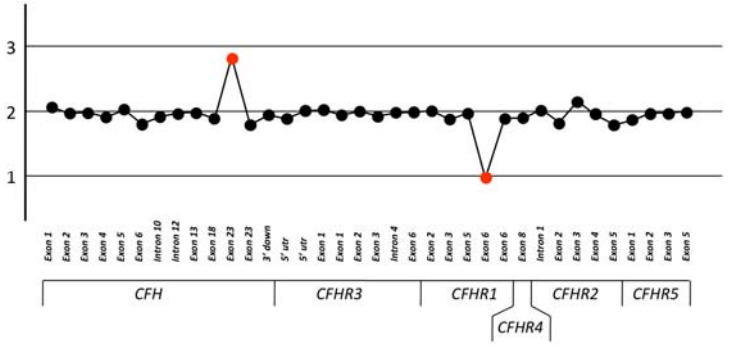
NGS panel

CFH	THBD
MCP	CFHR1
CFI	CFHR2
C3	CFHR3
CFB	CFHR4
DGKE	CFHR5

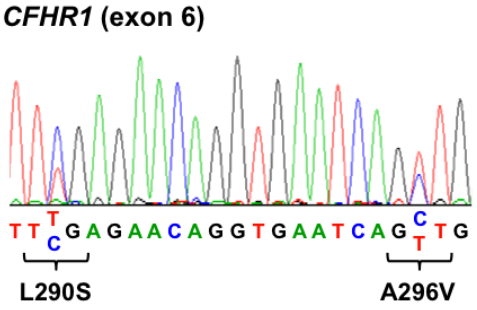
CFH: Ser411Thr



Not detected by NGS!

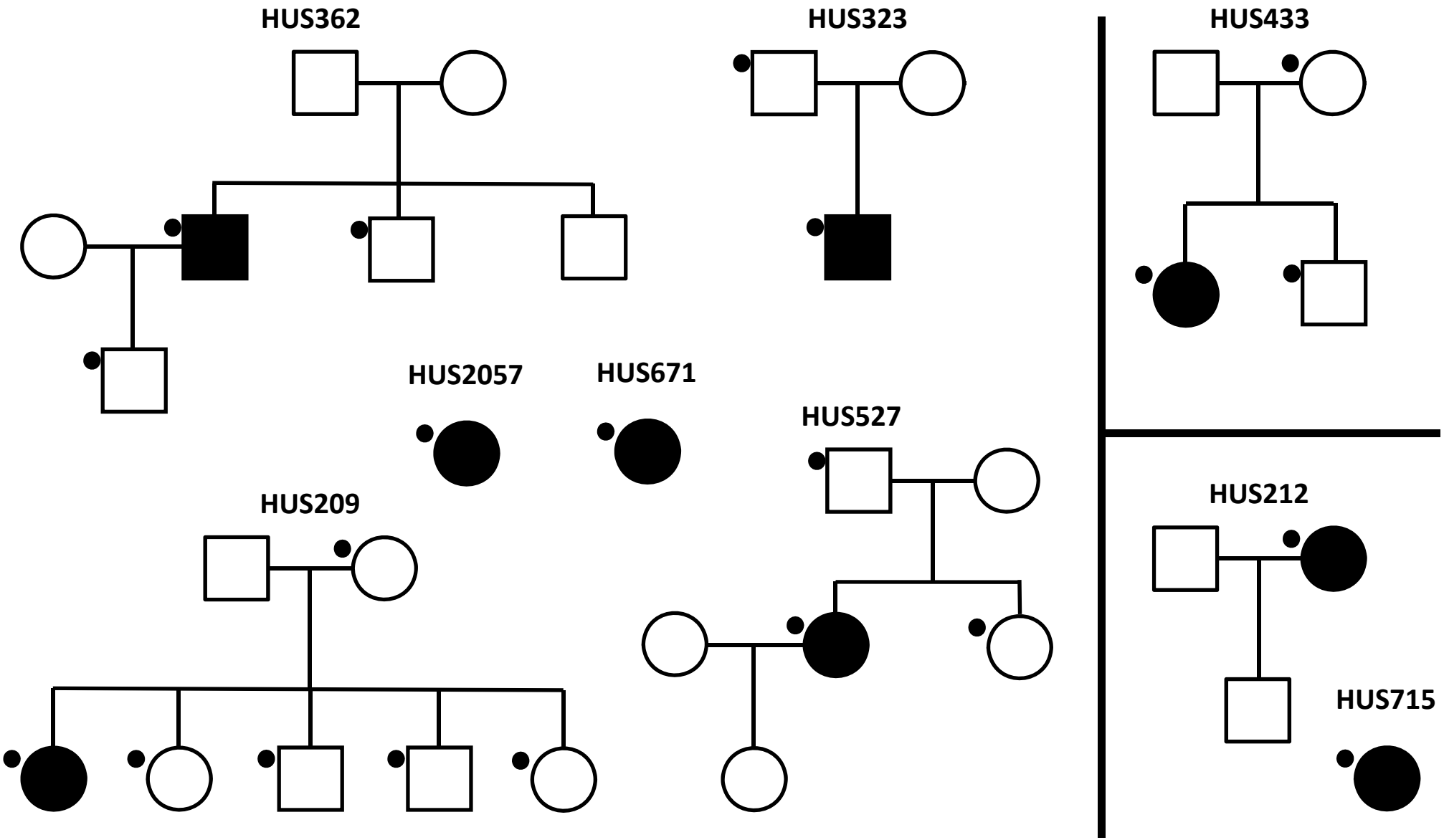


MLPA



Goicoechea de Jorge et al, JASN (2017)

Prevalence of *CFHR1* mutant in aHUS



Goicoechea de Jorge et al, JASN (2017)

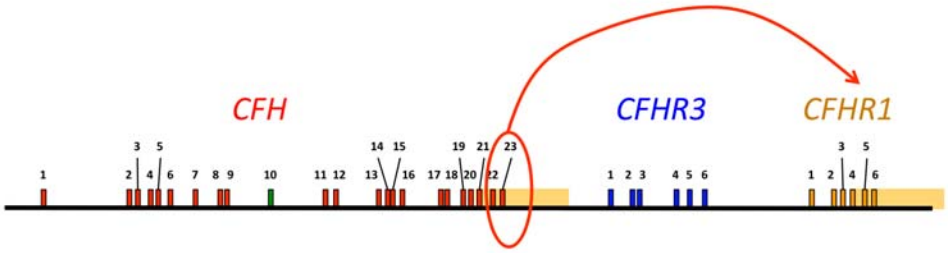
CFHR1$_{290Ser\text{-}296Val}$ is a recurrent variant associated with severe aHUS presentations.

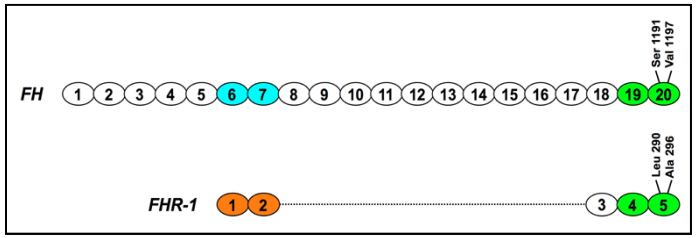
Patient	Age	Gender	Origin	Onset (Age)	ESRD (Age)	Recurrences	Transplants (Date)	Current status	Treatment
H209	43	Female	NW-Spain	32	33	-	1 (31-07-2016)	Functioning graft	Eculizumab
H212	30	Female	Spain	20	21	-	1 (01-03-2009)	Hemodialysis	Eculizumab
H323	21	Male	NW-Spain	17	-	1[a]	0	Normal renal function	Eculizumab
H362	53	Male	NW-Spain	49	-	-	0	End-stage renal disease[b]	Eculizumab
H433	25	Female	N-Spain	23	-	1[c]	0	Normal renal function (sCr 2.2mg/mL)	Eculizumab
H527	28	Female	NW-Spain	25	-	2[d]	0	Normal renal function	Plasmapheresis – steroids
H671	31	Female	NW-Spain	30	-	0	0	Normal renal function	Eculizumab
H715	47	Female	Spain	36	36	3[e]	1 (22/08/2007)	Hemodialysis	Eculizumab
H2057	50	Female	N-Portugal	47	47	-	Waiting list	End-stage renal disease	Plasmapheresis

