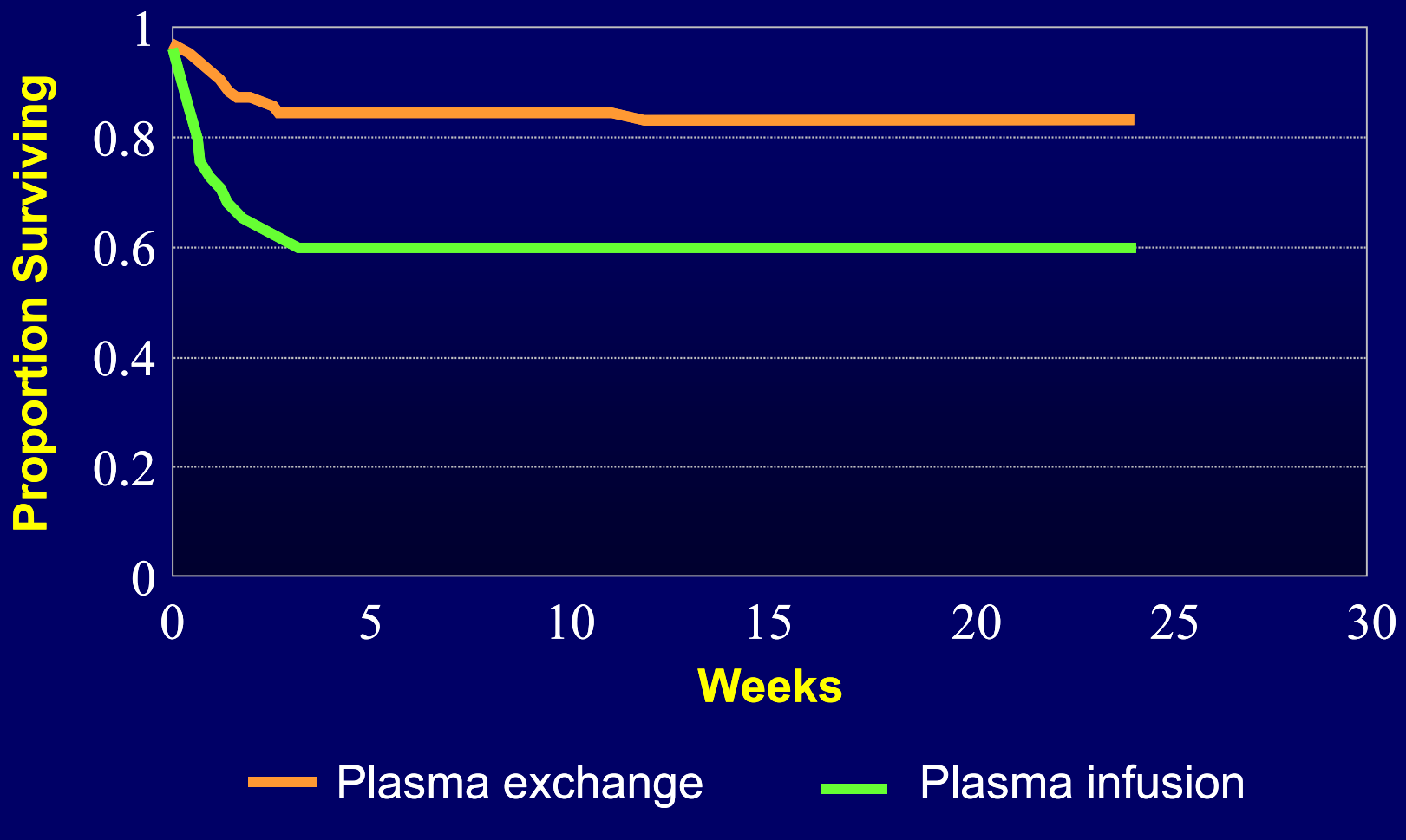
17° corso nazionale di aggiornamento
della SIdEM
Palermo 18/20 Ottobre 2012

Il paradigma dell'applicazione terapeutica del plasma-scambio: le microangiopatie trombotiche

Gerlando Quintini
UOC Ematologia TMO
AOUP Paolo Giaccone - Palermo

Plasma Infusion vs. Plasma Exchange



Rock et al. *NEJM* 1991 325(6): 396

What's the deal with ADAMTS 13?

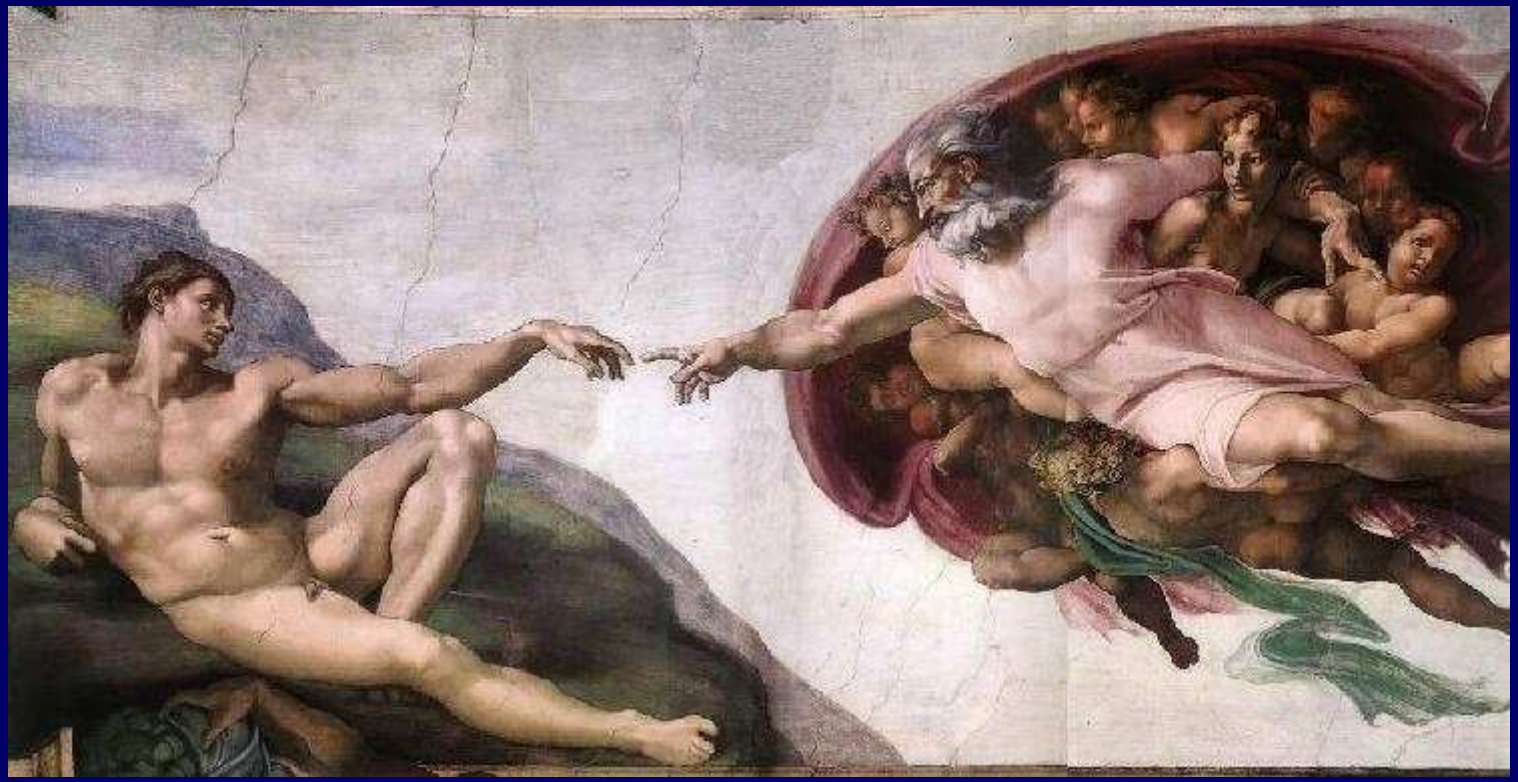
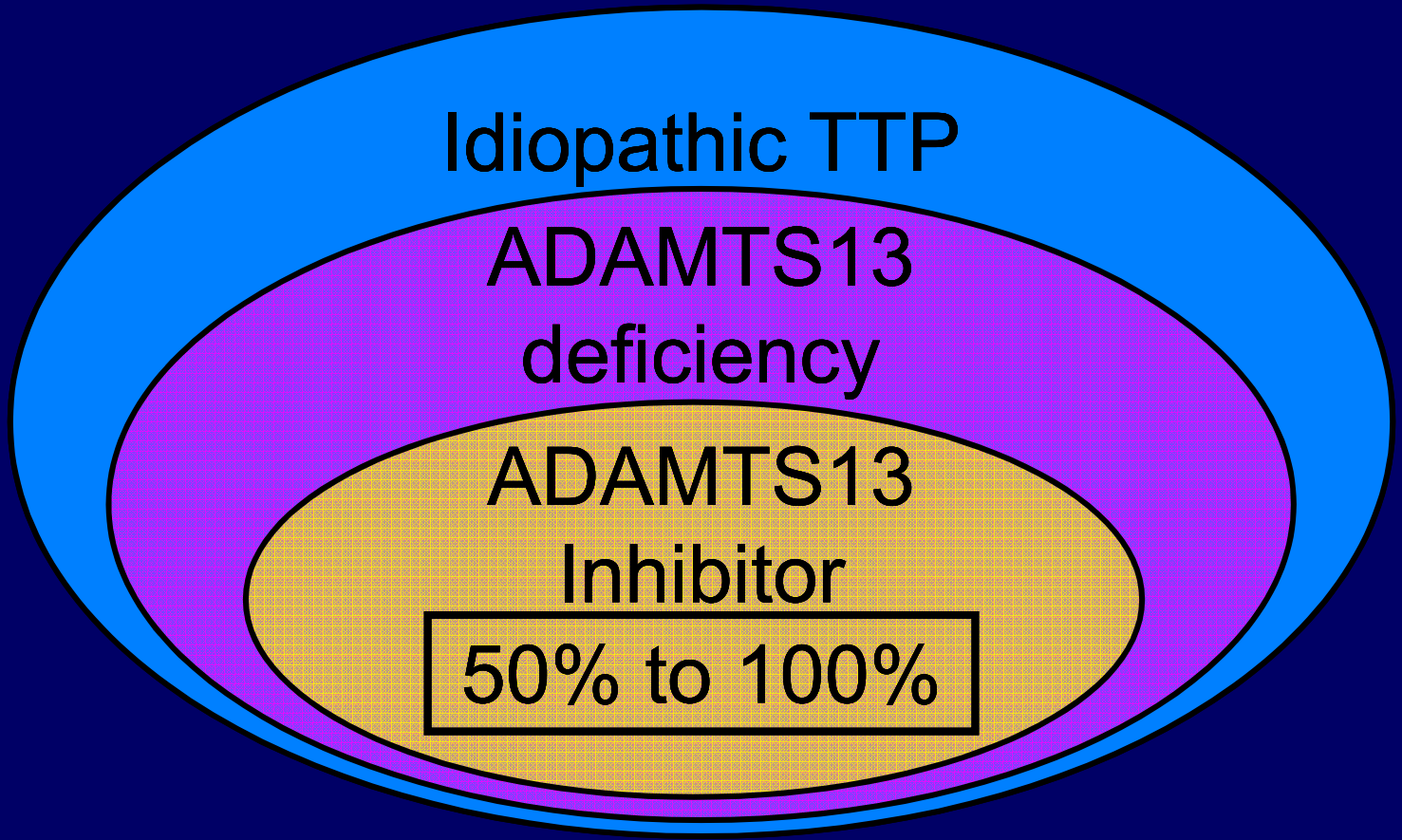


