UNIVERSITÀ CATTOLICA del Sacro Cuore

ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI IRCCS

Patogenesi della PTT e della SEU

Elena Bresin

IRCCS - Istituto Mario Negri, Bergamo

Convegno Microangiopatie Trombotiche UCSC 2016

Roma, 19 Febbraio 2016

THROMBOTIC THROMBOCYTOPENIC PURPURA

TTP is a thrombotic microangiopathy characterized by thrombocytopenia, microangiopathic hemolytic anemia, central nervous system involvement and/or renal impairment.

In the majority of patients deficiency of ADAMTS13, the von Willebrand factor-cleaving protease, is associated with autoantibodies to ADAMTS13.

These cases are at high risk of disease relapse when there is a persistence of anti-ADAMTS13 autoantibodies.

Congenital deficiency of ADAMTS13 cause Hereditary THROMBOTIC THROMBOCYTOPENIC PURPURA

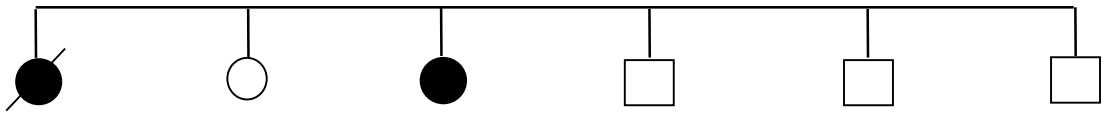
Hereditary TTP is very rare (< 10% of all TTP cases) and usually manifests in the postnatal period or during infancy, although in some cases (especially women) the onset is later at 20-30 years.

Hereditary TTP is caused by homozygous or double heterozygous mutations of *ADAMTS13* gene that determine congenital deficiency of ADAMTS13, the von Willebrand Factor (VWF)-cleaving protease.

Patients with hereditary TTP tipically exhibit a relapsing course and need regular plasma infusions to prevent relapses.

ADAMTS13 MUTATIONS IN TTP CAUSE SEVERELY REDUCED PLASMA ADAMTS13 CONCENTRATION

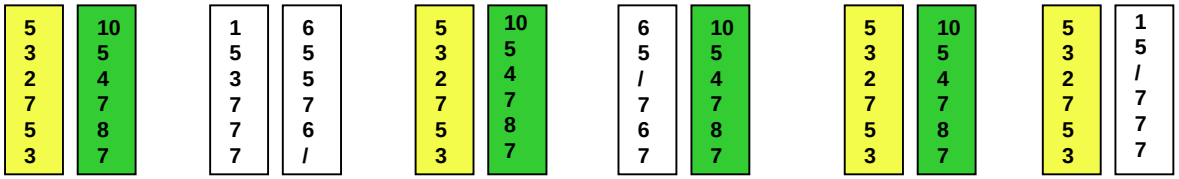
Family A



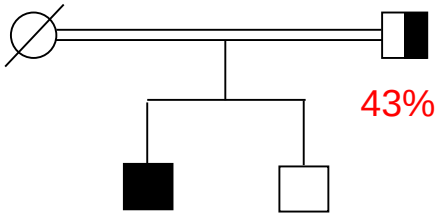
ADAMTS13 antigen *(ng/mL)*	**77**	**1580**	**79**	**731**	**82**	**786**
ADAMTS13 activity *(%)*	< 6%	108%	< 6%	62%	< 6%	67%

Heterozygous

- Val88Met(metalloprotease)
- Gly1239Val (CUB1)



Family B

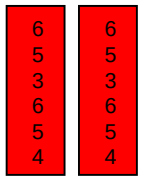


ADAMTS13 antigen *(ng/mL)* **< 62.5**

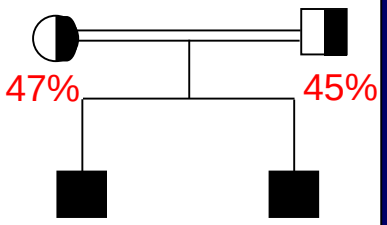
ADAMTS13 activity *(%)* < 6%

Homozygous

- Arg1123Cys (TSP8)



Family C

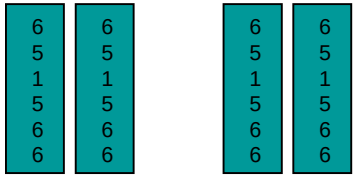


< 62.5 **< 62.5**

< 6% < 6%

Homozygous

- Arg1219Trp (CUB1)



REDUCED ADAMTS13 ACTIVITY IN HEALTH AND DISEASE

- elderly
- newborns
- third trimester of pregnancy
- uremia
- after major surgery
- inflammatory states
- liver cirrhosis

Mannucci et al., *Blood,* 2001

DIFFERENTIAL DIAGNOSIS BETWEEN TTP ASSOCIATED WITH GENETIC OR IMMUNE-MEDIATED ADAMTS13 DEFICIENCY IS IMPORTANT TO GUIDE SPECIFIC TREATMENTS

Genetic ADAMTS13 deficiency:

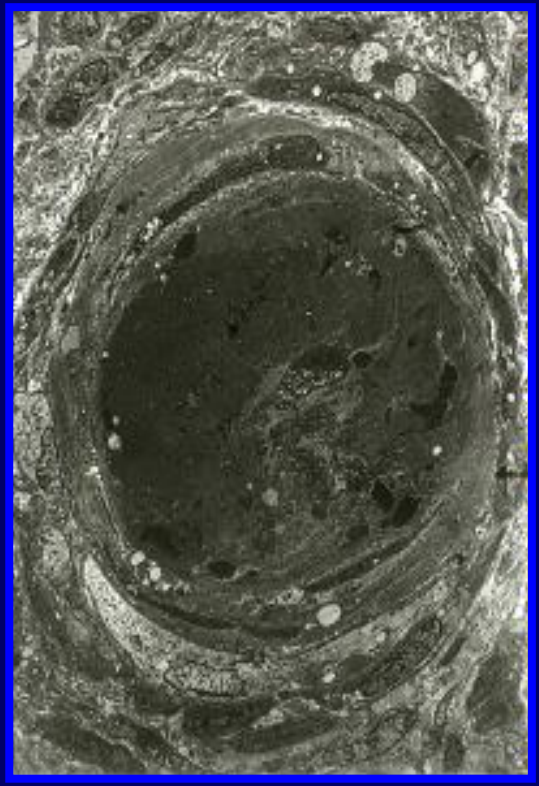
- replacement with plasma of the defective activity

Immuno-mediated ADAMTS13 deficiency:

- plasma exchange to remove anti-ADAMTS13 autoantibodies and to replace the metalloprotease
- inhibition of the autoantibodies production through treatment with glucocorticoids, immunosuppressive agents, or rituximab

Hemolytic Uremic Syndrome

THROMBOTIC MICROANGIOPATHY



Definition

A multisystem disease with predominant renal involvement, characterized by a triad of symptoms:

- microangiopathic hemolytic anemia,
- thrombocytopenia,
- formation of platelet-rich thrombi in the microcirculation

Classification of the different forms of Hemolytic Uremic Syndrome

- **Typical HUS**

  Infections by *E. coli* producing Shiga-like toxin/verotoxin and causing hemorrhagic colitis
  Typical HUS manifests expecially in children with diarrhea often bloody
  Typical HUS usually has a favorable outcome: only 10% of patients progress to ESRD

- **Atypical HUS**

  Atypical HUS is rare: 10% of all HUS cases

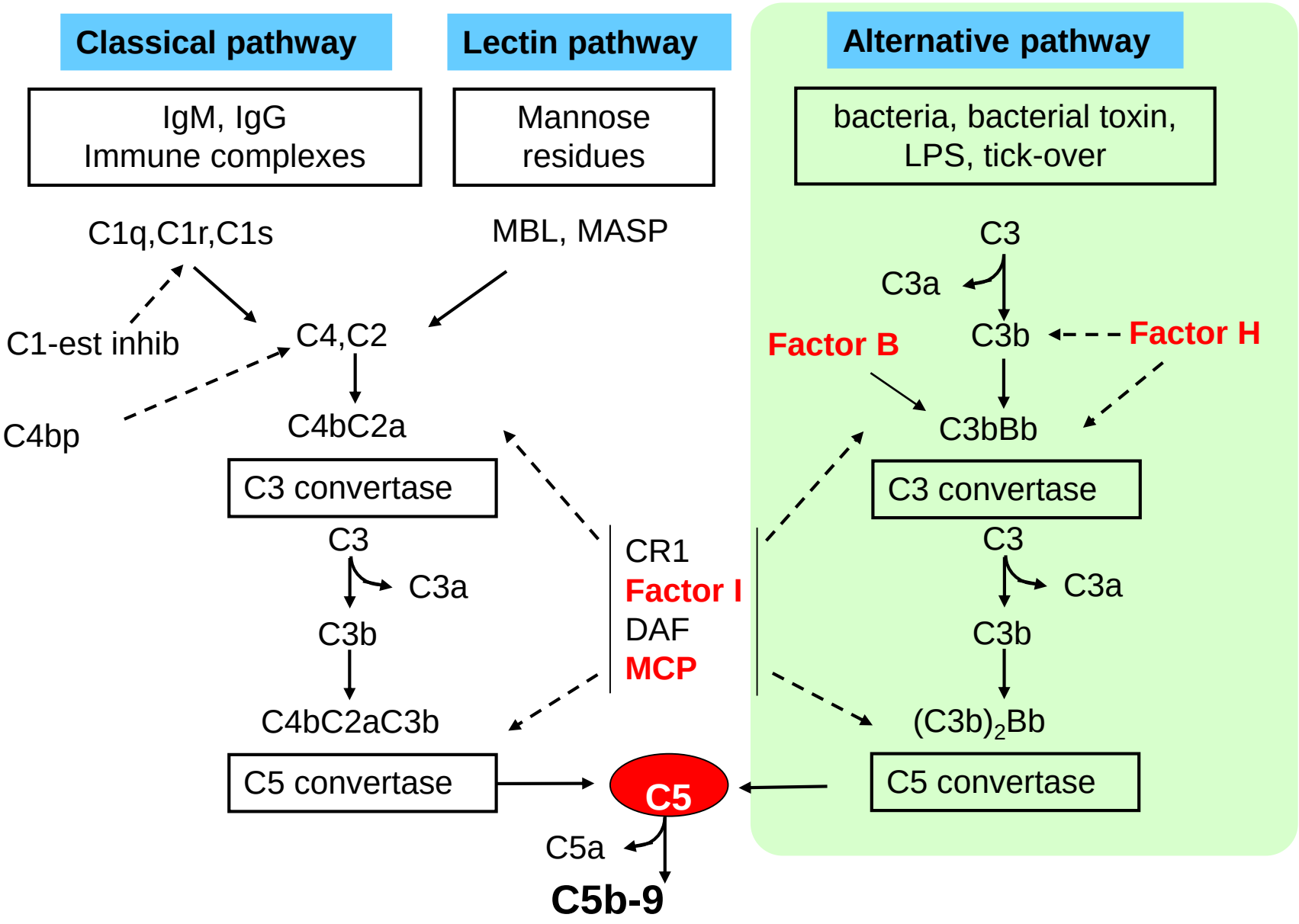
  Atypical HUS may be familial or sporadic and may manifest at all ages.