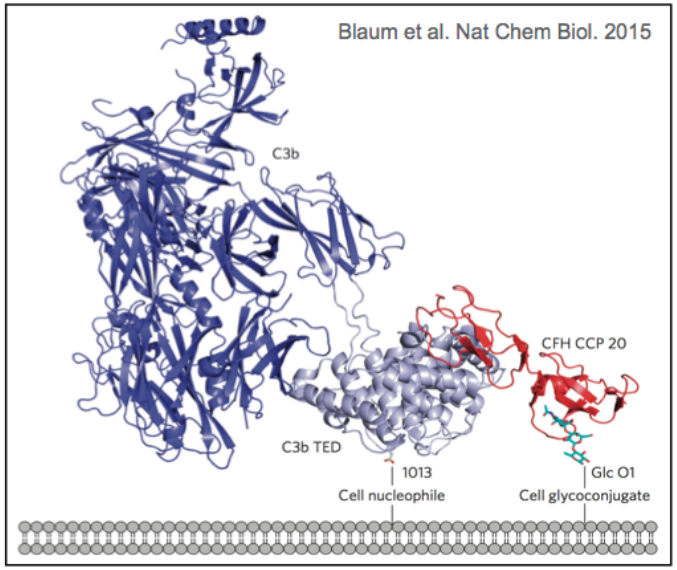
[a]) Recurrence when eculizumab dose was reduced
[b]) Acute presentation triggered by a Churg-Strauss vasculitis and evolution to ESRD, despite eculizumab treatment.
[c]) After one year of treatment, eculizumab was discontinued in 2015. She became pregnant and had a recurrence on March 2016 requiring again eculizumab. She gave birth in April 2016 by cesarean. Eculizumab was discontinued again August 26, 2016.
[d]) Onset associated with postpartum and first recurrence with pancreatitis. No clear cause for the second recurrence, which presented with neurological symptoms.
[e]) Onset associated with postpartum. The third recurrence was associated with a mesenteric thrombosis.

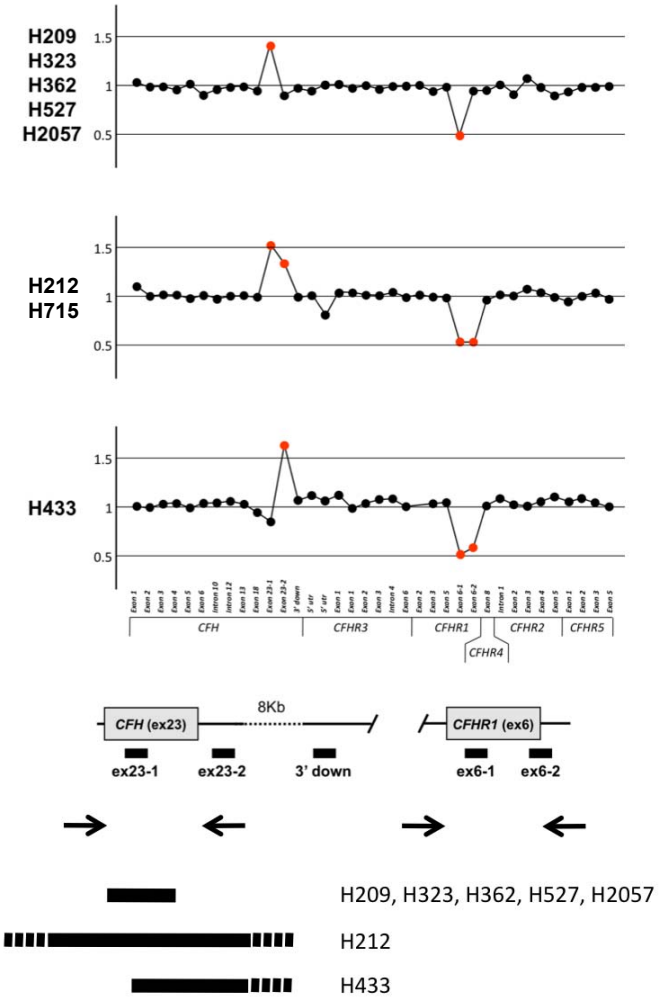
Goicoechea de Jorge et al, JASN (2017)

Mutant *CFHR1* originate by gene conversion events



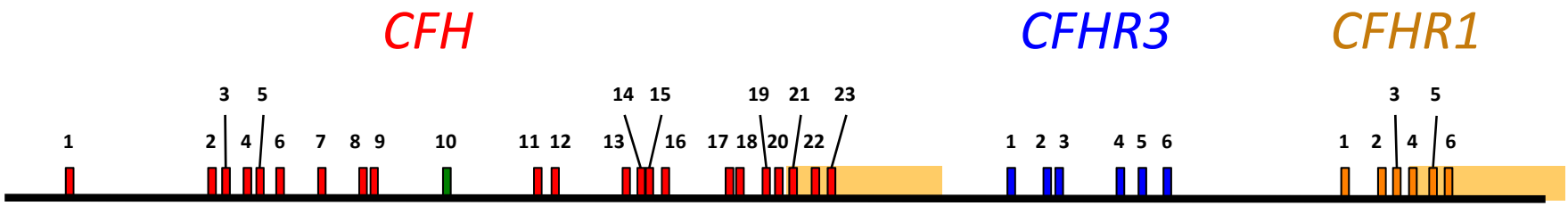






Goicoechea de Jorge et al, JASN (2017)