Image of Adam (looking fairly inactive at the moment): Michelangelo, Sistine Chapel.

Autoimmune Idiopathic TTP
Acquired ADAMTS13 Deficiency



ADAMTS13 Deficient, With Inhibitor

Response to Plasma Exchange

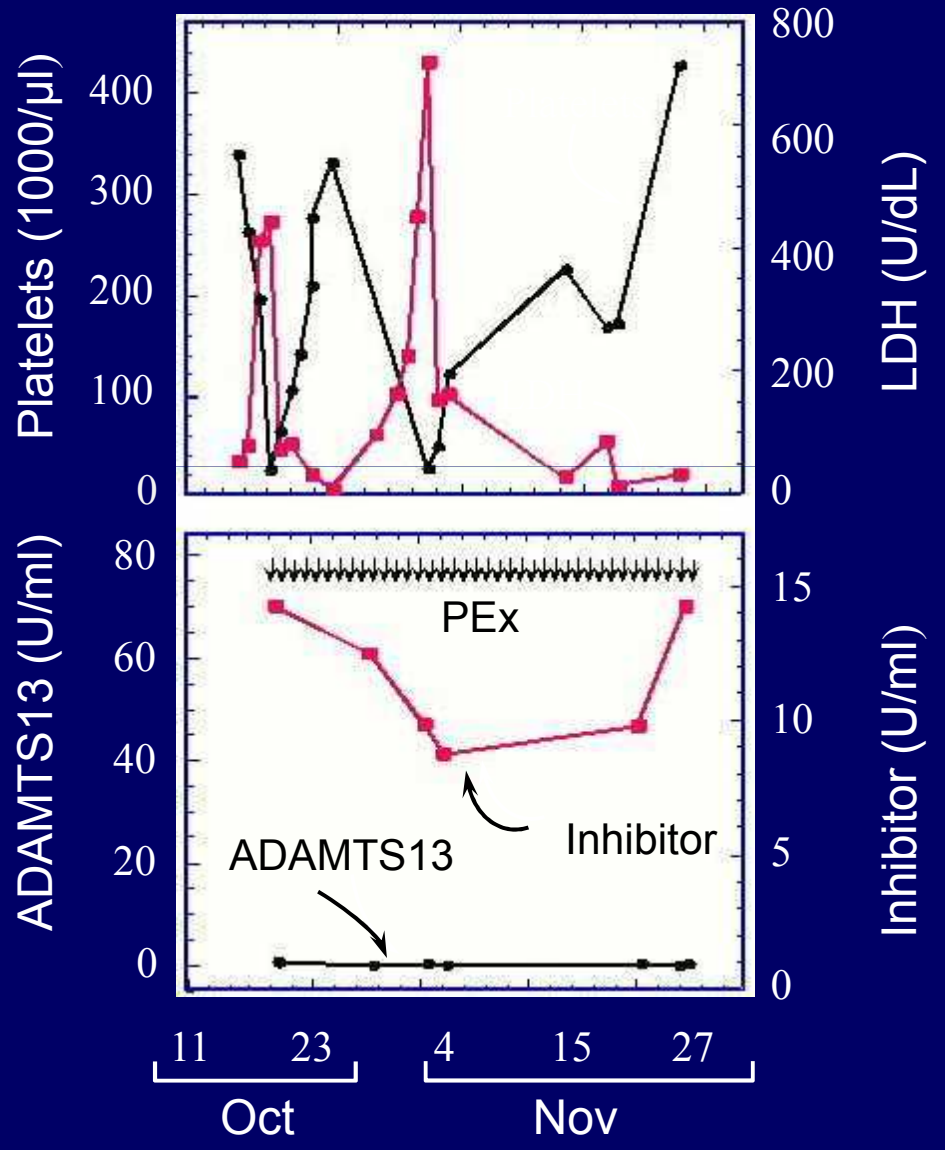
7 patients with:

- ADAMTS13 <5%
- Inhibitor present
- Multiple relapses in 4 and 2 deaths

Plasma exchange:

- Good response
- Unchanged ADAMTS13
- Persistent inhibitor

Why is PE effective?



Zheng et al, *Blood* 2004; **103:** 4043-4049

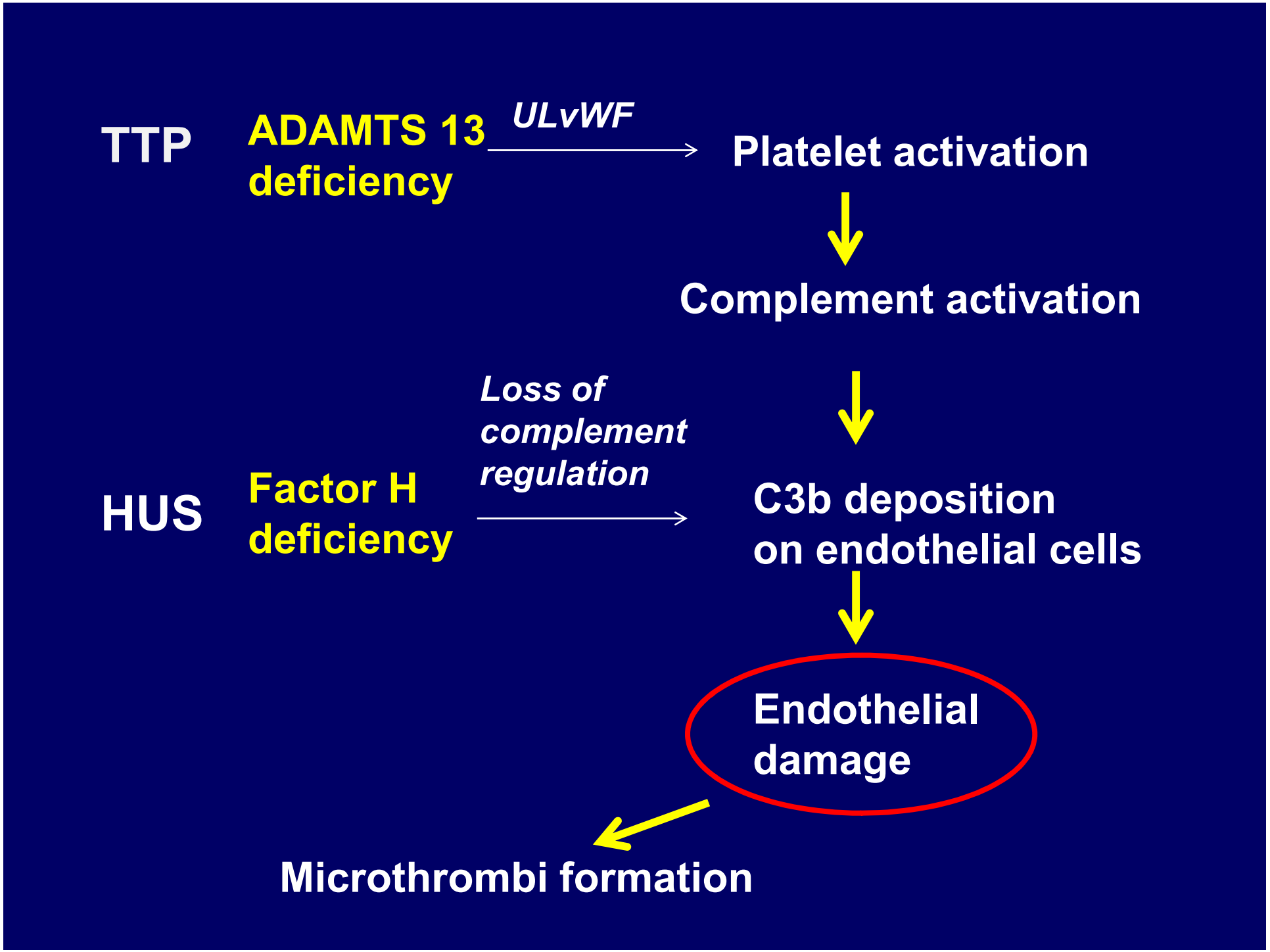
TTP/HUS: Why Does Plasma Exchange Work?

Stress precipitates TTP in familial ADAMTS13 deficiency

- Childhood triggers: vaginal delivery, upper respiratory infection, pneumonia, otitis media
- Adult triggers: infection, alcohol abuse, pregnancy

Resolution of stress may end an attack of familial (or acquired idiopathic) TTP

Similarly about 50% of heterozygous CFH mutation carriers never develop aHUS


TTP
ADAMTS 13 deficiency
ULvWF
Platelet activation
Complement activation
HUS
Factor H deficiency
Loss of complement regulation
C3b deposition on endothelial cells
Endothelial damage
Microthrombi formation

History

Dr. Eli Moschcowitz

1925 Eli Moschcowitz described “An acute febrile pleiochromic anemia with hyaline thrombosis of terminal arterioles and capillaries. Un undescribed disease” (Archives of Internal Medicine, Chicago, 1925, 36: 89).

