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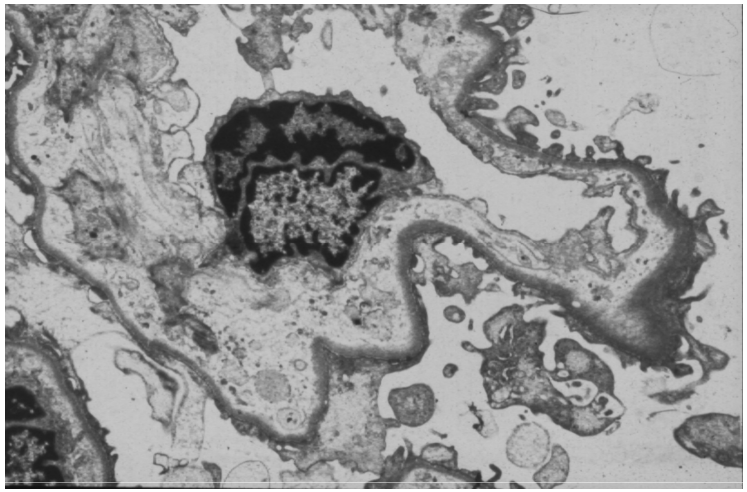
Unravelling the pathophysiology of HUS in light of recent discoveries on complement activation

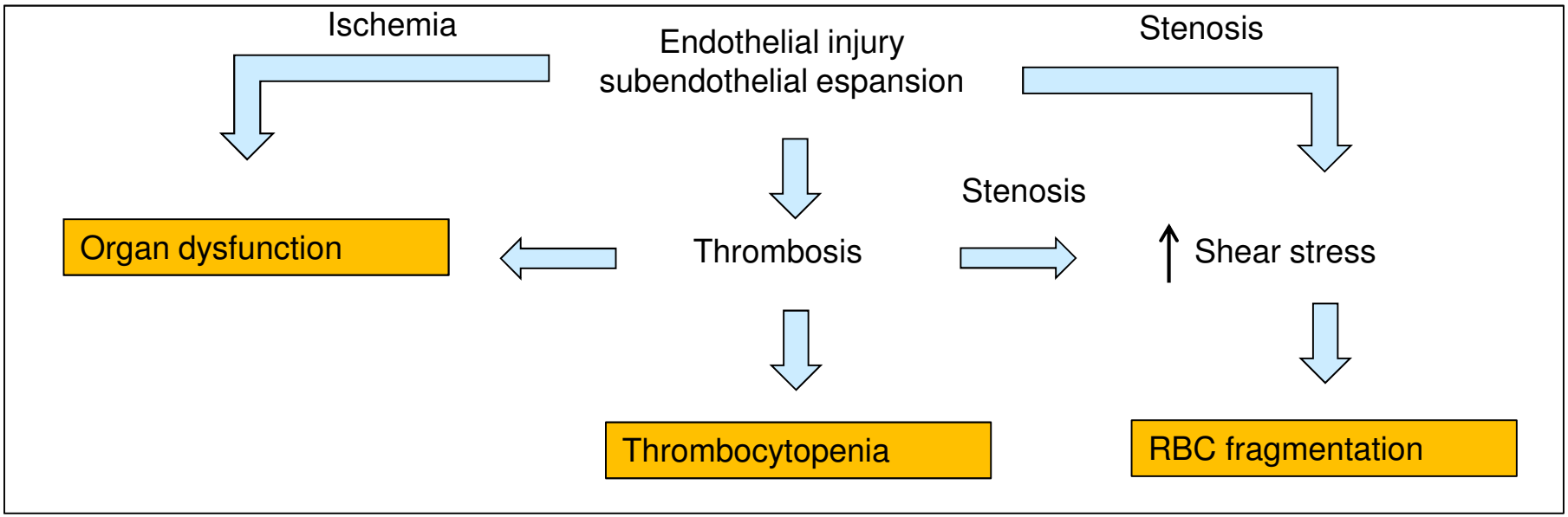
Marina Noris

November 13, 2018

THROMBOTIC MICROANGIOPATHY

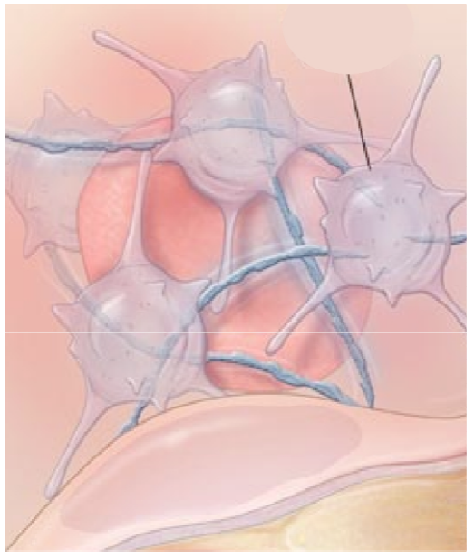
Histology lesions: Swelling and detachment of endothelial cells, accumulation of fluffy material in the subendothelium, thrombi and obstruction of the vessel lumina.





Ruggenenti and Remuzzi, *Kidney Int,* 2001

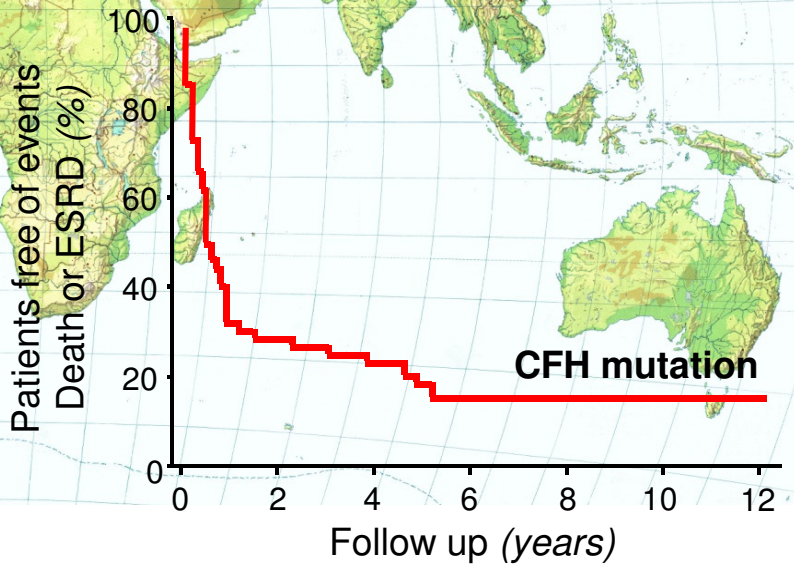
dHUS	**Stx**	*Adhesion molecules and P-selectin up-regulation*
aHUS	**Gene mutation**	*Defective complement regulation*
TTP	**ADAMTS13**	*UL-VWF multimers*
sTMA	**Autoimmune or systemic diseases, glomerulopathies, tx, infections**	*Complement activating conditions*



