Genetic diagnostics

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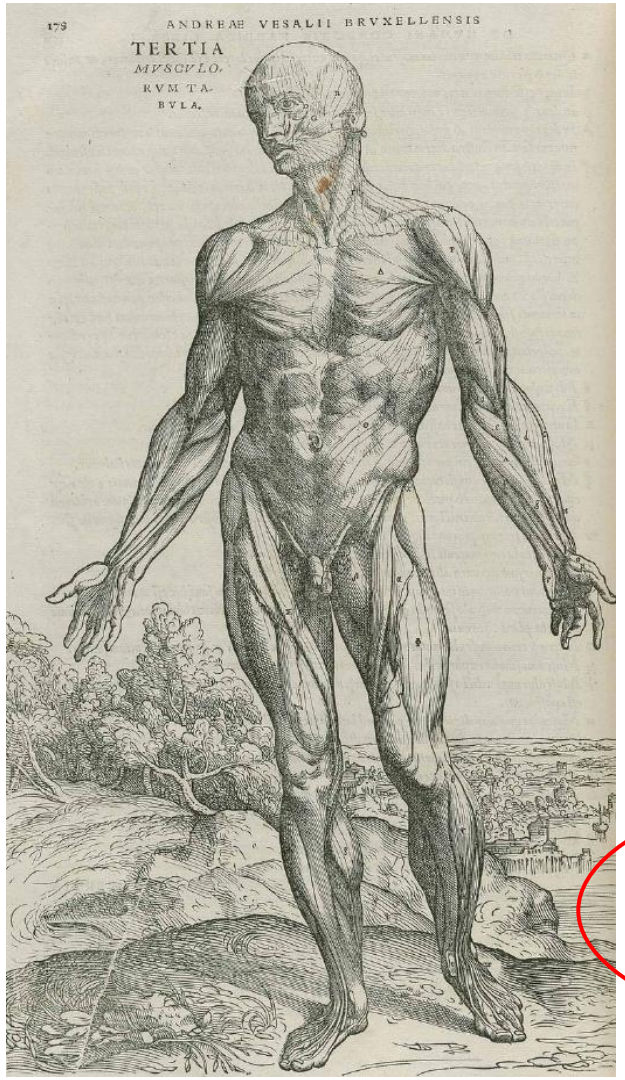
Florence, 12-14 March 2026

ISTITUTO DI RICERCHE
FARMACOLOGICHE
MARIO NEGRI · IRCCS

- **The presenter received grant/research support and/or honoraria from commercial entities:**

  - *Alexion (research grant and honoraria for educational talks)*
  - *Novartis (research grant and advisory board)*
  - *Sobi (research grant and advisory board)*
  - *Gemini (research and travel grants)*
  - *Eleva (research grant)*

DISEASES ASSOCIATED WITH COMPLEMENT DYSREGULATION



Stroke

Alzheimer's diseases

Age-related macular degeneration

Asthma

Myocardial infarction

Crohn's disease

Rheumatoid arthritis

Membranous nephropathy

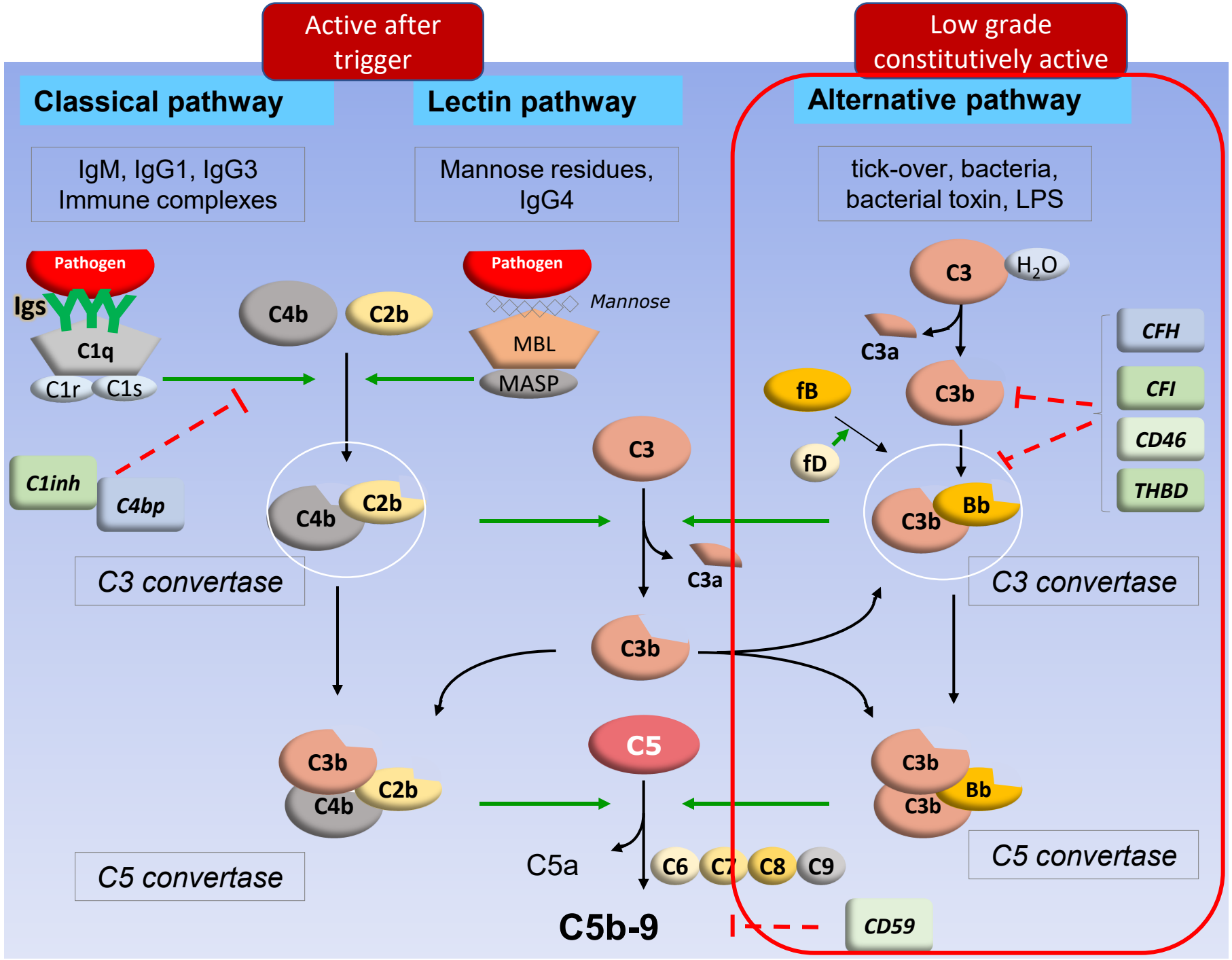
IgA nephropathy

SLE

ANCA vasculitis

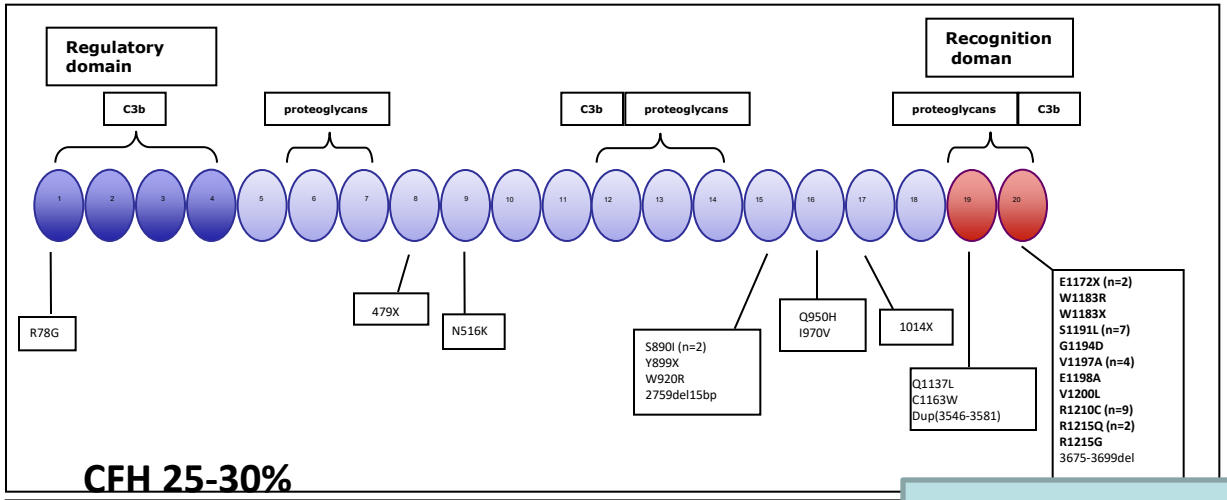
Hemolytic uremic syndrome

MPGN /C3 glomerulopathy

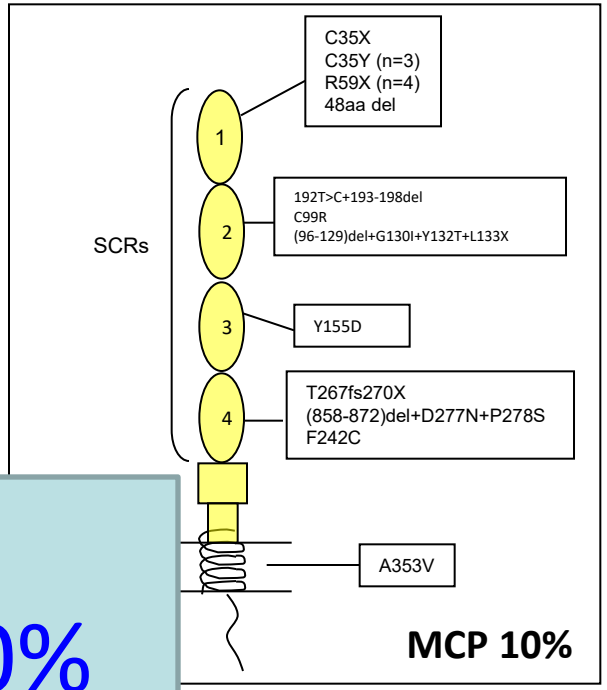

Active after trigger
Low grade constitutively active
Classical pathway
Lectin pathway
Alternative pathway
IgM, IgG1, IgG3 Immune complexes
Mannose residues, IgG4
tick-over, bacteria, bacterial toxin, LPS
Pathogen
Igs
C1q
C1r
C1s
C4b
C2b
Pathogen
Mannose
MBL
MASP
C3
H2O
C3a
C3b
fB
fD
CFH
CFI
CD46
THBD
C1inh
C4bp
C4b
C2b
C3
C3a
C3b
Bb
C3 convertase
C3 convertase
C3b
C4b
C2b
C5
C3b
Bb
C3b
C5a
C6
C7
C8
C9
C5 convertase
C5 convertase
C5b-9
CD59