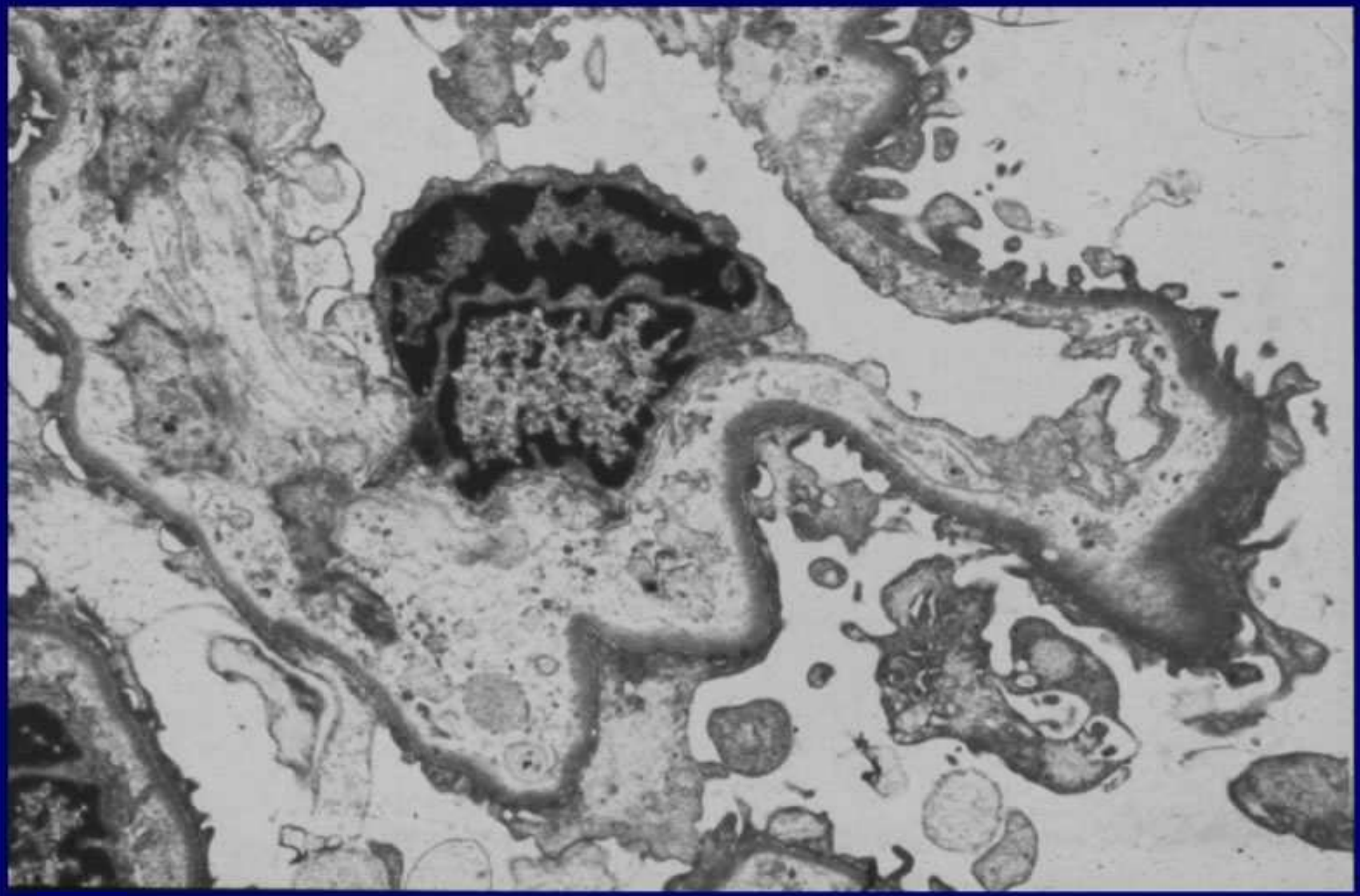
1936 Baehr found microthrombi composed of fibrin and platelets, the pathognomonic lesion.

1947 Singer named "TTP" to emphasize these lesions

1957 Named "Moschcowitz's disease" by Bernheim

Gasser's syndrome

The syndrome reported by Gasser in 1955 named the hemolytic-uremic syndrome, was in most respects similar to that previously reported by Moschcowitz and Baehr as thrombotic thrombocytopenic purpura



CAN TTP BE DIFFERENTIATED FROM HUS?

Differentiating criteria (from the books)	HUS	TTP
• Age	*Childhood*	*Adult*
• Organ involvement	*Renal*	*Multiple*
• Complications	*Renal failure*	*Neurological symptoms*
• Prognosis	*Good*	*Bad*

None of these are really discriminating	HUS	TTP
• Age	*Adult* Giromini, 1969 Shapiro, 1970 Utting, 1973 Karlsberg, 1977 Morel Maroger, 1979 Ponticelli, 1980 Cacoub, 1987 Prakash, 1987	*Newborn and small children* Amorosi, 1966 Monnens, 1967 Kennedy, 1980
• Organ involvement		*Renal failure* Dunea, 1966 Amorosi, 1966 Ridolfi, 1981 Eknoyan, 1986
• Complications	*Multiple* Gianantonio, 1983 Fong, 1982	
• Prognosis	*Can be poor in forms other than classic* Drummond, 1985	

The same patient classified as HUS or TTP during two different relapse episodes

Shumway, 1957
Mac Whinney, 1962
Mettler, 1969

A diagnosis of this disease, which I shall term "HUS/TTP", should be entertained whenever acute hemolytic anemia of the microangiopathy type is associated with thrombocytopenia and any degree of renal damage

Remuzzi et al., Kidney International **1987**

The term TTP/HUS should be avoided because it obscures differences among the various types of thrombotic microangiopathy

Tsai et al. Throm Haem **2003**

A Classification of TMA

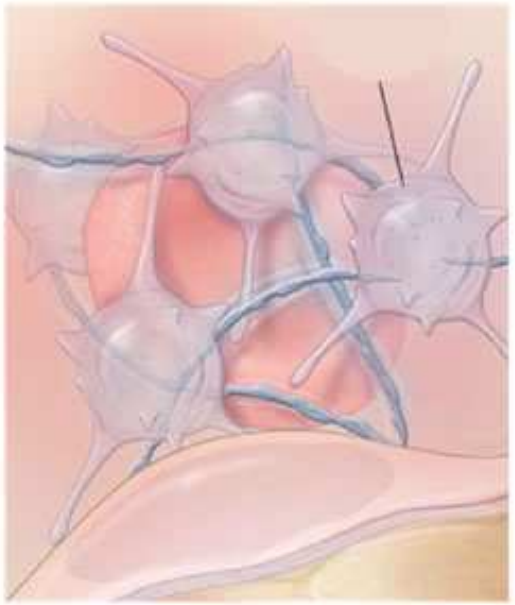
(Thrombotic Microangiopathy)

Typical / diarrheal	HUS or TTP (STX-induced TTP in ADAMTS13 deficient)
Complement defects	Atypical HUS
von Willebrand proteinase (ADAMSTS13) deficiency	Generally TTP
Cobalamin-C deficiency	TMA + multiorgan failure
Quinine-related	Abrupt TMA, exposure related
Post transplantation (calcineurin inhibitior related)	De-novo renal TMA May be renal "isolated"
Others: HIV, radiation, chemotherapy, HELLP, antiphospholipid Ab syndrome, unclassified	

Besbas et al. Kidney International 2006

dHUS Stx *P-selectin expression*

aHUS Gene mutation *Defective complement regulation*

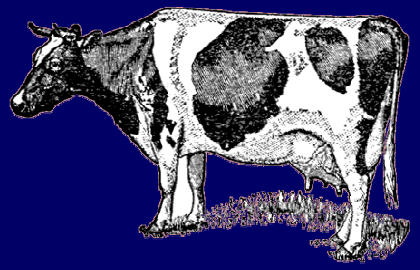


Microthrombi

Typical HUS

Triad of :

- **Microangiopathic hemolytic anemia**
- **Thrombocytopenia**
- **Acute renal failure**



Generally diarrhea-associated

- **Shiga toxin produced by *E coli* serotype O157:H7**
- **Shigella, Salmonella, others also**
- **Food borne disease: uncooked / unpasteurized products contaminated by animal wastes**

Or other infections (respiratory):