  Atypical HUS has a poor outcome: 50 to 60% of patients reach ESRD at the 1st episode

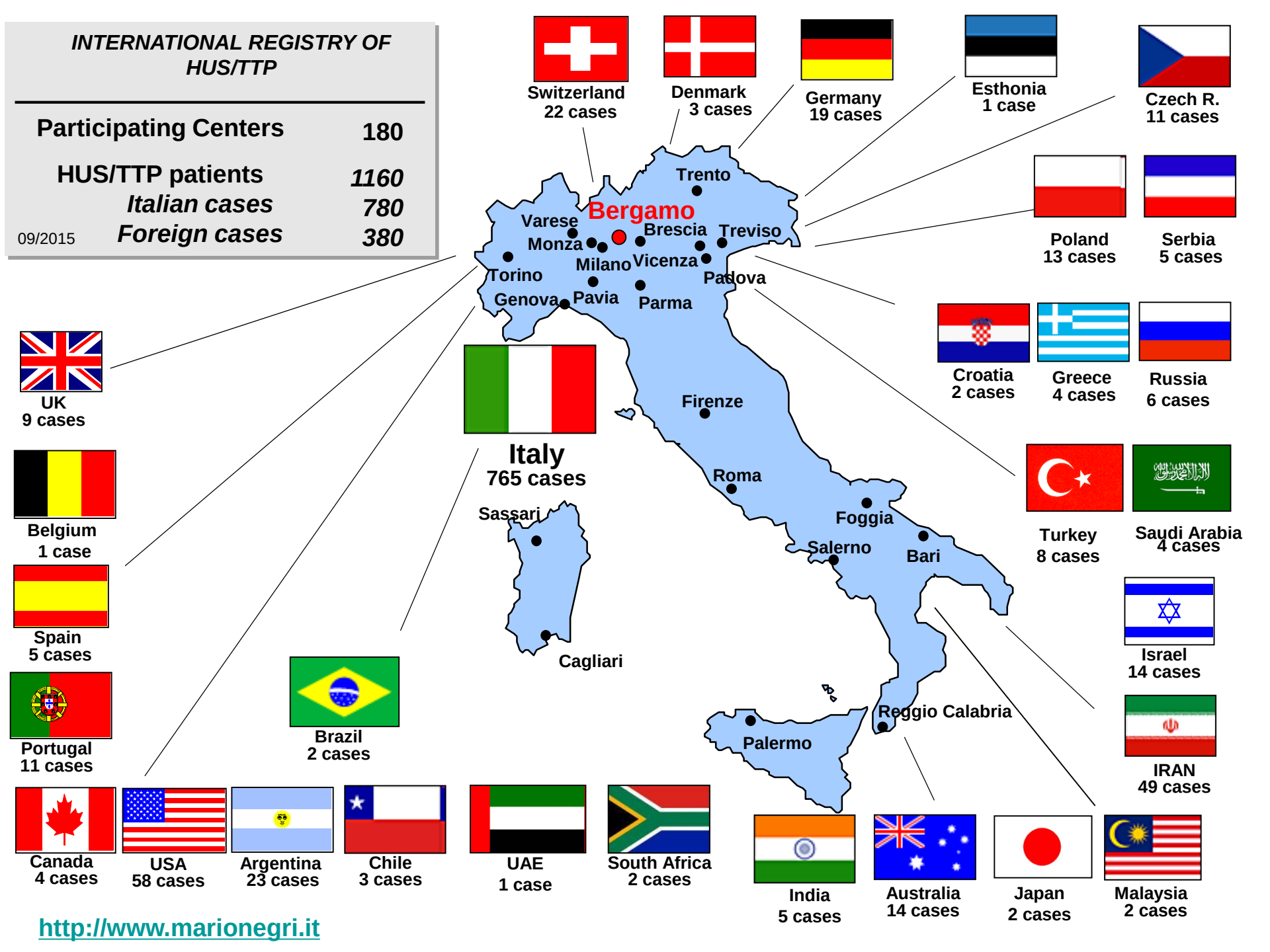
Atypical HUS

In the last 15 years, extensive research has established a clear link between aHUS and defects in regulation of the alternative complement pathway resulting in uncontrolled activation of the complement system

COMPLEMENT ACTIVATION PATHWAYS

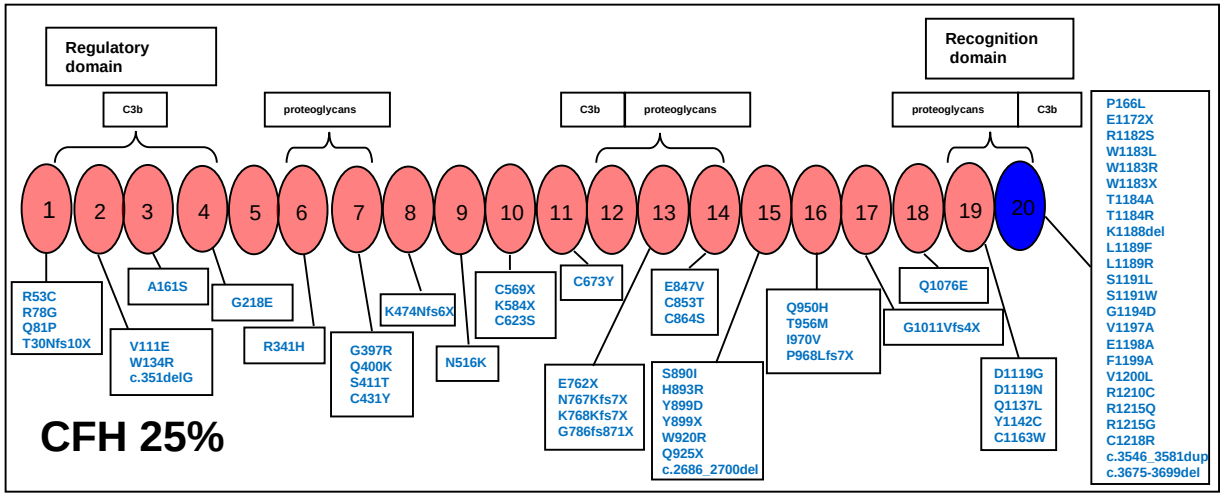


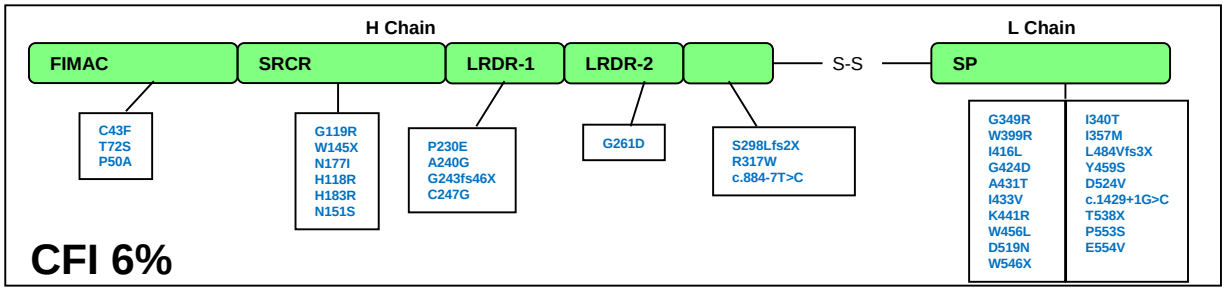
AP dysregulation on endothelial cells resulting in endothelial injury and loss of anti-thrombogenic properties.

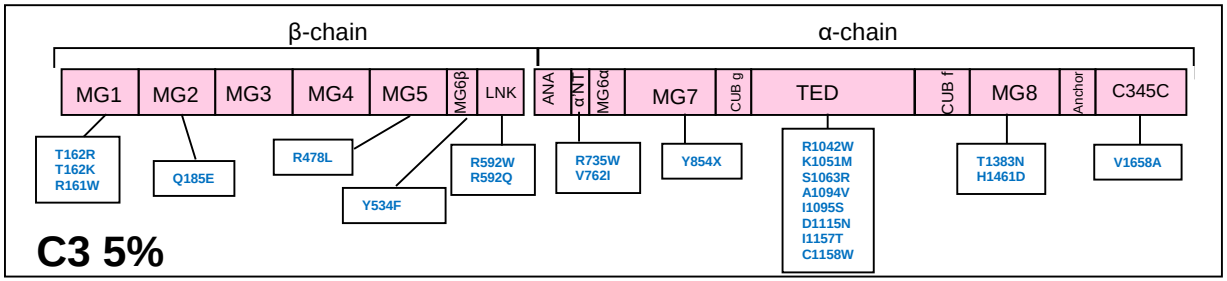

INTERNATIONAL REGISTRY OF HUS/TTP
Participating Centers 180
HUS/TTP patients 1160
Italian cases 780
Foreign cases 380
09/2015
Switzerland 22 cases
Denmark 3 cases
Germany 19 cases
Esthonia 1 case
Czech R. 11 cases
Poland 13 cases
Serbia 5 cases
Croatia 2 cases
Greece 4 cases
Russia 6 cases
Turkey 8 cases
Saudi Arabia 4 cases
Israel 14 cases
IRAN 49 cases
Australia 14 cases
Japan 2 cases
Malaysia 2 cases
India 5 cases
South Africa 2 cases
UAE 1 case
Chile 3 cases
Argentina 23 cases
USA 58 cases
Canada 4 cases
Brazil 2 cases
Portugal 11 cases
Spain 5 cases
Belgium 1 case
UK 9 cases
Italy 765 cases
Trento
Bergamo
Varese
Monza
Brescia
Treviso
Milano
Vicenza
Torino
Padova
Genova
Pavia
Parma
Firenze
Roma
Sassari
Foggia
Salerno
Bari
Cagliari
Reggio Calabria
Palermo
http://www.marionegri.it

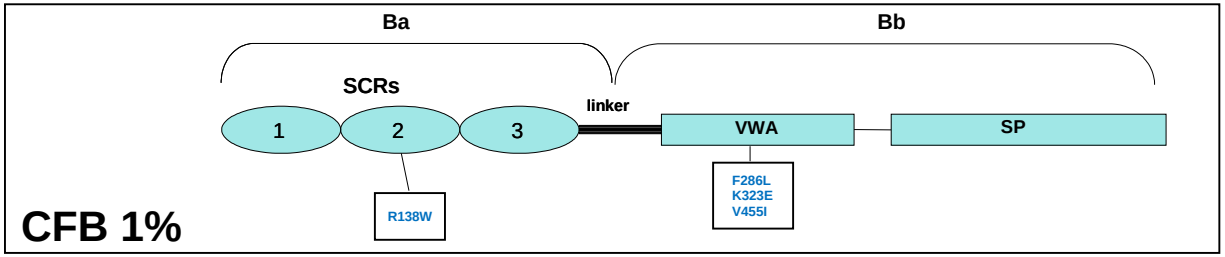
COMPLEMENT ABNORMALITIES IN aHUS PATIENTS

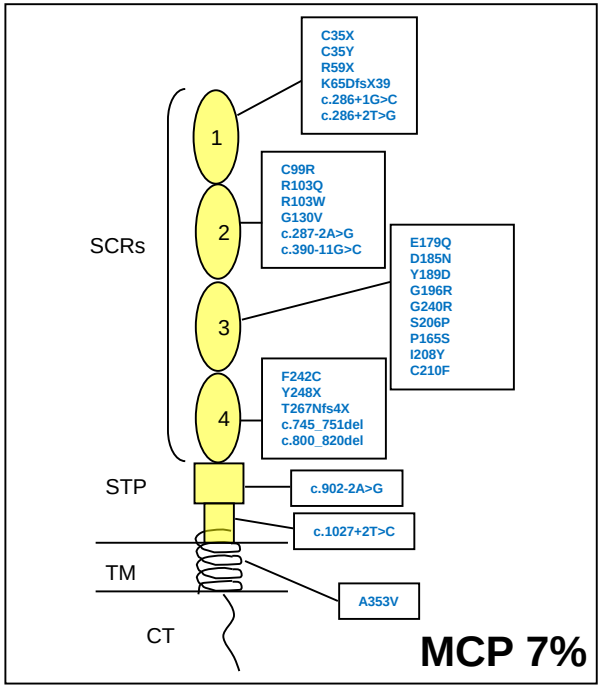
(Noris et al CJASN 2010; Bresin et al 2013)