Microthrombi

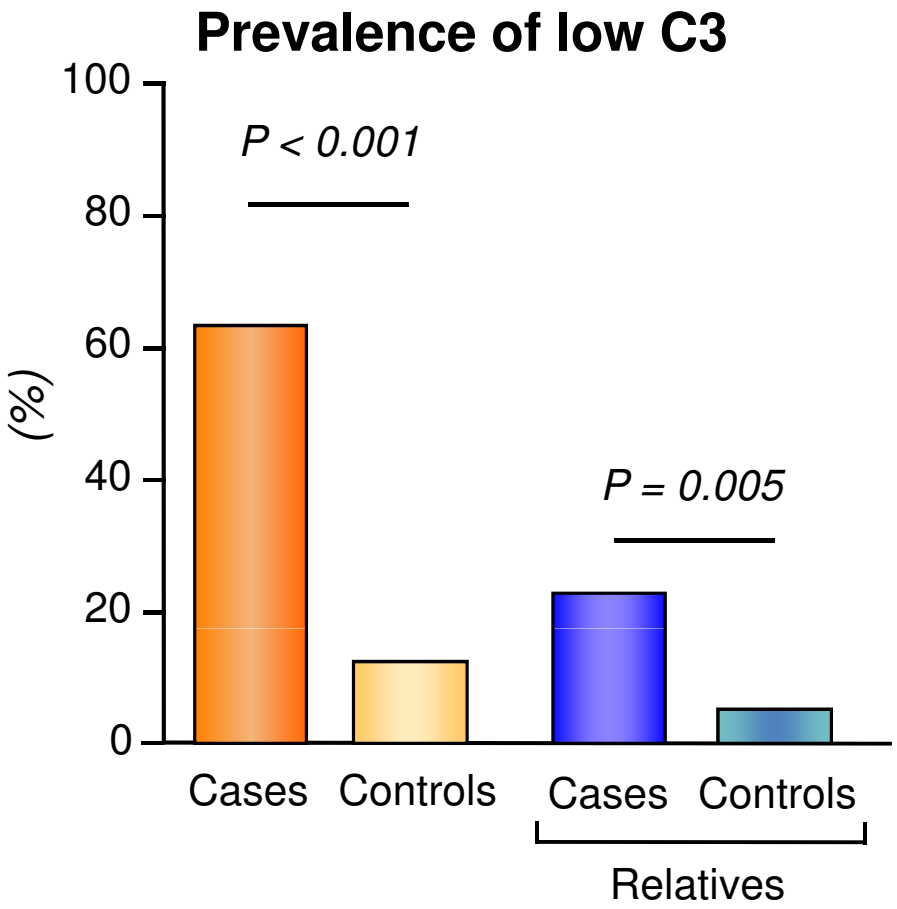
ATYPICAL HEMOLYTIC UREMIC SYNDROME

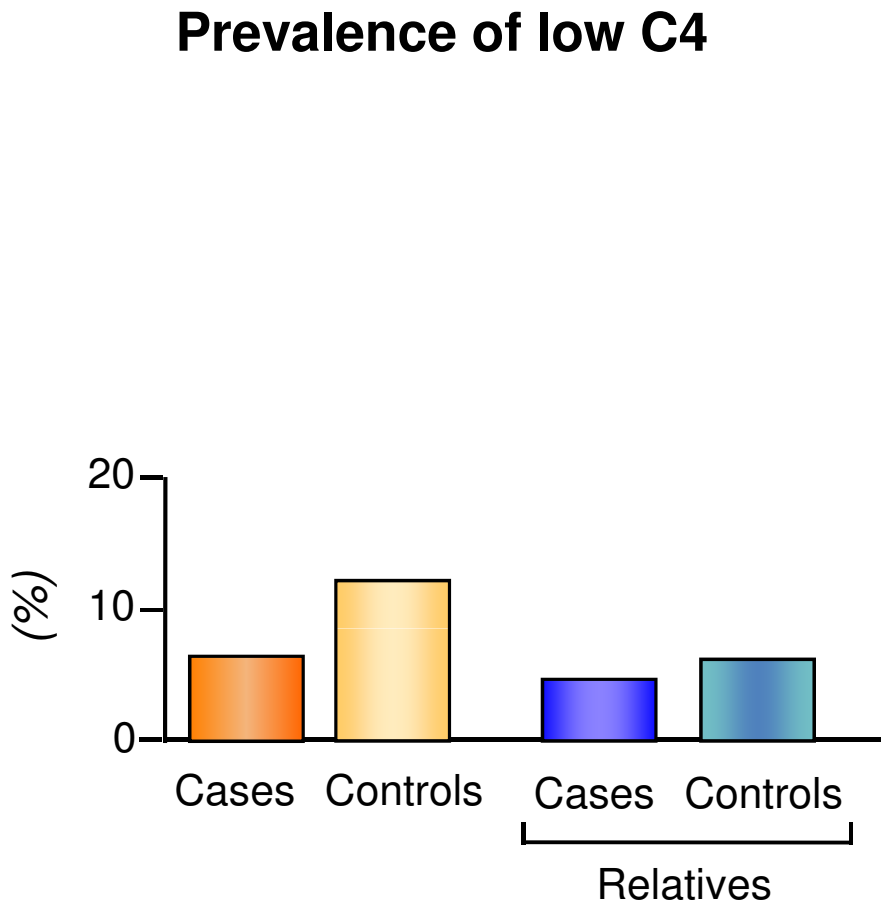
Ryan



aHUS
Incidence:
0.5-2/1,000,000/year

Sex: no difference

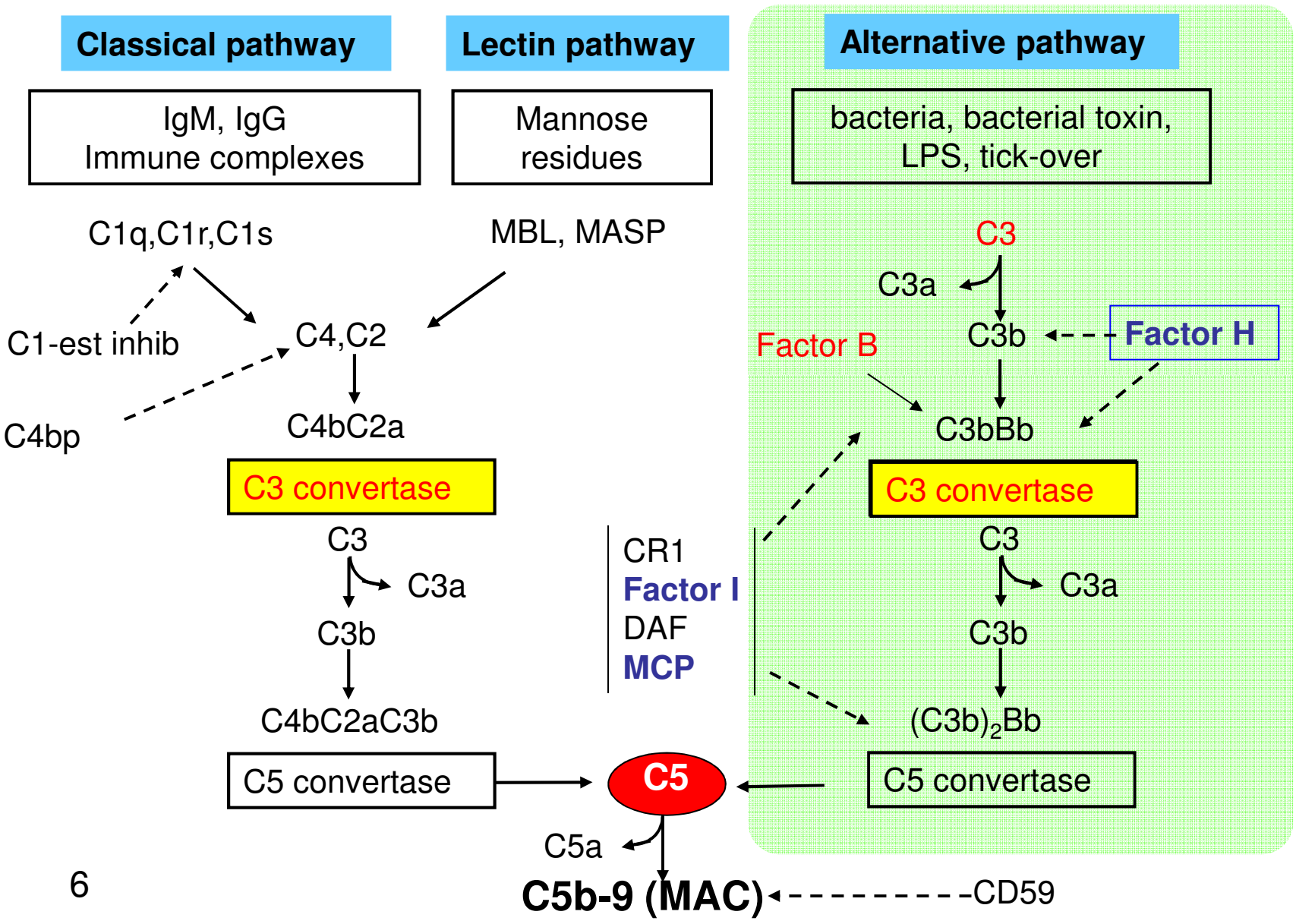




Within families, subjects with lower than normal C3 serum levels had a relative risk of HUS of 16.5 as compared to subjects with normal C3 levels

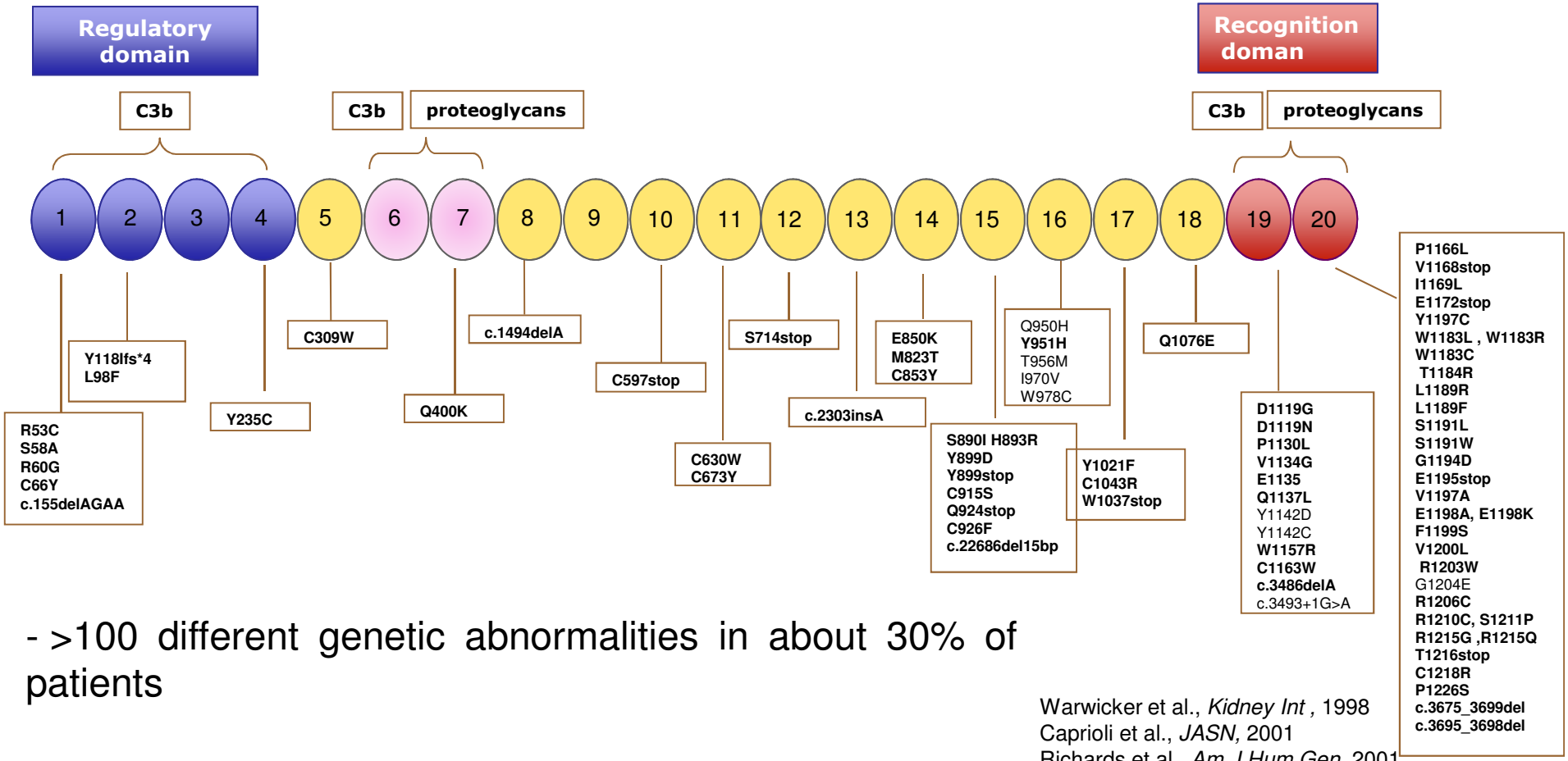
Noris et al., *J Am Soc Nephrol,* 1999

COMPLEMENT ACTIVATION PATHWAYS



Factor H plays a pivotal role in the regulation of the alternative pathway of complement activation