- **Invasive *S. Pneumoniae* or viral infections**

1982 E. coli of certain strains - unique for their fermentation properties - produce Shiga-like cytotoxins

O'Brien et al., *J Infect Dis*

1983 E. coli O157:H7 produces Shiga-like toxin/verotoxin and causes haemorrhagic colitis

Riley et al., *N Engl J Med*

1983 E. coli O157:H7 is associated with sporadic cases of classical HUS of children

Karmali et al., *Lancet*

dHUS - Prevalence

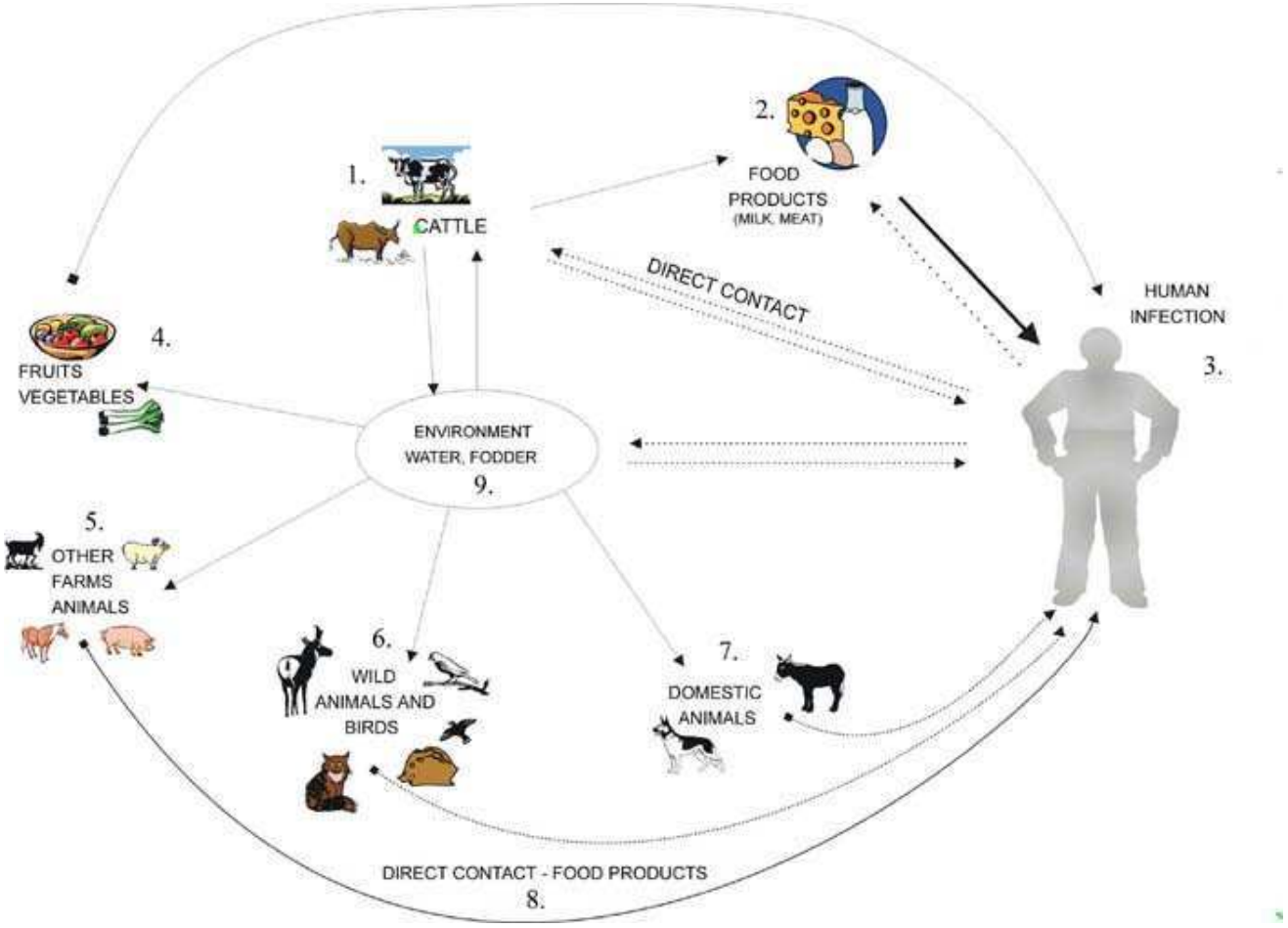
Incidence
(cases/100,000/year)

Overall: 2

Sex: no difference

A multisystem disease of microangiopathic haemolytic anemia and thrombocytopenia with predominant but not exclusive renal involvement

EPIDEMIOLOGY OF SHIGA-LIKE TOXIN – PRODUCING ESCHERICHIA COLI STRAINS (STEC)



The New York Times

SATURDAY, AUGUST 16, 1997

5 Million Hamburger Patties May Be Tainted, U.S. Warns

By DAVID STOUT

WASHINGTON, Aug. 15 — The Agriculture Department issued a far broader alert about tainted hamburger today, warning that about five million patties sold across the country may have been contaminated with potentially deadly E. coli bacteria.

The hamburgers were made on June 4, June 5 and June 9 at a plant in Columbus, Neb., owned by the Hud-

Agriculture Department inspectors reviewed the records of the company's Nebraska plant, it found that more than 1.2 million pounds of frozen quarter-pound patties might be tainted.

Mr. Amontree said that 16 people were sickened in Colorado from the contaminated hamburger, but that no one had died or become seriously

But when the Agriculture Department inspectors reviewed the records of the company's Nebraska plant, it found that more than 1.2 million pounds of frozen quarter-pound patties might be tainted

Corriere della Sera LUNEDÌ 9 DICEMBRE 1996

GRAN BRETAGNA / Manzo infetto, strage in Scozia

Carne, è terrore infinito
Arriva il batterio-killer

DAL NOSTRO CORRISPONDENTE

LONDRA — Dopo il «morbo della mucca pazza», la meningite. E, dopo la meningite, il colibacillo 0-157. La salute pubblica in Gran Bretagna diventa un problema nazionale, se le epidemie si moltiplicano. Non c'è per ora da allarmarsi, anche se Sir Kenneth Calman, il maggior ufficiale medico britannico, mette in guardia i connazionali dall'«adagiarsi in un falso senso di sicurezza potenzialmente pericoloso». Stupisce la quantità di focolai degli ultimi mesi: ieri un anziano è morto in una casa di riposo in Scozia, colpito dall'enterocolite provocata dal batterio 0-157. E non sarà l'ultimo.

Quella scozzese è ormai una strage: nove persone hanno già perso la vita, 386 persone sono state infettate, 57 sono ancora in ospedale, 31 delle quali «danno grave preoccupazione ai medici» e almeno una dozzina sono in prognosi riservata. Non si può escludere che altri malati stiano incubando il letale batterio, noto già da una ventina d'anni, ma «difficilmente affrontabile dalla medicina: è il batterio che uccise 7 persone in Giappone l'estate scorsa, contro il quale, dice Sir Kenneth, «non sempre gli antibiotici funzionano».

L'epidemia è stata provocata dalla carne avariata di una rinomata macelleria di Wishaw: la John Barr and Son, già premiata per la qualità delle sue merci. Lì una partita di manzo malamente conservato era stata smerciata due settimane fa per preparare «meat pie», il pasticcio di carne che è un piatto centrale della cucina britannica, da servire a un pranzo per pensionati. Po-

Dopo il [illegible] dell[illegible] la mening[illegible] «colibacillo 0-15[illegible] agli antibiotici. I[illegible] ha colpito in [illegible]

che ore dopo dozzine di persone hanno cominciato a sentirsi male.

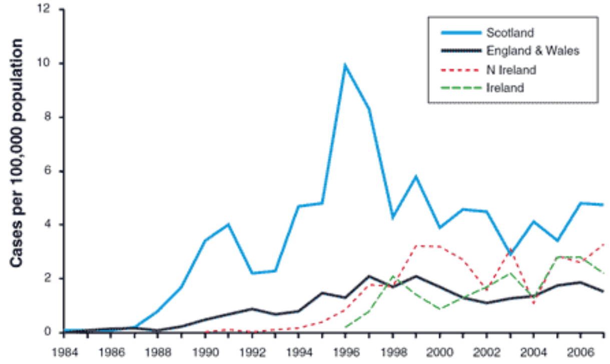
E la questione sta diventando politica. Non solo perché anche sette dipendenti del macellaio di Wishaw sono rimasti colpiti dal batterio, il che dimo- [illegible]

[illegible] altri decessi si sono verificati nel Suffolk, a Brighton, nello Yorkshire. Le vittime dovrebbero essere meno di dieci.

Secondo l'ufficiale medico Calman, non ci sarebbe motivo d'allarme: ogni anno si registrano nel Regno Unito duemila casi di meningite, di cui circa duecento con esito mortale, e finora le cifre non si discostano dalla norma. Nel Paese che ha visto esplodere il «morbo della vacca pazza» queste [illegible] non suonano del [illegible] convincenti.

Alessio Altichieri

FIGURE 1: *E. coli* O157 cases: rates per 100,000 population, 1984-2007



Data from outwith Scotland courtesy of Health Protection Agency Belfast & London and Health Protection Surveillance Centre Dublin (2007 figures are provisional)

L'epidemia è stata provocata dalla carne avariata di una rinomata macelleria di Wishaw: la John Barr and Son, già premiata per la qualità delle sue merci. Lì una partita di manzo malamente conservato era stata smerciata due settimane fa per preparare "meat pie", il pasticcio di carne che è un piatto centrale della cucina brittannica, da servire a un pranzo per pensionati

HUS OUTBREAK IN JAPAN

Sakay City (July 12, - July 14, 1996)

> 6,000 school children with acute enterocolitis due to enterohemorrhagic E. coli 0157:H7

Admitted children		629
- HUS	68*	
- deaths		4
- encephalopathy and intracerebular hemorrhage		2

* Including a couple of twins treated with hemodialysis (20,000-30,000 ptl/mm^3)

- Bacteria have been isolated from white radish sprout linked to three new cases observed in Nagoya and Yokohama: identical E. coli O157 genotypes were demonstrated in sprouts and affected patients

- Seeds used to grow the sprouts in Jokohama and Sakay City, the center of the Japanese outbreak, were imported from the same source in Oregon

CORRIERE DELLA SERA *27 maggio 10 giugno 2011*

Epidemia di Sindrome Emolitica Uremica in Germania

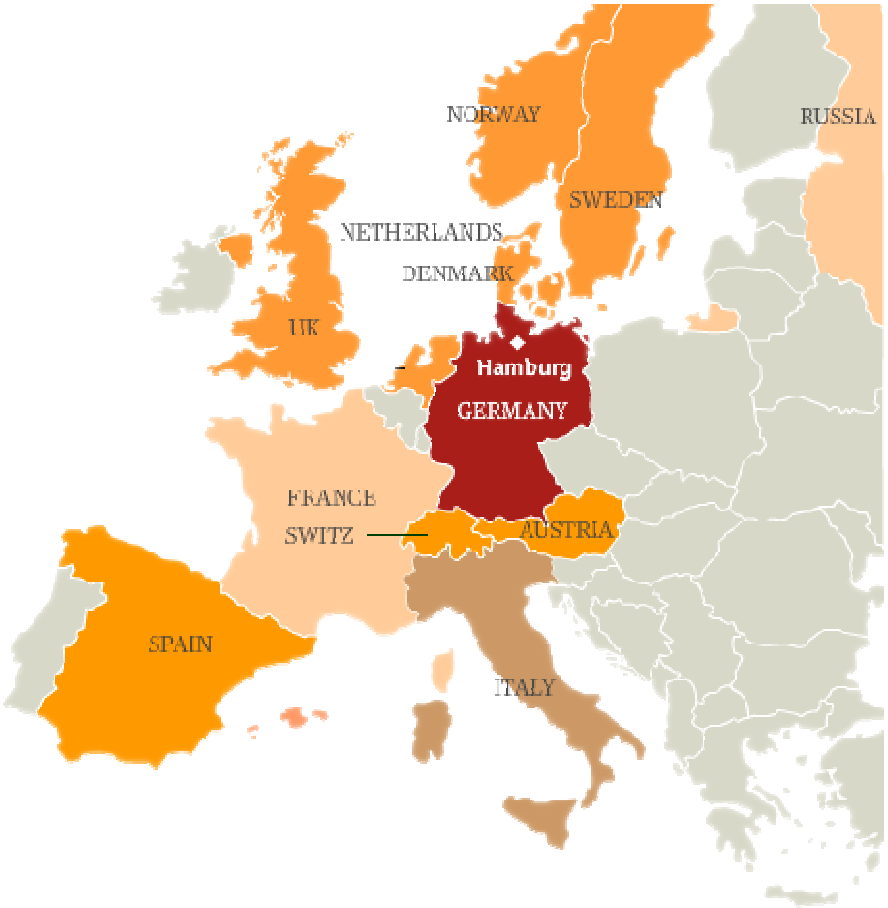
Una epidemia causata da una grave malattia sta causando preoccupazione in Germania, dove tre donne sono morte e **276** persone hanno sviluppato la sindrome emolitica uremica (HUS), sin dalla seconda metà di maggio.