Functional analysis of *CFH S411T* variant and mutant *CFHR1*

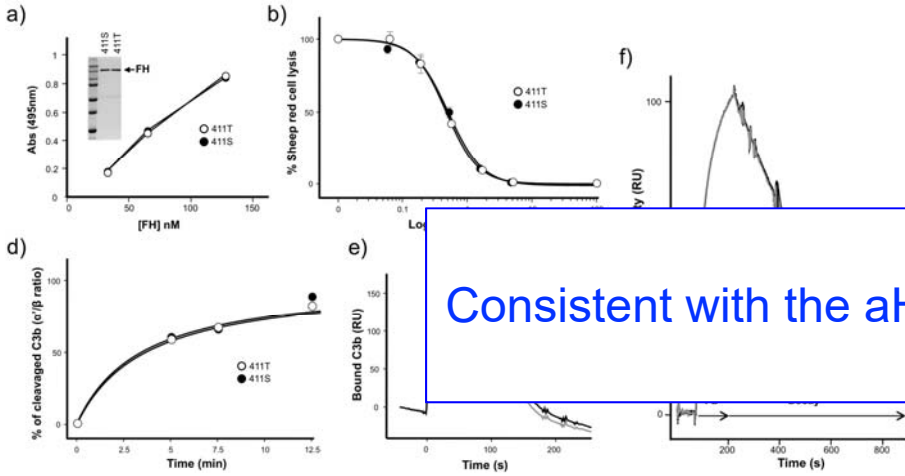


Ser411Thr

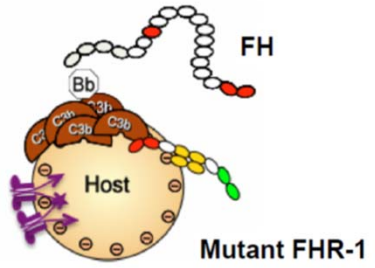
Leu290Ser
Ala296Val

CFH Ser411Thr is a polymorphism without functional consequences

Mutant FHR-1 competes complement regulation by FH on sheep erythrocytes

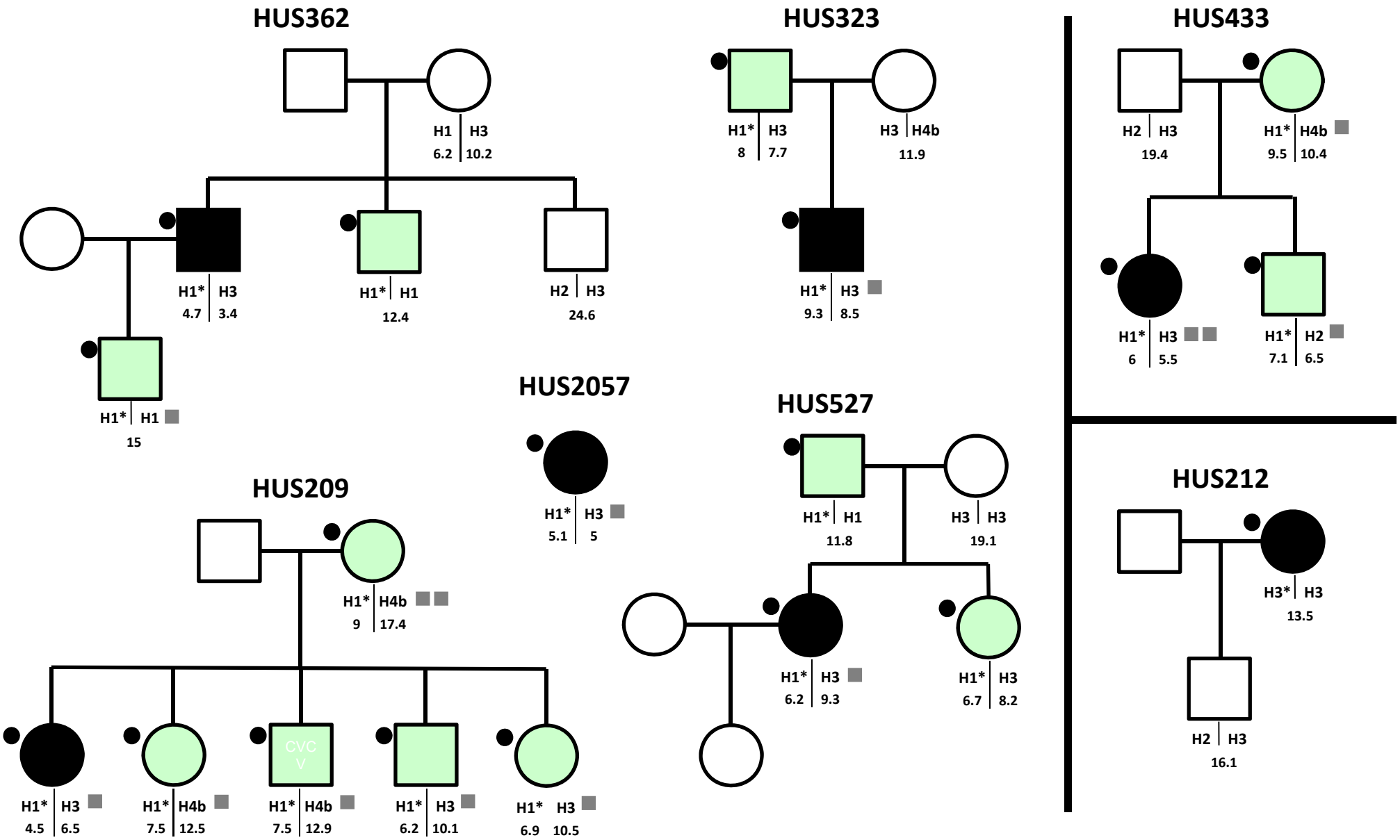


Consistent with the aHUS pathogenic mechanism!

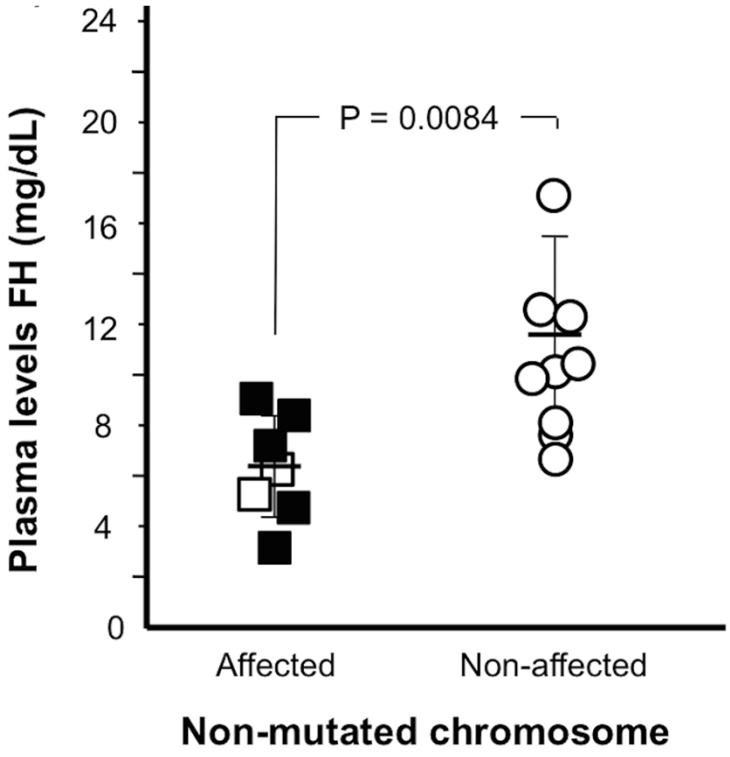


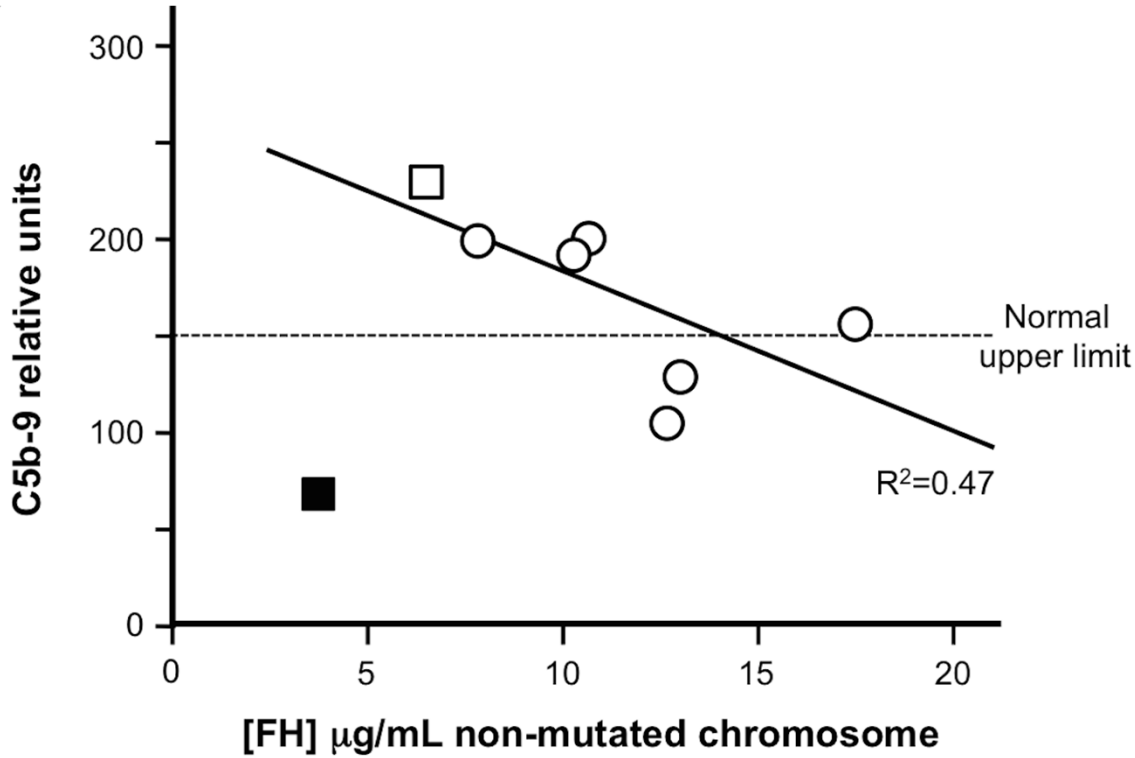
)17)

Incomplete penetrance of aHUS in carriers of the CFHR1 mutant



Levels of FH modulate penetrance of aHUS in mutant CFHR1 carriers.