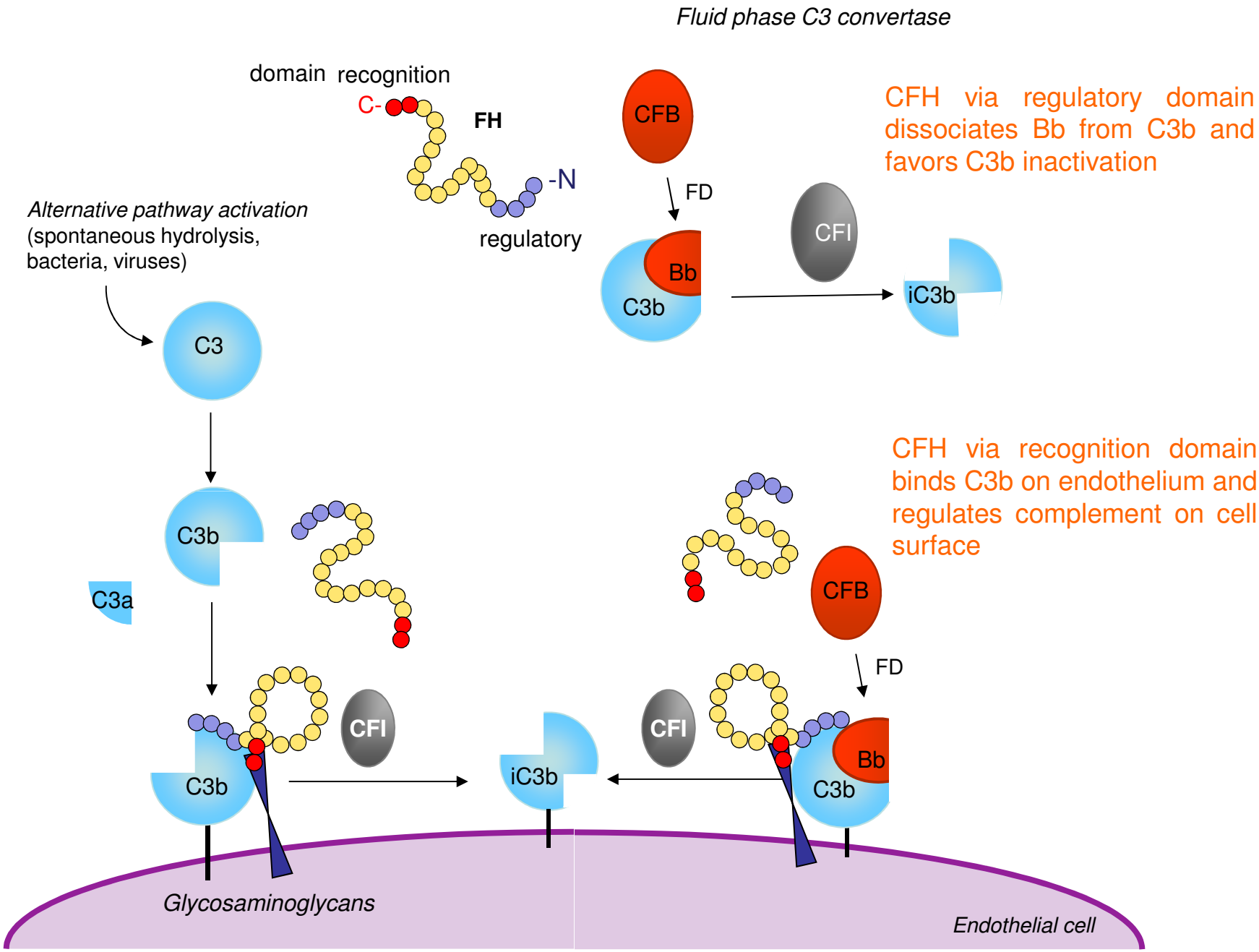
Produced mainly in the liver as a single peptide glycoprotein, factor H circulates in plasma at a concentration of 50 mg/dl



- >100 different genetic abnormalities in about 30% of patients

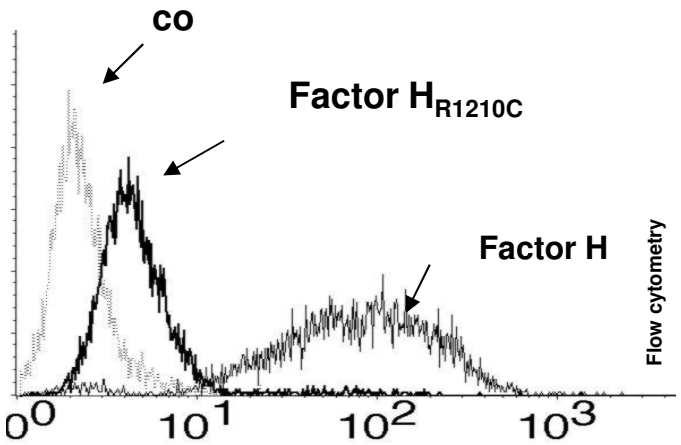
- 90% are heterozygous, most in the recognition domain

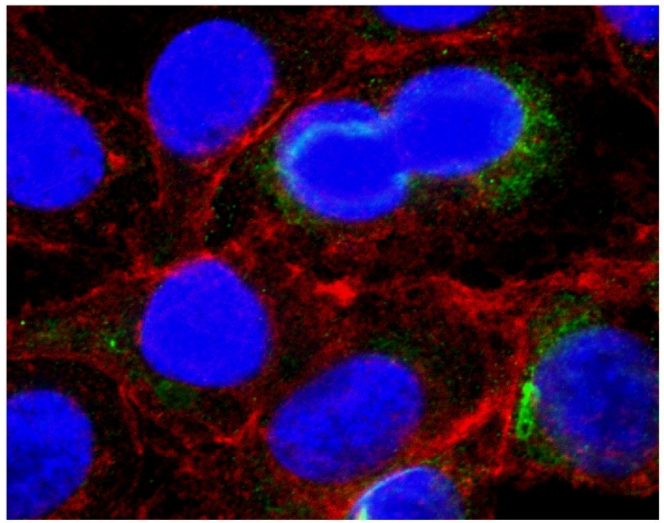
Warwicker et al., *Kidney Int* , 1998
Caprioli et al., *JASN,* 2001
Richards et al., *Am J Hum Gen,* 2001
Perez-Caballero et al., *Am J Hum Gen,* 2001
Dragon-Durey et al., *J Am Soc Nephrol,* 2004
Caprioli et al., *Blood*, 2006
Noris et al, CJASN 2010


Fluid phase C3 convertase
domain recognition
C-
FH
-N
regulatory
CFB
FD
CFI
Bb
C3b
iC3b
CFH via regulatory domain dissociates Bb from C3b and favors C3b inactivation
Alternative pathway activation
(spontaneous hydrolysis,
bacteria, viruses)
C3
C3b
C3a
CFH via recognition domain binds C3b on endothelium and regulates complement on cell surface
CFB
FD
CFI
C3b
iC3b
CFI
Bb
C3b
Glycosaminoglycans
Endothelial cell

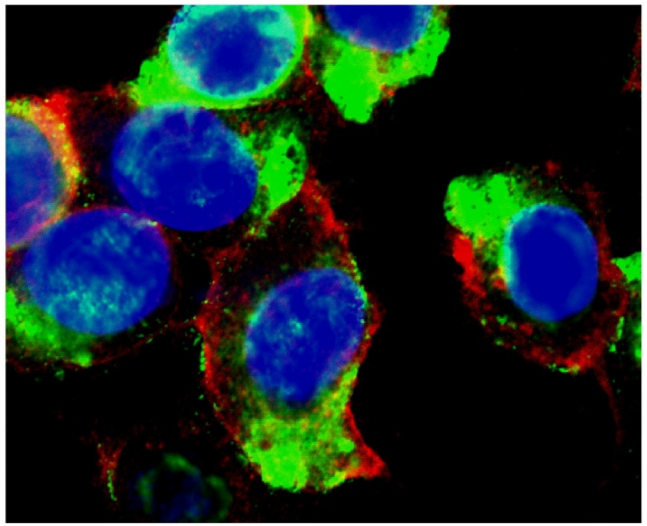
SINGLE AMINO ACID CHANGES IN SCR 20 OF FACTOR H AFFECT ENDOTHELIAL CELL BINDING

HUVEC incubated with recombinant wild type or mutated factor H stained with fluorescinated anti-factor H antibody and analyzed by FACS