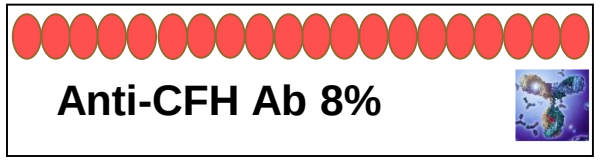


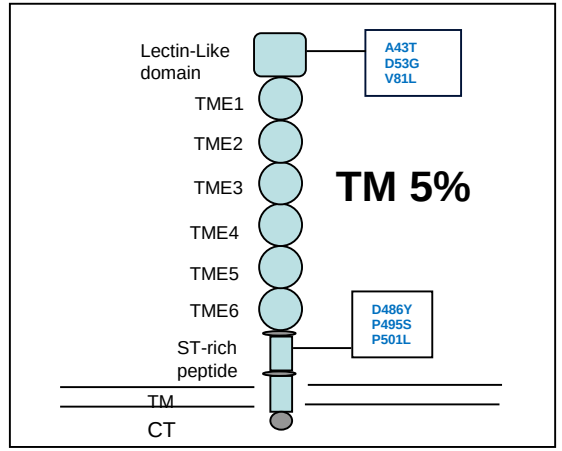




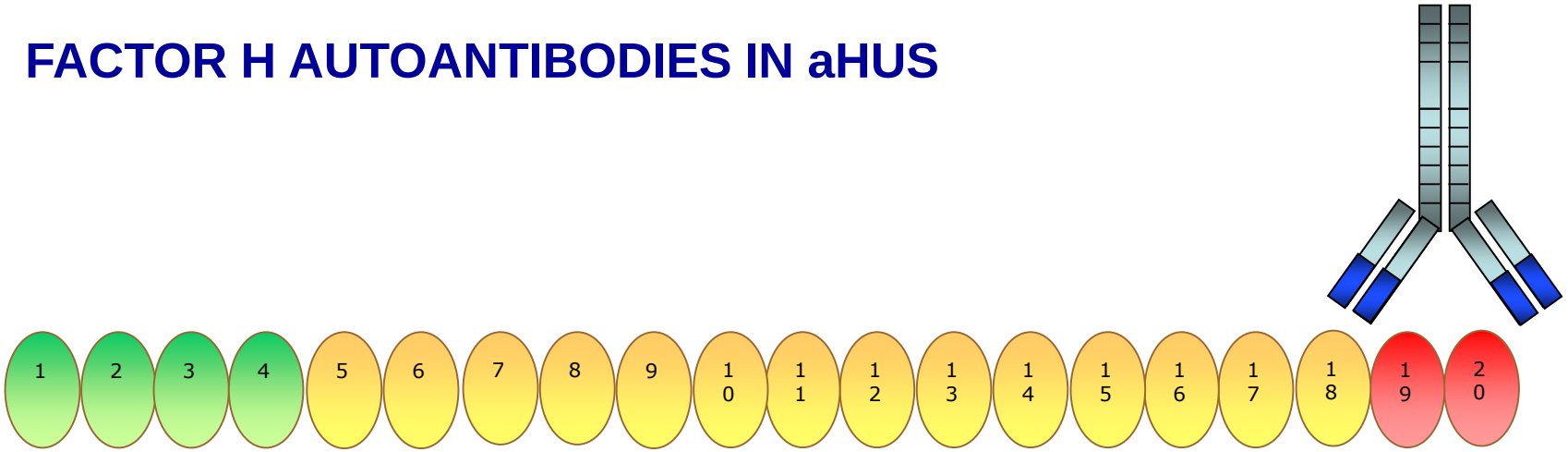








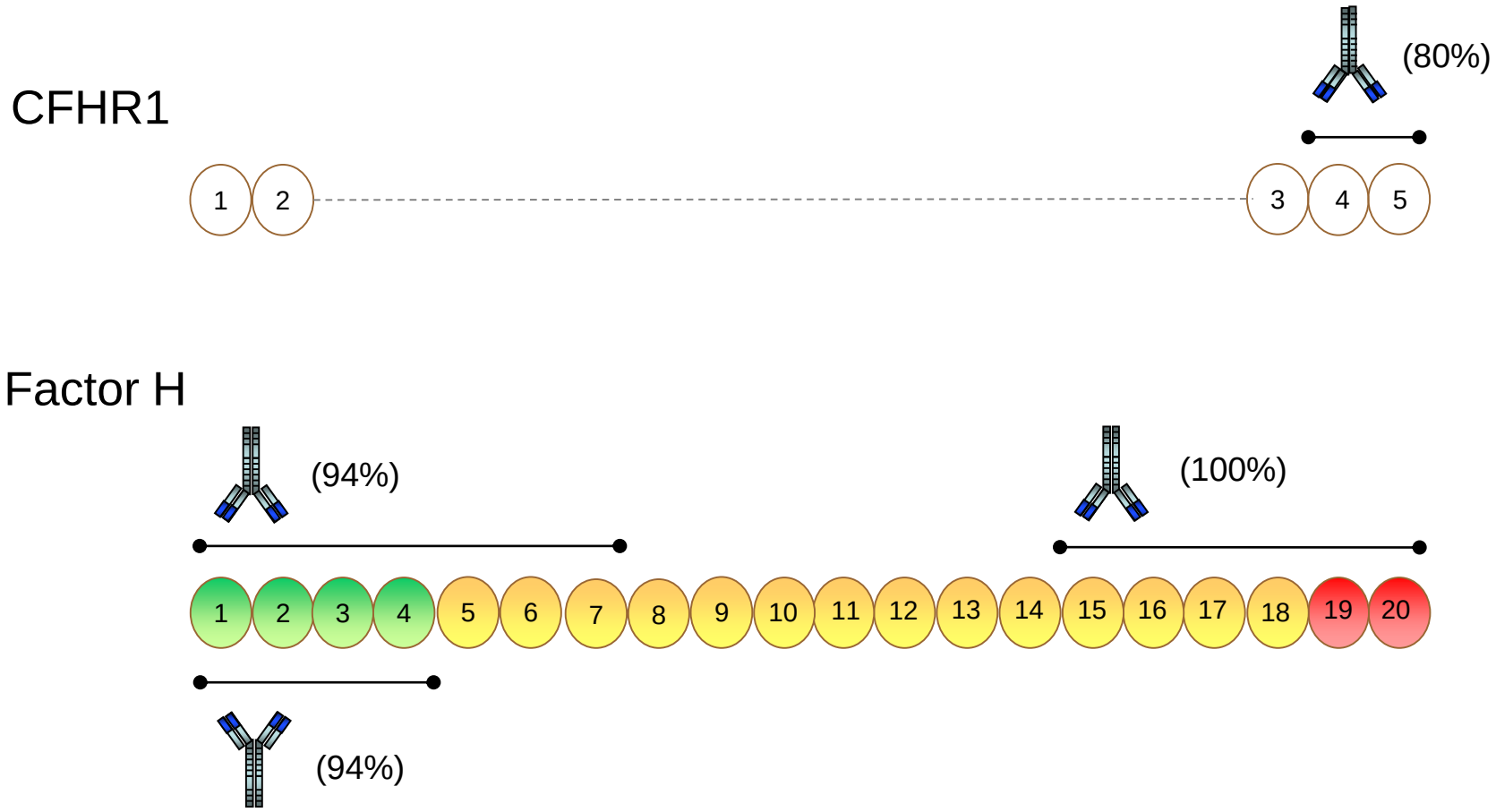
FACTOR H AUTOANTIBODIES IN aHUS



HUS associated factor H autoantibodies (described in 10% of aHUS cases, mainly in children) mimic the effect of C-terminal factor H mutations, as they inhibit the regulatory function of factor H at cell surfaces by blocking its C-terminal recognition region

Dragon-Durey et al, *JASN,* 2005
Jozsi et al., *Blood,* 2007

RECOGNITION SITES OF THE ANTI-FH AUTOANTIBODIES TO CFHR1 AND FH IN aHUS



Blanc C. et al. J Immunol, 2012

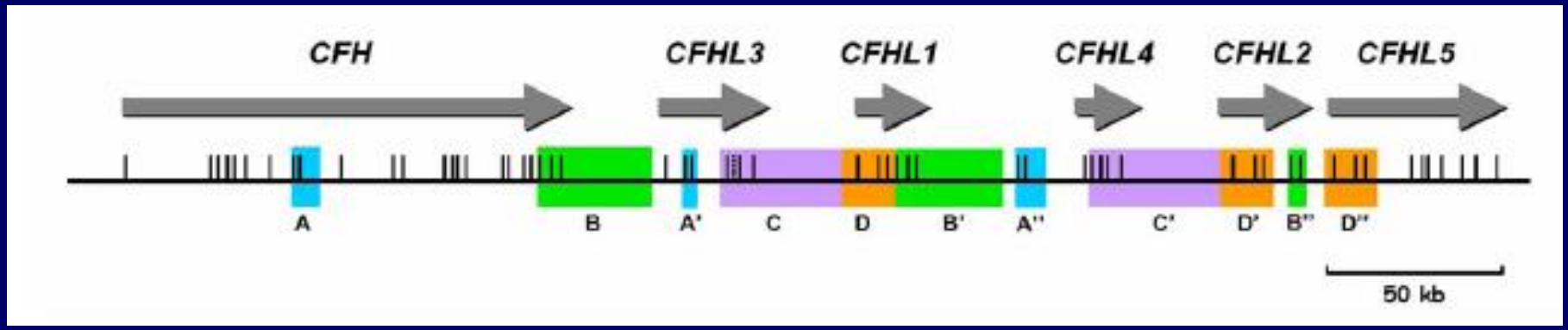
Among 104 aHUS patients tested both for the *CFHR1* deletion and anti-CFH autoantibodies, 23 out of 27 patients with autoantibodies (85%) were also homozygous for the *CFHR1* deletion.

Valoti et al. Immunobiology 2012

The Hom Δ*CFHR1* is a strong predisposing factor for the generation of anti-CFH autoantibodies.

Blanc C. et al. J Immunol 2012

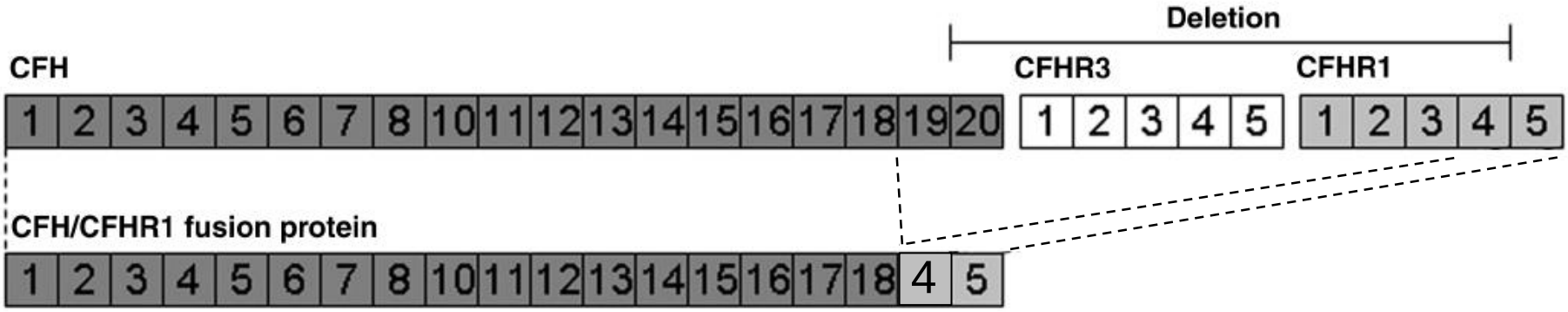
The formation of hybrid genes is a recently described mechanism for CFH abnormalities in HUS



High degree of sequence identity between the gene for factor H (CFH) and the genes for the five factor H-related proteins (CFHR1-5)

CFH consists of 20 SCR and CFHR1 of 5 SCR. CFH SCR20 and CFHL1 SCR 5 differ at only 2 aminoacids

ATYPICAL HEMOLYTIC UREMIC SYNDROME ASSOCIATED WITH A HYBRID COMPLEMENT GENE



Affected individuals carry a heterozygous CFH/CFHL1 hybrid protein in which SCR 1-18 are derived from CFH and SCR 19/20 from SCR 4/5 of CFHR1 by non allelic homologous recombination

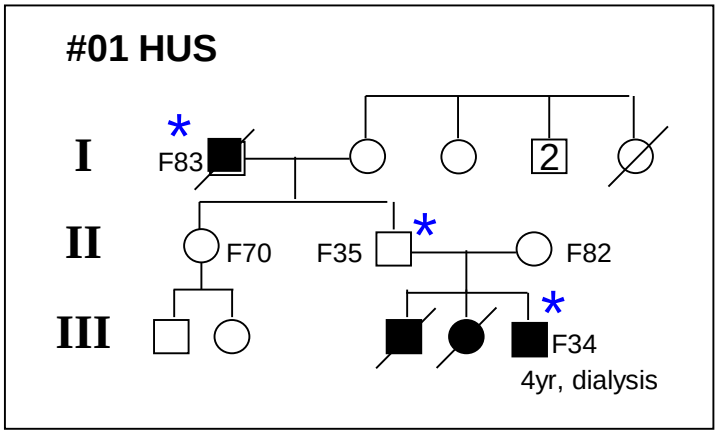
The protein product of the hybrid gene is a protein that lacks any complement regulatory activity on endothelial cell surface