COMPLEMENT GENE VARIANTS IN **aHUS** PATIENTS

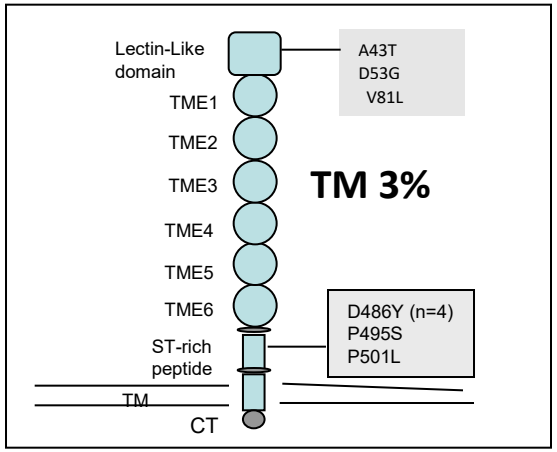
Published data



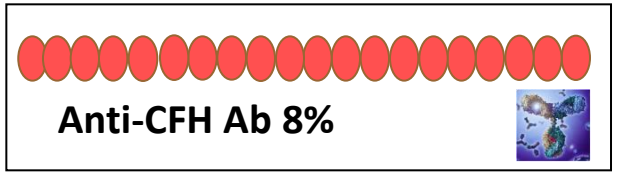
CFH 25-30%

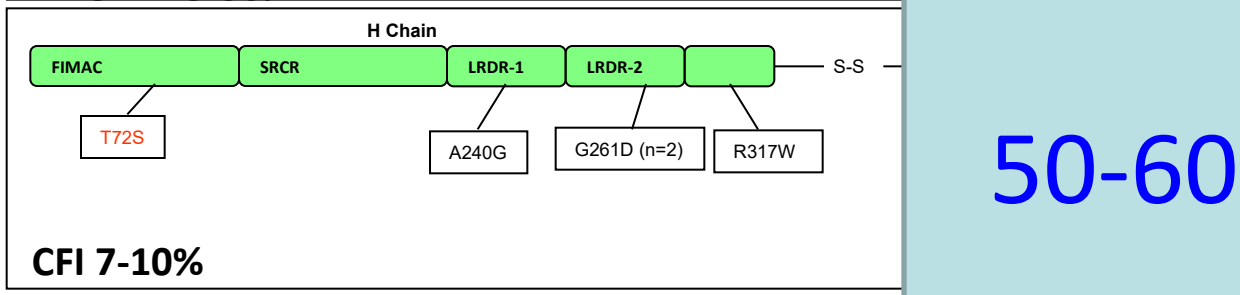


MCP 10%



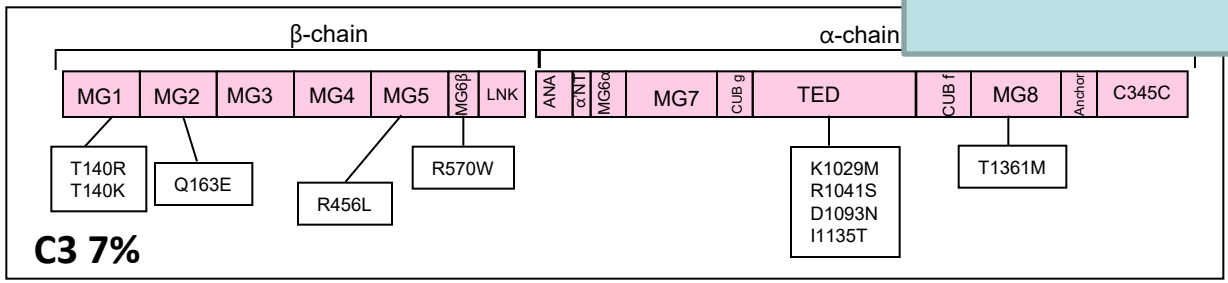
TM 3%



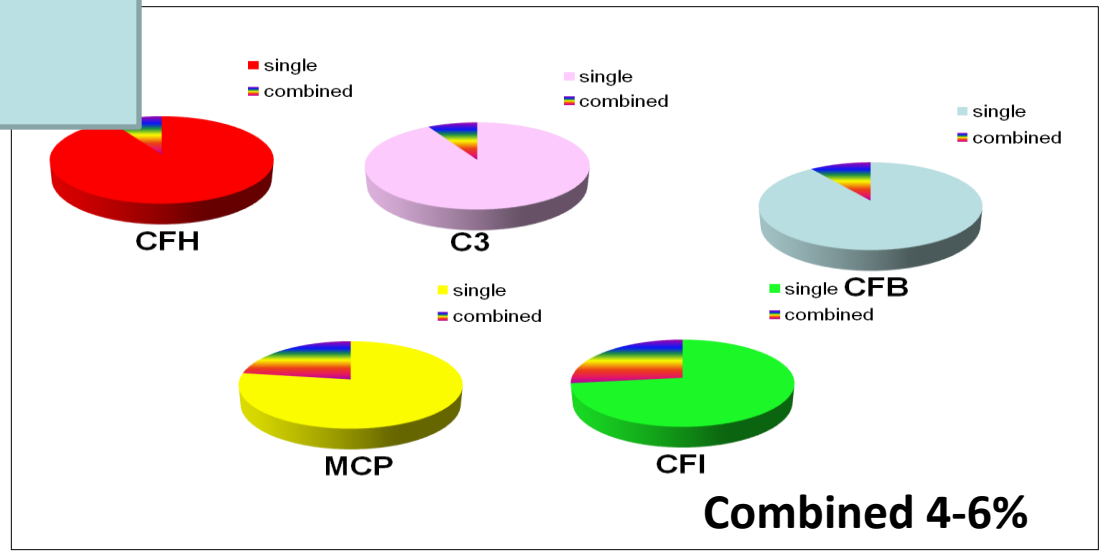


CFI 7-10%

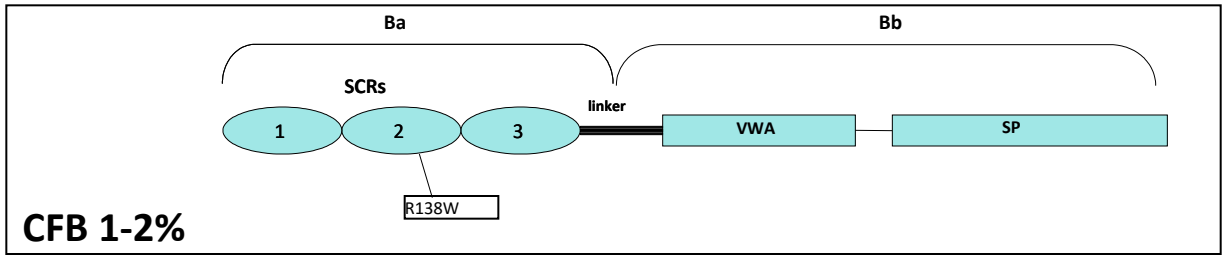
50-60%



C3 7%



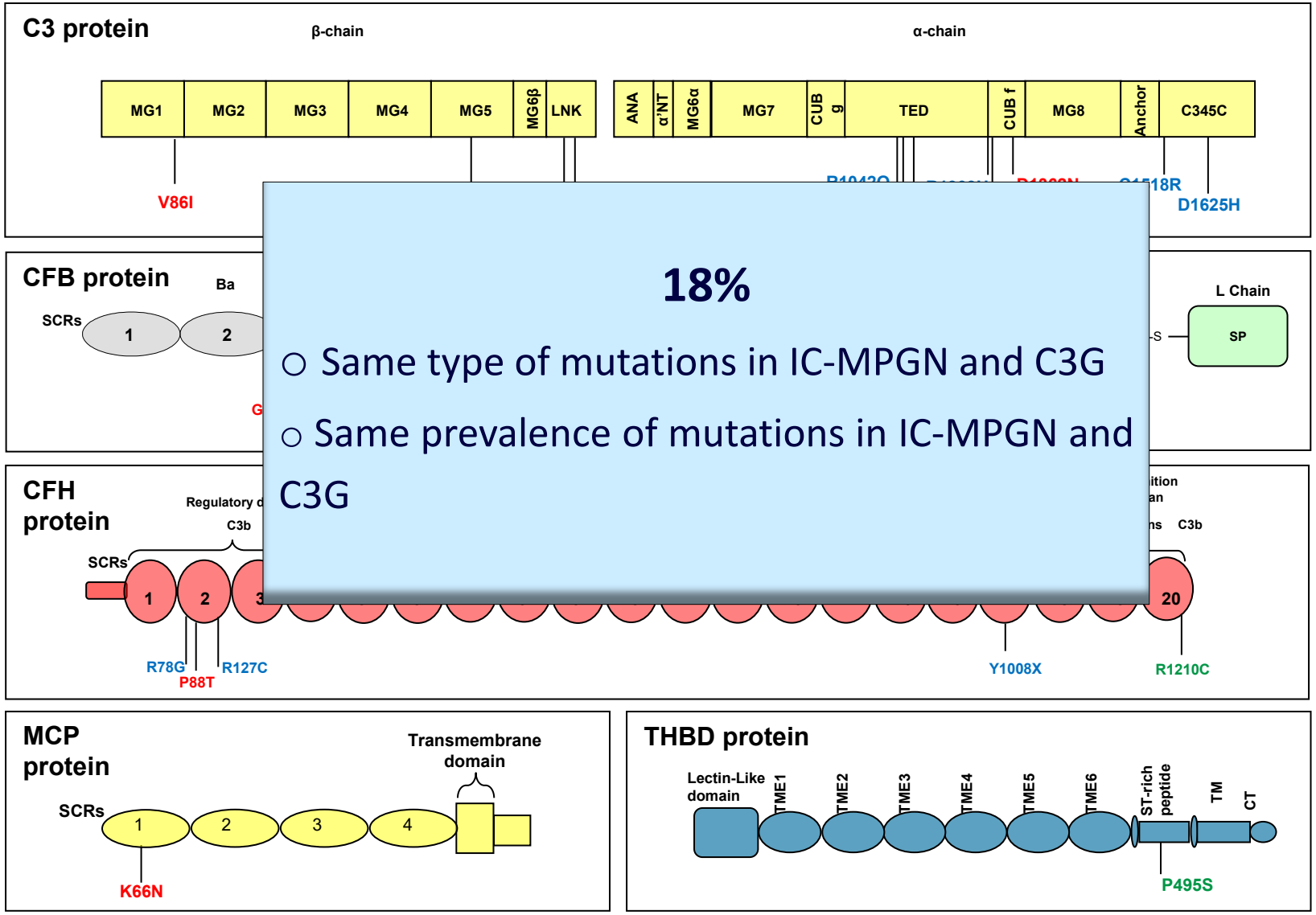
Combined 4-6%



CFB 1-2%

Noris et al, CJASN 2010

COMPLEMENT GENE MUTATIONS IN IC-MPGN C3GN DDD PATIENTS



18%

- Same type of mutations in IC-MPGN and C3G
- Same prevalence of mutations in IC-MPGN and C3G

Iatropoulos et al, *Mol Immunol,* 2016

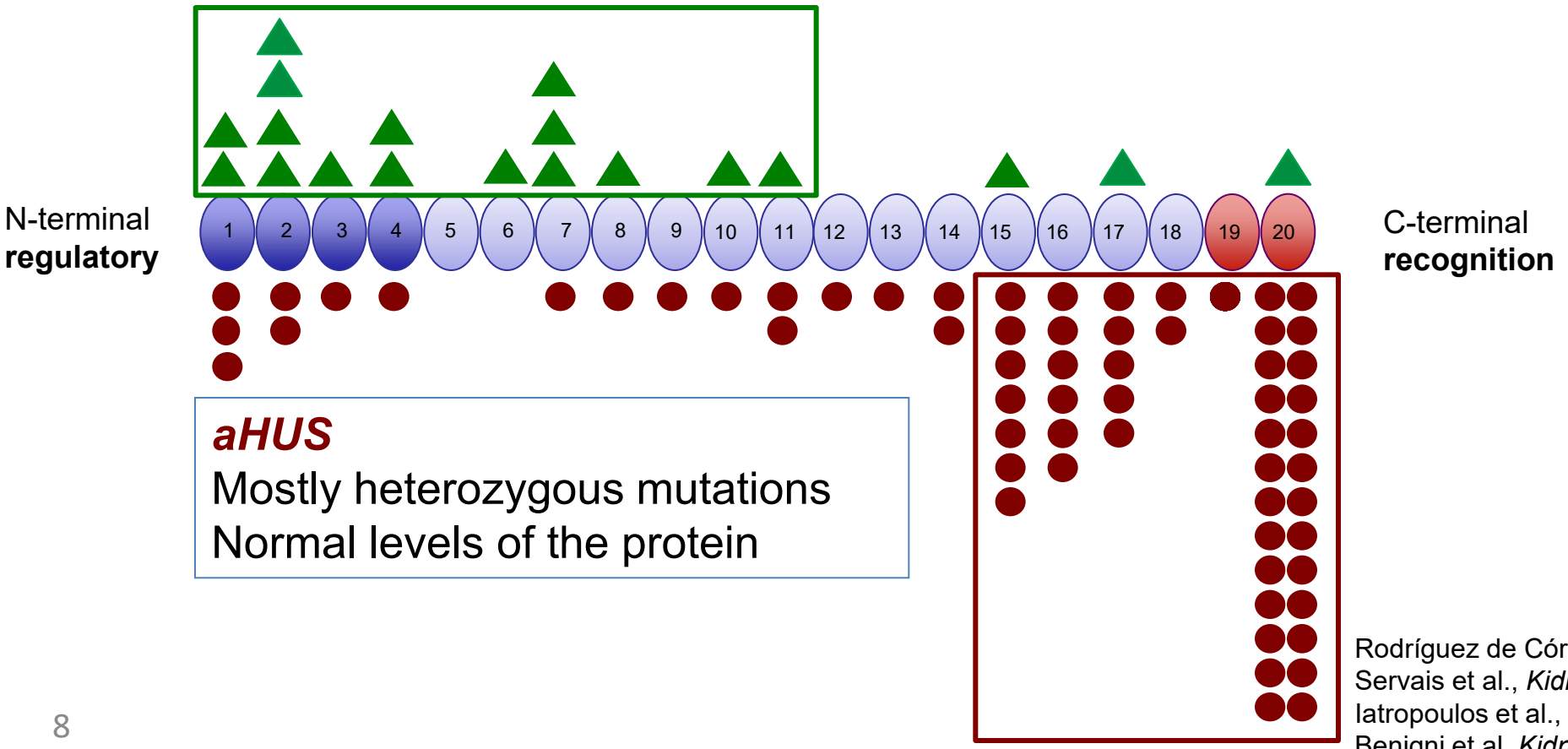
	C3G/IC-MPGN	aHUS
C3	9%	8%
MCP	1%	10%
CFI	2%	7%
CFB	1%	/
CFH	4%	25%
THBD	1%	3%
LPVs (total)	18%	53%