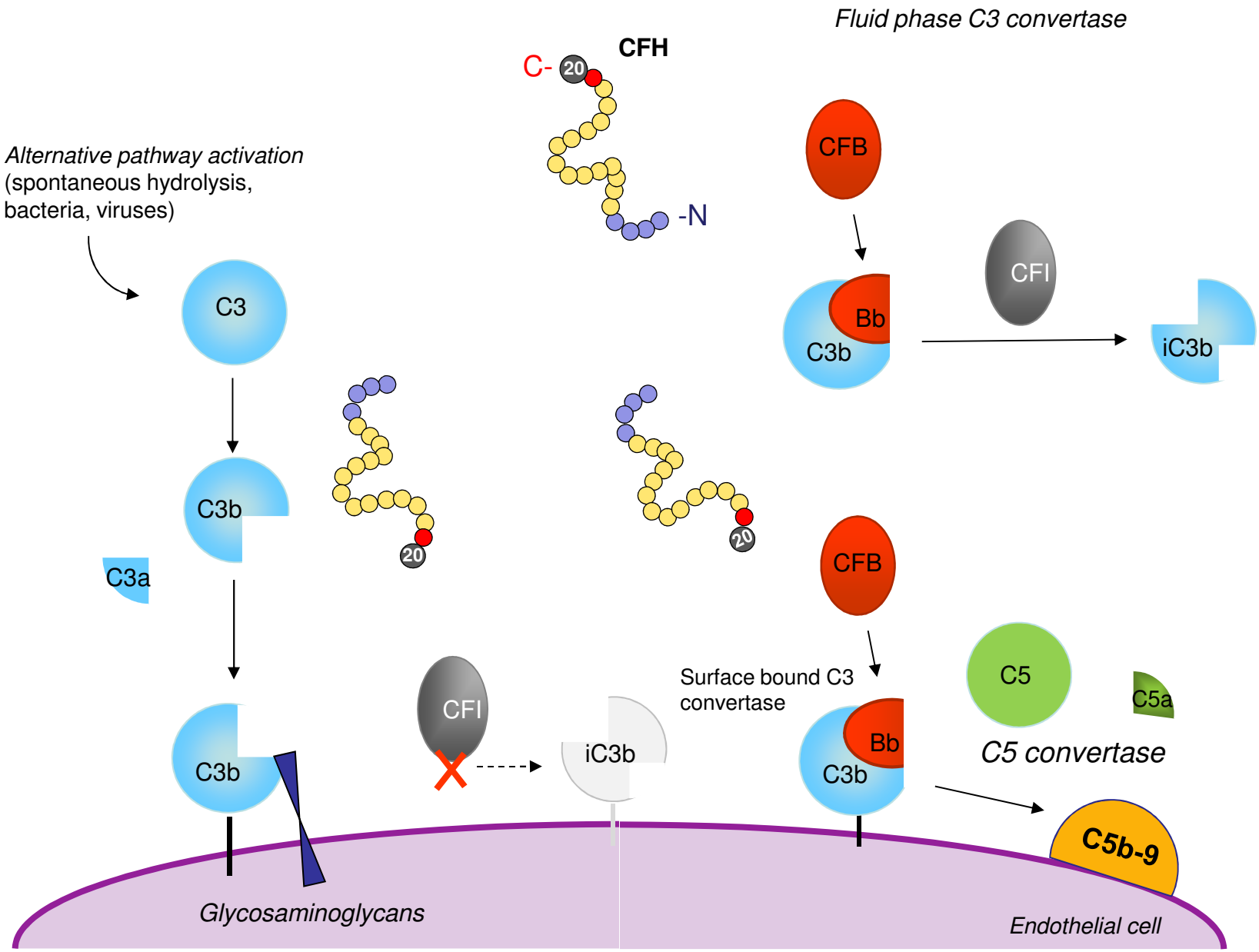
Factor H R1210C



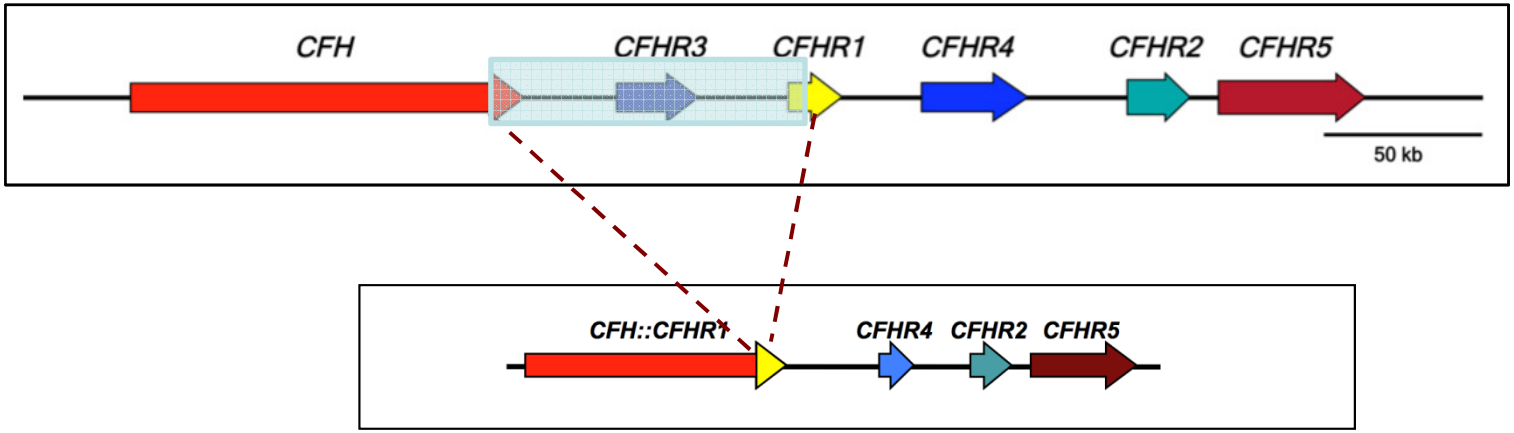
WT Factor H

Manuelian et al, *J Clin Invest,* 2003

CONSEQUENCES OF CFH MUTATIONS



HIGH HOMOLOGY IN THE REGULATORS OF COMPLEMENT ACTIVATION GENE CLUSTER



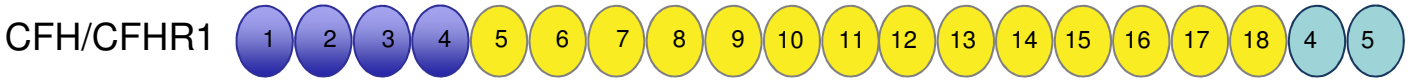
***CFH/CFHR1* hybrid gene**

- High degree of sequence identity between the gene for factor H and the genes for the five factor H-related proteins (CFHR1 to 5) which favors non-allelic homologous recombinations giving rise to hybrid genes.

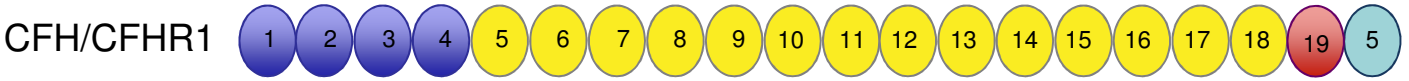
- Copy number variation assays are required to detect hybrid genes

Venables et al., Plos Medicine 2006
Valoti et al, JASN 2015

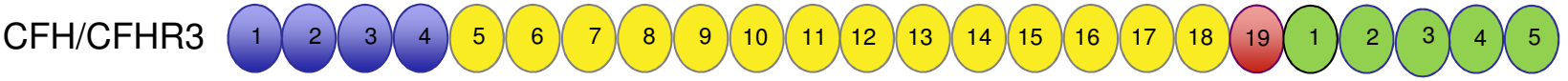
CFH/CFHR HYBRID PROTEINS FOUND IN PATIENTS WITH aHUS



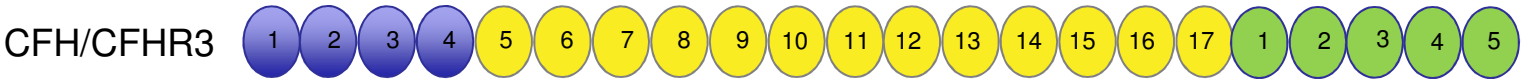
Venables et al., PLoS Med 2006



Valoti et al., *JASN*, 2015



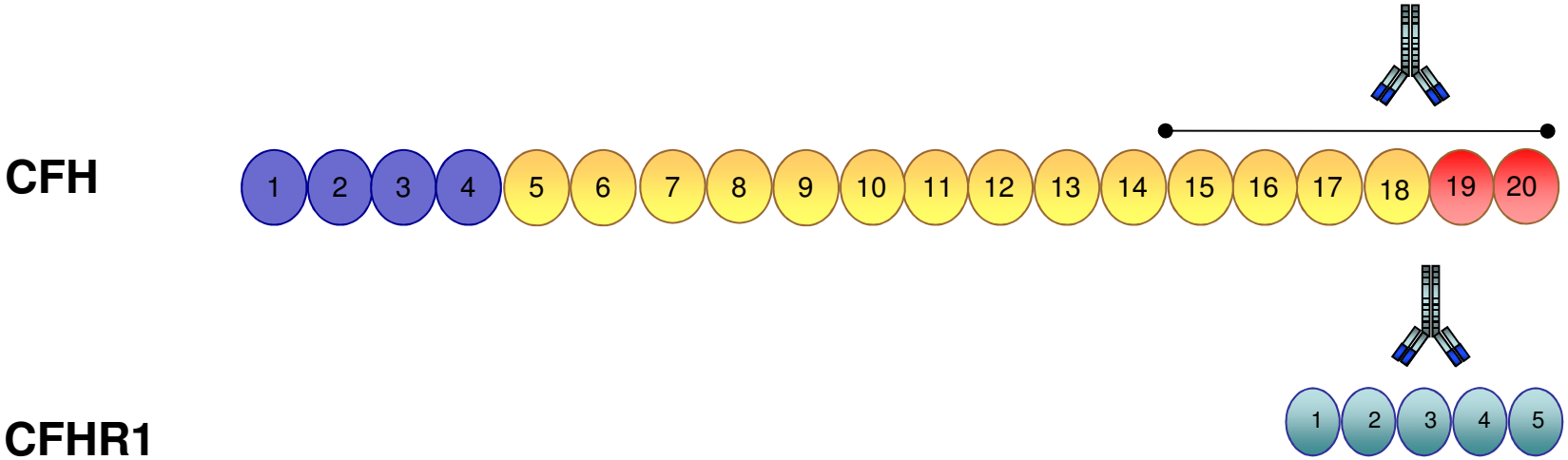
Francis et al., *Blood*, 2012



Challis et al., *Blood*, 2015

In the CFH/CFHR hybrid molecules the C-terminal SCRs of CFH are substituted with those of CFHR1 or with the entire CFHR3, resulting in decreased complement regulatory activity on endothelial cell surface

HOMOZYGOUS DELETION OF CFHR1-CFHR3 GENES IS ASSOCIATED WITH FORMATION OF ANTI-CFH AUTOANTIBODIES



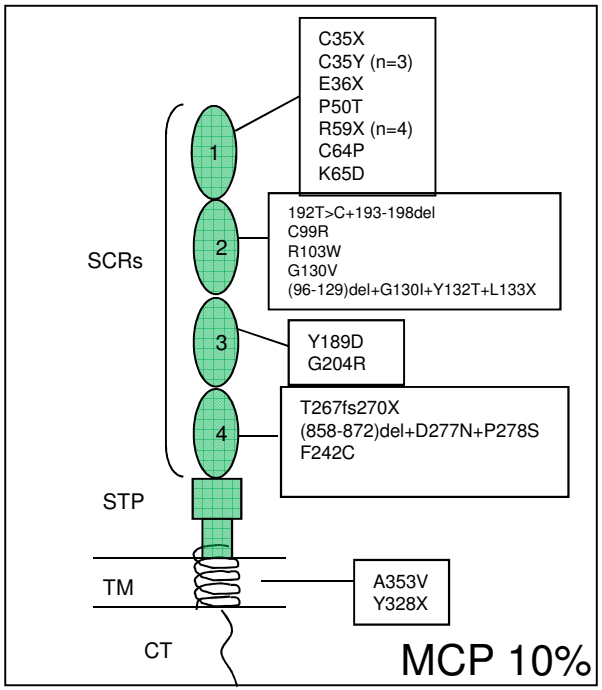
Blanc et al., *J Immunol,* 2012

- Eight percent of patients (mostly children) develop anti-CFH autoantibodies
- Most patients with aHUS and CFH autoantibodies are homozygous for a deletion of genes encoding CFH related proteins 1 and 3

Jozsi et al, Blood, 2008
Moore et al, Blood 2010

Failure of central and/or peripheral tolerance to FH related 1 in subjects completely lacking CFHR1?