Venables et al., PLoS Med, 2006
Noris et al., CJASN, 2010

INCOMPLETE PENETRANCE OF aHUS IN CARRIERS OF COMPLEMENT GENE MUTATION

CFH mutations: 48%
MCP mutations: 53%
CFI mutations: 50%
C3 mutations: 56%

Incomplete penetrance of aHUS indicates that additional genetic and/or environmental hits are necessary for disease manifestation



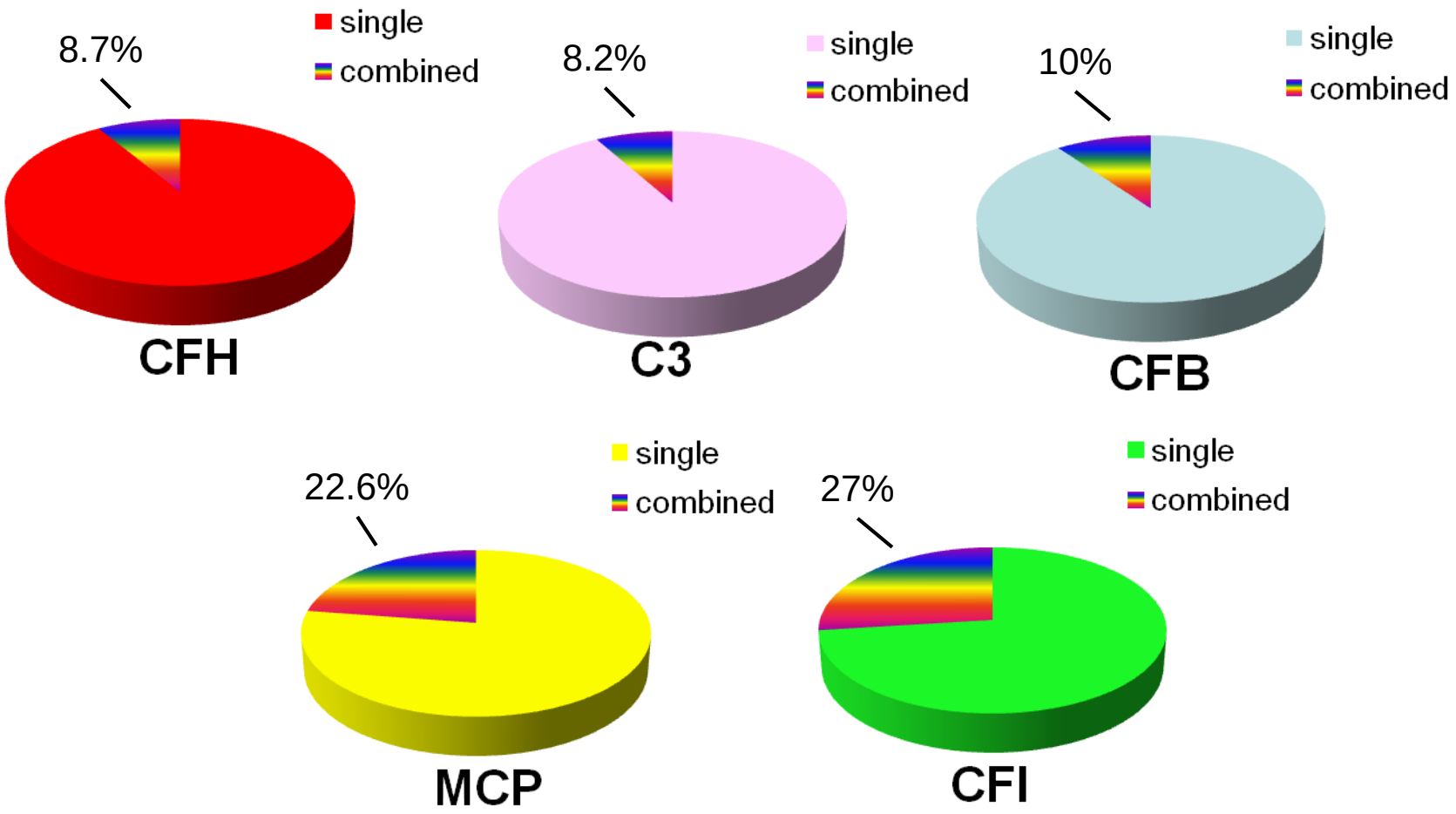
- 3 subjects in the III generation developed aHUS in infancy: 2 died and 1 reached ESRD
- father F35 never developed aHUS
- grandfather F83, carrier of the R1215Q/CFH mutation, developed aHUS and died at 82 years of age

Noris et al., *CJASN* 2010

#	Cohort	CFH	MCP	CFI	C3	CFB
1	S	T30Nfs10X	I208Y			
2	I	G1194D	F242C			
3	I	G1194D	F242C			
4	F	R1210C	Y29X			
5	I	R1210C	C35Y and R59X			
6	I	R1210C	C35Y and R59X			
7	S	R1215Q	R103Q			
8	F	N767Kfs7X		H183R		
9	S	P968fs947X		I340T		
10	I	S1191L		E554V		
11	I	V1197A			G1094R	
12	F	R341H			R161W	
13	F		R103W	P50A		
14	F		IVS2+2	H118R		
15	S		R103W	N151S		
16	S		R103W+c.800-820del	N151S		
17	S		C210F	C247G		
18	I		(96-129)del+ G130I+Y131I+Y132T+L133X	L484V+Q485G+ W486X		
19	S		P165S	T538X		
20	S		P165S	T538X		
21	UK		A353V	P553S		
22	F		A353V		H1464V	
23	F			D524V	P1114L	
24	F			Y459S		V455I
25	F	R1210C	Y29X	P553S		
26	I	R1210C	IVS2+2	I357M		

Bresin et al, JASN 2013

COMBINED complement gene MUTATIONS in aHUS



Combined complement gene mutations were found in 3% of aHUS patients, more frequently in those with *MCP* or *CFI* mutation than in patients with CFH or C3 or CFB mutations.

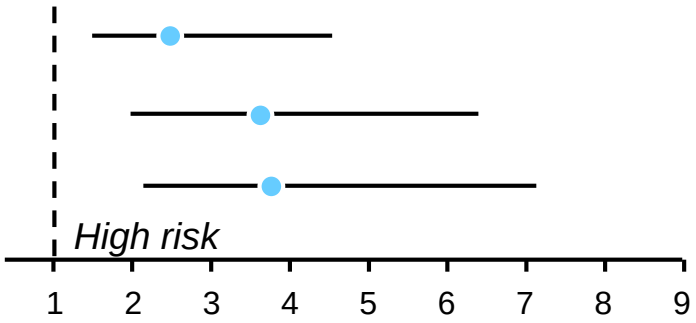
Bresin et al, JASN 2013

Human Molecular Genetics, 2003, Vol. 12, No. 24 **3385–3395**
DOI: 10.1093/hmg/ddg363

Complement factor H mutations and gene polymorphisms in haemolytic uraemic syndrome: the C-257T, the A2089G and the G2881T polymorphisms are strongly associated with the disease

Jessica Caprioli[1], Federica Castelletti[1], Sara Bucchioni[1], Paola Bettinaglio[1], Elena Bresin[1], Gaia Pianetti[1], Sara Gamba[1], Simona Brioschi[1], Erica Daina[1], Giuseppe Remuzzi[1,2] and Marina Noris[1,*] for the International Registry of Recurrent and Familial HUS/TTP

Genotypes	*Odds Ratios (95 % C.I.)*	*P value*
-257**T** Promoter		*0.0015*
2089**G**/SCR11, 2881**T**/SCR16		*< 0.0001*
Promoter + SCR16		*< 0.0001*



Risk haplotype *CFH-tgt*

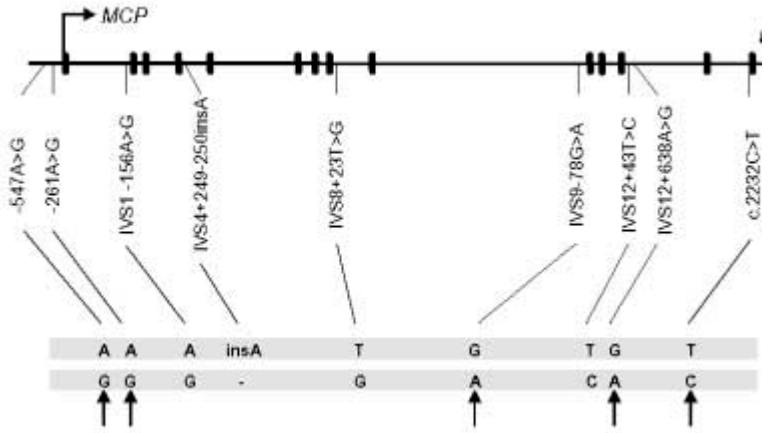
Caprioli et al., *Hum Mol Gen,* 2003

Human Molecular Genetics, 2005, Vol. 14, No. 5

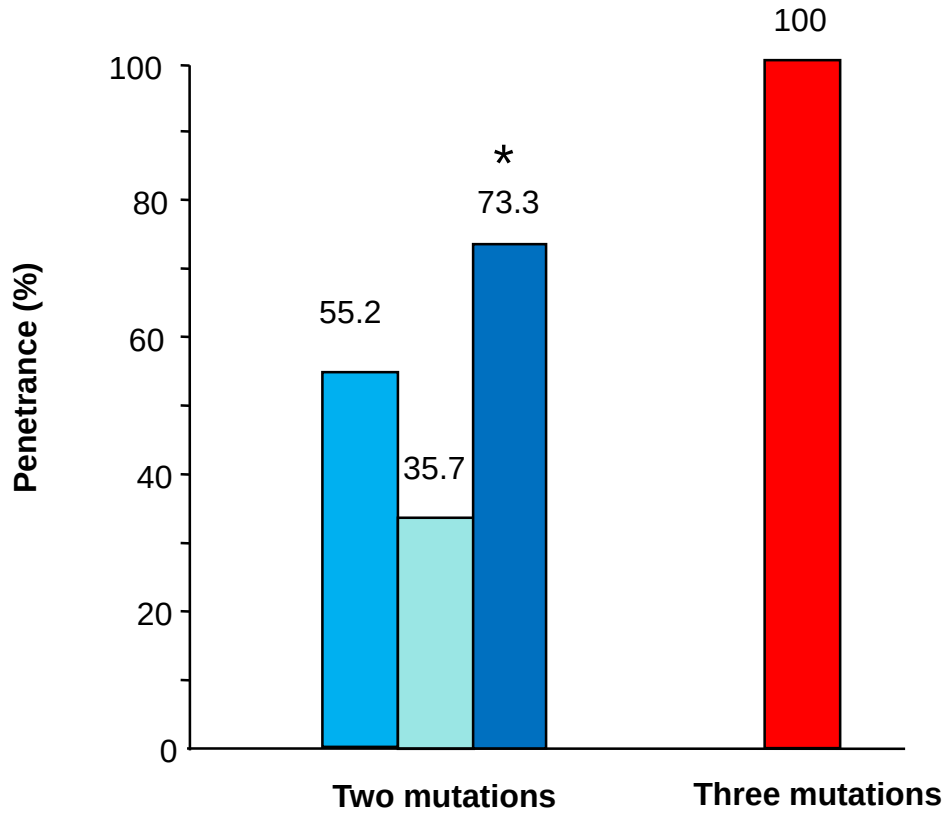
Predisposition to atypical hemolytic uremic syndrome involves the concurrence of different susceptibility alleles in the regulators of complement activation gene cluster in 1q32

Jorge Esparza-Gordillo[1], Elena Goicoechea de Jorge[1], Alfonso Buil[2], Luis Carreras Berges[3], Margarita López-Trascasa[4], Pilar Sánchez-Corral[4] and Santiago Rodríguez de Córdoba[1,*]

A specific SNP haplotype block, spanning the MCP gene in the regulators of complement activation gene cluster, which is over-represented in aHUS patients and strongly associates with the severity of the disease.