Paura in Germania: «germe killer» fa le prime vittime

Quattro morti e oltre **600** casi per un'epidemia causata dal batterio fecale, *Escherichia Coli*

Batterio killer, 37 i morti in Germania

L'ultima vittima un bimbo di due anni. Epidemia in calo, in tutto **3.254** i casi di infezione.



Andamento dell'epidemia	numero casi	nuovi casi	decessi
domenica 5 giugno 11	2335		22
lunedì 6 giugno 11	2431	96	24
martedì 7 giugno 11	2745	314	24
mercoledì 8 giugno 11	2904	159	27
giovedì 9 giugno 11	3092	188	31
venerdì 10 giugno 11	3255	163	35
lunedì 13 giugno 11	3343	88	36
martedì 14 giugno 11	3362	19	37
mercoledì 15 giugno 11	3412	50	39

Batterio Killer del cetriolo

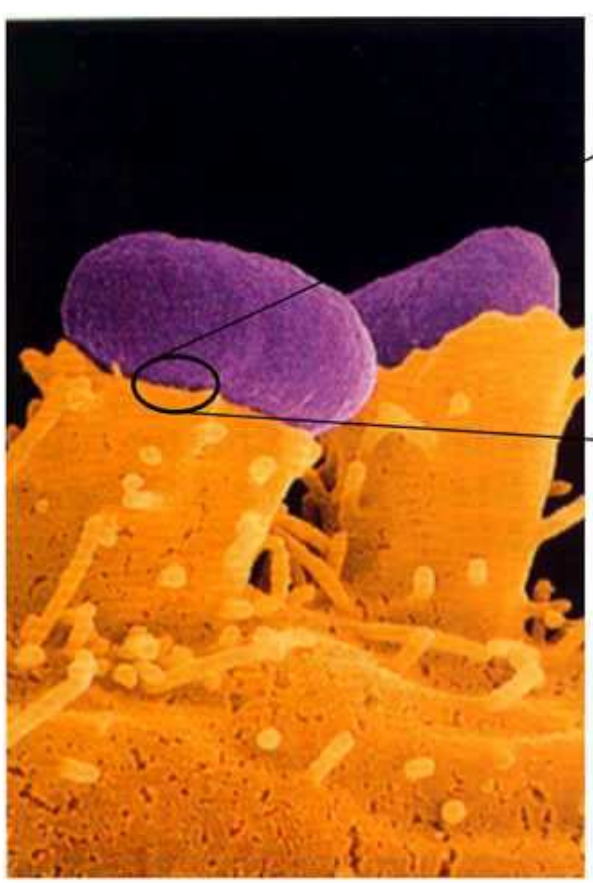
Batterio killer, germogli di soia colpevoli: trovato il focolaio in un'azienda agricola

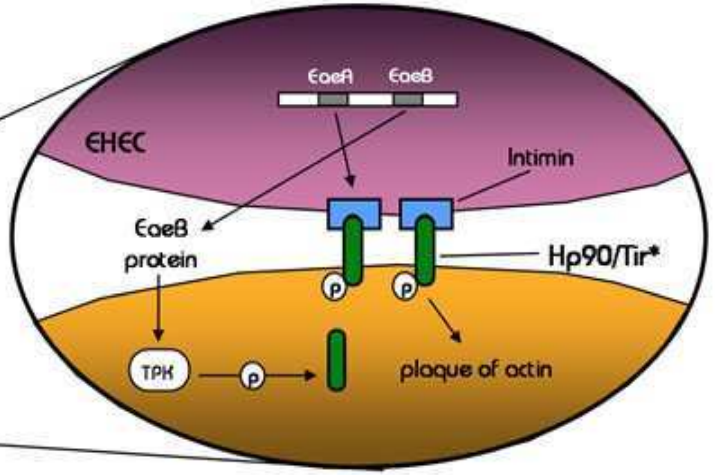

BATTERIO KILLER, DOPO I CETRIOLI CADONO LE ACCUSE ALLA SOIA
ORA SOSPETTIAMO LE VERZE...
LA FACCENDA PUZZA!


CRIME SCENE
E. coli E.coli E.coli


Salve, siamo
Escherichia Coli,
non cetrioli

Escherichia coli O157:H7



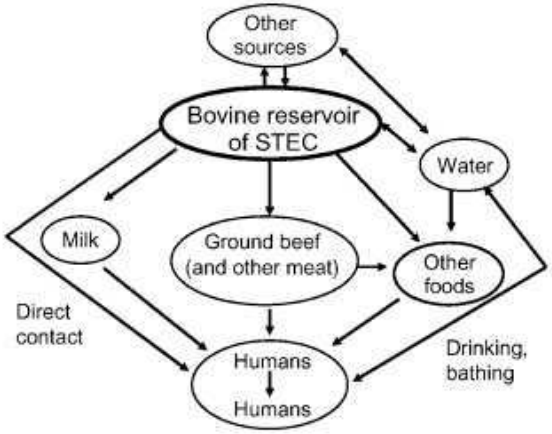


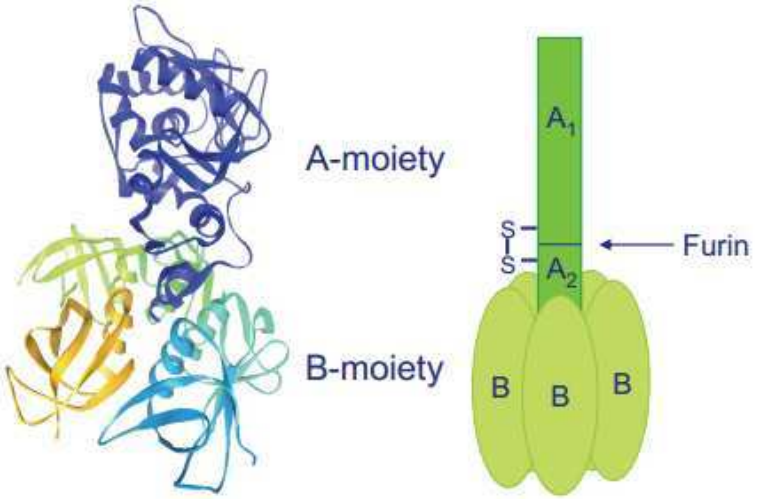
Eae gene: E. coli attaching effacing gene

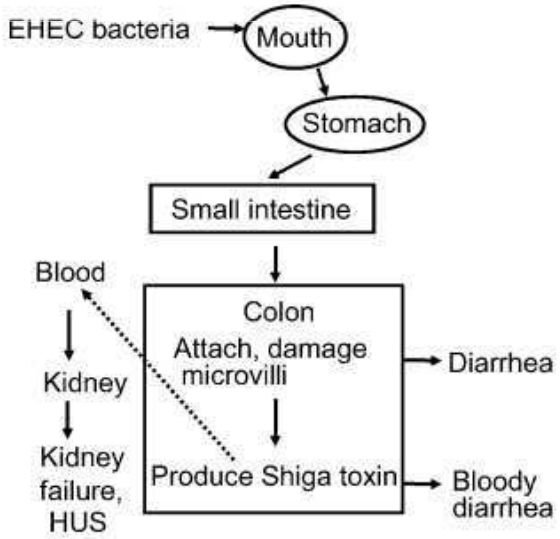
Hp90/Tir: host protein intimin counter receptor secreted by EHEC