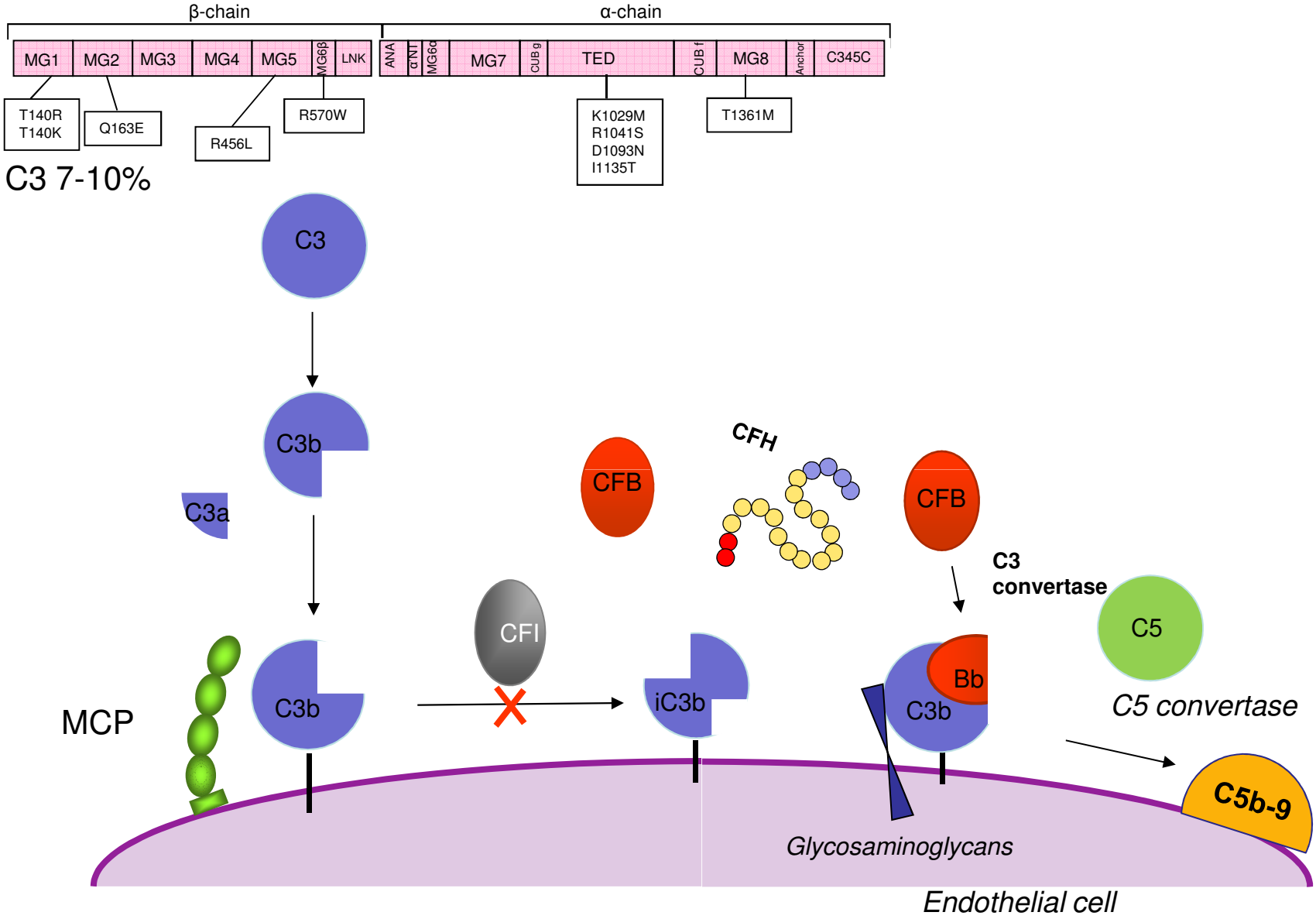
MCP VARIANTS IN aHUS



Loss of function heterozygous variants: low expression or reduced C3b binding and cofactor activity

Noris et al, Lancet 2003
Richards et al, PNAS 2003
Caprioli et al, Blood 2006

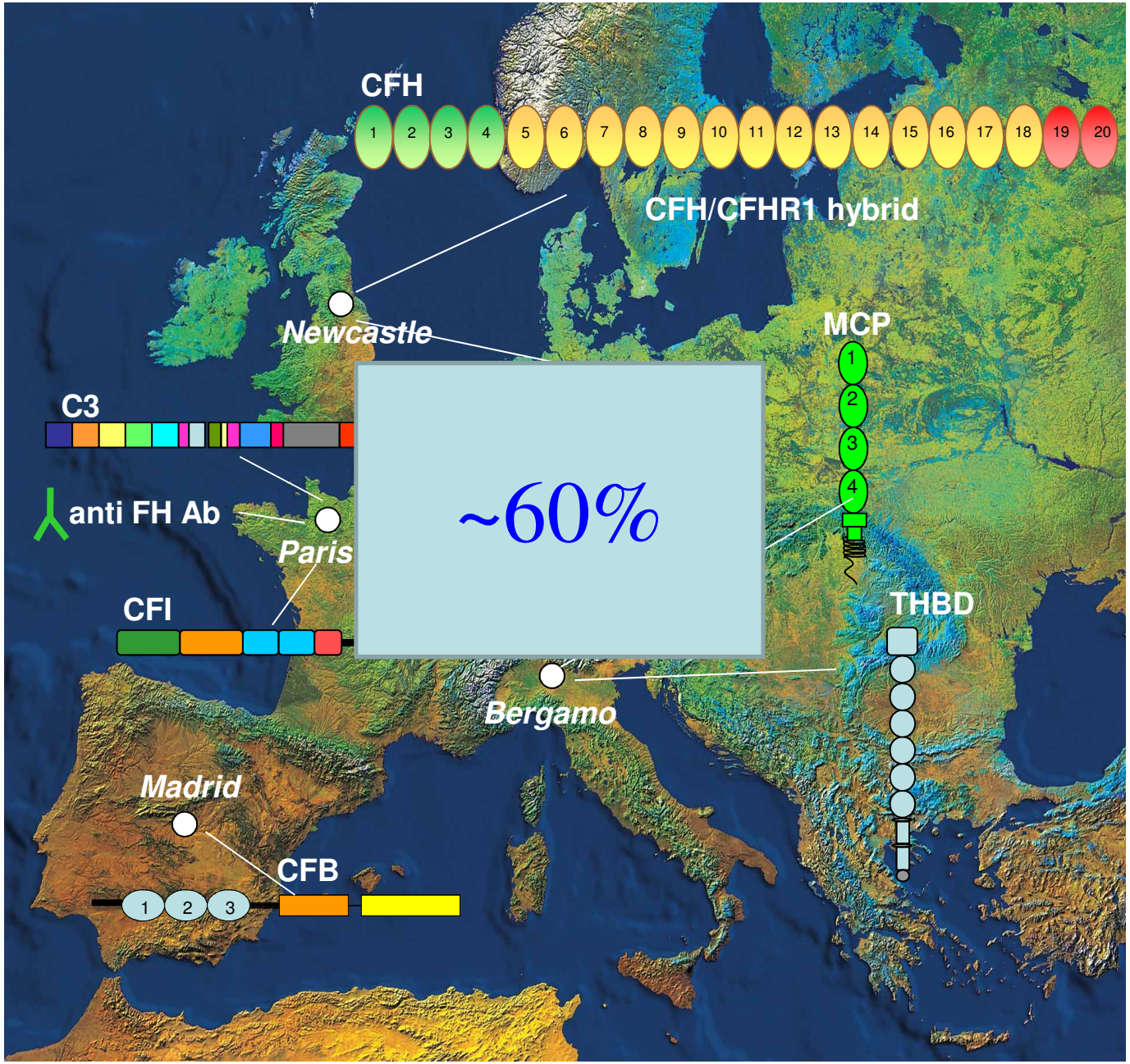
C3 IN aHUS



Gain of function C3 mutations: reduced binding to MCP and CFH , increased affinity to CFB

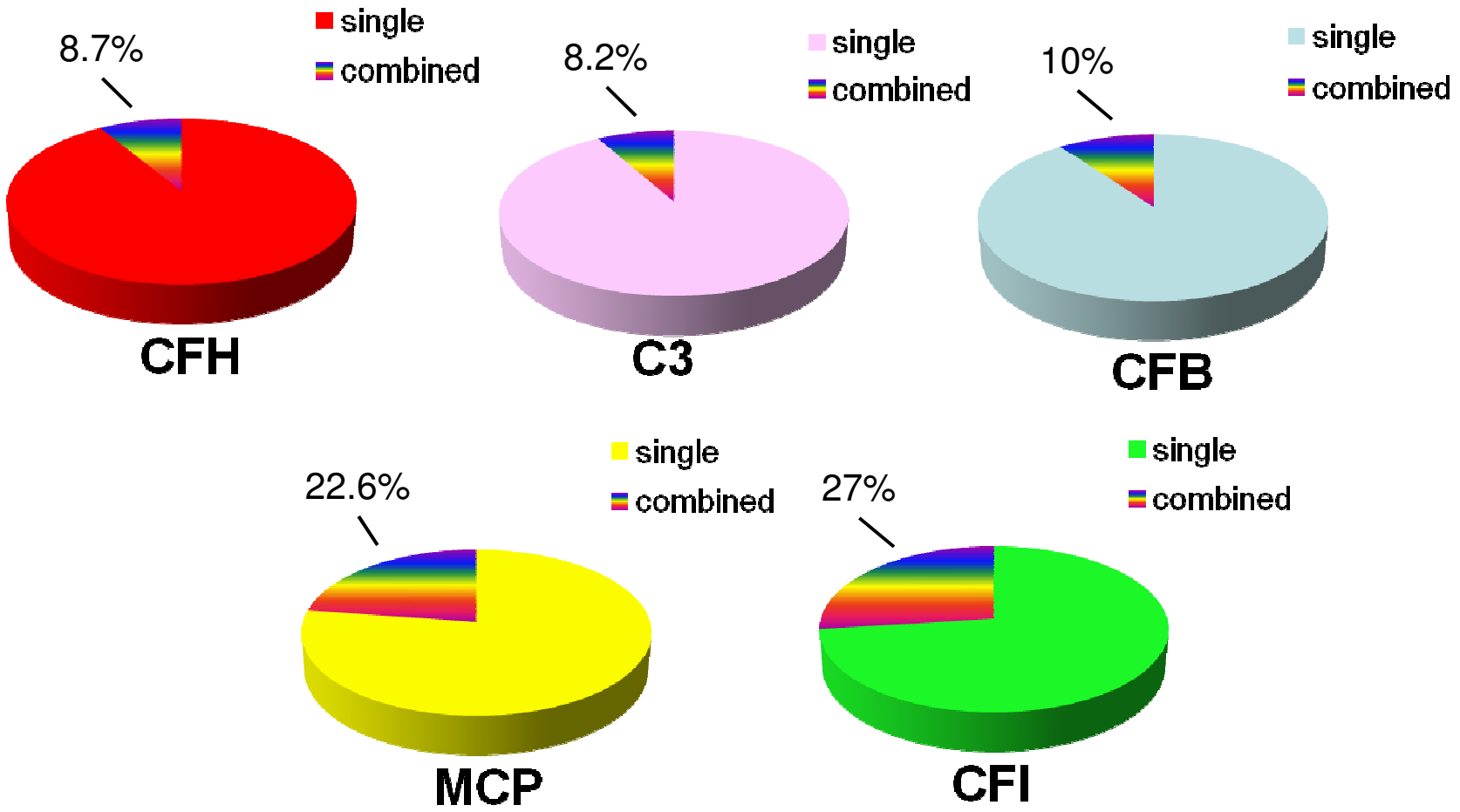
Goicoechea et al., *PNAS,* 2007
Roumenina et al., *Blood,* 2009
Fremeaux-Bacchi et al, Blood 2008