LPV: Likely Pathogenic Variant

Noris et al., *2026*

CFH GENE MUTATIONS IN IC-MPGN/C3G AND aHUS

IC-MPGN/C3G
Mostly heterozygous mutations
Often low levels of the protein

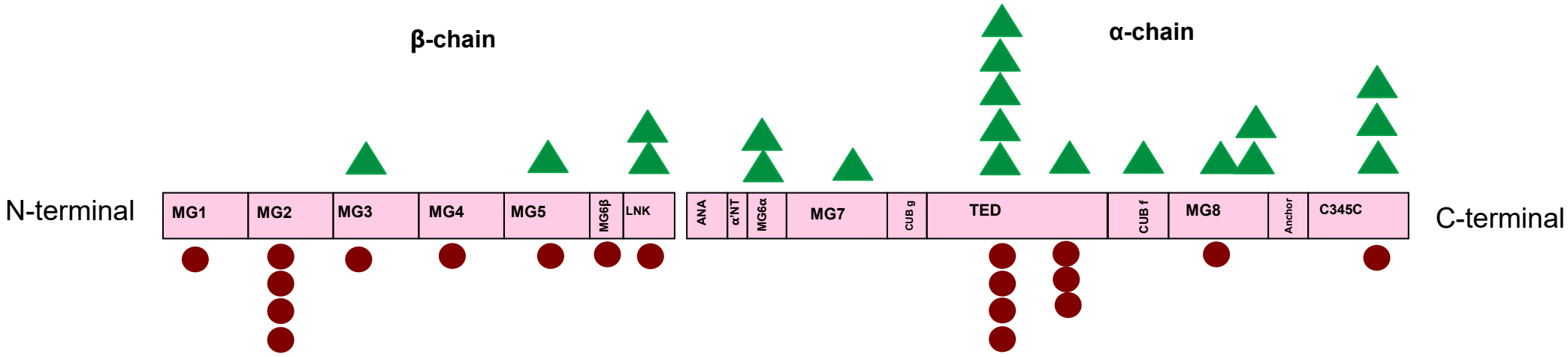


aHUS
Mostly heterozygous mutations
Normal levels of the protein

Rodríguez de Córdoba et al., *Biochem Biophys Acta,* 2011
Servais et al., *Kidney Int,* 2012
Iatropoulos et al., *JASN,* 2018
Benigni et al, *Kidney Int*, 2025

C3 GENE ABNORMALITIES IN IC-MPGN/C3G AND aHUS

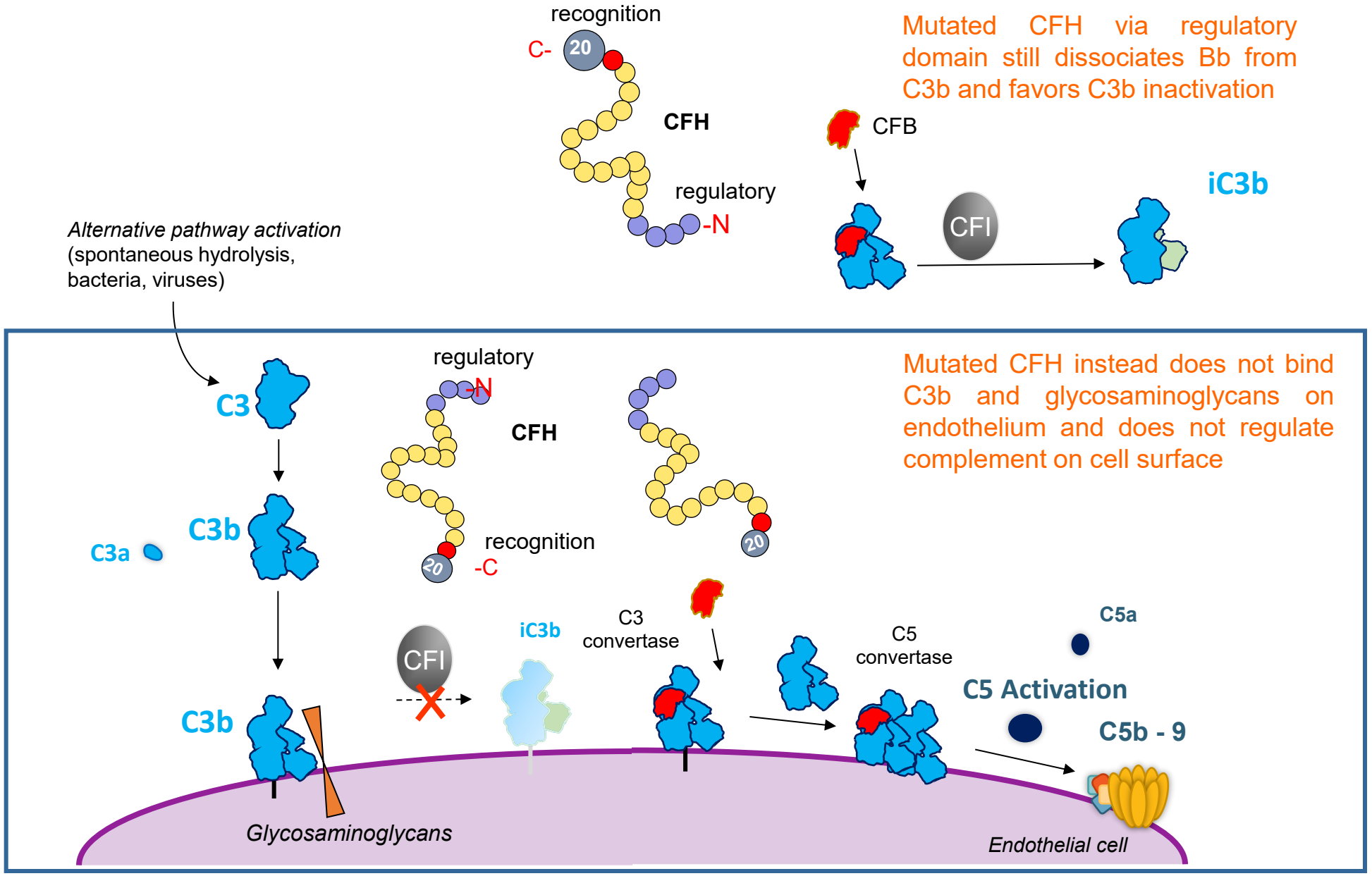
IC-MPGN/C3G
All heterozygous mutations
Cluster in the alpha chain



aHUS
All heterozygous mutations
More broadly distributed

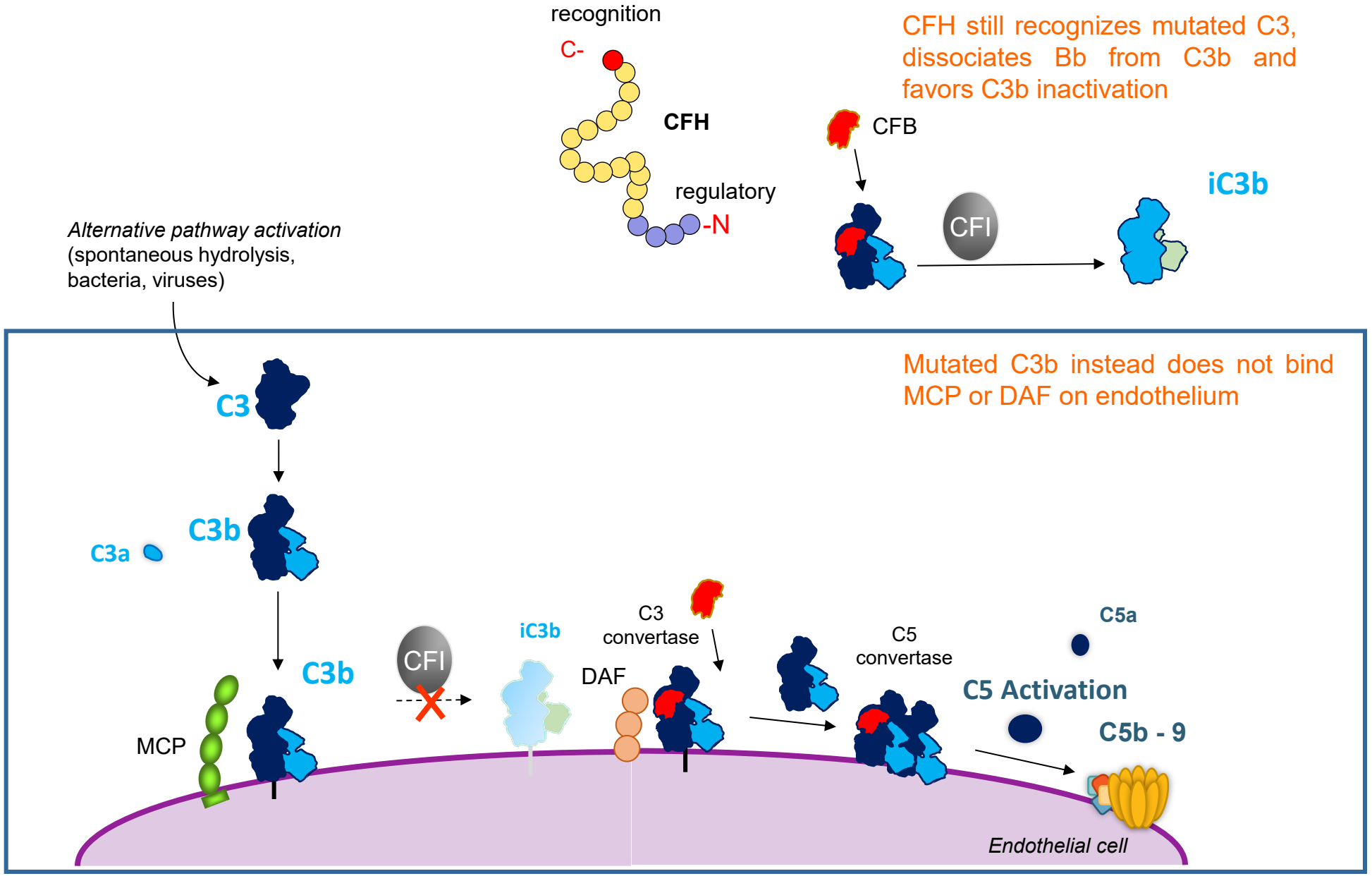
Scharamm et al., *Blood* 2015
Servais et al., *Kidney Int,* 2012
Iatropoulos et al., *JASN,* 2018
Benigni et al, *Kidney Int*, 2025