IMPACT of presence of *CFH* and *MCP* RISK HAPLOTYPES on aHUS PENETRANCE



*P<0.05 vs 0-1 risk haplotype

Risk haplotypes

***CFH-tgt*:**
c.1-332C>**T** (rs3753394) and c.2808G>**T**, p.E936D (rs1065489)

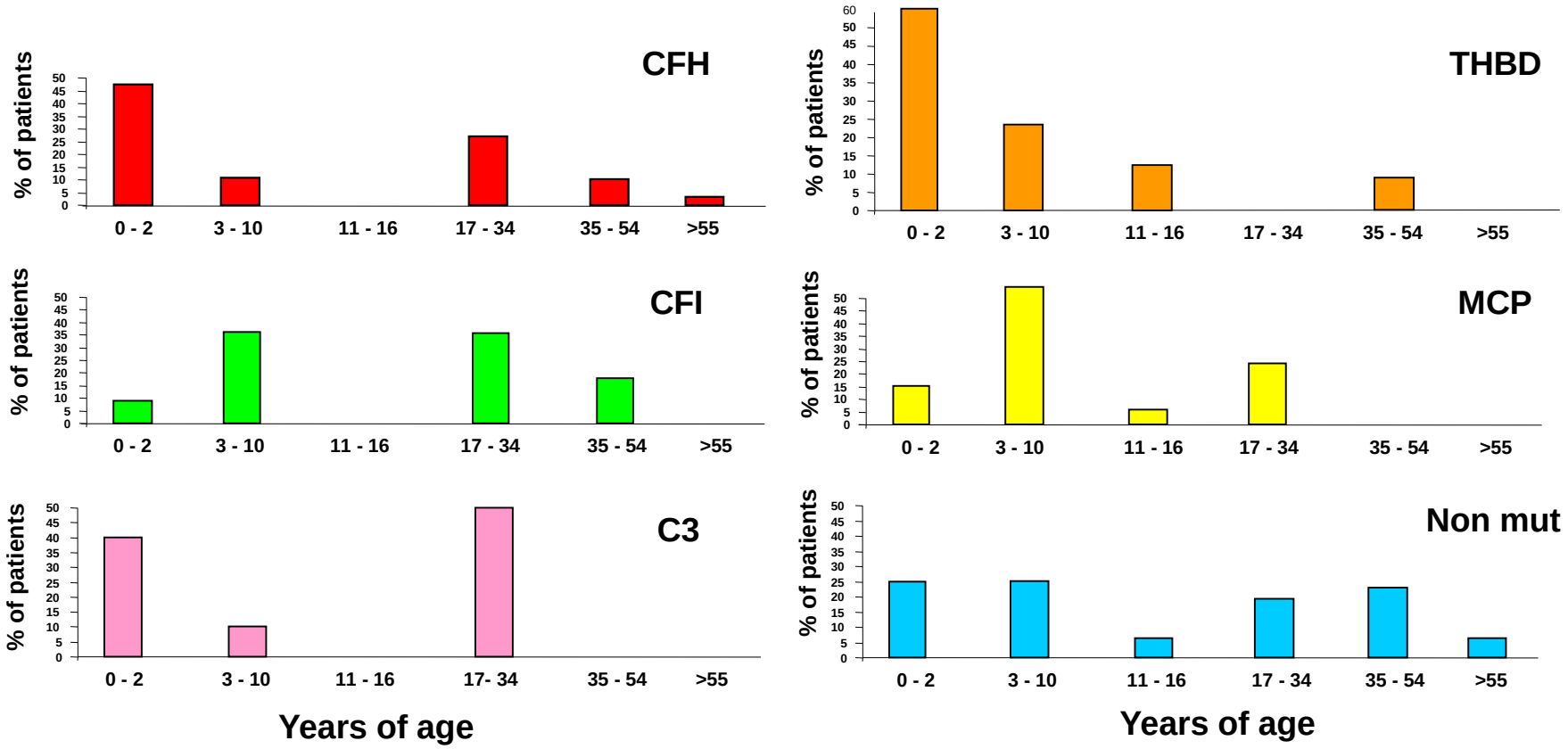
***MCPggaac*:**
c.*897T>C (rs7144)

The concomitant presence of CFH and MCP risk haplotypes increased aHUS penetrance in carriers of combined mutations

Bresin et al, JASN 2013

Abnormalities of complement regulators in aHUS: how do they impact clinical phenotype and management of patients?

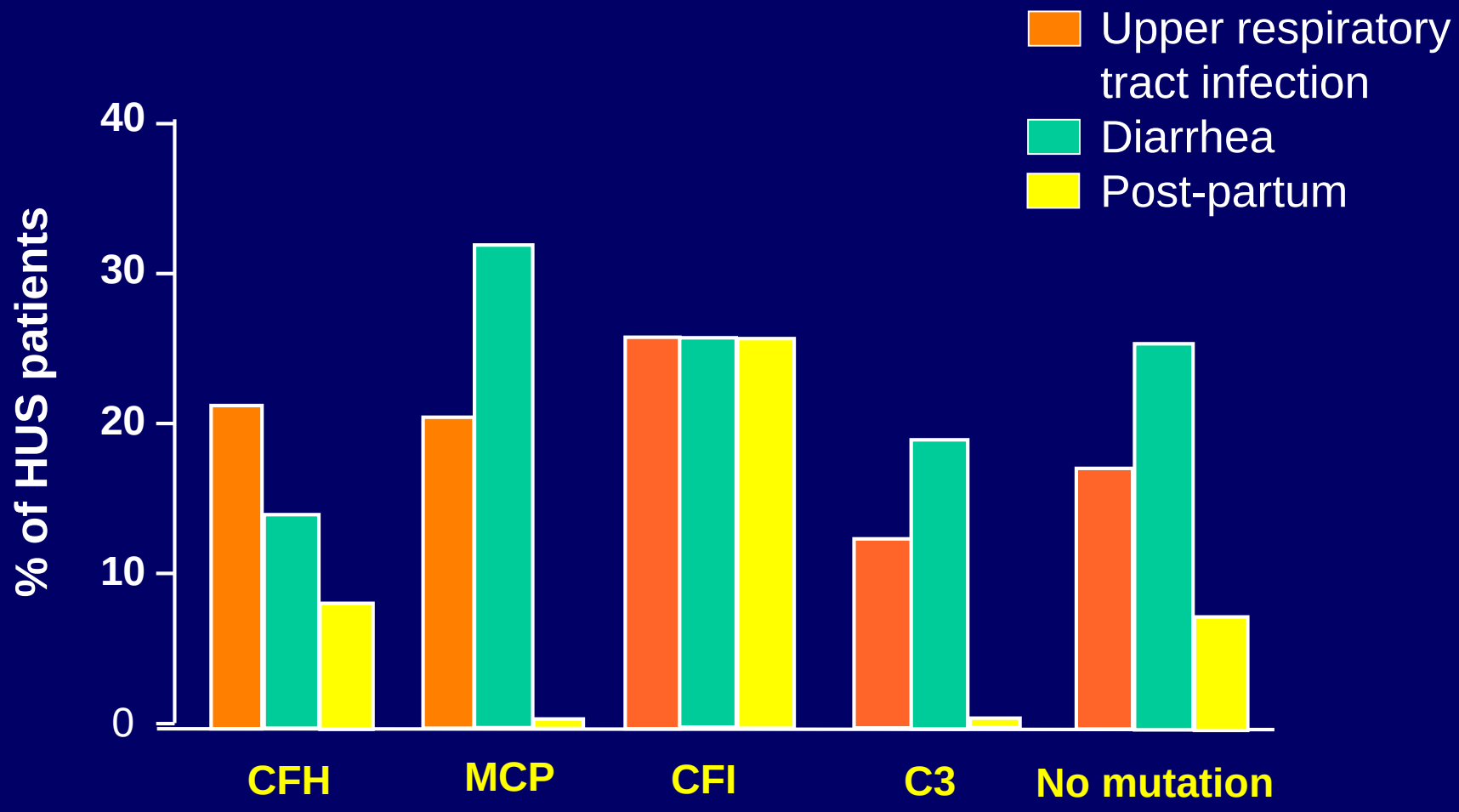
AGE AT ONSET



- aHUS manifested during infancy in most patients
- The earliest onset was in subjects with CFH or THBD mutations
- A second pick was at 20-30 years of age, often in association with post-partum