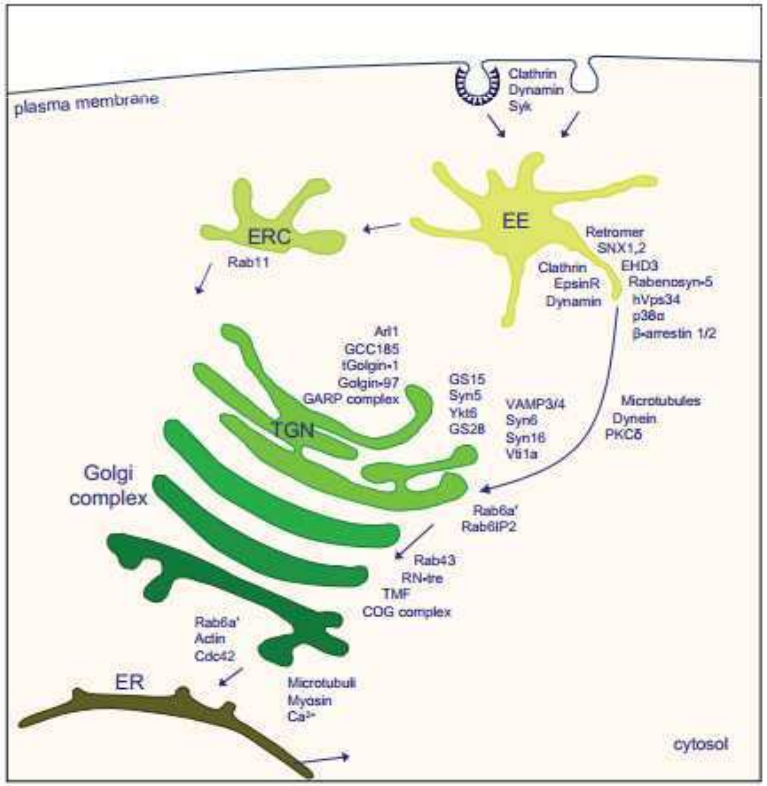
Tir: Transmembrane intimin receptor

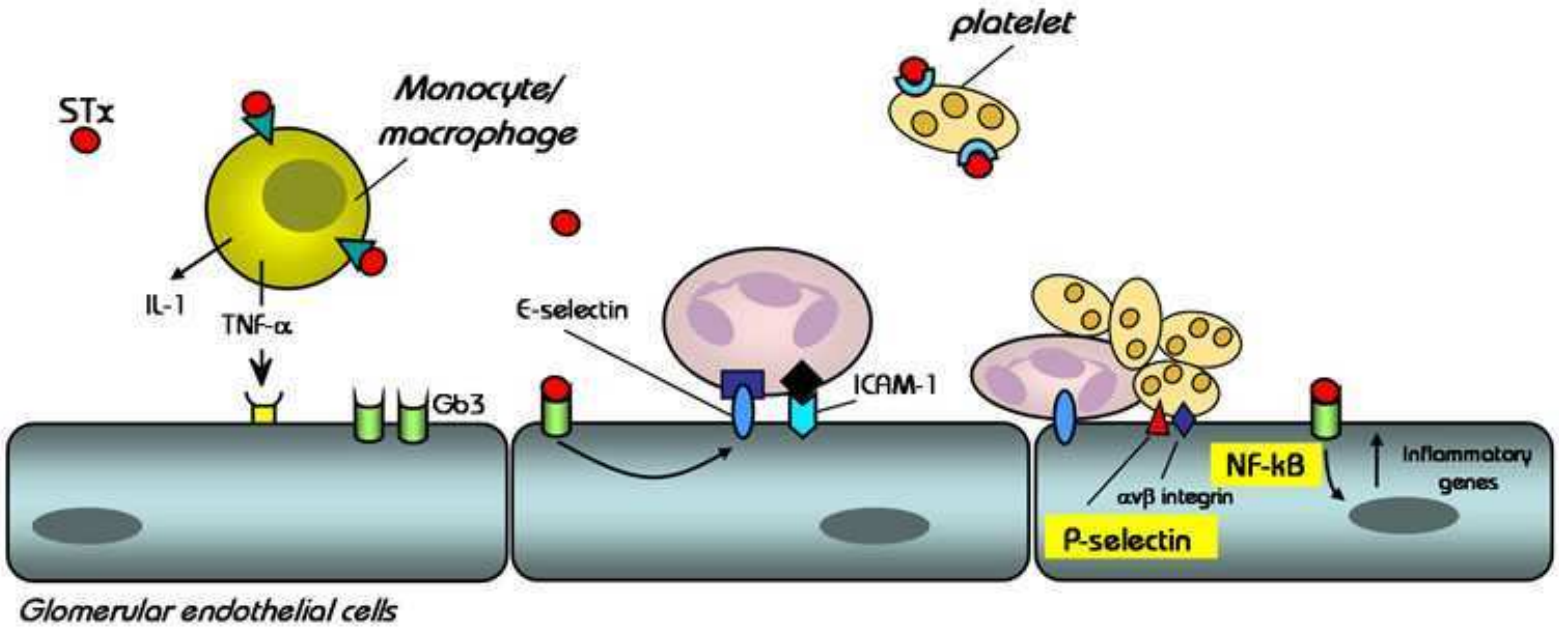
Rosenshine et al., *EMBO J*, 1996


Other sources
Bovine reservoir of STEC
Water
Milk
Ground beef (and other meat)
Other foods
Direct contact
Humans
Humans
Drinking, bathing


A-moiety
B-moiety
A1
S
S
A2
Furin
B
B
B


EHEC bacteria
Mouth
Stomach
Small intestine
Blood
Colon
Attach, damage microvilli
Diarrhea
Kidney
Kidney failure, HUS
Produce Shiga toxin
Bloody diarrhea


plasma membrane
Clathrin
Dynamin
Syk
ERC
Rab11
EE
Retromer
SNX1,2
Clathrin
EpsinR
Dynamin
EHD3
Rabenosyn-5
hVps34
p38α
β-arrestin 1/2
Arl1
GCC185
tGolgin-1
Golgin-97
GARP complex
GS15
Syn5
Ykt6
GS28
VAMP3/4
Syn6
Syn16
Vti1a
Microtubules
Dynein
PKCδ
TGN
Golgi complex
Rab6a'
Rab6IP2
Rab43
RN-tre
TMF
COG complex
Rab6a'
Actin
Cdc42
ER
Microtubuli
Myosin
Ca2+
cytosol



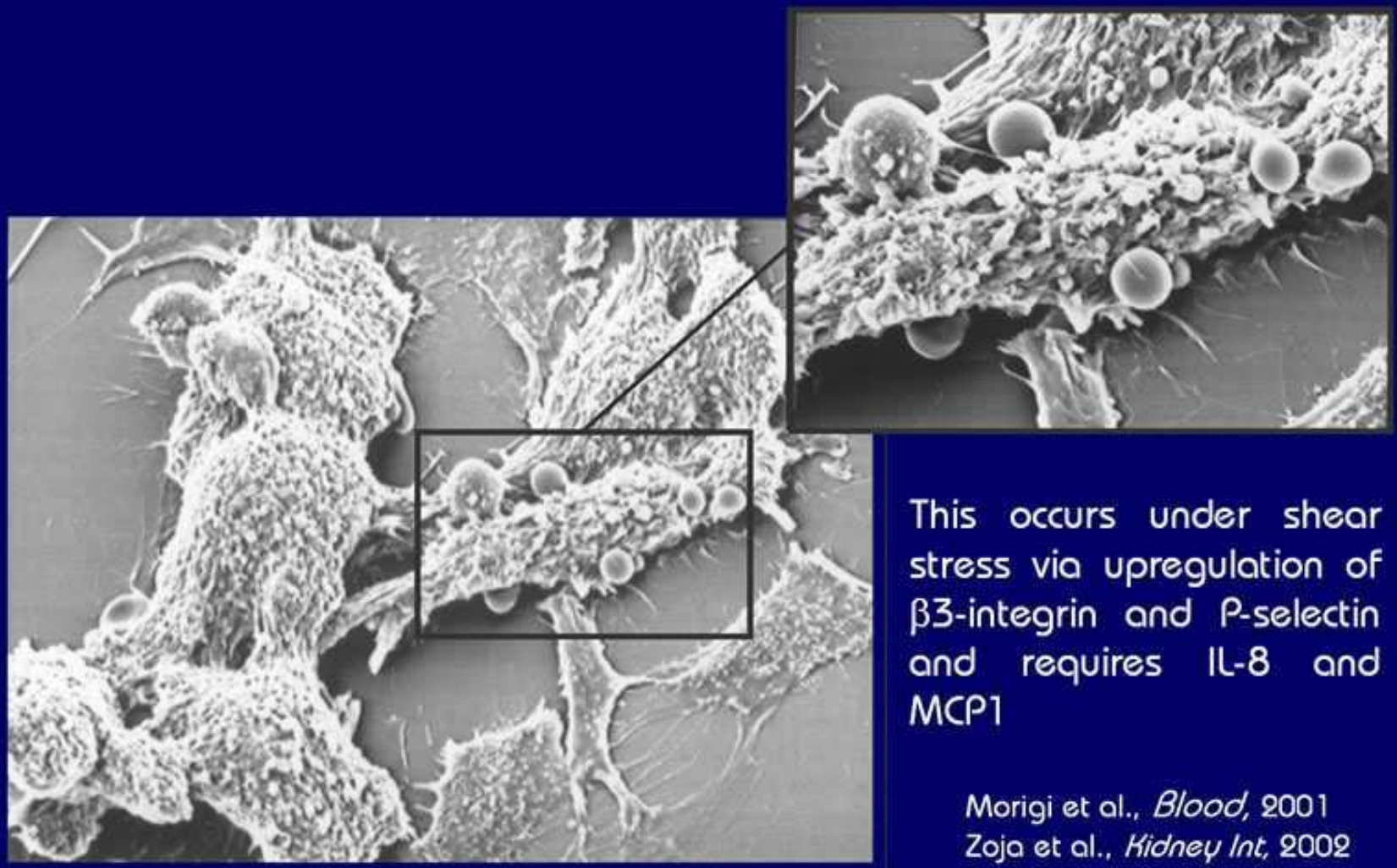
- STx promotes leukocyte adhesion and thrombus formation on vascular endothelium via upregulation of β3-integrin and P-selectin

  Morigi et al., *Blood,* 2001

- Activated endothelial cells express NF-kB dependent inflammatory genes in response to STx

  Zoja et al., *Kidney Int,* 2002
  Zanchi et al., *J Immunol,* 2008

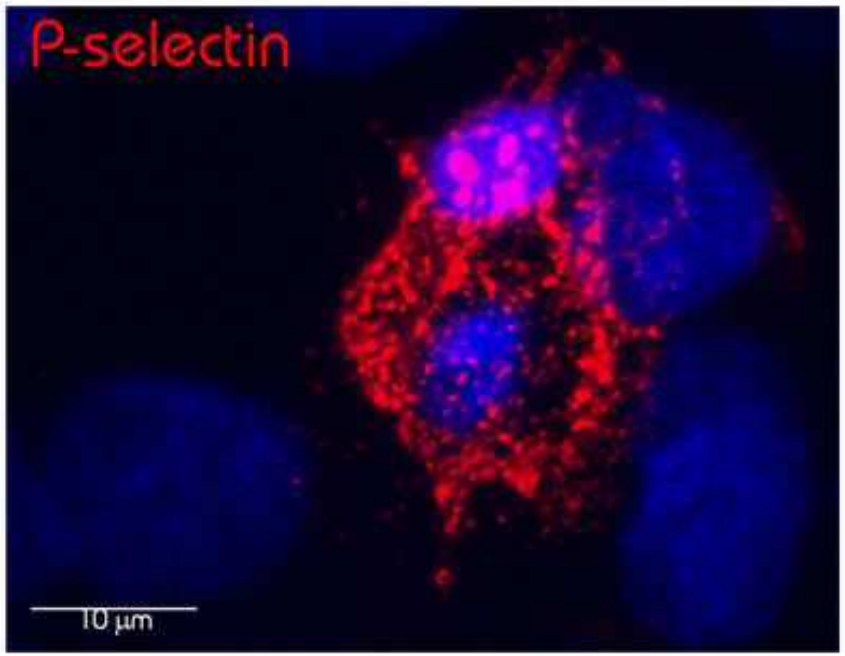
STX-1 PROMOTES PLATELET ADHESION AND THROMBUS FORMATION ON CULTURED MICROVASCULAR ENDOTHELIUM