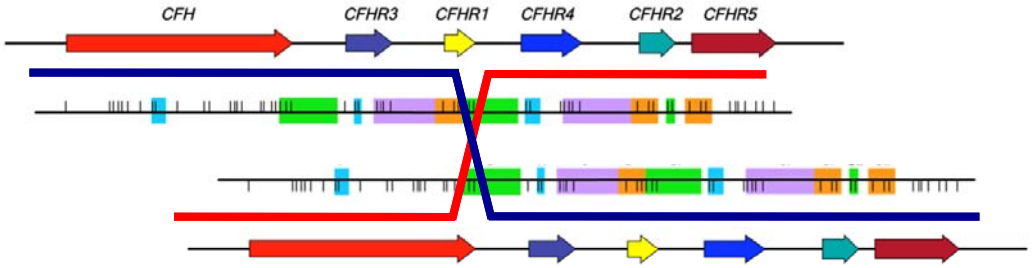


Goicoechea de Jorge et al, JASN (2017)

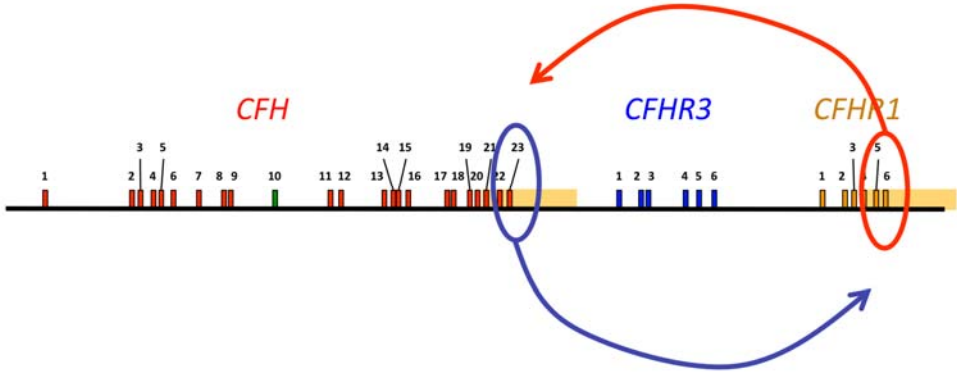
Exchanging C-ter regions between FH and FHR-1 is a major cause of aHUS.

1) *CFH::CFHR1 hybrid*

Unequal crossover *(DelCFHR3-CFHR1)* (Venables et al. 2006)
***CFH* > *CFHR1* gene conversion** (Heinen et al. 2005)



Unequal crossover



Gene conversion

2) *CFHR1::CFH hybrid*

Unequal crossover *(DupCFHR3-CFHR1)* (Valoti et al. 2015)
***CFHR1* > *CFH* gene conversion** (Goicoechea de Jorge et al. 2017)

Novel *CFHR1* mutant associated with aHUS (caso C. Fdez-Ribera)

HUS259/642

Male, 54 y-old, without family history of renal disease.

2008, aHUS onset that evolved to ESRD and HD.

2009, episode of MAT with neurological involvement.

2010, Cadaveric renal TX.

Complement study 1

2010, aHUS recurrence day 7 post TX.

2010, No response to PE. Biopsies show progressive deterioration.

2010, eculizumab is finaly obtained 2mo post TX.

2010, treated for 3mo. No renal response despite hematological improvement

2010, restarts HD.

2013, graft removed.

2016, renal TX from live donor (wife), eculizumab profilaxis.

Complement study 2

2017, excellent evolution of renal function. Biopsy with normal glomeruli

COMPLEMENT STUDY 2010

CFH, CFI MCP: No complement pathogenic gene variants
C3, normal.
C4, normal
FH, normal
MCP, normal
FI, normal
anti-Factor H, negative
WB analysis, normal.

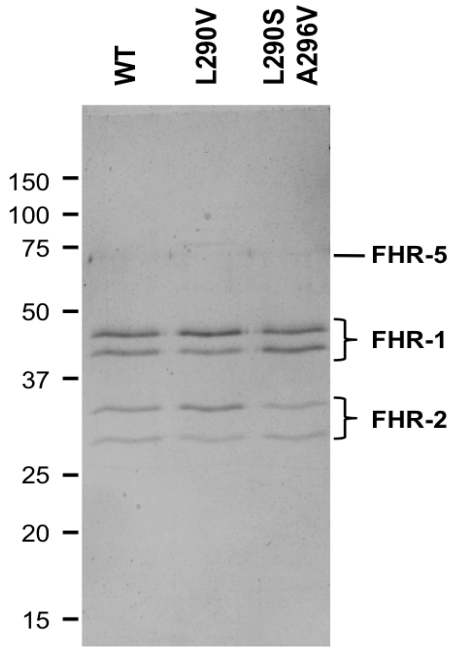
COMPLEMENT STUDY 2016

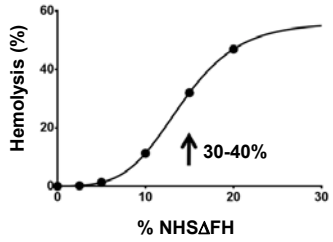
NGS panel: CFH, MCP, CFI, C3, CFB, DGKE, THBD, CFHR1, CFHR2, CFHR3, CFHR4 and CFHR5.

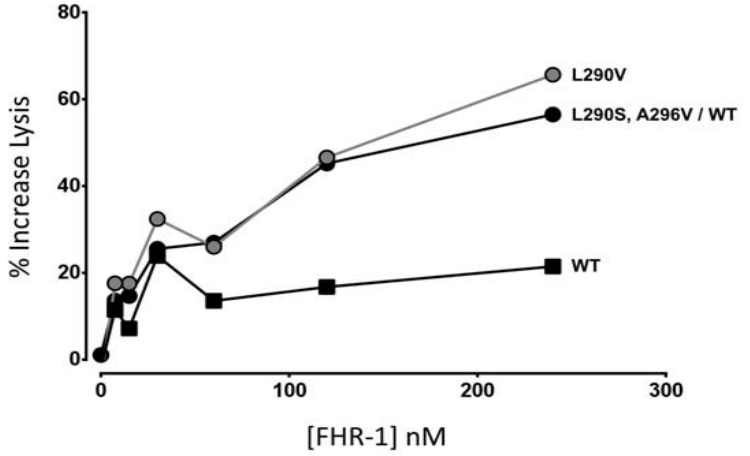
Novel gene variant identifed in *CFHR1* (L290V) involving a position that suggest a relevant functional impact.

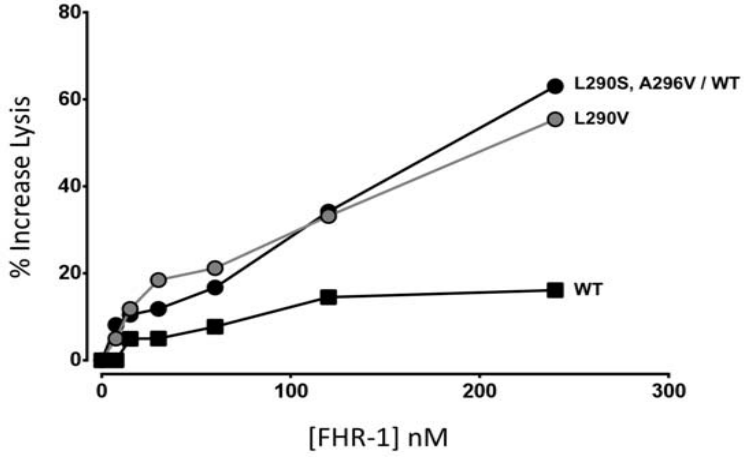
Purification of mutant FHR-1 protein and fuctional analysis confirm pathogenicity: competion with FH for surface regulation.