GENETICS OF AHUS: AN EUROPEAN DISCOVERY



COMBINED COMPLEMENT GENE ABNORMALITIES IN aHUS

795 patients from 4 European cohorts

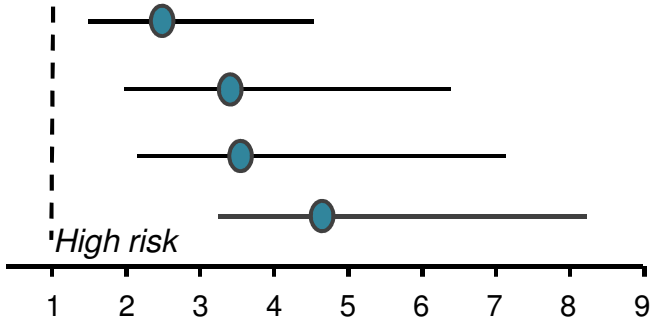


Combined complement gene abnormalities were found in about one quarter of patients with MCP or CFI mutations, and in around 10% of patients with CFH or C3 or CFB mutations

Bresin et al, JASN 2013

THREE CFH COMMON POLYMORPHISMS TAGGING THE H3 HAPLOTYPE ARE STRONGLY ASSOCIATED WITH AHUS

Genotypes	*Odds Ratios (95 % C.I.)*	*P value*
-332C/T Promoter		*0.0015*
c.2016A/G (p.Q672Q, SCR11)		*< 0.0001*
c.2808G/T (p.E936D,SCR16)		*< 0.0001*
-332T+c.2016G+p.936D		*< 0.0001*

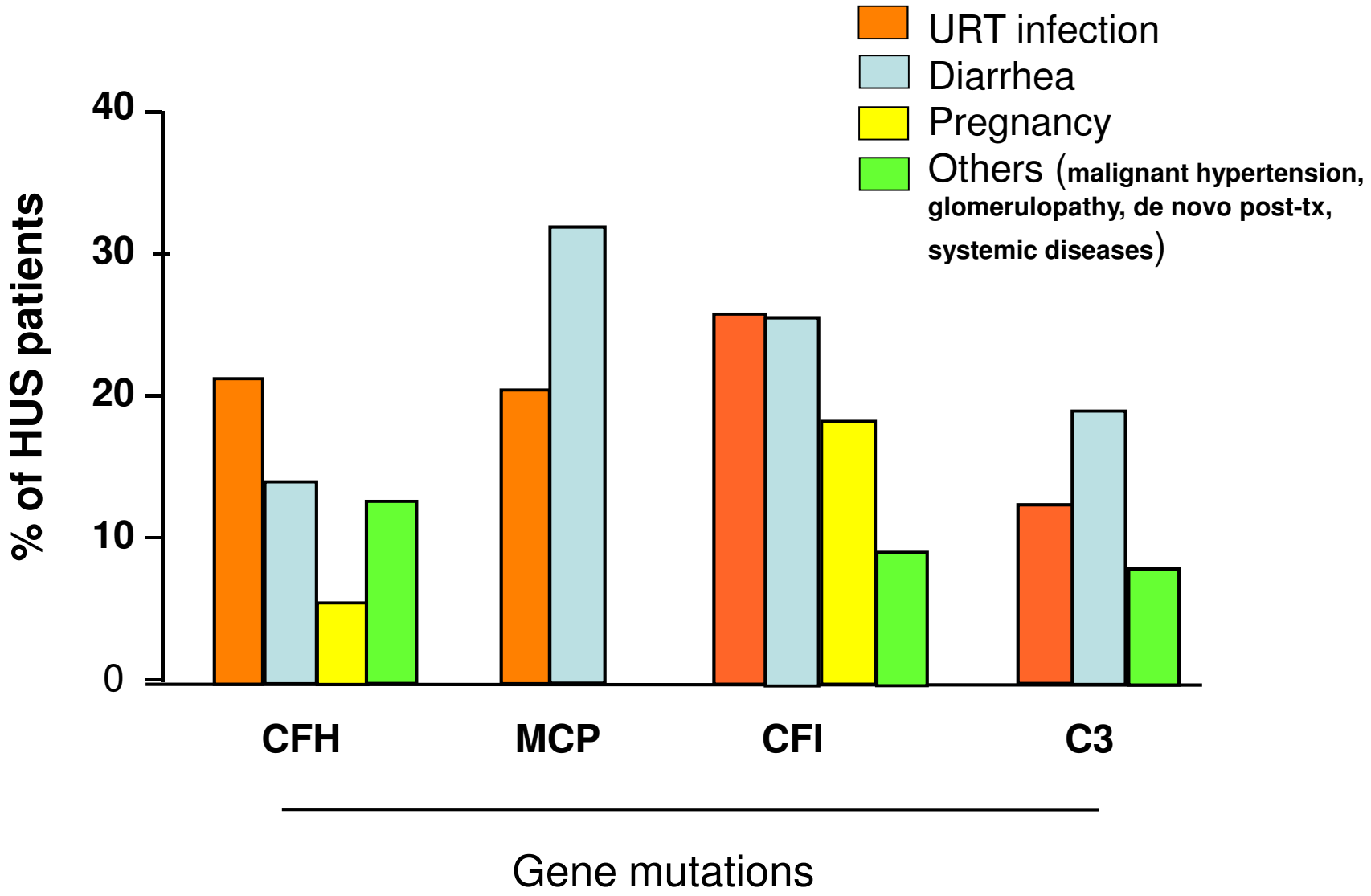


Caprioli et al., *Hum Mol Genet,* 2003

	-332C/T Promoter (rs3753394)	c.184G/A V62I (rs800292)	c.1204T/C Y402H (rs1061170)	c.2016A/G Q672Q (rs3753396)	c.2808G/T E936D (rs1065489)	Control (n=139)	aHUS (n=94)		
						Freq.	Freq.	OR (95% CI)	P value
H1	C	G	C	A	G	0.228	0.21	-	NS
H2	C	A	T	A	G	0.266	0.10	0.30 (0.18-0.53)	$< 10^{-5}$
H3	T	G	T	G	T	0.192	0.31	1.89 (1.23-2.91)	0.0039
H4	C	G	T	A	G	0.167	0.17	-	NS
H5	T	G	C	A	G	0.075	0.12	-	NS
H6	C	G	T	G	T	0.039	0.02	-	NS