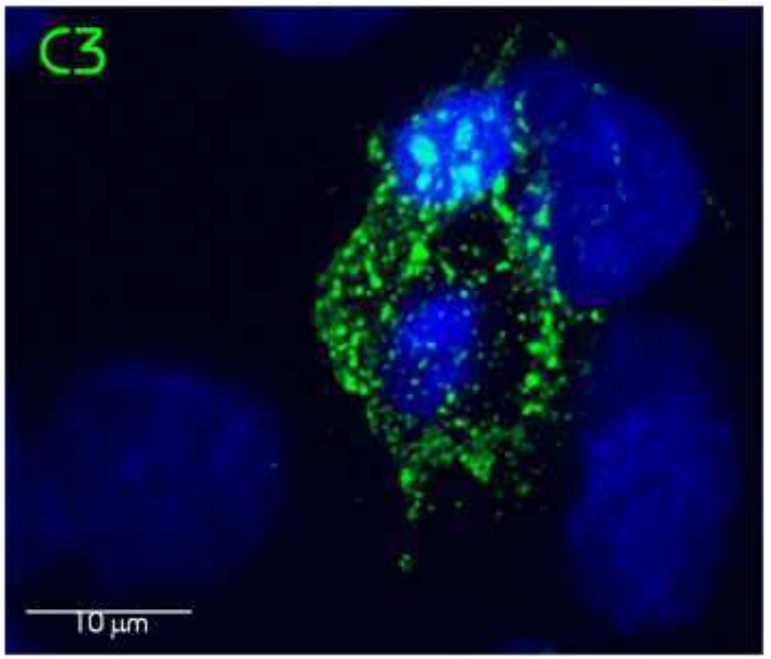


This occurs under shear stress via upregulation of β3-integrin and P-selectin and requires IL-8 and MCP1

Morigi et al., *Blood*, 2001
Zoja et al., *Kidney Int*, 2002

STX-TREATED MICROVASCULAR ENDOTHELIAL CELLS

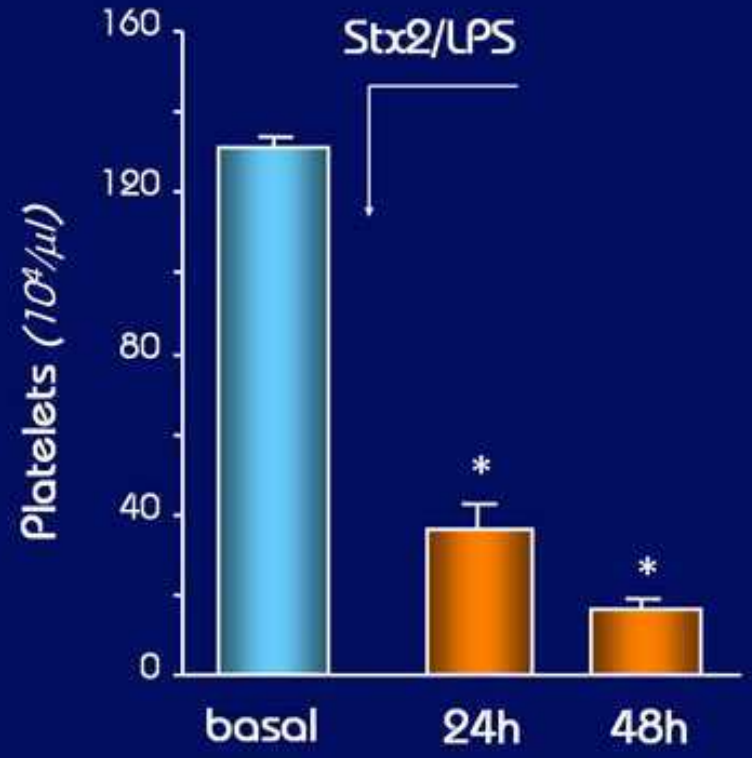


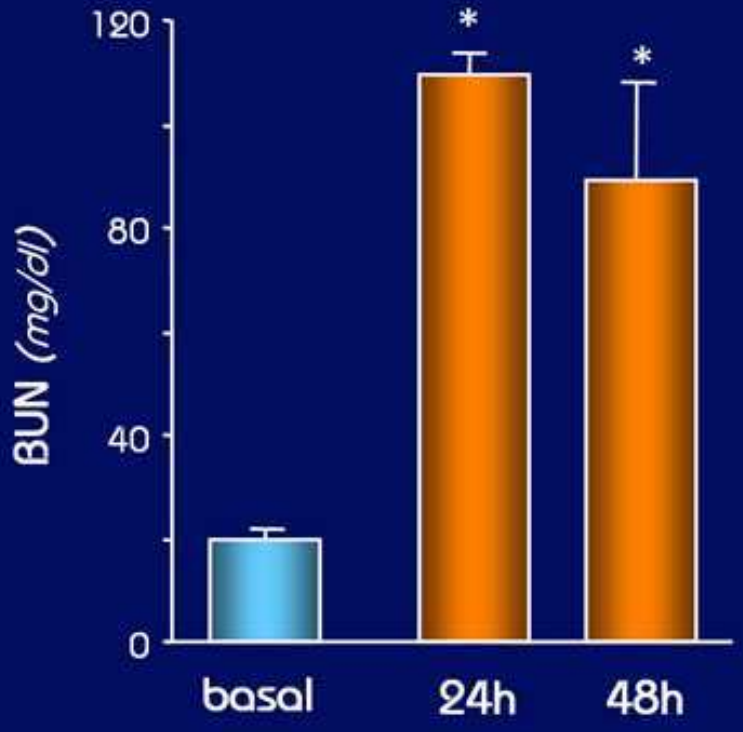


P-selectin
C3 deposits
DAPI in cell nuclei

A MURINE MODEL OF HUS

C57 BL/6 mice received i.p. injections of Stx2 (200ng/mouse) plus LPS (75µg/mouse)



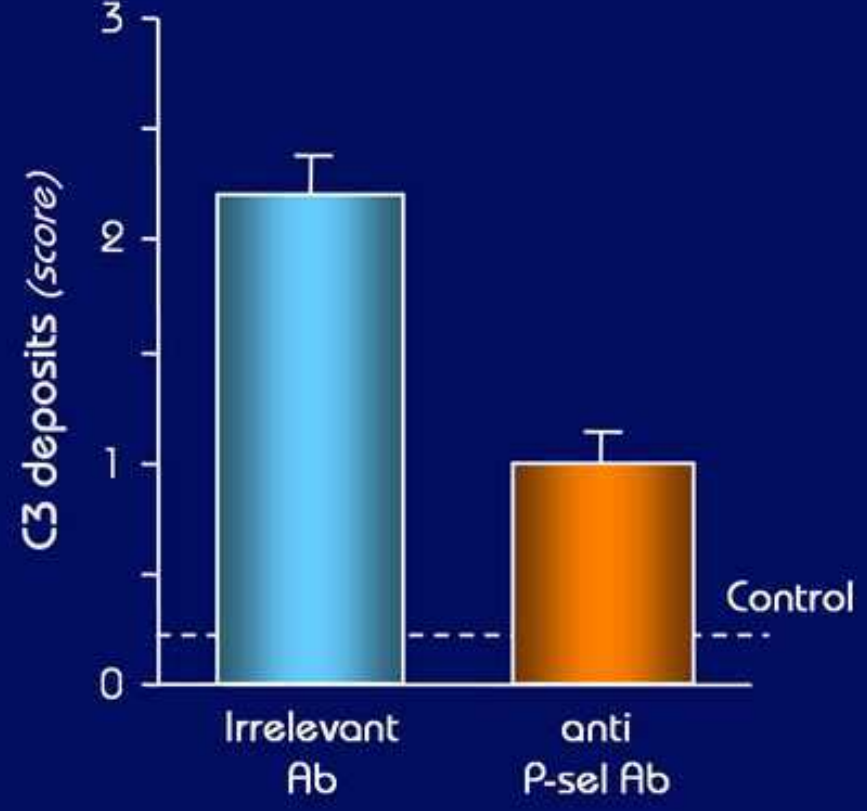


*P<0.01 vs basal

Morigi et al., *J Am Soc Nephrol*, 2008

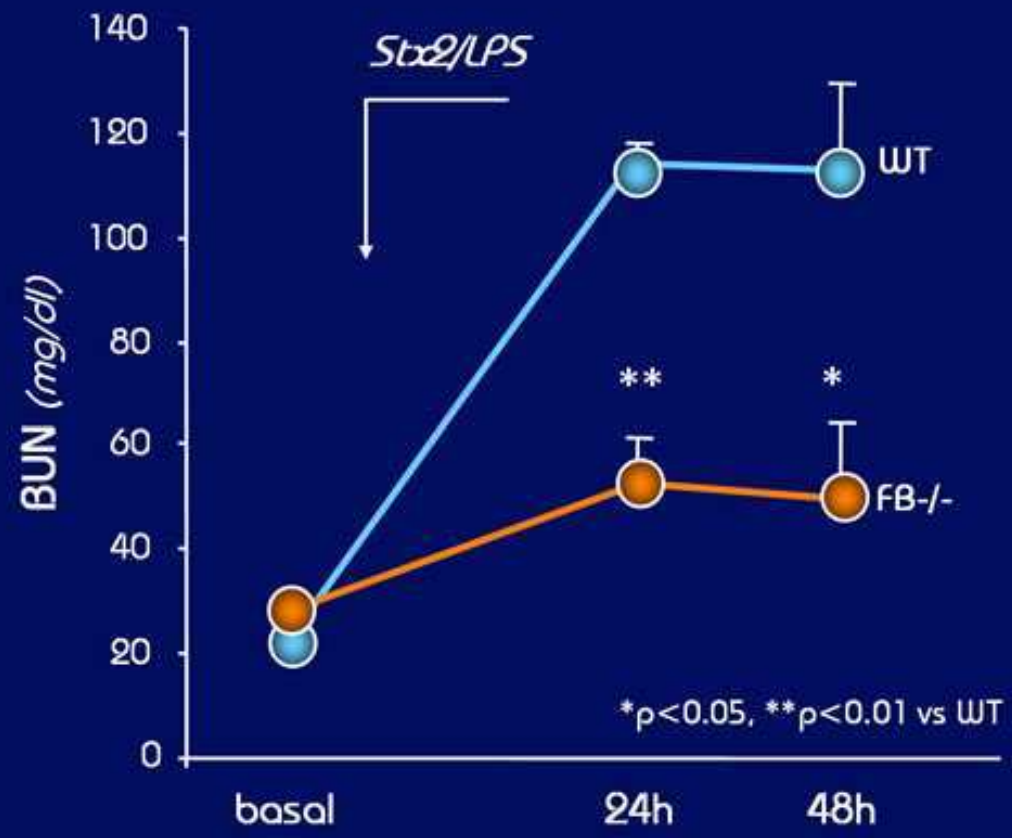
P-SELECTIN BLOCKADE LIMITS GLOMERULAR C3 DEPOSITS IN HUS MICE