L290V FHR-1 mutant competes FH in a sheep red cell assay

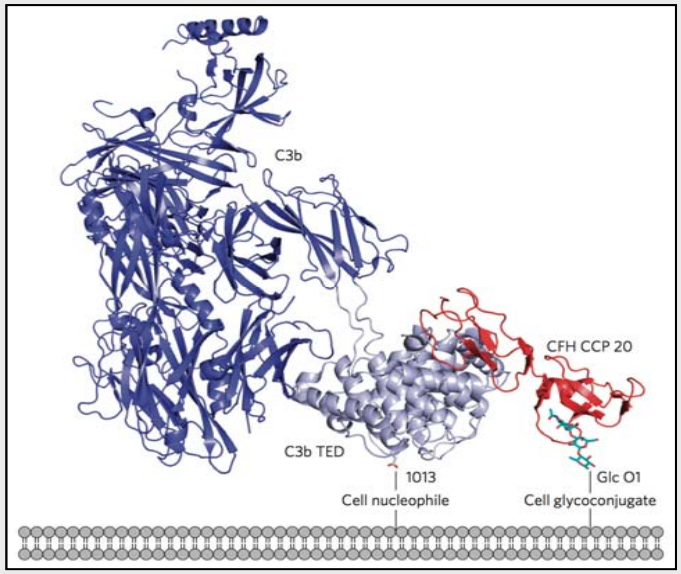


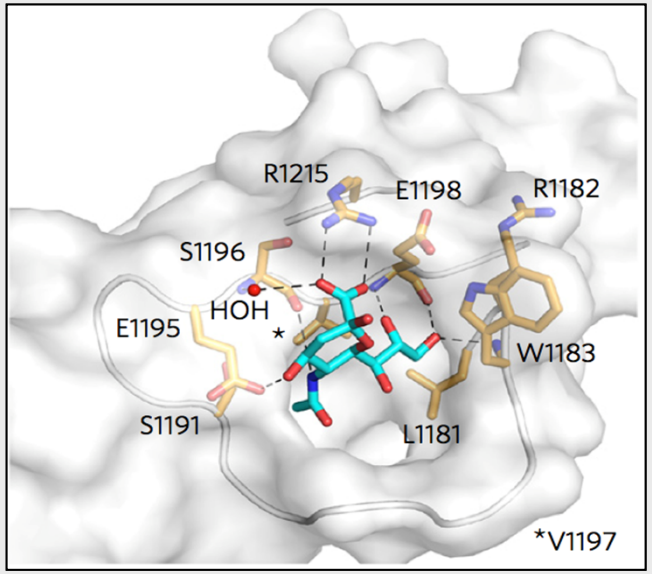


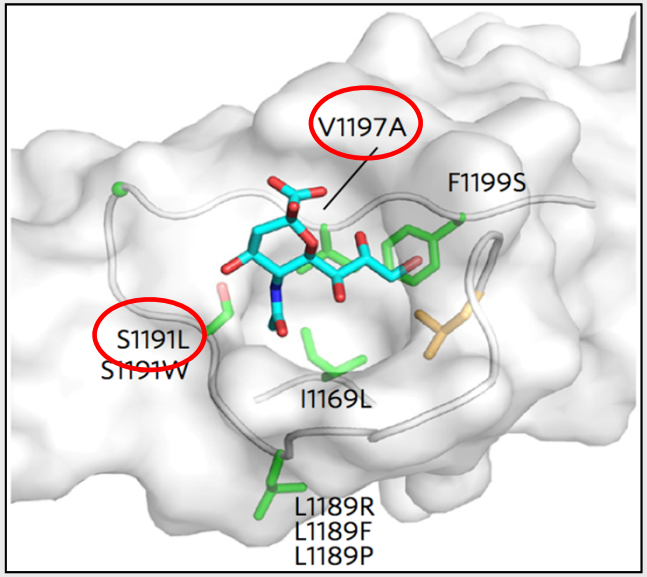


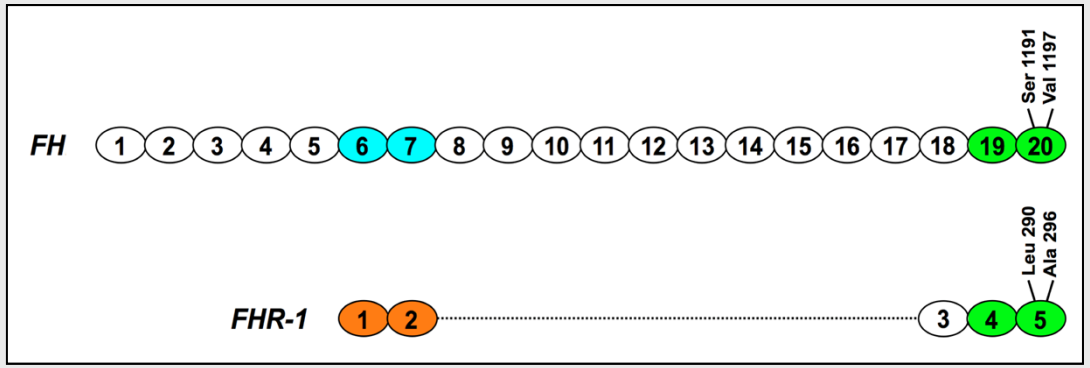


Structural basis for sialic acid-mediated self-recognition by FH









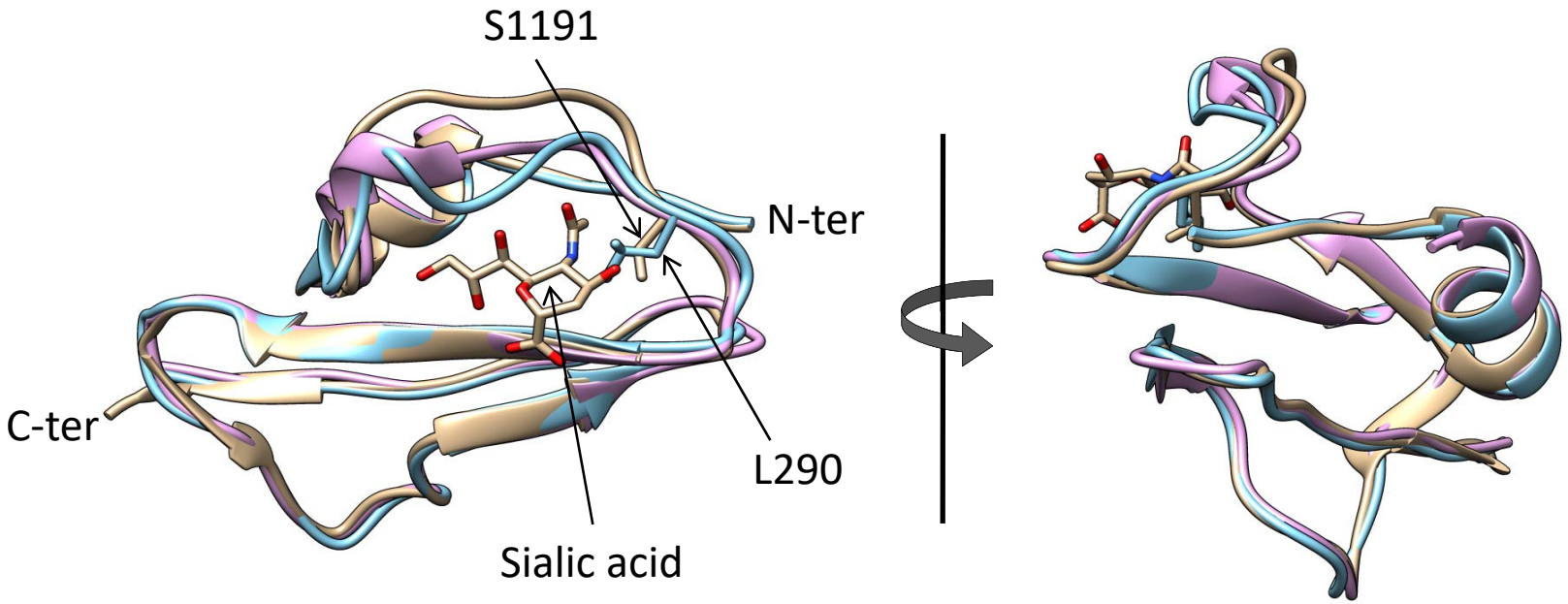
L290V

Blaum et al. Nat Chem Biol. 2015

Structural basis for the competition between FHR-1(L290V) mutant and FH