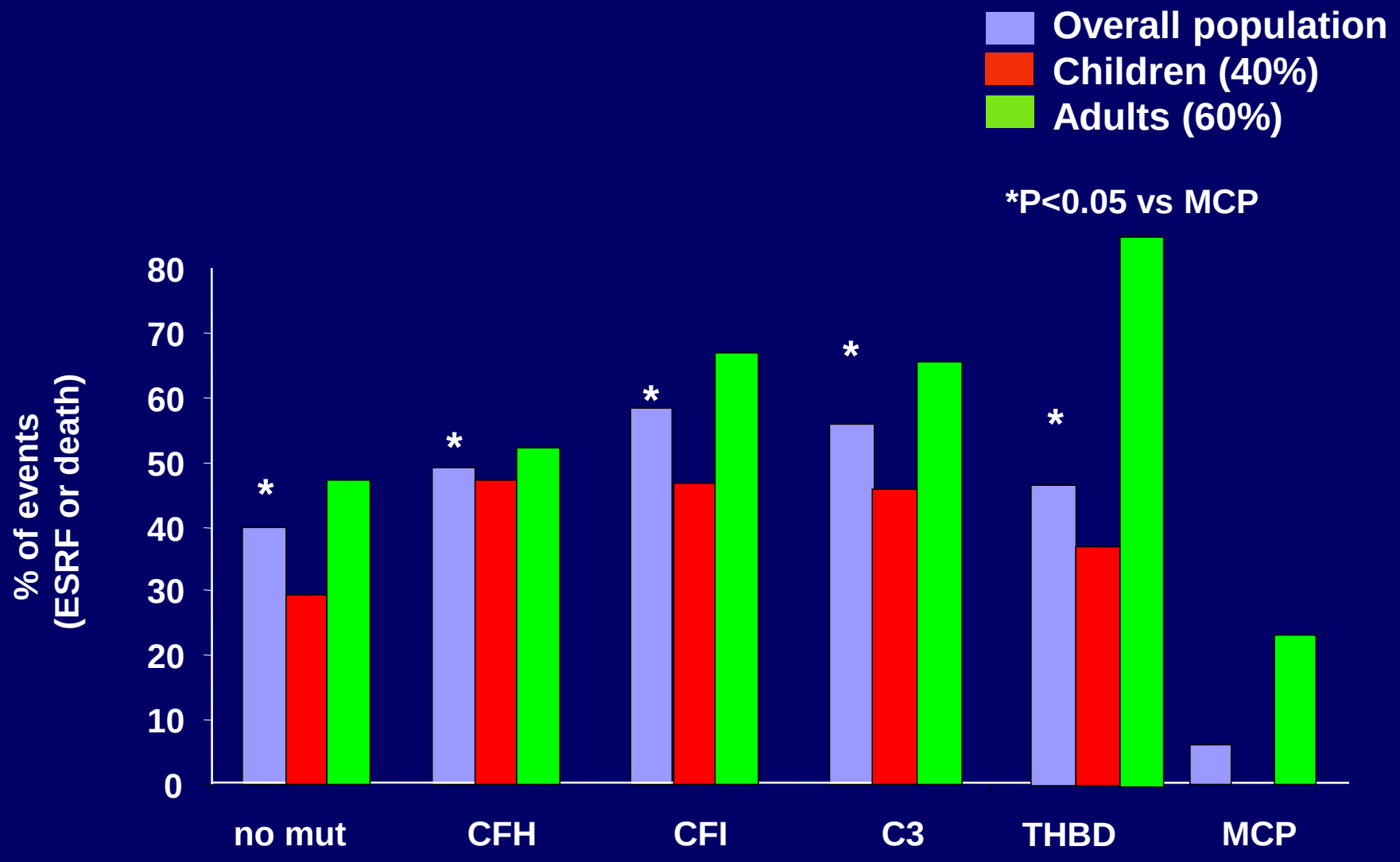
Noris et al *CJASN* 2010

TRIGGERING /UNDERLYING CONDITION



Noris et al, *CJASN* 2010

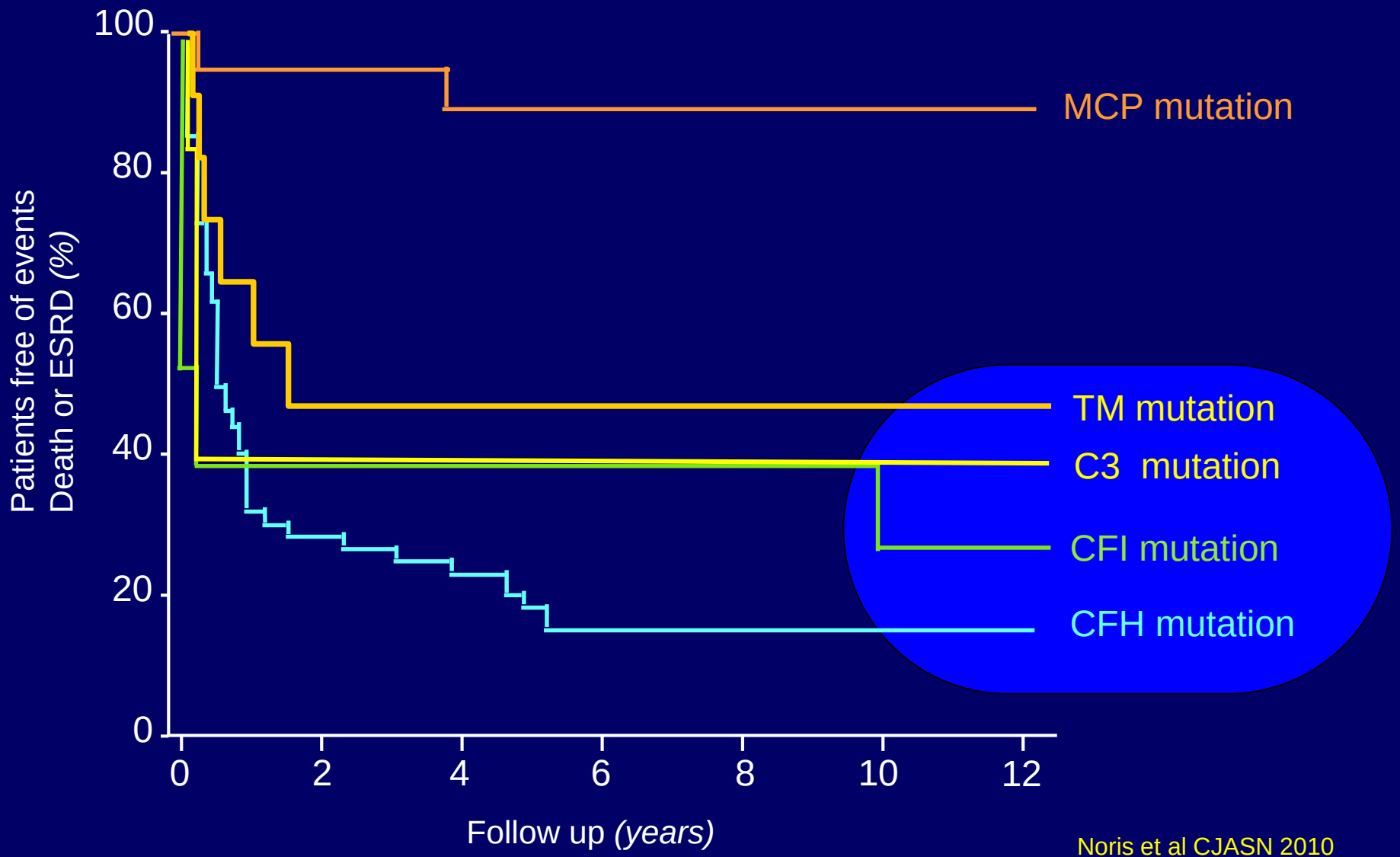
OUTCOME OF THE FIRST EPISODE



Noris et al *CJASN* 2010

LONG TERM OUTCOME OF aHUS PATIENTS

Mutations in CFH, CFI, C3 and THBD were associated with a worse long-term outcome than mutations in MCP.



Noris et al CJASN 2010

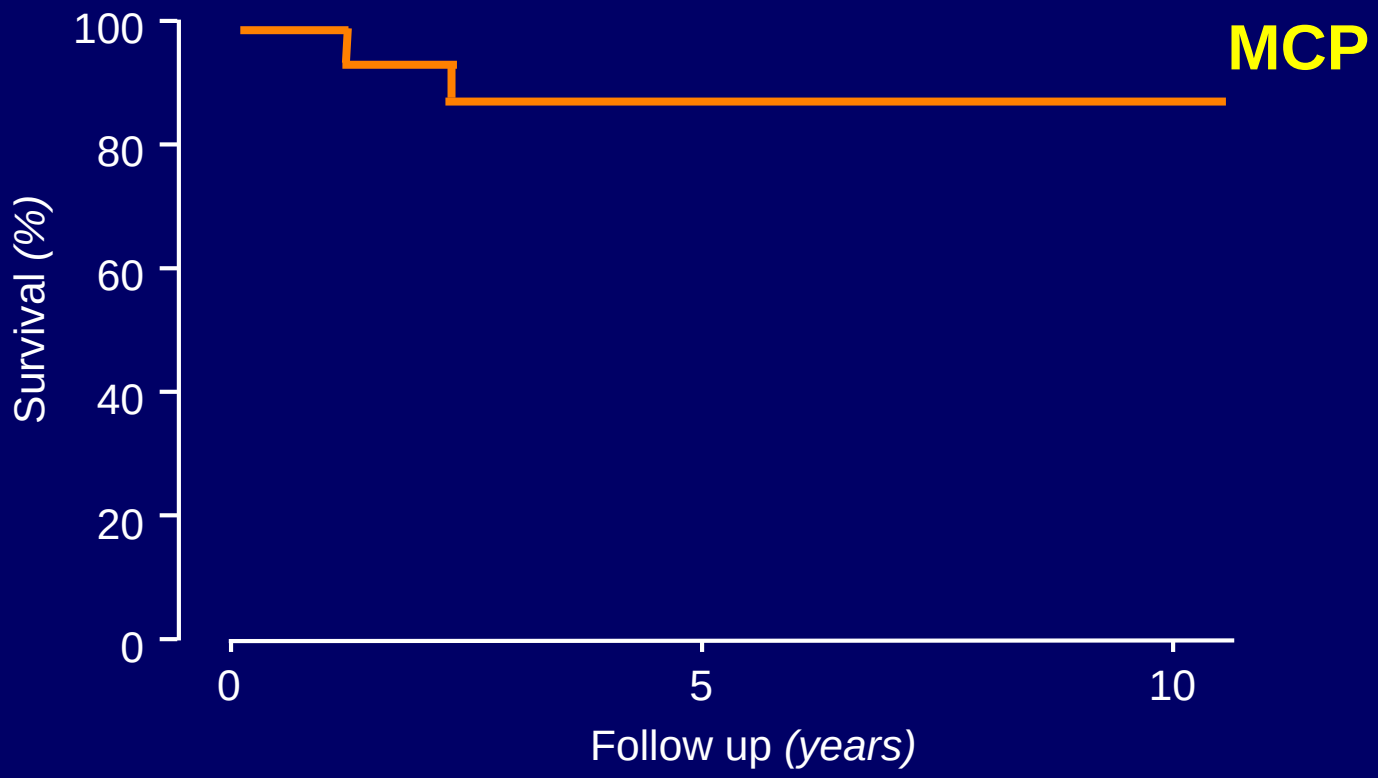
RENAL TRANSPLANTATION OUTCOMES

	patients/grafts	recurrences (% grafts)	graft failure for aHUS recurrence
Patients with:			
- CFH mutations	60/76	49/76 (64%)	40 out 49 (82%)
- CFI mutations	23/26	19/26 (73%)	18 out 19 (95%)
- C3 mutations	18/30	16/30 (53%)	12 out 16 (75%)
- CFB mutations	3/4	4/4 (100%)	4 out 4 (100%)

Noris & Remuzzi, *Am J Transplant* 2010
Zuber Jet al. Nat Rev Nephrol 2011
Le Quintrec M et al. Am J Tranpl 2013

In patients with alterations in circulating complement proteins, the risk of graft failure for aHUS recurrence is high since the altered protein is produced by the liver and persists in the circulation after kidney transplantation.

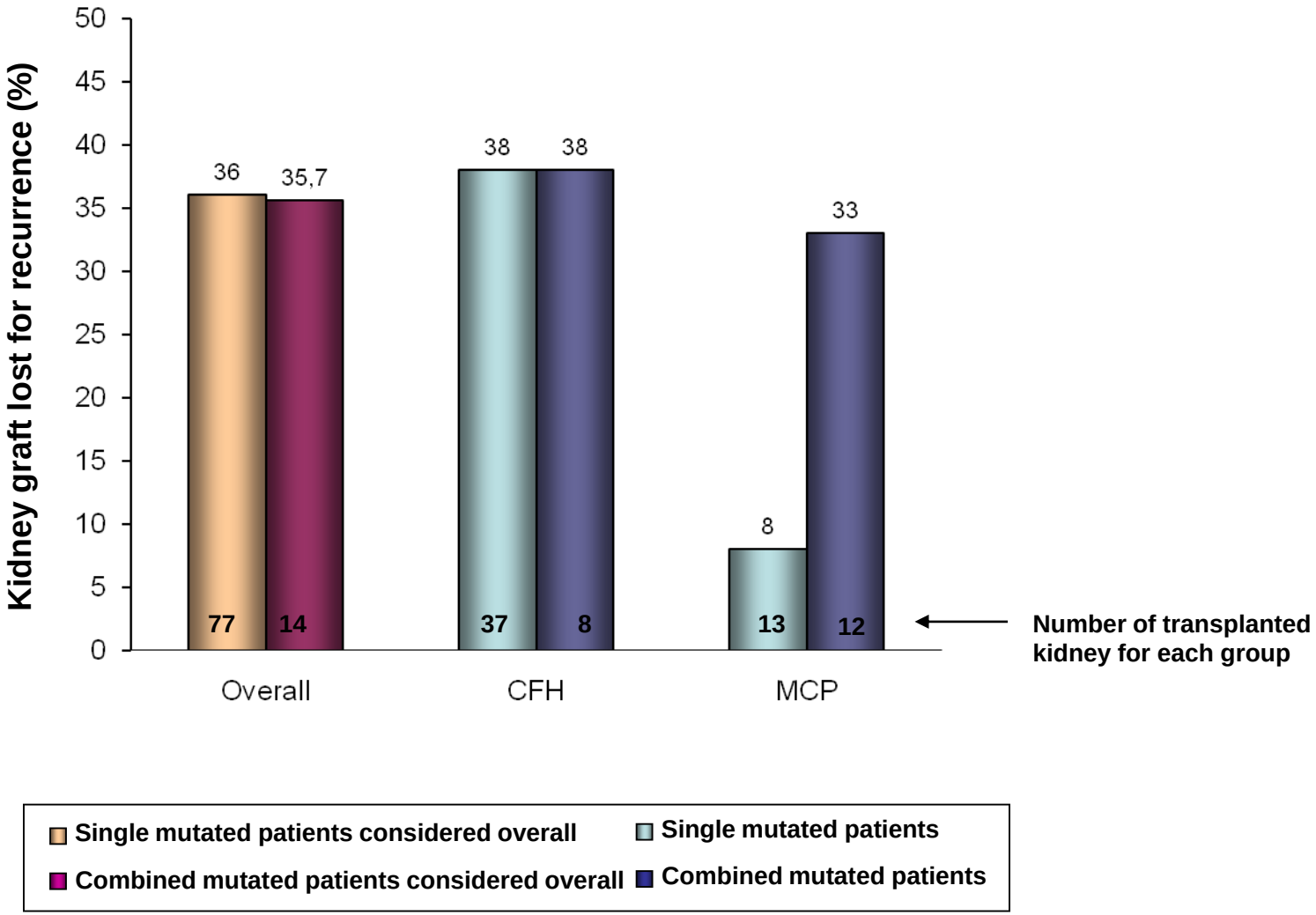
SURVIVAL TIME OF THE KIDNEY GRAFT IN PATIENTS WITH SINGLE MCP MUTATIONS



No HUS recurrence in 10 out of 12 kidney grafts.
MCP is a membrane-bound protein highly expressed in the kidney.
A dysfunction in MCP can be corrected by transplanting a normal kidney.