SOLID-PHASE RESTRICTED COMPLEMENT ACTIVATION IN aHUS



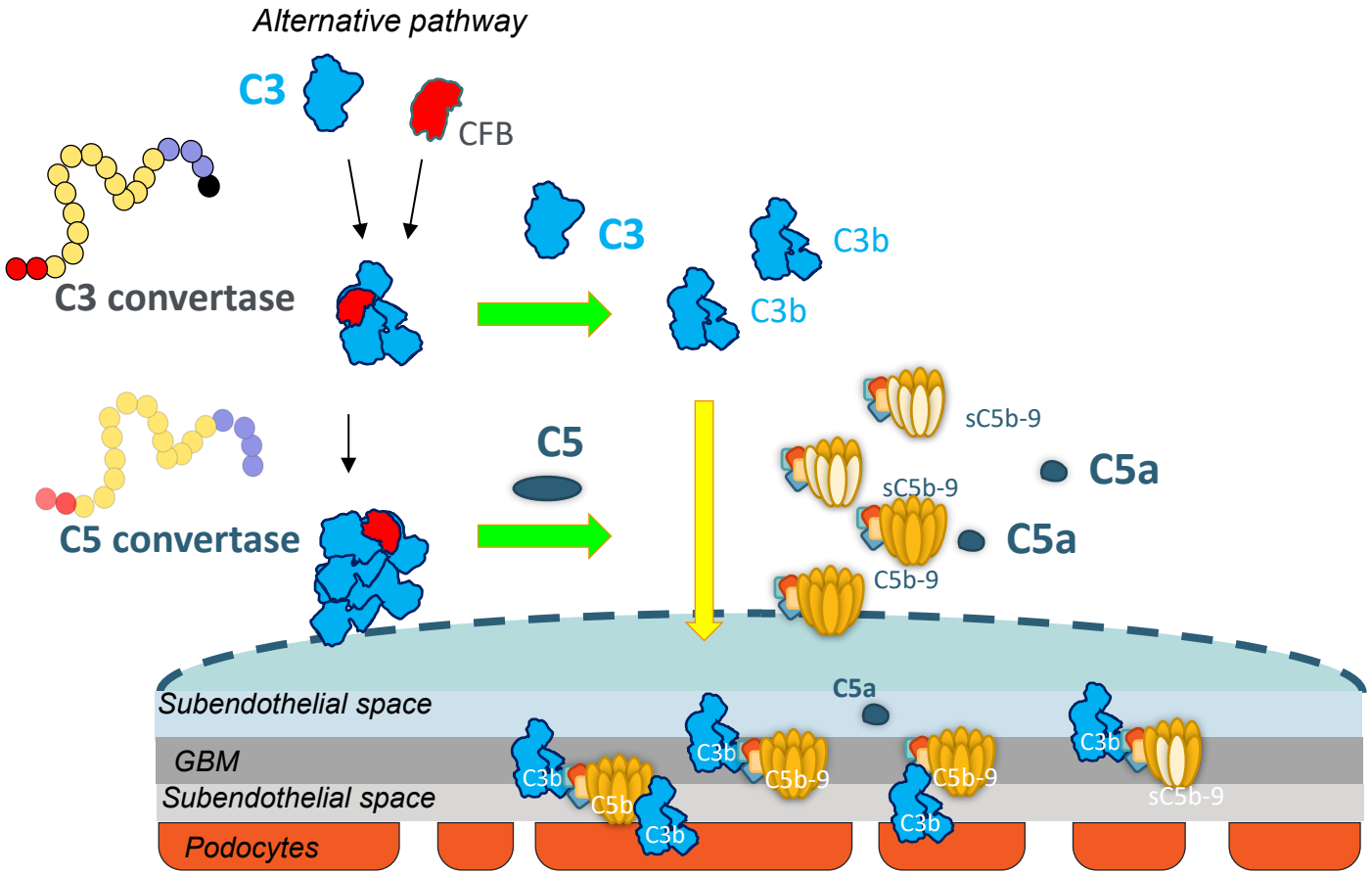
Manuelian et al., *JCI,* 2003
Sanchez-Corral et al., *Am J Hum Gen,* 2002
Heinen et al., *JASN,* 2007
Noris et al, Blood 2014
Merinero HM et al, Kidney Int 2018

C3 GAIN OF FUNCTION VARIANTS IN aHUS



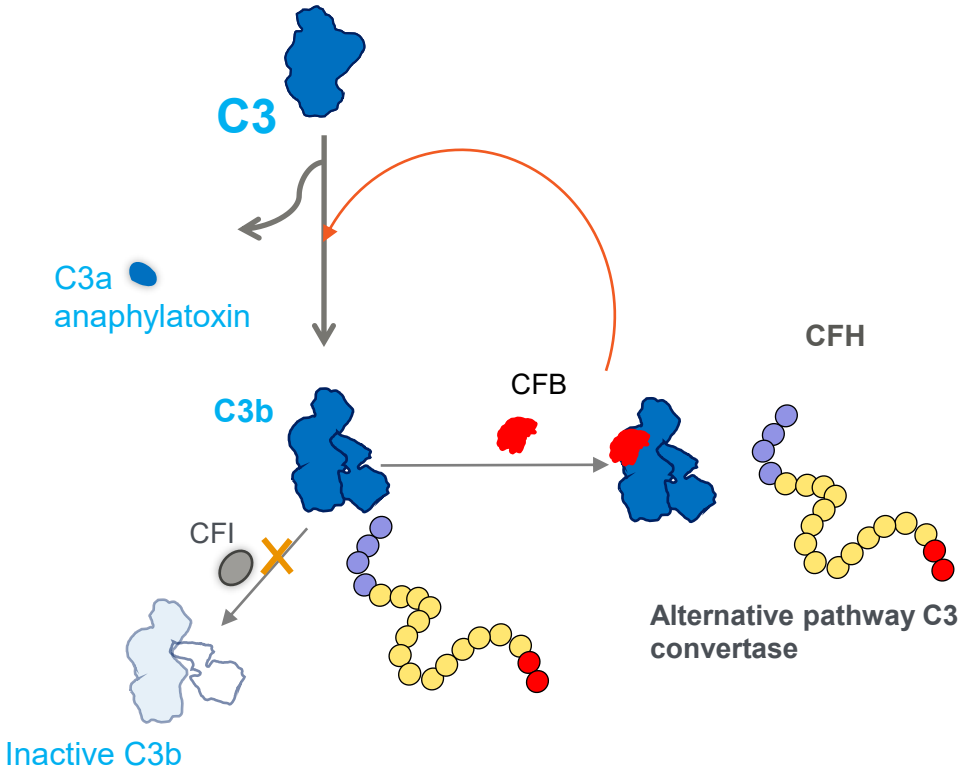
Manuelian et al., *JCI,* 2003
Sanchez-Corral et al., *Am J Hum Gen,* 2002
Heinen et al., *JASN,* 2007
Noris et al, Blood 2014
Merinero HM et al, Kidney Int 2018

FLUID-PHASE COMPLEMENT ACTIVATION IN C3G/IC-MPGN



C3 variants in C3G and primary IC-MPGN cause alternative pathway dysregulation

$C3_{1636A}$mutant



Fluid phase dysregulation

Sartz L. *et al., J Immunol , 2016*

$C3_{734T}$mutant

Fluid phase

- Interactions with FB and FH normal
- Less efficiently cleaved by FI in presence of FH

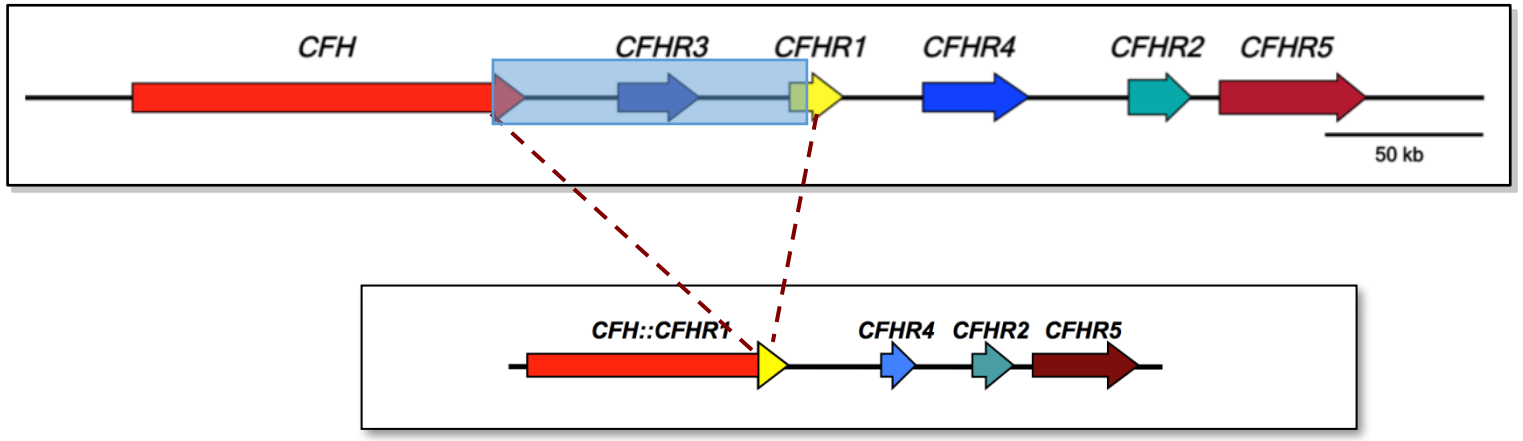
Solid phase

- Decreased binding to CR1, a complement regulator highly expressed on podocytes

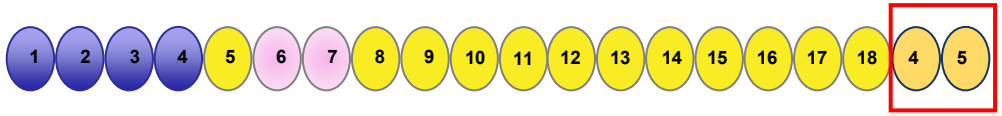
Cell surface dysregulation on podocytes

Chauvet S. *et al., JASN, 2016*

HIGH HOMOLOGY IN THE REGULATORS OF COMPLEMENT GENE CLUSTER



Venables et al., *Plos Medicine,* 2006

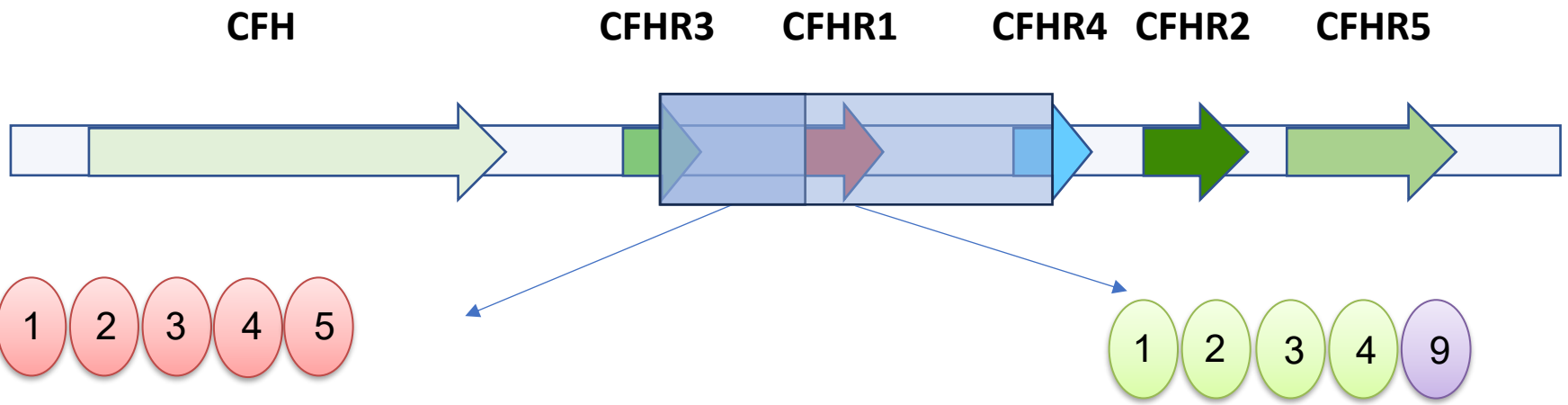


FH $_{1-18}$/FHR1 $_{4-5}$ hybrid protein

- 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 (CGH arrays or MLPA) are required to detect hybrid genes
- Identified in 20/258 aHUS patients (8%).