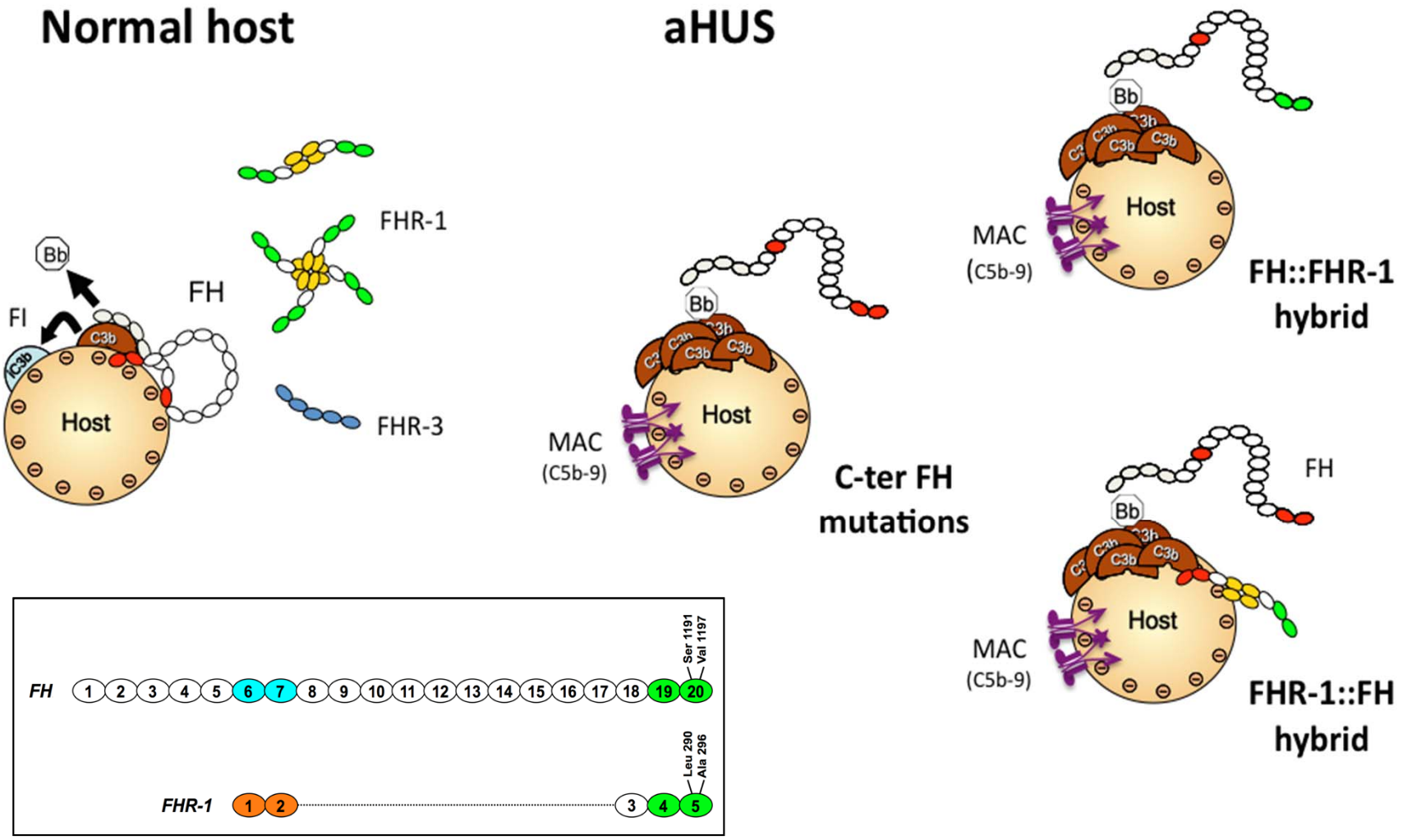
S1191L substitution closes the sialic acid pocket

The L290V substitution reverses this situation returning to a more FH-like conformation

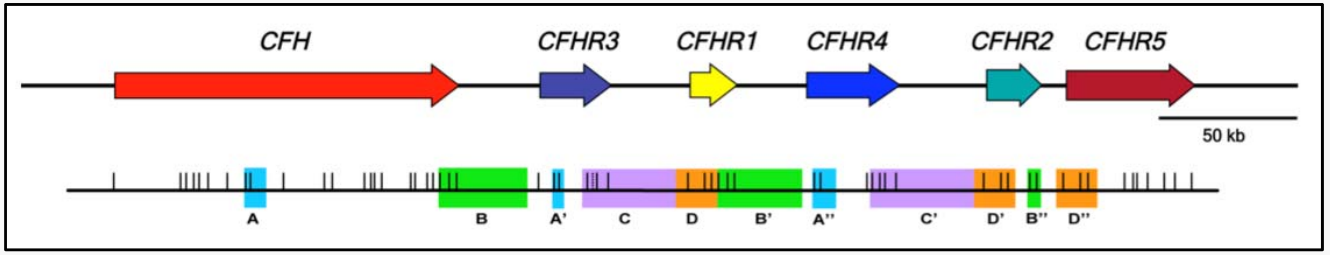


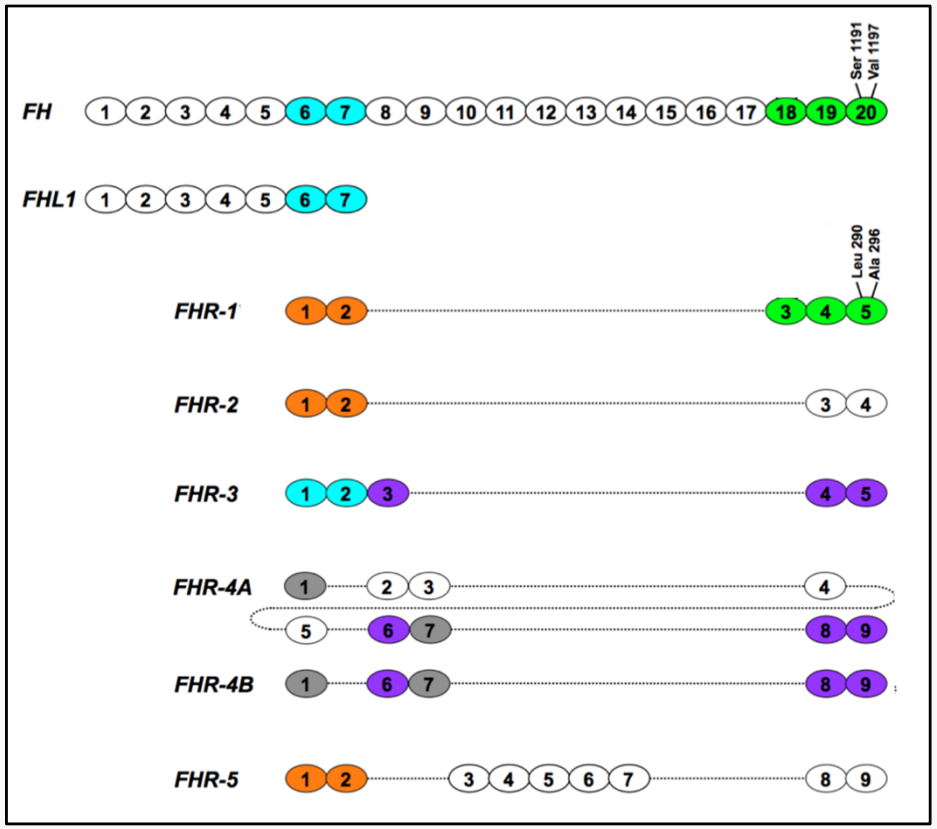
Brown: FH SCR20; Blue: FHR-1 SCR5; Pink: FHR-1 SCR5 (L290V)

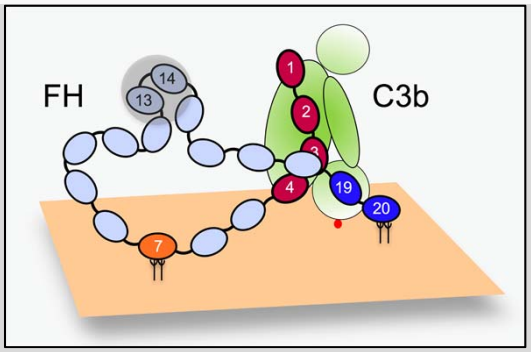
Exchanging C-terminal regions between FH and FHR-1 is pathogenic