Noris and Remuzzi, *Am J Transplant* 2010

OUTCOME OF KIDNEY TRANSPLANTATION OF COMBINED AND SINGLE MUTATED PATIENTS FROM 4 COHORTS OF aHUS PATIENTS



Bresin et al, JASN 2013

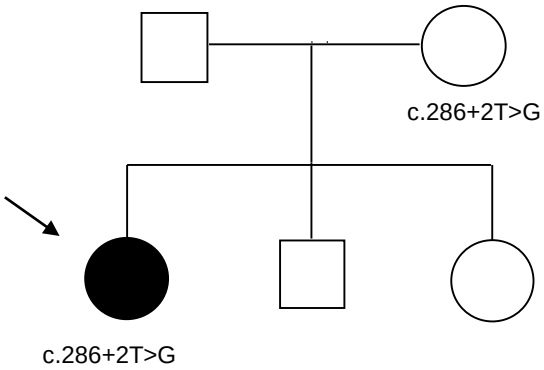
Recurrence of HUS after live-related renal transplantation associated with subsequent *de novo* disease in the donor

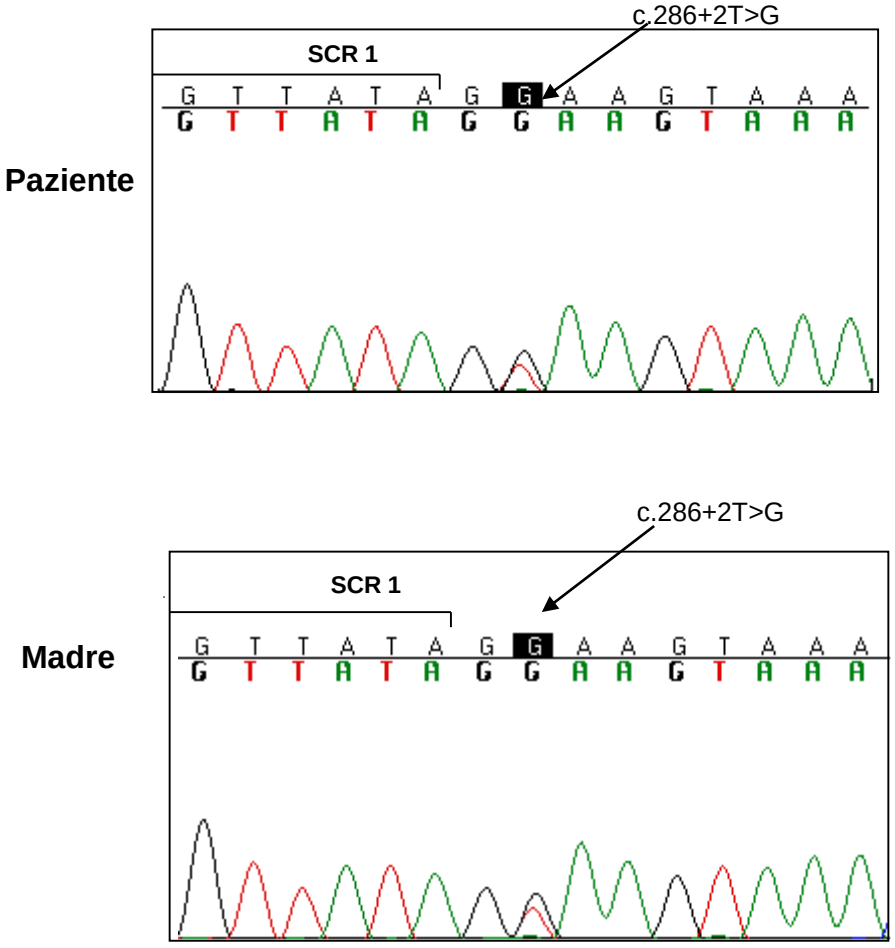
- Proband: a 4 month-old female with ESRD following an episode of aHUS and no familial history of the disease
- Living-related renal transplant from the father with graft loss in 7 weeks for HUS
- The donor developed HUS 10 months after donation and subsequently progressed to ESRD
- Genetic screening revealed that both the proband and her donor father carried a heterozygous *S1191L* mutation in *CFH*

In the case of CFH associated HUS, living related kidney transplant is risky not only for the recipient, but also for the donor

Donne et al., *Am J Kidney Dis,* 2002

Patiente con mutazione MCP e recidiva di SEU post-trapianto





La paziente aveva ricevuto il rene dalla madre, portatrice dello stessa mutazione MCP.

Il rene trapiantato produceva la stessa proteina MCP disfunzionale come il rene nativo e non era abbastanza protetto dalla iperattivazione del complemento.

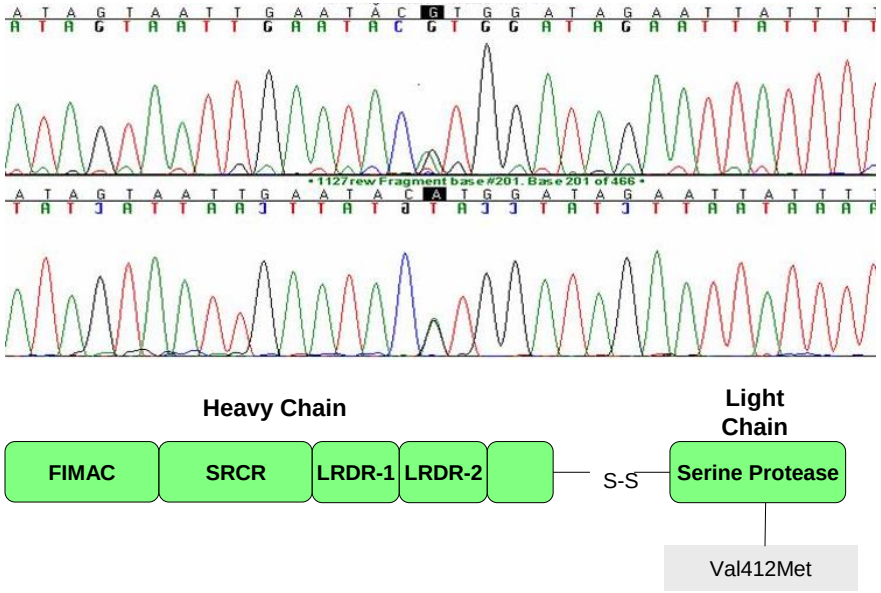
Alberti M. et al. *Am J Transpl* 2013

La SEU tipica ha un basso tasso di recidiva post-trapianto (0-5%)

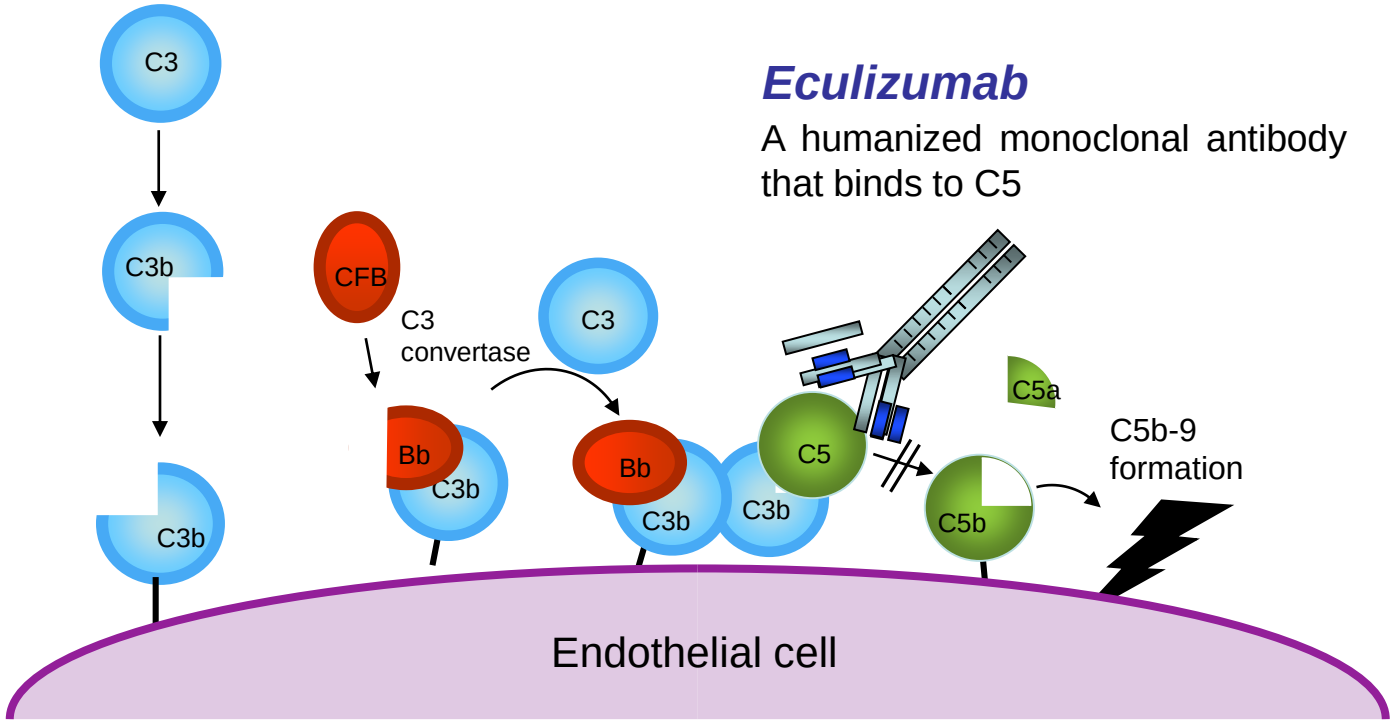
Caso

Una giovane donna con IRT dopo grave episodio di SEU tipica aveva ricevuto un trapianto di rene da cadavere che era fallito dopo 1 anno, per ricorrenza della SEU.
Lo screening genetico eseguito prima di pianificare 2° trapianto rivelava una mutazione di *CFI*.

Alberti M. et al. *Am J Transpl* 2013



Questo caso indica che lo screening dei geni associati alla SEU si dovrebbe eseguire anche in pazienti in dialisi a seguito di gravi episodi di SEU tipica.
Ci possono essere casi non diagnosticati di SEU atipica, innescati da infezione da *Escherichia Coli* che hanno una predisposizione genetica.



Eculizumab

A humanized monoclonal antibody that binds to C5

Eculizumab was safe and well tolerated in patients with Paroxistic Nocturnal Hemoglobinuria (PNH).

This antibody against terminal complement protein 5 reduced intravascular hemolysis, hemoglobinuria, and the need for transfusion, with an associated improvement in the quality of life.

TRIALS OF ECULIZUMAB IN ATYPICAL HUS (62 weeks data)

- **Patients resistant to plasma therapy**

-Platelet normalization	15/17 patients
-TMA event-free status	15/17 patients
-Change in renal function	
One-stage improvement	11/17 patients
>25% creatinine reduction	13/17 patients

- **Patients on chronic plasma therapy**

-TMA event-free status	17/20 patients
-Hematological normalization	18/20 patients
-One-stage renal function improvement	9/20 patients

Ten patients had no identified genetic abnormalities or anti-CFH antibodies

Licht et al., ***J Am Soc Nephrol*** *(Abstract),* 2011
Greenbaum et al., ***J Am Soc Nephrol*** *(Abstract),* 2011
Nester CM and Brophy PD, ***Curr Opin Pediatr***, 2013
Legendre CM et al., ***NEJM***, 2013

Managing and preventing aHUS recurrence after kidney tx

- **Patients with aHUS recurrence in the kidney graft (n=13)**

Mutation	Response to plasma	Response to Ecu	Loss
CFH	2/8	6/8	*1/8
C3	2(partial)/2	2/2	0/2
not identified	0/3	*1/3	2/3

*Single dose of Eculizumab

Two patients were plasma-dependent whereas the other had plasma-resistant forms.
A single-dose eculizumab and eculizumab discontinuation after several months were associated with subsequent relapses and graft loss in 3 patients despite re-initiation of eculizumab.