Piras et al., *Frontiers in Medicine,* 2020

CFHR STRUCTURAL VARIANTS IN C3G/IC-MPGN

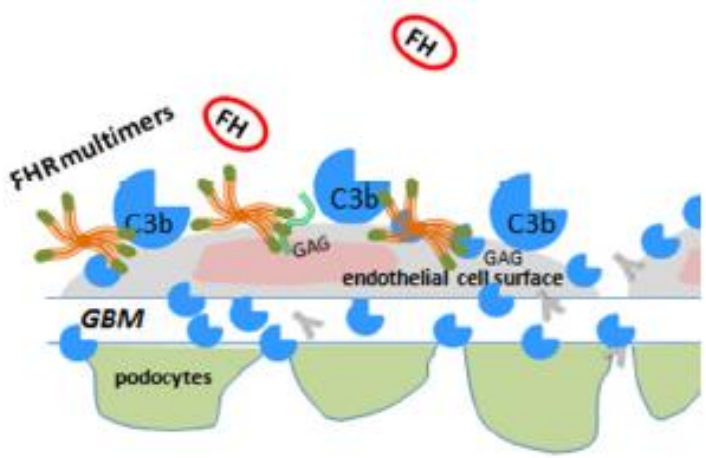


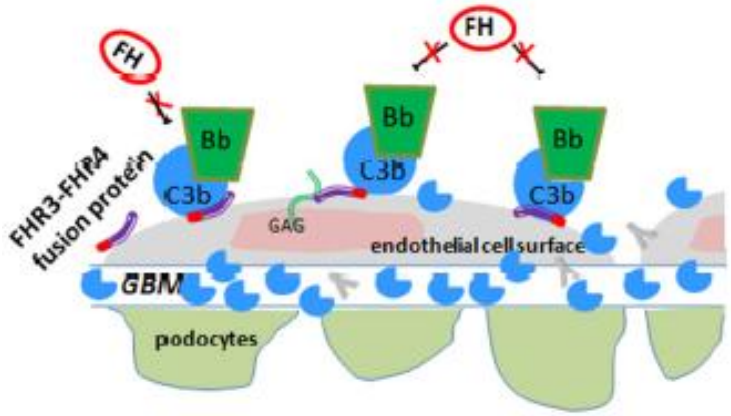
FHR3 SCR12/FHR1 SCR12345

FHR3 SCR1234/FHR4 SCR9

Oligomerization and deregulation of complement locally in the glomeruli. Normal C3 levels

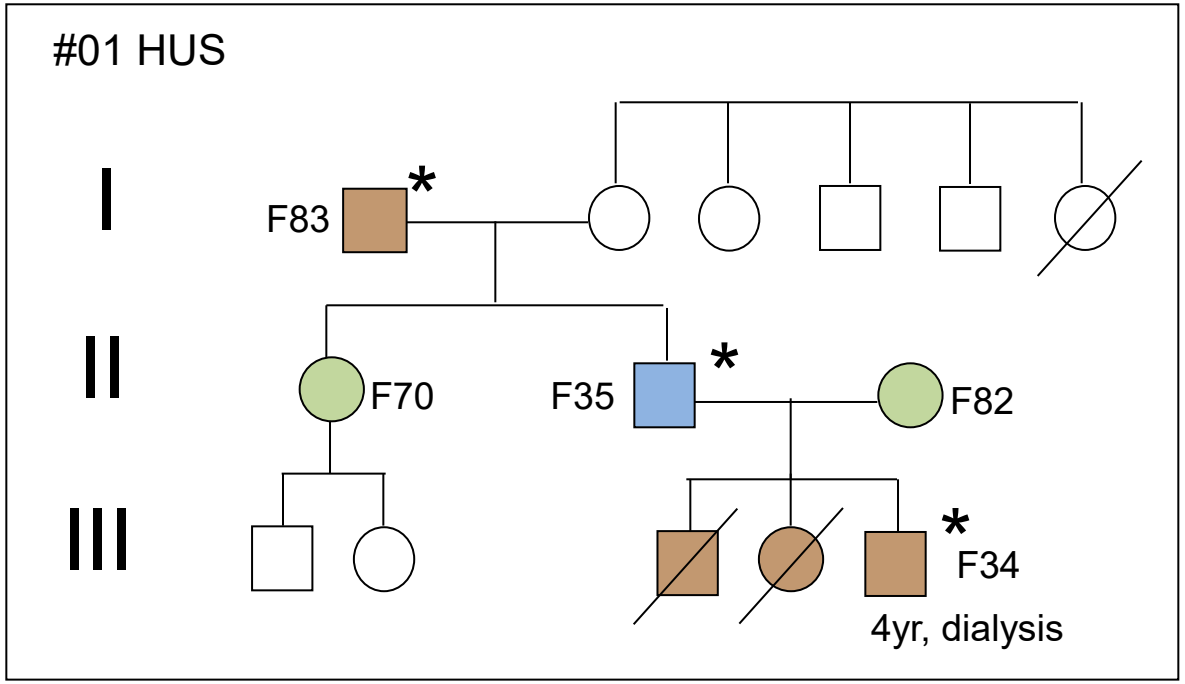
High avidity for C3b and cell surface ligands and enhanced C3 convertase activity .





Malik et al., JASN *2012*
Piras et al ., Front Genet 2021

INCOMPLETE PENETRANCE OF aHUS IN CARRIERS OF COMPLEMENT GENE VARIANTS

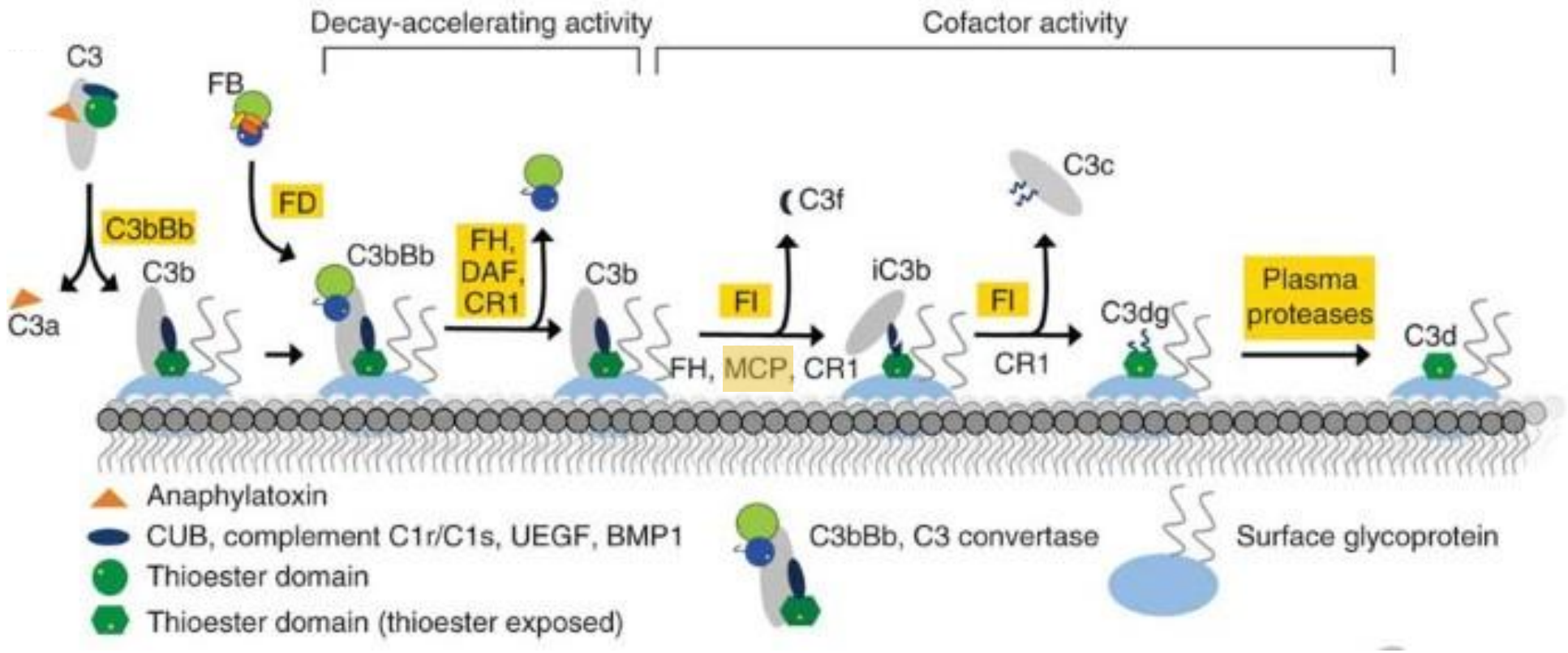


* R1215Q pathogenetic change in CFH

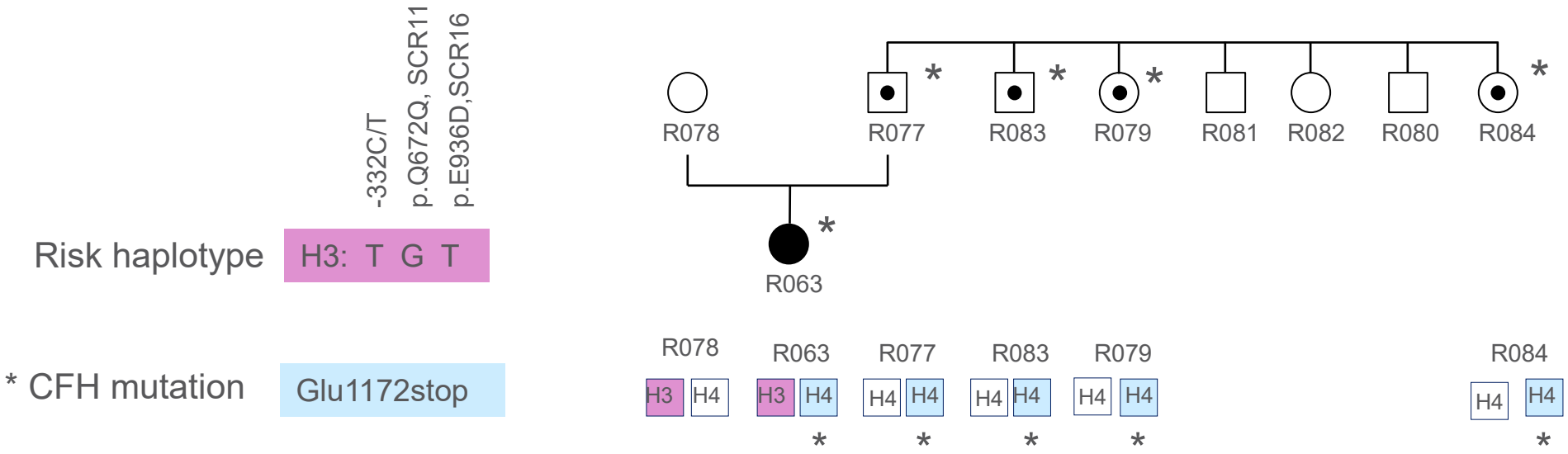
- 3 subjects in the III generation developed aHUS in infancy: 2 died, 1 reached ESRD
- F35 never developed aHUS
- Subject F83, carrier of the R1215Q mutation developed aHUS and died at 82 years of age

Noris et al., C*JASN,* 2010

IN NORMAL CONDITIONS HUMAN CELLS ARE PROTECTED FROM COMPLEMENT ATTACK BY SEVERAL REGULATORY PROTEINS WITH REDUNDANT



THE CASE OF GENE POLYMORPHISMS IN CARRIERS OF PATHOGENETIC VARIANTS TO EXPLAIN DISEASE PENETRANCE



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

• In 80 pedigrees with a mutation in complement genes (CFH, or MCP or C3 or CFB) aHUS penetrance was 58% in subjects who also carried the CFH-H3 and the MCPggaac risk haplotypes, while only 18% of mutation carriers with neither risk haplotypes developed the disease.

Arjona E et al., *Blood 2020*

HOW TO CLASSIFY A COMPLEMENT GENE VARIANT?