Pickering et al., *J Exp Med,* 200

TRIGGERING /UNDERLYING CONDITIONS



Noris et al, CJASN 2010

Trigger

C3

C3b

C3a

CFH

CFI

CFB

C3 convertase

C3

TM

C3b

MCP

Bb

C3b

Bb

C3b

C5 convertase

C5

C5b

C5a

Eculizumab

A humanized monoclonal antibody that binds to C5

PMP

CD40L

TM shedding

Coagulation

Platelet aggregation

C5b-9

TM

VWF

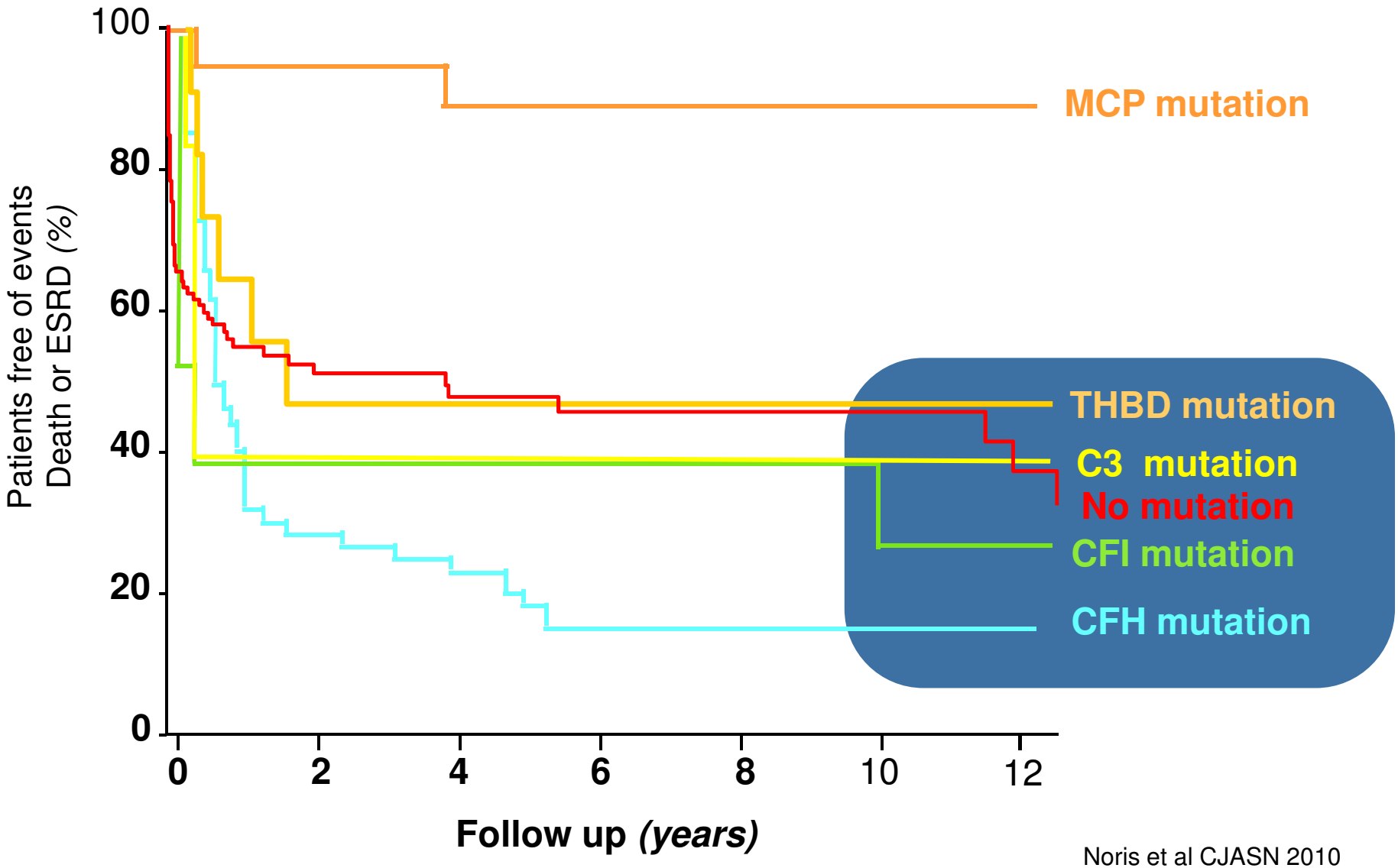
P-sel

Endothelial cell

Endothelial cell

Modified from Noris and Remuzzi, NEJM 2009

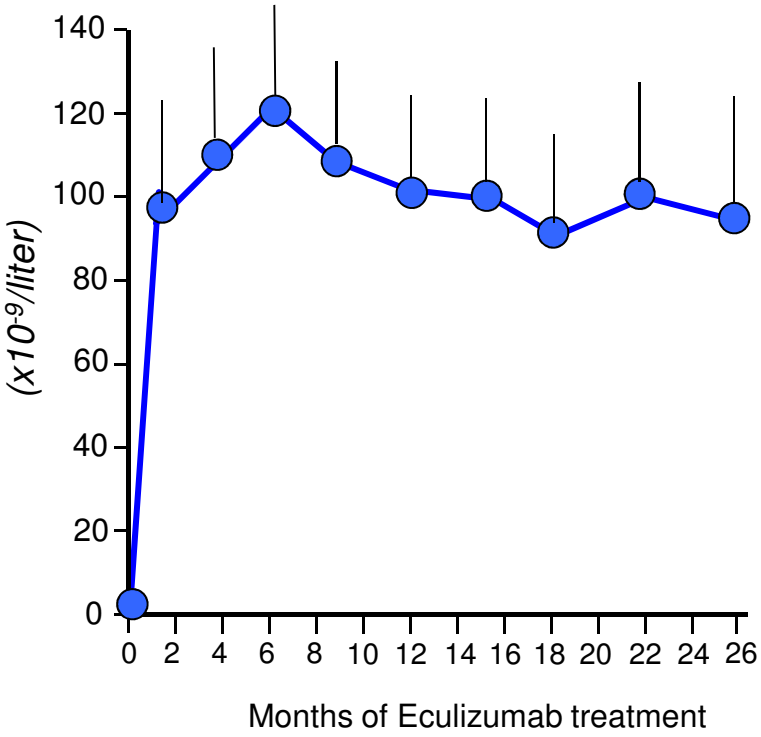
LONG TERM OUTCOME OF aHUS PATIENTS



Noris et al CJASN 2010

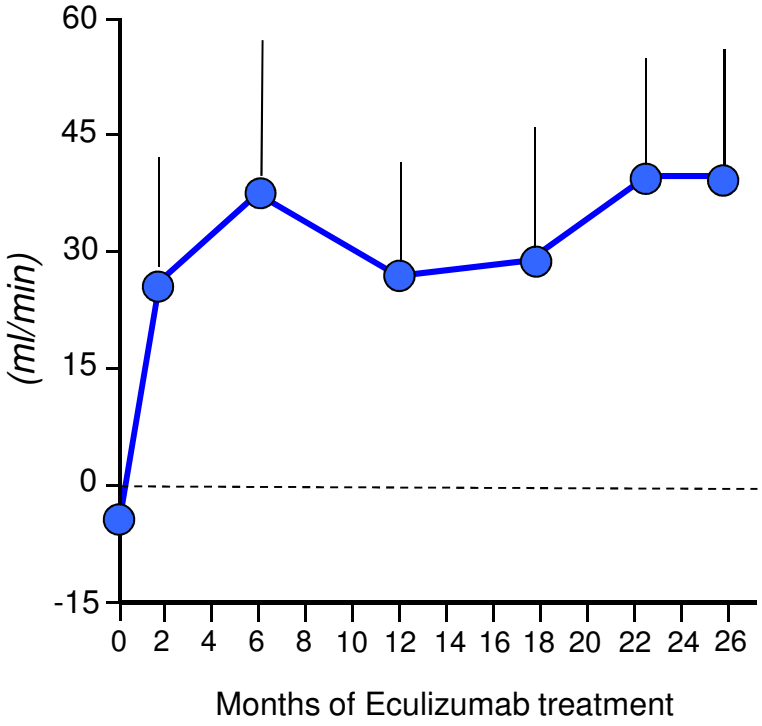
Platelet count

mean change from baseline



Estimated GFR

mean change from baseline



Treatment effect was sustained for up to 26 months

Licht et al., *Kidney Int,* 2015

BLOOD, 11 SEPTEMBER 2014

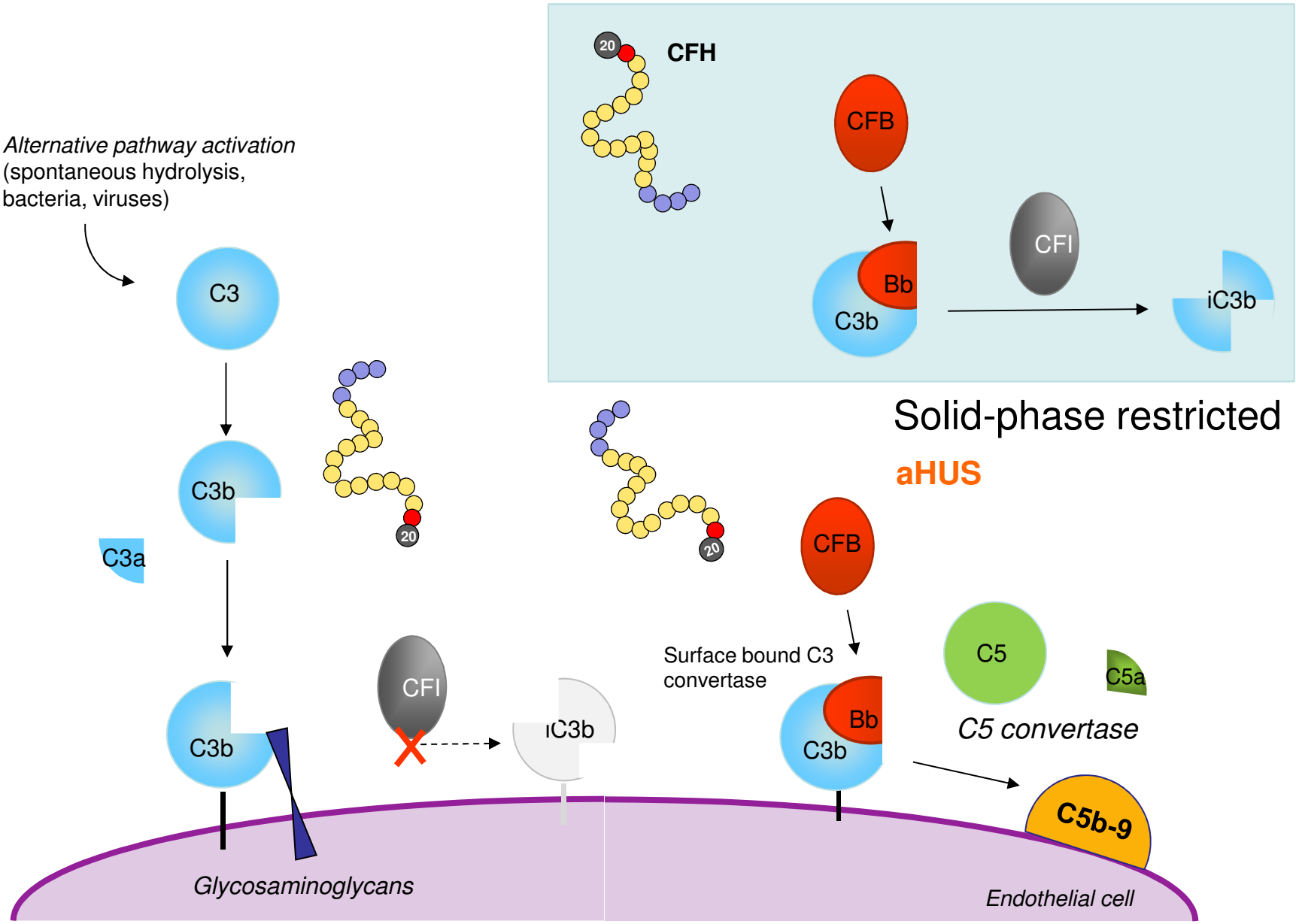
CLINICAL TRIALS AND OBSERVATIONS

Dynamics of complement activation in aHUS and how to monitor eculizumab therapy

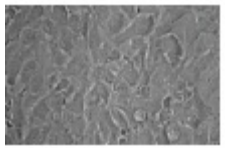
Marina Noris,[1] Miriam Galbusera,[1] Sara Gastoldi,[1] Paolo Macor,[2] Federica Banterla,[1] Elena Bresin,[1] Claudio Tripodo,[3] Serena Bettoni,[1] Roberta Donadelli,[1] Elisabetta Valoti,[1] Francesco Tedesco,[4] Alessandro Amore,[5] Rosanna Coppo,[5] Piero Ruggenenti,[6] Eliana Gotti,[6] and Giuseppe Remuzzi[1,6]

Both during the acute phase of the disease and at remission about half of aHUS patients had normal serum C3 and plasma sC5b-9 levels