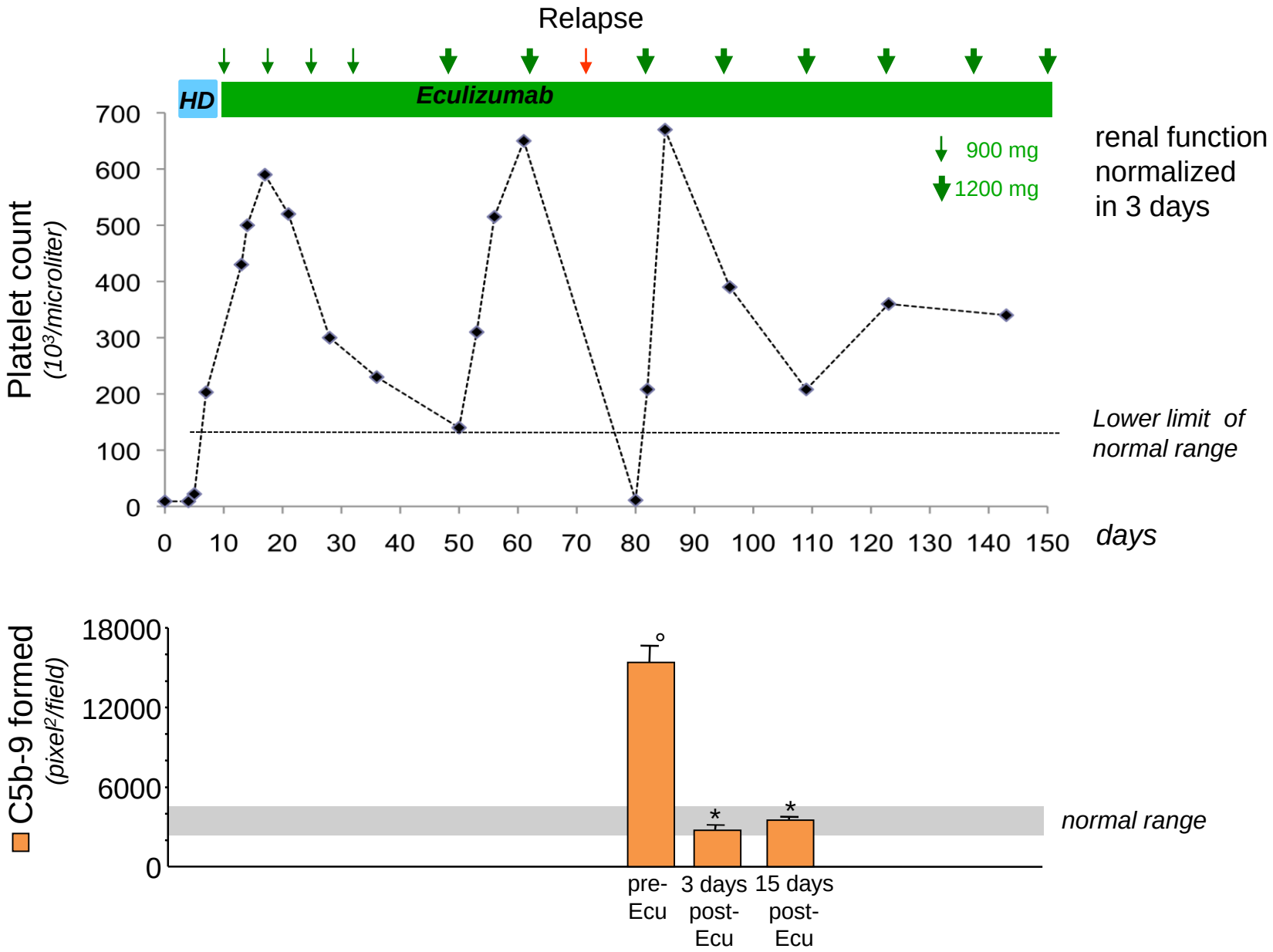
- **Patients with post-transplant Eculizumab prophylaxis (n=10)**

Mutation	Plasma therapy	Recurrence	Loss
-CFH	4/9	0/9	1/9
-C3	0/1	0/1	0/1

Two pts received PEX just before tx and were switched to eculizumab at day 5 and 10 post-tx.
In 3 pts eculizumab was started 1 week or more before tx, preceded (in 2 pts) by PEX.
The more common strategy was based on eculizumab alone, started immediately before tx.
Only one patient lost the graft for immediated arterial thrombosis at day 1 post-tx.

Congenital TTP and eculizumab

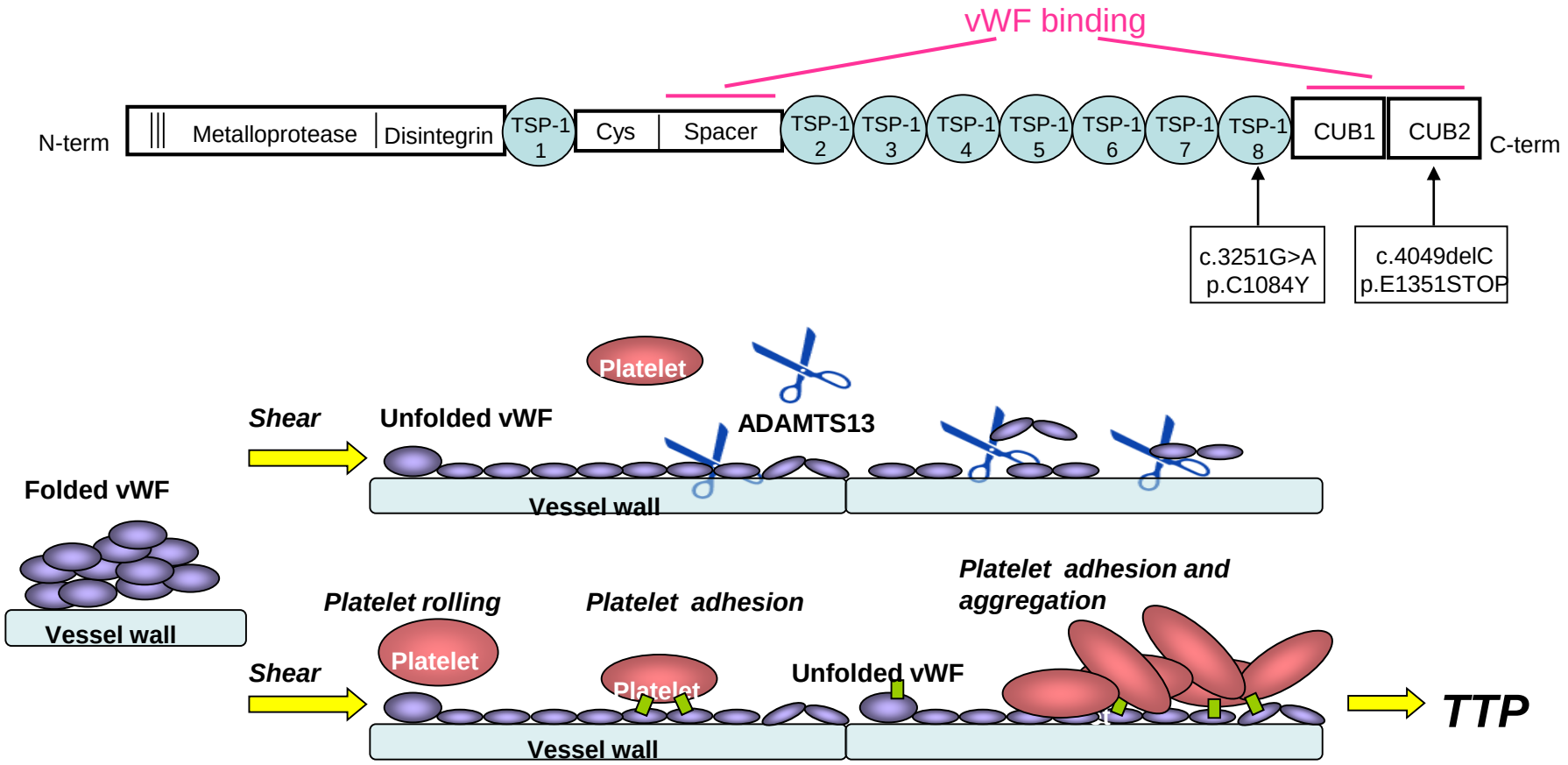
- A 12-year-old Italian boy was admitted to the Hospital with hemolytic anemia, thrombocytopenia, acute renal failure requiring hemodialysis and generalized seizures, preceded by an upper respiratory tract infection
- Absence of prodromal diarrhoea and negative results (by stool culture and sierology) for Shiga-like toxin-producing E.coli
- Elevated plasma levels of the terminal complement complex sC5b-9 (520 ng/ml, n.v.<400 ng/ml)
- Diagnosis of complement-mediated atypical hemolytic uremic syndrome (aHUS)



Serum-induced C5b-9 deposits on HMEC-1 ex-vivo normalized after Eculizumab

Pecoraro C, Remuzzi G, 2015

- No mutations in CFH, MCP, CFI, C3, CFB, THBD, and no anti-CFH antibodies.
- Undetectable plasma ADAMTS13 activity (<6% by CBA, without inhibitory antibodies).
  Two compound heterozygous ADAMTS13 mutations:



Pecoraro C, Remuzzi G, 2015

The clinical evolution of this patient and the intense C5b-9 deposits suggested a role of complement activation in the pathogenesis of TTP and opens a perspective for the treatment of this disease

RECESSIVE MUTATIONS IN DGKE CAUSE ATYPICAL HEMOLYTIC-UREMIC SYNDROME

- Disease presentation before the age of 1 year, with multiple relapsing episodes, often progressing to CKD by the second decade of life.

- Mutations in DGKE encoding diacylglycerol kinase ε. Recessive inheritance. Explained 27% of aHUS cases in the first year of life.

- No evidence linking DGKE deficiency to complement cascade.

  One of 7 patients with DGKE mutation on maintenance Eculizumab therapy had an HUS relapse.

- Renal transplantation can be efficacious in patients with DGKE mutations. No aHUS recurrence after transplantation in 3/3 subjects.

Lemaire M et al, Nature Genet 2013

Conclusioni

- La PTT è causata da deficienza acquisita o congenita di ADAMTS13 ed è importante definire la causa per scegliere la terapia appropriata.
- Importante lo screening di mutazioni del complemento nella SEU atipica, e nelle forme severe di SEU tipica.
- Elevato rischio di recidiva post-tx in pazienti con mutazioni in geni che codificano per proteine del complemento circolanti (CFH, CFI, C3, CFB).
- Pazienti con mutazioni in MCP e DGKE hanno una prognosi favorevole e mostrano buon esito del trapianto.

Laboratory

Marina Noris

Roberta Donadelli

Elisabetta Valoti

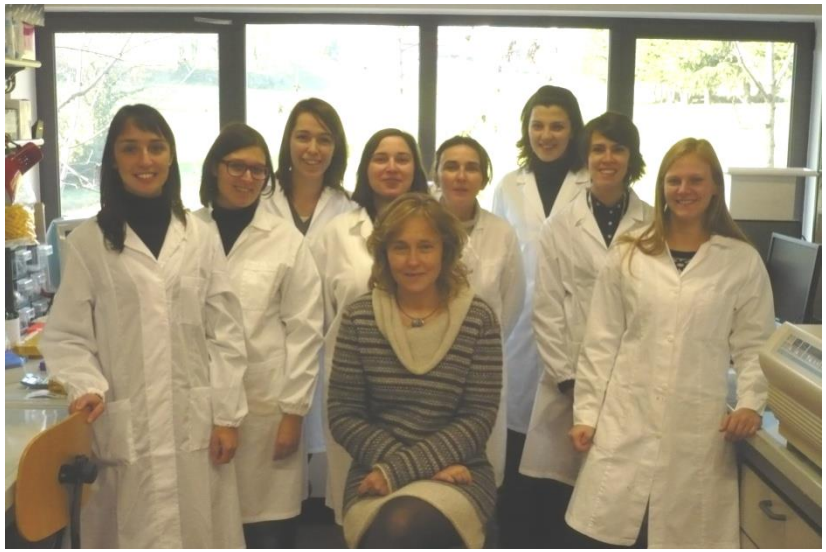
Rossela Piras

Caterina Mele

Marta Alberti

Matteo Breno

Serena Bettoni



Clinical

Giuseppe Remuzzi

Erica Daina

Piero Ruggenenti

Sara Gamba

Paraskevas Iatropoulos

Manuela Curreri

Collaborations

Santiago Rodriguez DeCordoba

Veronique Fremeaux-Bacchi

Tim Goodship

Peter Zipfel