Rules for combining criteria to classify sequence variants

Pathogenic

- 1 Very strong (PVS1) *AND*
  - (a) ≥1 Strong (PS1–PS4) *OR*
  - (b) ≥2 Moderate (PM1–PM6) *OR*
  - (c) 1 Moderate (PM1–PM6) *AND* 1 supporting (PP1–PP5) *OR*
  - (d) ≥2 Supporting (PP1–PP5)
- ≥2 Strong (PS1–PS4)
- 1 **Strong** (PS1–PS4) *AND*
  - (a) ≥3 Moderate (PM1–PM6) *OR*
  - (b) **2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5)** *OR*
  - (c) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)

Likely pathogenic

- 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR*
- 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR*
- 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR*
- ≥3 Moderate (PM1–PM6) *OR*
- 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR*
- 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)

Benign

- 1 Stand-alone (BA1) *OR*
- ≥2 Strong (BS1–BS4)

Likely benign

- 1 Strong (BS1–BS4) and 1 supporting (BP1– BP7) *OR*
- ≥2 Supporting (BP1–BP7)

Very strong: PVS1: null variant
Strong: PS1: same aa as in previously PV; PS2: de novo; **PS3: functional studies**; PS4: prevalence increased than in controls;
Moderate: PM1: in mut hot spot or funct domain; PM2: absent from ctrs; PM3: rec in trans with a PV; PM4: protein length change; PM5 novel change at an aa with previous PV; PM6: presumed de novo
Supporting: PP1: cosegregation with disease ; PP2: missense in a gene with low rate of BV; **PP3: in silico deleterious**; PP4: patient phenotype specific for single gen etiology; **PP5: PV reported by other source**

Richards et al, *Genet Med* 2015, ACMG/AMP Guidelines

CFH c.C3628T, p.R1210C (aHUS)

gnomAD

SNV: 1-196747245-C-T(GRCh38)

Copy variant ID | Gene page | Dataset: gnomAD v4.1.0

	Exomes	Genomes	Total
Filters	Pass	Pass	Discrepant frequencies
Allele Count	194	27	221
Allele Number	1461694	152178	1613872
Allele Frequency	0.0001327	0.0001774	0.0001369
Grpmax Filtering AF (95% confidence)	0.0001388	0.0002428	0.0001502
Number of homozygotes	0	0	0

External Resources

- dbSNP (rs121913059)
- UCSC
- ClinVar (16558)
- ClinGen Allele Registry (CA128563)
- All of Us

Feedback

Report an issue with this variant

Genetic Ancestry Group Frequencies

gnomAD | HGDP | 1KG | Local Ancestry

Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
European (non-Finnish)	200	1179908	0	0.0001695
Remaining	10	62472	0	0.0001601
Admixed American	6	59998	0	0.0001000
African/African American	3	74916	0	0.00004004
European (Finnish)	1	64028	0	0.00001562
South Asian	1	91080	0	0.00001098
Ashkenazi Jewish	0	29598	0	0.000
East Asian	0	44880	0	0.000
Middle Eastern	0	6080	0	0.000
Amish	0	912	0	0.000
XX	106	812366	0	0.0001305
XY	115	801506	0	0.0001435
Total	**221**	**1613872**	**0**	**0.0001369**

missense

1. CFH
   1. ENST00000367429.9
      MANE Select transcript for CFH
      HGVSp: p.Arg1210Cys
      HGVSc: c.3628C>T
      Domains: PF00084 (Pfam), and 23 more

non coding transcript exon

1. CFH
   1. ENST00000466229.5
      HGVSc: n.6726C>T

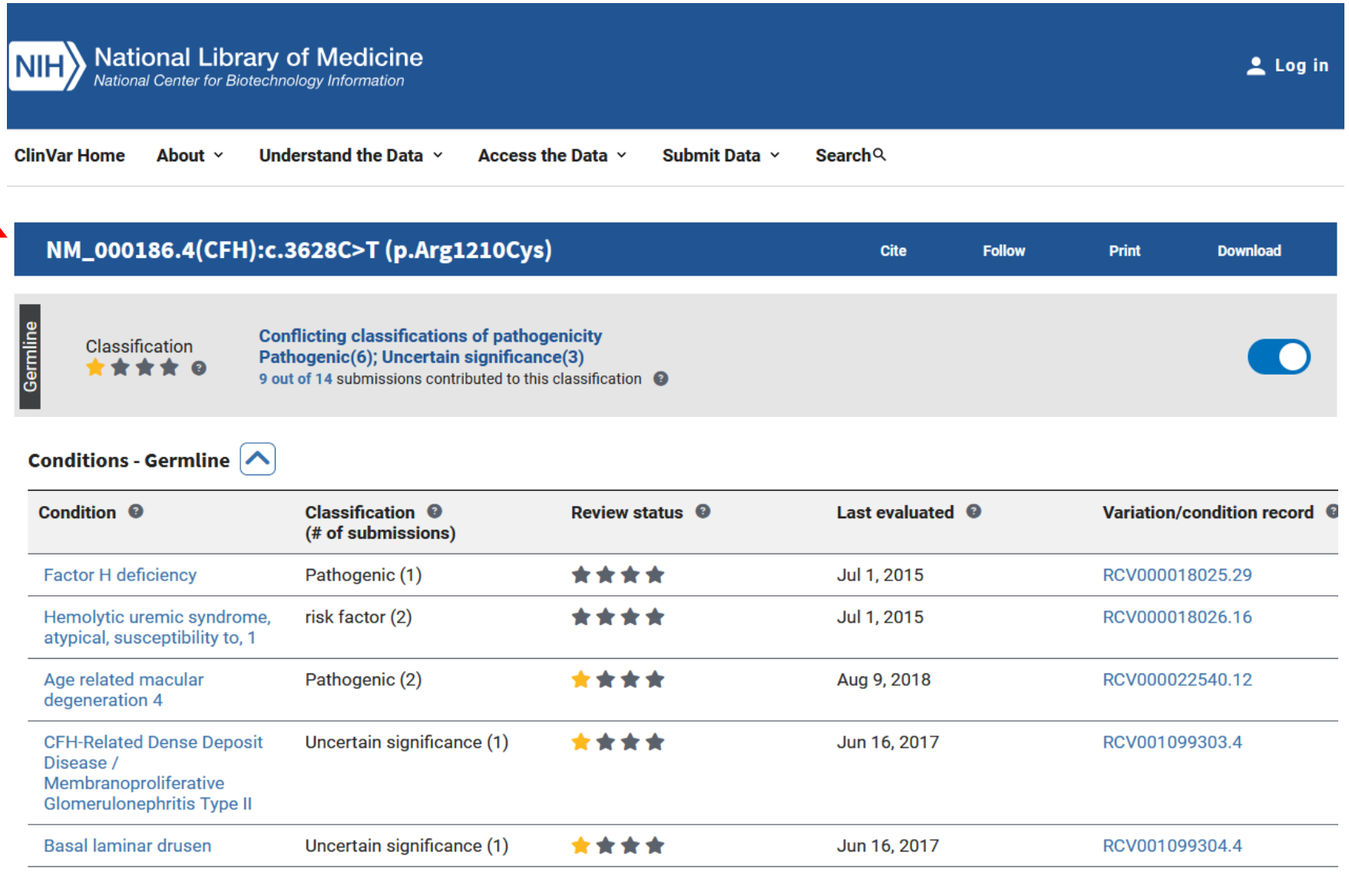
In Silico Predictors

CADD: 0.181	**Benign**
REVEL: 0.280	**Benign**
SpliceAI: 0.00	No effect
Pangolin: 0.0300	No effect
phyloP: -4.47	Low conservation
PolyPhen (max): 0.280	**Likely Benign**

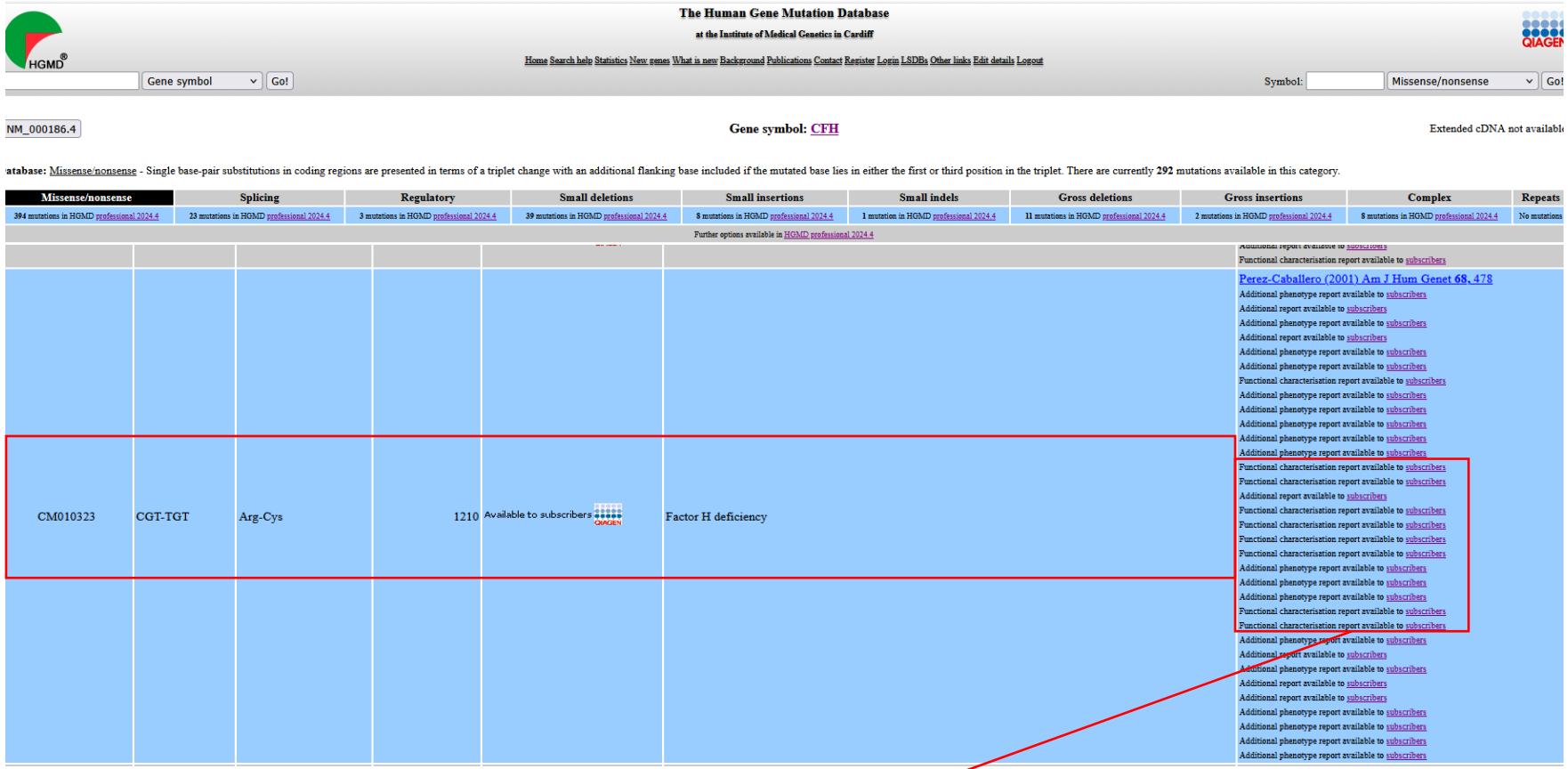
Note The SpliceAI and Pangolin predictions displayed here were precomputed by Illumina and Invitae. For more detailed and up to date SpliceAI and Pangolin predictions, please visit our SpliceAI Lookup browser.

! Present in controls : not so rare

! In silico : BV/LBV



- In a mut hot spot: PM1
- PV reported by other sources: PP5
- Prevalence increased than in controls: PS4



The Human Gene Mutation Database

at the Institute of Medical Genetics in Cardiff

Home Search help Statistics New genes What is new Background Publications Contact Register Login LSDBs Other links Edit details Logout

Gene symbol Go! Symbol: Missense/nonsense Go!

NM_000186.4 **Gene symbol: CFH** Extended cDNA not available

atabase: Missense/nonsense - Single base-pair substitutions in coding regions are presented in terms of a triplet change with an additional flanking base included if the mutated base lies in either the first or third position in the triplet. There are currently **292** mutations available in this category.