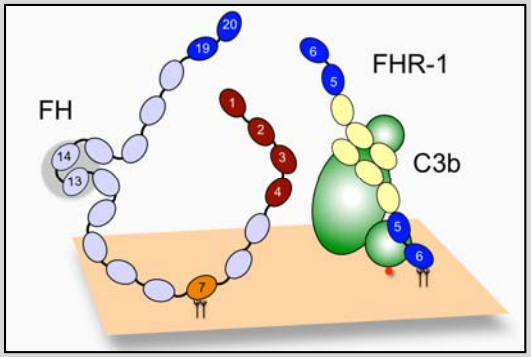
The *CFH-CFHRs* locus





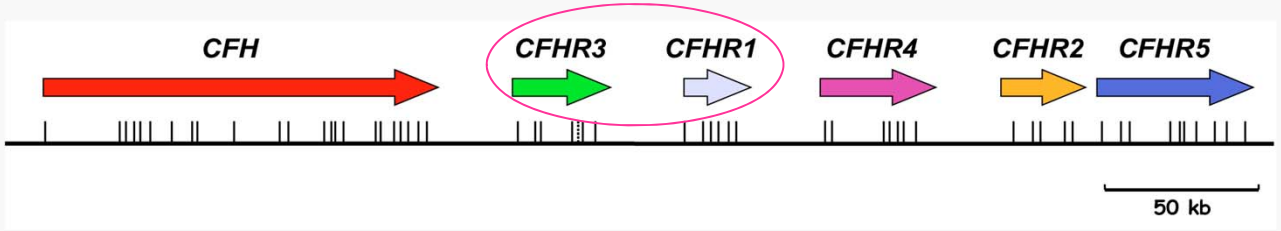


“ FH de-regulation”



Józsi,…, Rodriguez de Cordoba. Trends Immunol (2015)

$\Delta_{CFHR3\text{-}CFHR1}$ protects from AMD



	$\Delta_{CFHR3\text{-}CFHR1}$ (allele frequency)	
USA		
Columbia (n=357)	0.26	Hageman *et al.*, 2006
European		
Ireland (n=170)	0.19	Hughes et al., 2006
Spain (n=269)	0.25	(Martinez-Barricarte *et al.* unpublished)
UK (n=238)	0.18	Holmes et al., 2013
Italy (n=49)	0.22	Holmes et al., "013
African		
Nigeria (n=21)	0.55	Holmes *et al.*, 2013
Algeria (n=29)	0.23	Holmes *et al.*, 2013
South Saharan (n=83)	0.34	Holmes *et al.*, 2013
South American		
Brasil, Colombia (n=29)	0	Holmes *et al.*, 2013
Asia		
Japan (n=29)	0	Holmes *et al.*, 2013
China (n=50)	0.06	Holmes *et al.*, 2013

Frequencies of $\Delta_{CFHR3\text{-}CFHR1}$, in general, correlate well with the prevalence of AMD, IgAN and SLE.

$\Delta_{CFHR3\text{-}CFHR1}$, also protects from C3G.

Hughes et al., Nat Genet 38: 1173-77 (2006), Gharavi et al., Nat Genet 43 321-7 (2011), Kiryluk et al., PLoS Genetics 8: e1002767 (2011), Zhoa et al., PloS Genetics 7: e1002079 (2011), Goicoechea de Jorge, Unpublished

www.kidney-international.org

clinical investigation

Elevated factor H–related protein 1 and factor H pathogenic variants decrease complement regulation in IgA nephropathy

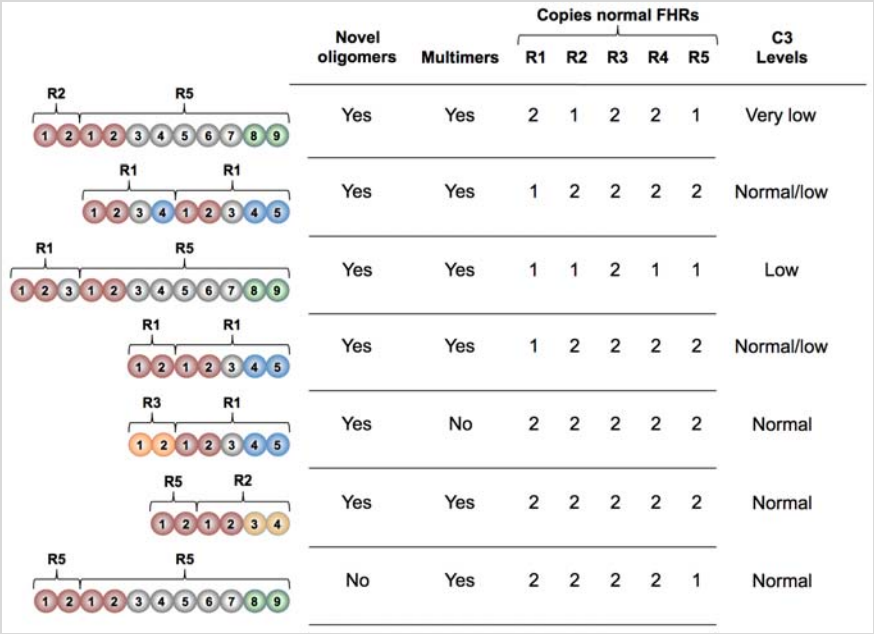
see commentary on page 790

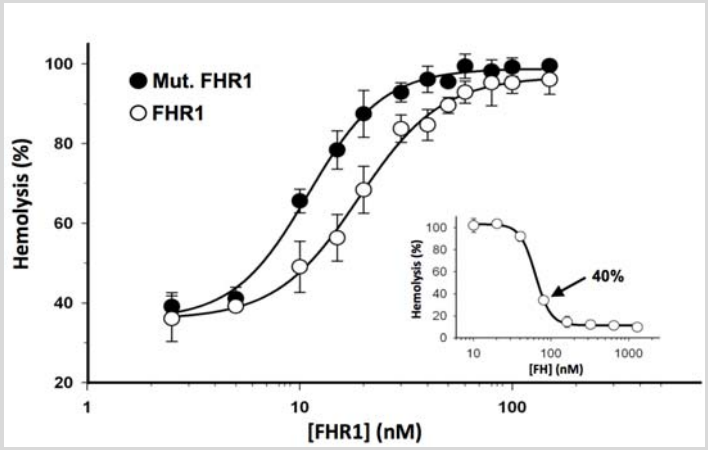
Agustín Tortajada[1,11], Eduardo Gutiérrez[2,11], Elena Goicoechea de Jorge[3,11], Jaouad Anter[1], Alfons Segarra[4], Mario Espinosa[5], Miquel Blasco[6], Elena Roman[7], Helena Marco[8], Luis F. Quintana[6], Josué Gutiérrez[3], Sheila Pinto[1], Margarita Lopez-Trascasa[9], Manuel Praga[2,10] and Santiago Rodriguez de Córdoba[1]

IgA nephropathy (IgAN), a frequent cause of chronic kidney disease worldwide, is characterized by mesangial deposition of galactose-deficient IgA1-containing immune complexes. Complement involvement in IgAN pathogenesis is suggested by the glomerular deposition of complement components and the strong protection from IgAN development conferred by the deletion of the CFHR3 and CFHR1 genes ($\Delta_{CFHR3\text{-}CFHR1}$). Here we searched for correlations between clinical progression and levels of factor H (FH) and FH-related protein 1 (FHR-1) using well-characterized patient cohorts consisting of 112 patients with IgAN, 46 with non-complement-related autosomal dominant polycystic kidney disease (ADPKD), and 76 control individuals. Patients with either IgAN or ADPKD presented normal FH but abnormally elevated FHR-1 levels and FHR-1/FH ratios compared to control individuals. Highest FHR-1 levels and FHR-1/FH ratios are found in patients with IgAN with disease progression and in patients with ADPKD who have reached chronic kidney disease, suggesting that renal function impairment elevates the FHR-1/FH ratio, which may increase FHR-1/FH competition for activated C3 fragments. Interestingly, $\Delta_{CFHR3\text{-}CFHR1}$ homozygotes are protected from IgAN, but not from ADPKD, and we found five IgAN patients with low FH carrying CFH or CFI pathogenic variants. These data support a decreased FH activity in IgAN due to increased FHR-1/FH competition or pathogenic CFH variants. They also suggest that alternative pathway complement activation in patients with IgAN, initially triggered by galactose-deficient IgA1-containing immune complexes, may exacerbate in a vicious circle as renal function deterioration increase FHR-1 levels. Thus, a role of FHR-1 in IgAN pathogenesis is to compete with complement regulation by FH.