SOLID-PHASE RESTRICTED COMPLEMENT ACTIVATION IN aHUS



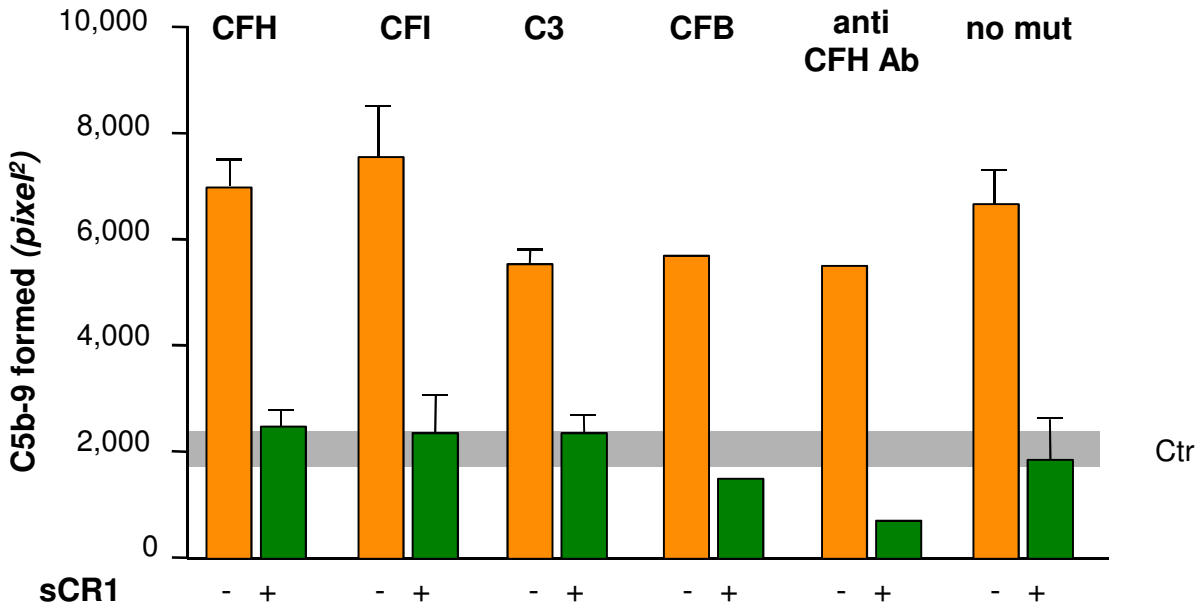
SERUM FROM aHUS PATIENTS CAUSES C5b-9 FORMATION ON *ACTIVATED* HMEC-1 REGARDLESS ON MUTATION STATUS



HMEC-1 — ADP 10µM — *10 min* — Static incubation — *4 hours* → ***C5b-9 deposition***

-Control or aHUS serum
(n=29, in remission no eculizumab)
(n=7 in acute phase)

- anti-C5b-9 Ab staining
- Confocal microscopy



Excessive C5b-9 deposits were observed with serum from all aHUS patients (with or without identified complement abnormalities) during acute or remission phase.

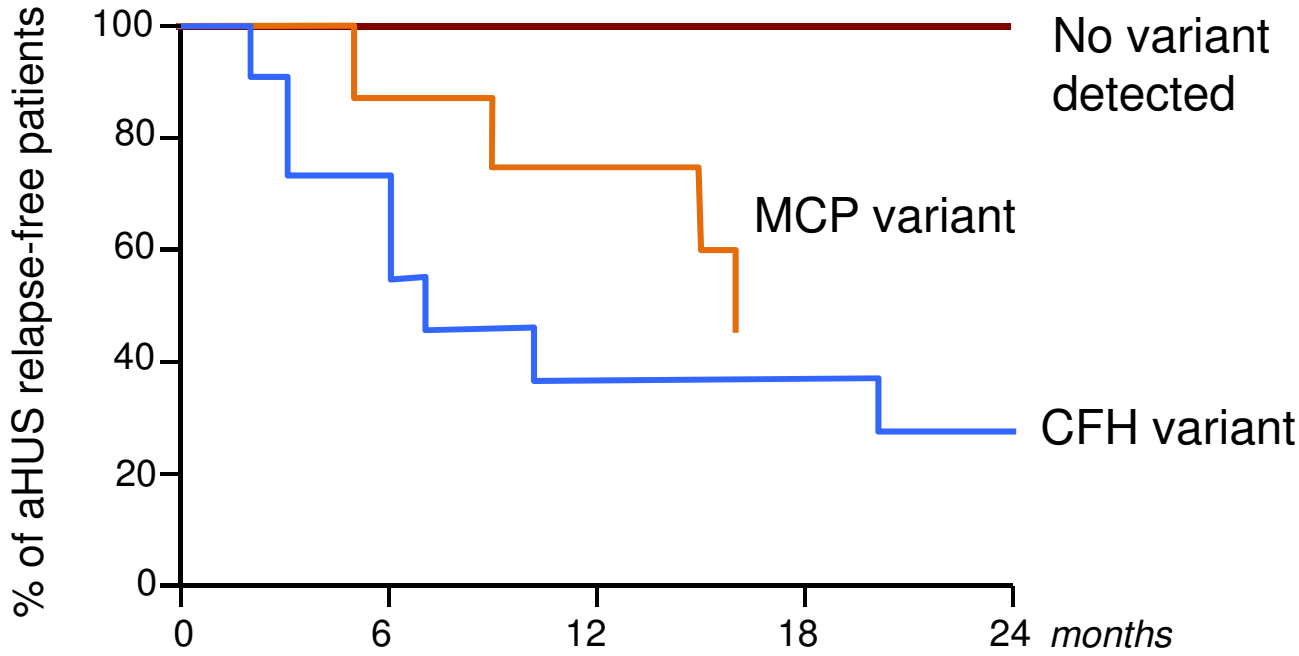
Noris et al., *Blood*, 2014

460.000

Every 15 days forever?

RISK OF ATYPICAL HEMOLYTIC UREMIC SYNDROME RELAPSE AFTER ECULIZUMAB DISCONTINUATION

38 patients (24 women - 9 children and 29 adults)



Fakhouri et al, *CJASN*, 2017

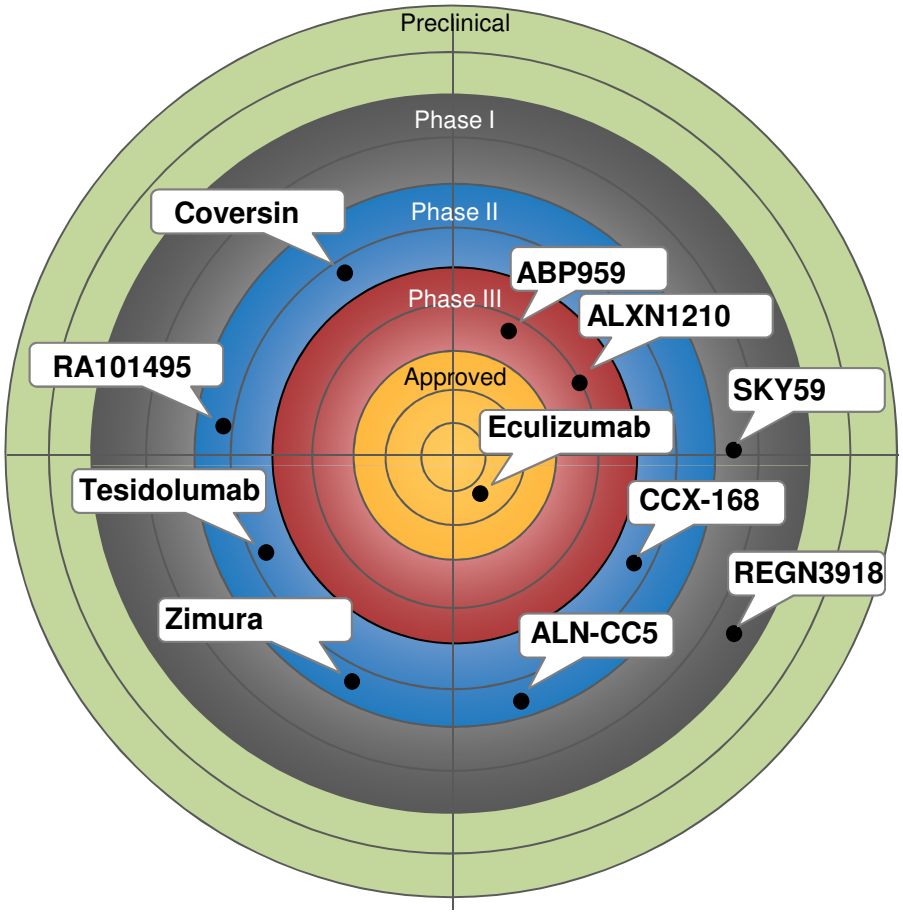
•Among 52 case reports discontinuing eculizumab 16 (31%) had a subsequent TMA. In eculizumab clinical trials 12/61 patients who discontinued eculizumab experienced 15 TMA complications.

•TMA relapse occurred irrespective of identified genetic mutations, high risk polymorphism or auto-antibodies.

Macia et al, *Clin Kidney J* , 2017

How to monitor and possibly tapering?

Terminal complement inhibitors



11 complement-targeted drug candidates in preclinical

Eculizumab: anti-C5 Ab blocks C5 cleavage *(Alexion)*

ALXN1210: Anti-C5 antibody *(Alexion)*

ALN-CC5: siRNA Targeting C5 *(Alnylam)*

CCX-168: C5aR antagonist *(Chemocentrix)*

Coversin: *Small C5 inhibitor (Akari)*

RA101495: cyclic peptide, C5 inhibitor *(RaPharma)*

Tesidolumab: Anti-C5 antibody *(Novartis)*

Zimura: aptamer-based C5 inhibitor *(Ophthotech)*

ABP959: Anti-C5 antibody *(Amgen)*

REGN3918: Anti-C5 antibody *(Regeneron)*

SKY59: Anti-C5 antibody *(Roche)*

Multiple choice questions

1) **Which of the following statements is correct? Atypical hemolytic uremic syndrome is caused by:**
a) fluid phase complement activation
b) activation of the complement classical pathway on cell surface
c) activation of the alternative complement pathway on cell surface
d) impaired complement activation

2) **Which among the following genes is <u>not</u> altered in atypical hemolytic uremic syndrome?**
a) Complement factor H (CFH)
b) ADAMTS13
c) Complement C3 (C3)
d) Membrane cofactor protein (MCP)

Multiple choice questions

3**) Which among the following approaches is correct to diagnose atypical hemolytic uremic syndrome?**

a) Exclude an ADAMTS13 deficit

b) Exclude an ADAMTS13 deficit and shiga-toxin producing bacteria

c) Wait for the results of genetic screening

d) Measure serum C3 levels

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Next webinar:

Membranous Nephropathy

Jack Wetzels (Nijmegen)

November 27, 2018

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