Missense/nonsense	Splicing	Regulatory	Small deletions	Small insertions	Small indels	Gross deletions	Gross insertions	Complex	Repeats
394 mutations in HGMD professional 2024.4	23 mutations in HGMD professional 2024.4	3 mutations in HGMD professional 2024.4	39 mutations in HGMD professional 2024.4	8 mutations in HGMD professional 2024.4	1 mutation in HGMD professional 2024.4	11 mutations in HGMD professional 2024.4	2 mutations in HGMD professional 2024.4	8 mutations in HGMD professional 2024.4	No mutations

Further options available in HGMD professional 2024.4

						Functional characterisation report available to subscribers
						Perez-Caballero (2001) Am J Hum Genet 68, 478 Additional phenotype report available to subscribers Additional report available to subscribers Additional phenotype report available to subscribers Additional report available to subscribers Additional phenotype report available to subscribers Additional phenotype report available to subscribers Functional characterisation report available to subscribers Additional phenotype report available to subscribers Additional phenotype report available to subscribers Additional phenotype report available to subscribers Additional phenotype report available to subscribers Additional phenotype report available to subscribers
CM010323	CGT-TGT	Arg-Cys	1210	Available to subscribers	Factor H deficiency	Functional characterisation report available to subscribers Functional characterisation report available to subscribers Additional report available to subscribers Functional characterisation report available to subscribers Functional characterisation report available to subscribers Functional characterisation report available to subscribers Functional characterisation report available to subscribers Additional phenotype report available to subscribers Additional phenotype report available to subscribers Additional phenotype report available to subscribers Functional characterisation report available to subscribers Functional characterisation report available to subscribers
						Additional phenotype report available to subscribers Additional report available to subscribers Additional phenotype report available to subscribers Additional report available to subscribers Additional report available to subscribers Additional phenotype report available to subscribers Additional phenotype report available to subscribers Additional phenotype report available to subscribers Additional phenotype report available to subscribers

Functional studies: PS3

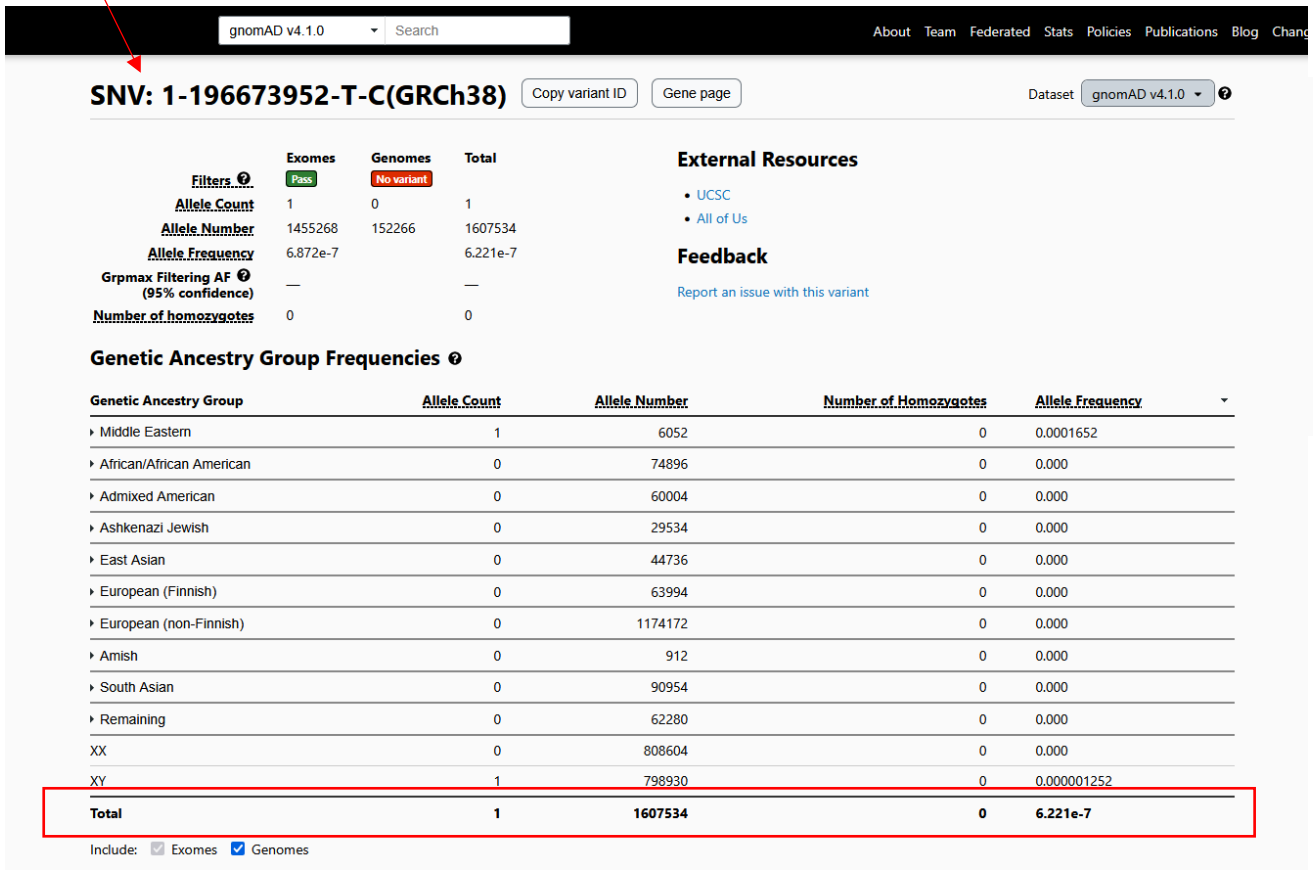
In a mut hot spot: PM1

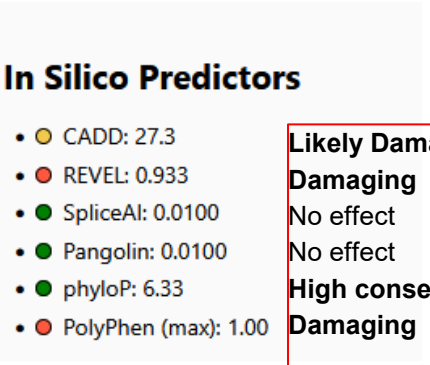
PV reported by other sources: PP5

Prevalence increased than in controls: PS4

⟹ **Pathogenic**

CFH c.G341A, p.C114Y (C3G)





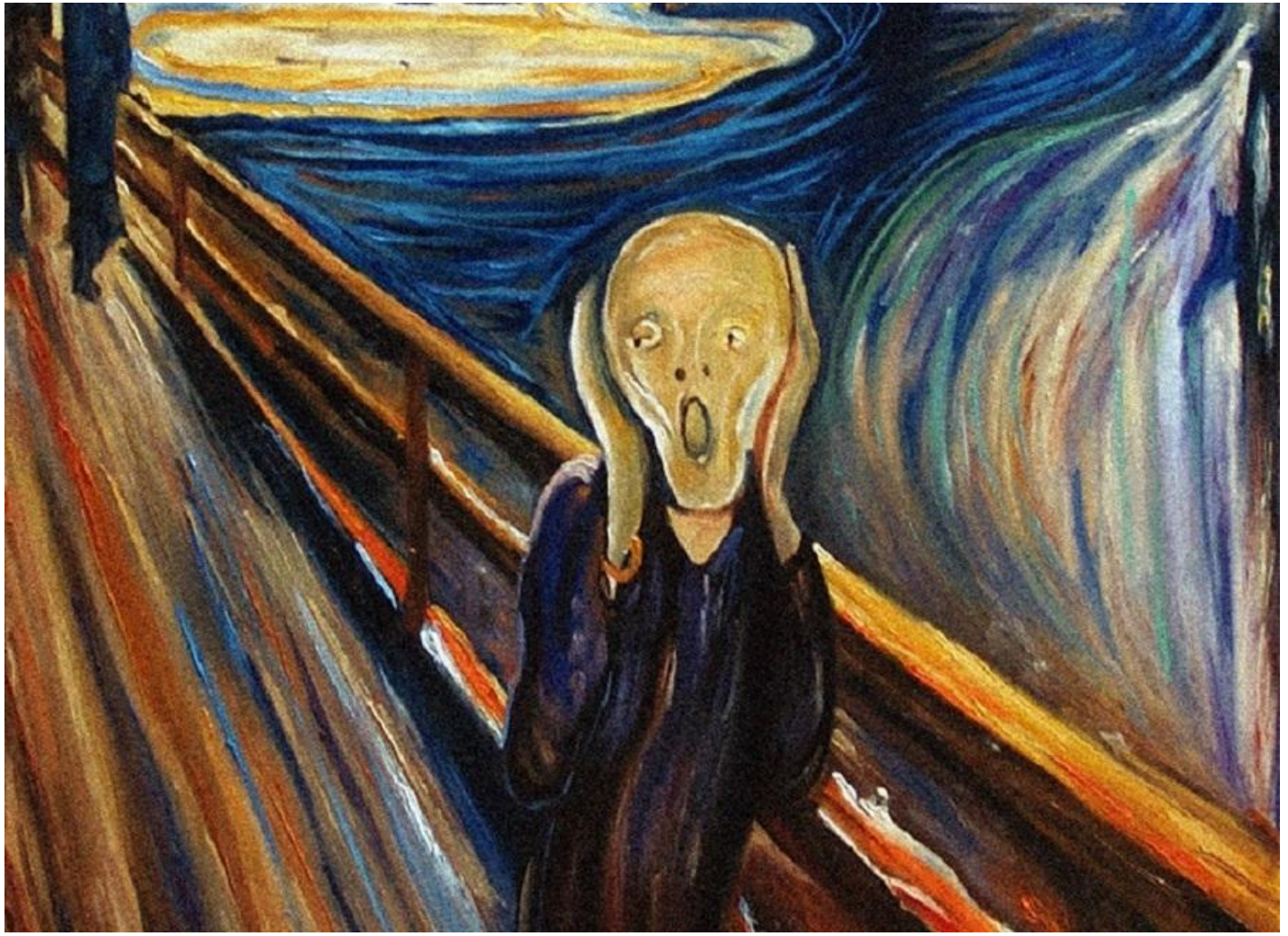
Likely Damaging
Damaging
No effect
No effect
High conservation
Damaging

Almost absent in controls : PM2
In silico deleterious: PP3
! Not present in HGMD, nor in ClinVar
! No functional studies

- the criteria for benign and pathogenic are contradictory.

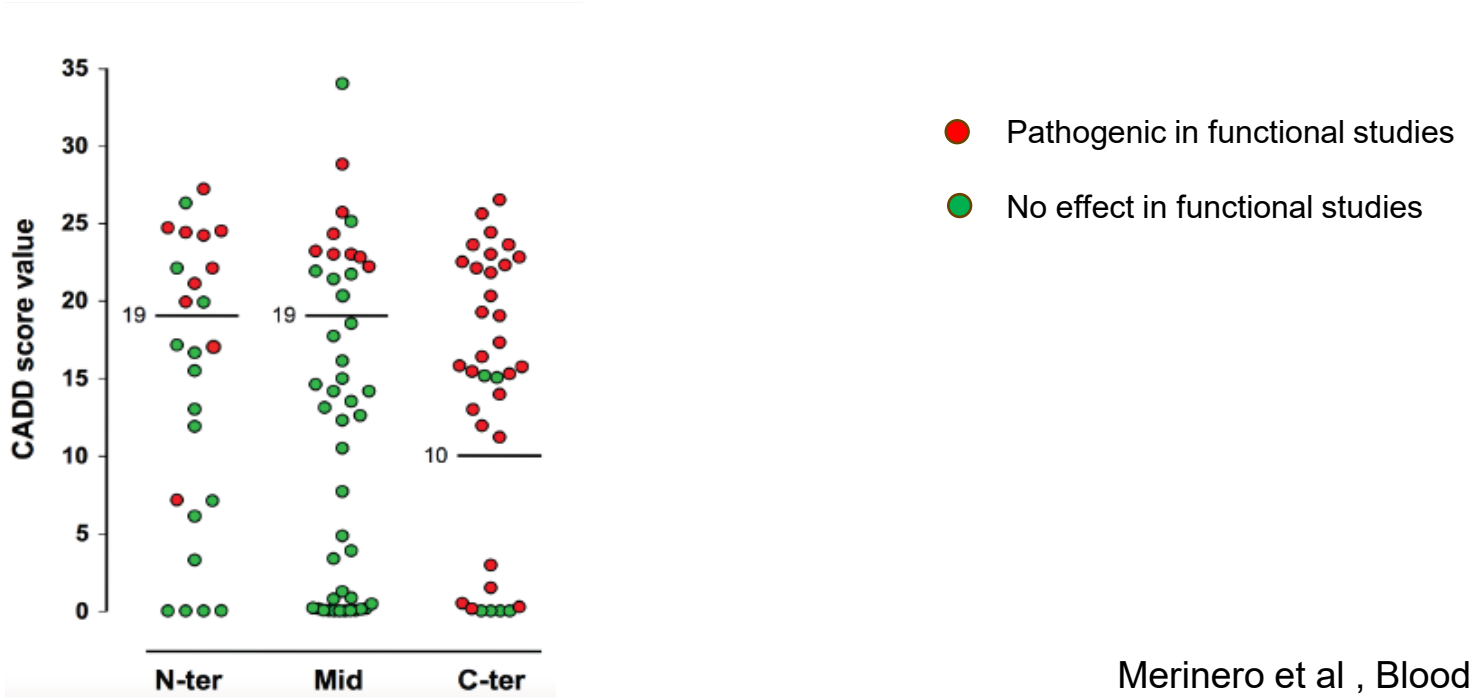
Uncertain significance

VUS !