Gain of function CFHRs mutations cause C3G

C3G	DDD	CFHR2::CFHR5 hybrid gene	Hybrid protein containing SCR1-2 of FHR-2 followed by the whole FHR-5 molecule.	Abnormal oligomerization. Increased competition with FH.	Risk	Very rare
	DDD / C3-GN	DupCFHR1	Mutant FHR-1 with SCR123412345	Abnormal oligomerization. Increased competition with FH.	Risk	Very rare
		CFHR1::CFHR5 hybrid gene	Hybrid protein containing SCR1-3 of FHR-1 followed by the whole FHR-5 molecule.	Abnormal oligomerization. Increased competition with FH.	Risk	Very rare
	C3-GN	DupCFHR1	Mutant FHR-1 with SCR1212345	Abnormal oligomerization. Increased competition with FH.	Risk	Very rare
		CFHR3::CFHR1 hybrid gene	Hybrid protein containing SCR1-2 of FHR-3 followed by the whole FHR-1 molecule.	Increased levels of FHR-1 Increased competition with FH?	Risk	Very rare
		CFHR5::CFHR2 hybrid gene	Hybrid protein containing SCR1-2 of FHR-5 followed by the whole FHR-2 molecule.	Increased levels of FHR-2 Increased competition with FH?	Risk	Very rare
		DupCFHR5	Mutant FHR-5 with SCR12123456789	Abnormal oligomerization. Increased competition with FH.	Risk	Several related cases described, 1 unrelated

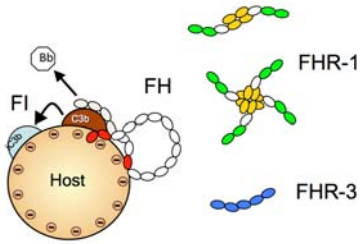




Gale et al. Lancet 2010
Malik et al. JASN 2012
Goicoechea de Jorge et al PNAS 2013
Tortajada et al, JCI 2013
Chen et al. JCI 2014
Zhang et al. MIMM 2016
Shambhuprasad et al. KI 2017
Goicoechea de Jorge et al JASN 2017

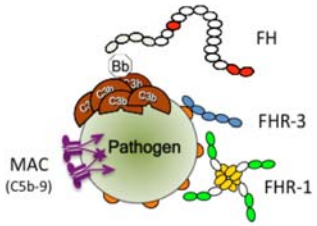
“Misleading” complement regulation

Host surfaces

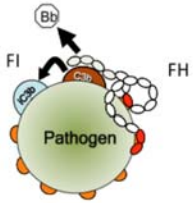


Pathogens

PROTECTION



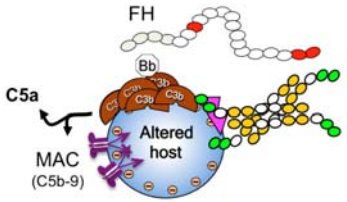
RISK



$\Delta_{CFHR3\text{-}CFHR1}$

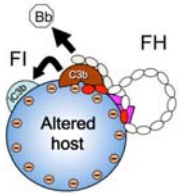
Altered surfaces

RISK



FHRs ↑
Gain-of-function FHRs

PROTECTION



$\Delta_{CFHR3\text{-}CFHR1}$

Goicoechea de Jorge et al. PNAS 2013; 110: 4685-90.
Tortajada et al. JCI 2013; 123: 2434–2446.
Józsi et al. Trends Immunol (2015)
Tortajada el at KI 2017;

▲ Altered host surfaces refer, for example, to ECM and other cell surface components modified by aging, microbial and chemical agents, or by deposition of immunecomplexes (like those containing galactose deficient-IgA), or even to iC3b, C3dg opsonised surfaces.

Benefits of genetic testing and molecular diagnostics in aHUS and C3G

In aHUS there is a clear benefit for the individual patients

It will provide you **understanding of the aetiological factor**, which will reinforce diagnosis, provide information about prognosis and assist in therapeutic decisions, including long-term treatment.

Not needed to start treatment; If you have reached a diagnosis of aHUS, initiate treatment, then consider performing a complete complement genetics and molecular analysis in your patient.

In C3G (and other complement-related diseases), currently the benefit for the individual patient is questionable.

However advances in understanding of the pathology have emerged from these analysis.

Clear benefit for the disease cohort as a whole

Goodship et al., Atypical hemolytic uremic syndrome and C3 glomerulopathy: Conclusions from a KDIGO Controversies Conference Kidney International (2016)

Genetic testing and molecular analyses

The minimum set of genes that should be screened in aHUS includes *CFH, CD46, CFI, C3, CFB, THBD, CFHR1-5* and *DGKE*.

Because of the frequent concurrence of genetic risk factors in aHUS, this analysis should also include genotyping for the risk haplotypes *CFH-CFHR3* and *MCPggaac*.

Genetic analyses must include suitable technologies to detect copy number variation, hybrid genes and other complex genomic rearrangements in the *CFH/CFHRs* genomic region.

DNA sequencing

NGS analysis (We use a in house NGS aHUS/C3G panel (40 genes/Nestera/Illumina/160bp/1500x).
Sanger sequencing.

CNV analyses

MLPA (We use commercial and custom developments).
NGS (OncoCNV, Nextgene).
CGH arrays (We developed one for 1q32 with high density probes).

Laboratory analyses

Protein levels
Auto antibodies
Functional analyses

Goodship et el. Kidney Int. 2017 91:539-551

Understanding genetic variants

Current level of knowledge allow experts to interpret most new genetic changes as pathogenic or not.

Expert laboratories that interpret the genetic results perform additional analyses to assist this interpretation.

Molecular pathogenicity *vs* functional alteration relevant to the pathology

Genetic variants are classified as ‘benign,’ ‘likely benign,’ ‘variant of uncertain significance (VUS),’ ‘likely pathogenic,’ or ‘pathogenic’ following international guidelines.

In aHUS, pathogenic variants specifically impair the capacity to protect host endothelial cells and platelets from complement damage.

We need to know whether a variant is pathogenic and relevant to the pathology

Conclusions I

- Genetics analyses reveal a crucial role of complement in aHUS, C3G and IgAN

- From a pathogenic point of view aHUS is very homogeneous. All pathogenic variants associated with aHUS (including those in the CFHRs) specifically impair the capacity to protect host endothelial cells from complement damage.
  - Underlying causes of secondary TMA are triggering factors of aHUS in carriers of complement pathogenic variants.
  - Eculizumab efficiently blocks C5 activation preventing endothelial damage independently of the complement gene mutated.
  - The overall individual predisposition to aHUS influences disease progression, responses to therapies and recurrences after transplantation. The genetic makeup also influences recurrences after eculizumab discontinuation.

- Genetic and molecular analyses will provide understanding of the aetiological factor, which will reinforce diagnosis, prognosis and assist in therapeutic decisions, including long-term treatment.

Conclusions II

- Genetics explain roughly ¼ of C3G cases and suggests that pathogenic mechanisms in C3G are complex and heterogeneous
- In some C3G cases, associated with FH and C3 mutations, or with C3Nef, the pathogenic mechanism likely involves massive activation of C3 in plasma.
- In other C3G cases a potentially undesired FH/FHRs competition has been identified. This alternative pathogenic mechanism likely involves uncontrolled complement activation at the GBM.
- Severe, progressing cases of IgAN may involve an potentially undesired FH/FHRs competition with the consequence of a complement-related TMA.
- Currently, the benefit of genetic analyses for C3G and IgAN patients is questionable. However, important advances in understanding these pathologies have emerged from these analysis.

CSIC

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COMPLEMENTO

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