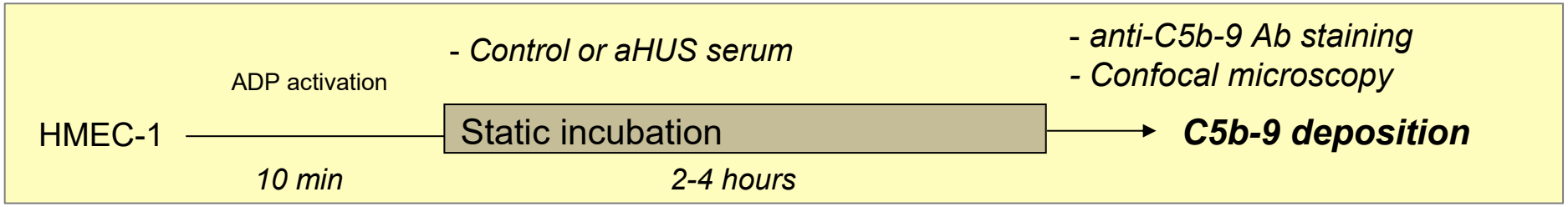
EMERGING TECHNOLOGIES: FUNCTIONAL STUDIES AND CADD THRESHOLDS

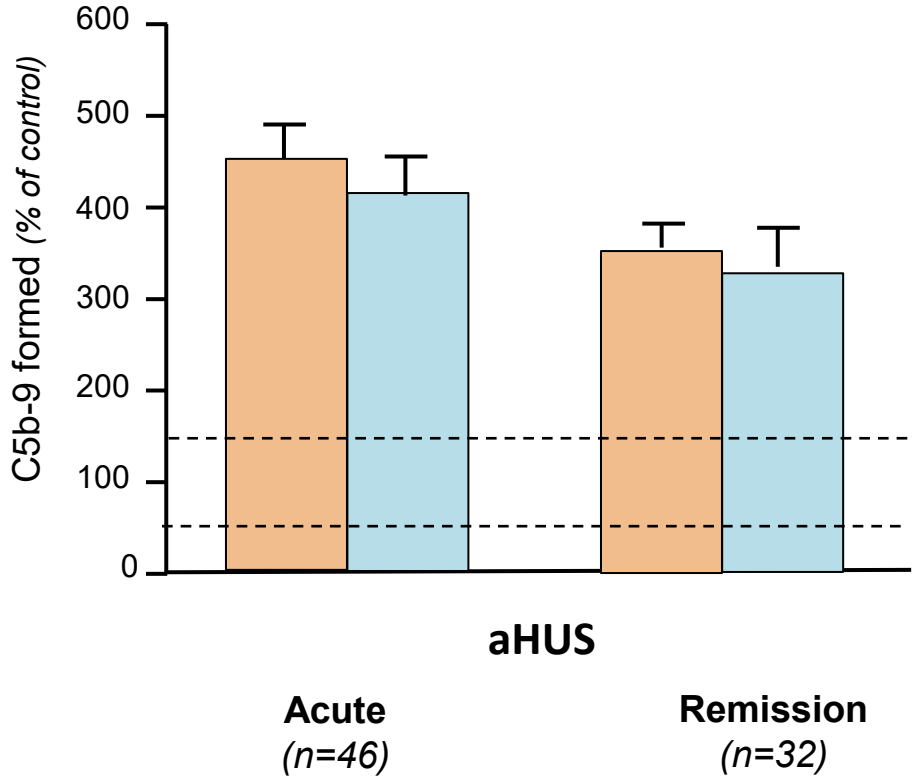
- Of 79 uncharacterized variants, only 29 (36.7%) alter FH *in vitro* expression or function and are proposed to be pathogenic.
- Rarity in databases is not informative for variant classification.
- There are limitations in applying prediction algorithms to FH variants.
- We recommend applying specific CADD thresholds to the different domains of FH. This approach has limitations because it only improves the classification from 73% to 83% and should be validated with additional studies.



Merinero et al , Blood, 2021

EMERGING TECHNOLOGIES: THE EX VIVO C5B-9 FORMATION ASSAY



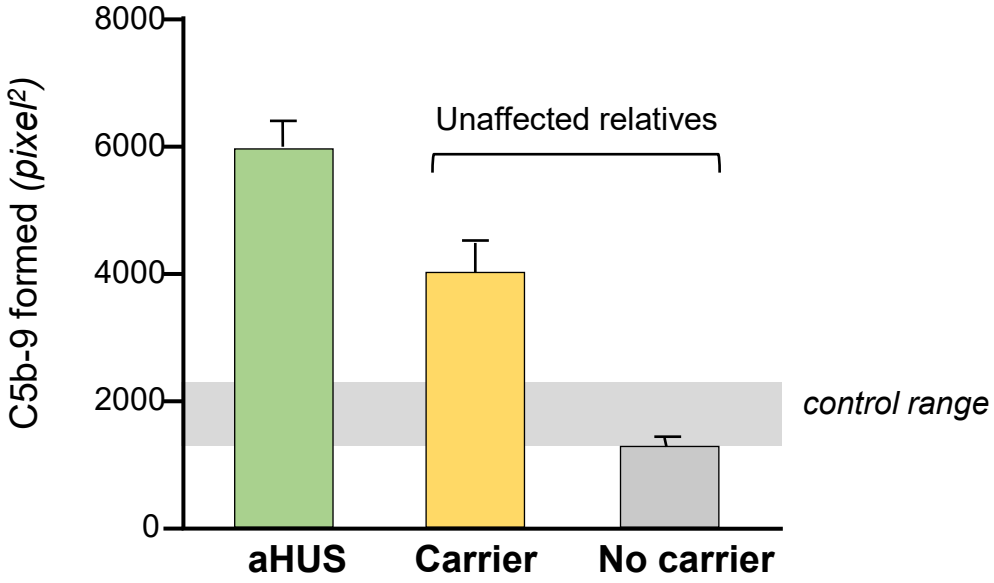


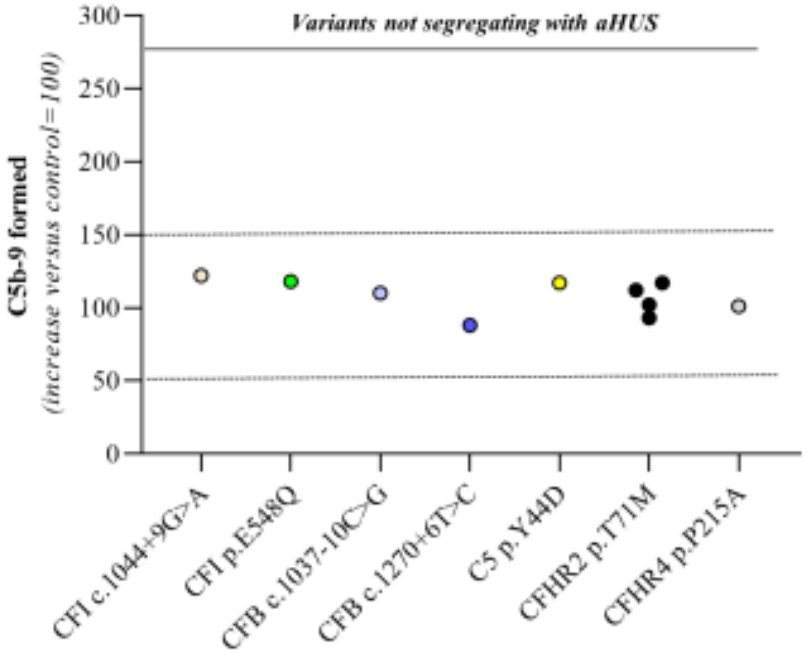
mutations
no mutations

- Complement dysregulation: sensitivity 100 %
- Positive both in acute phase and in remission
- Positive regardless from mutation status

Galbusera et al, *Am J Kidney Dis,* 2019

EMERGING TECHNOLOGIES: THE EX VIVO C5B-9 FORMATION ASSAY





- 92.7% (38/41) of unaffected relatives with known PV exhibited positive serum-induced C5b-9 formation test.
- The test was negative in all non-carrier relatives (28) and in relatives (10) with variants non-segregating with aHUS.

Gastoldi et al., *Frontiers Immunol* , 2023

COMPLEMENT-MEDIATED HUS: A DISEASE IN A DISH

Diagnosis

Monitoring

Genetic characterization

Thanks!

Caterina Mele

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Rossella Piras

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Marta Alberti

Zahra Imanifard

Annamaria Morotti

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Sistiana Aiello

Miriam Galbusera

Federica Basile

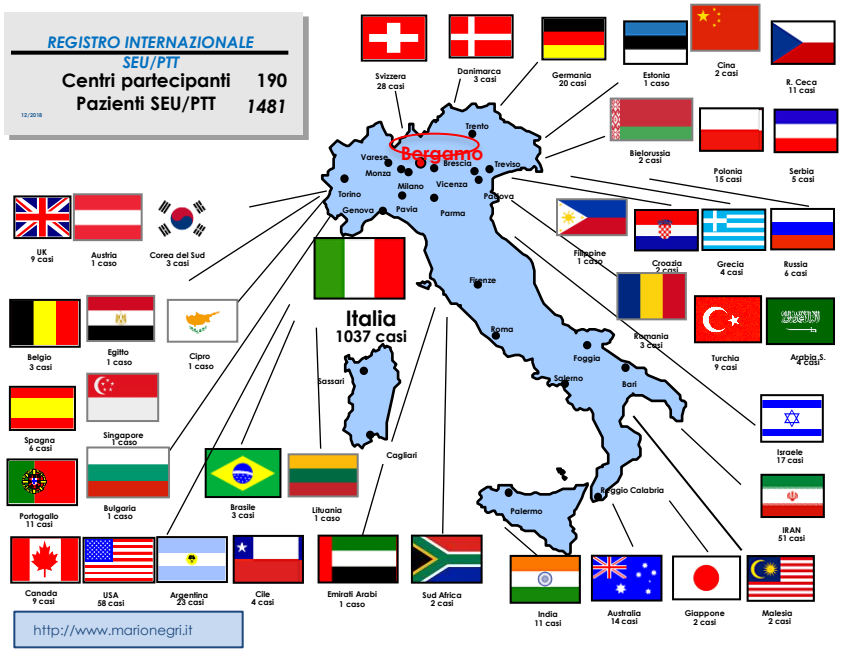
Francesca Penati

Erica Daina

Elena Bresin

Miriam Rigoldi

Sara Gamba



Ariela Benigni

Giuseppe Remuzzi

INTERNATIONAL C3 SUMMIT
TIME IS GLOM

These slides belong to
Marina Noris, *PhD.*
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sobi