Stx2/LPS mice were treated with anti P-selectin or irrelevant Ab and sacrificed at 24h



Morigi et al., *J Am Soc Nephrol*, 2008

ALTERNATIVE PATHWAY IN THE MURINE MODEL OF HUS



Factor B deficient mice did not show platelet clumps in glomeruli and were protected from renal function deterioration

Morigi et al., *J Am Soc Nephrol*, 2008

A CASE of STX-HUS WITH ACTIVATION OF THE ALTERNATIVE PATHWAY OF COMPLEMENT

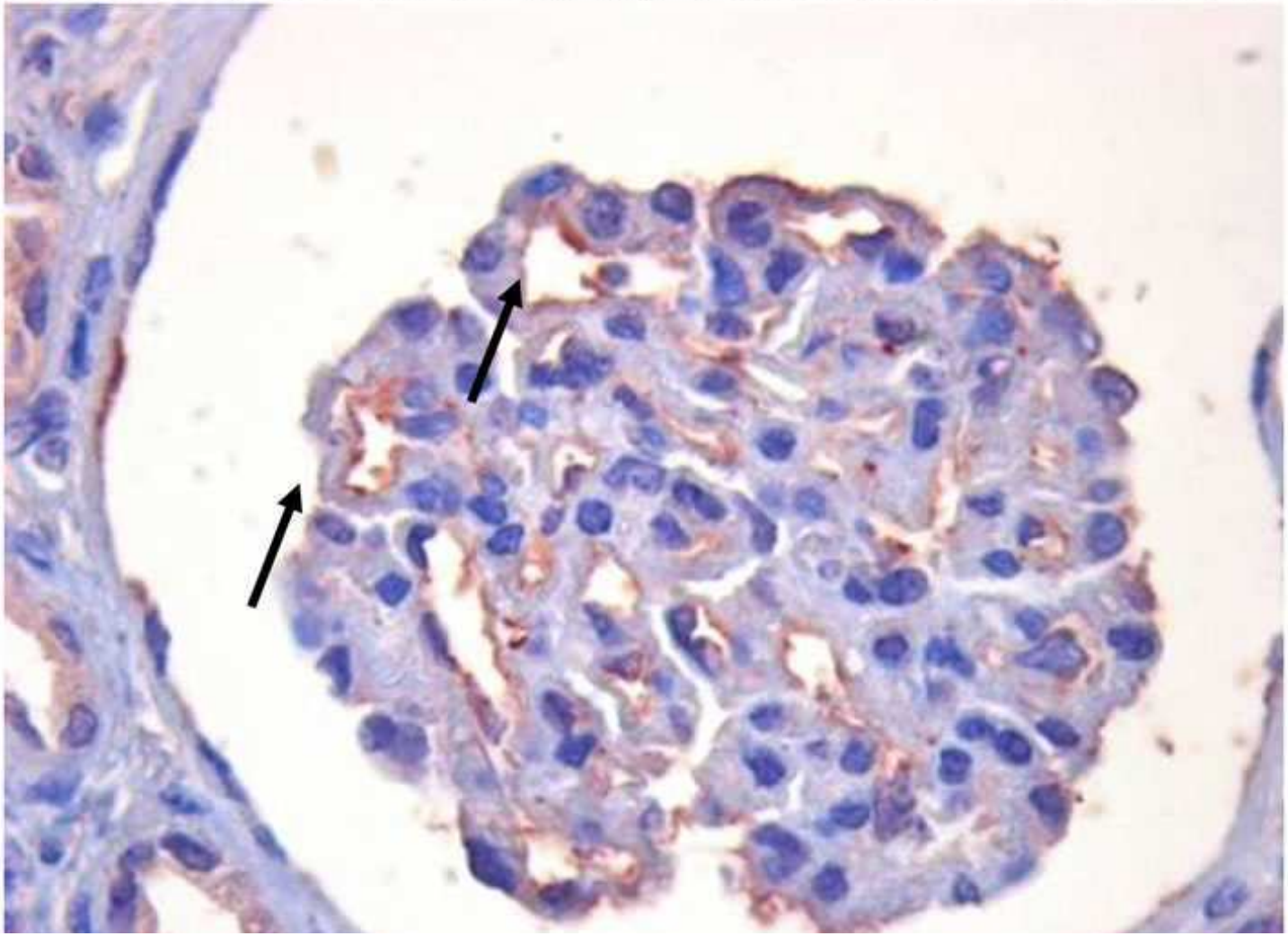
- A 26 year-old-woman admitted to the Hospital with
  - watery diarrhea and profuse vomiting
  - anemia, thrombocytopenia
  - renal insufficiency (creatinine 2 mg/dl)

- Anti-Stx and anti-E. coli O157:H7 LPS antibodies in blood

  Low C3 levels: *65 mg /dl*
  Normal C4 levels: *15 mg/dl*

- Renal biopsy showed classic features of TMA

C3 DEPOSITION IN Stx HUS KIDNEY



Typical HUS

Outcome

Acute episode

•Red blood cells transfusion	70%
•Dyalisis	50%
•Neurologic involvement	25%
•Death	3-5%

Long term results (10-20 years after HUS*)

63%	Complete recovery
12%	Recovery with proteinuria
6%	Recovery with proteinuria and HTN
16%	Recovery with low GFR ± proteinuria or HTN
3%	ESRD

**** Diarrheal or URI- related only, pediatric***

Spizzirri et al. Pediatric Nephrology 1996

dHUS Stx *P-selectin expression* **Complement activation**

Microthrombi

ACUTE GLOMERULONEPHRITIS IN INFANCY

Two children and their male cousin who all died at 5 months of age

Fison, *Arch Dis Child*, 1956

Clin Nephrol, 1991

The successful treatment of atypical hemolytic uremic syndrome with plasmapheresis

Robson WL., Leung AK.

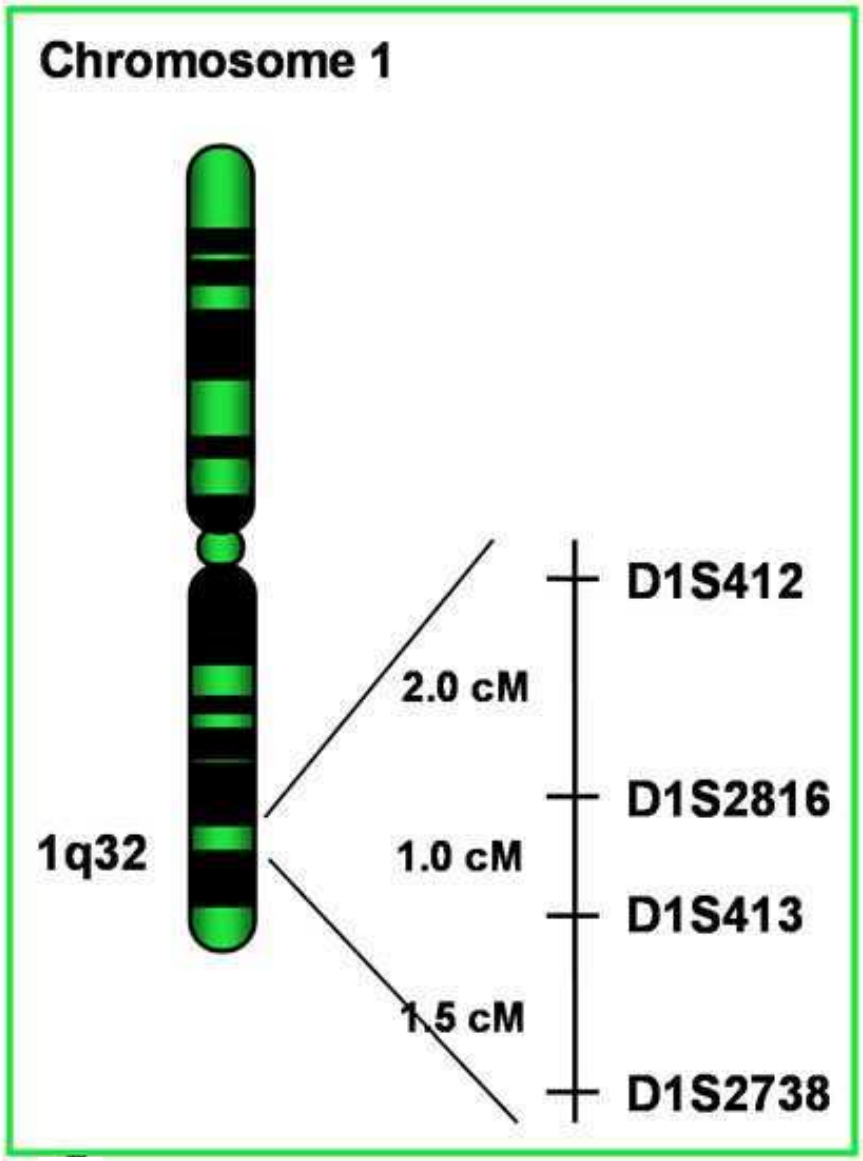
Prevalence: 0,07/10,000 persons

FAMILIAL TTP/HUS

***Definition**: at least two cases in the same family*

	Autosomal recessive	*Autosomal dominant*
Affected families	43	13
End stage renal failure	90-100 %	90-100 %
Mortality rate	65 %	> 90 %

Kaplan BS, Kaplan P, 1992



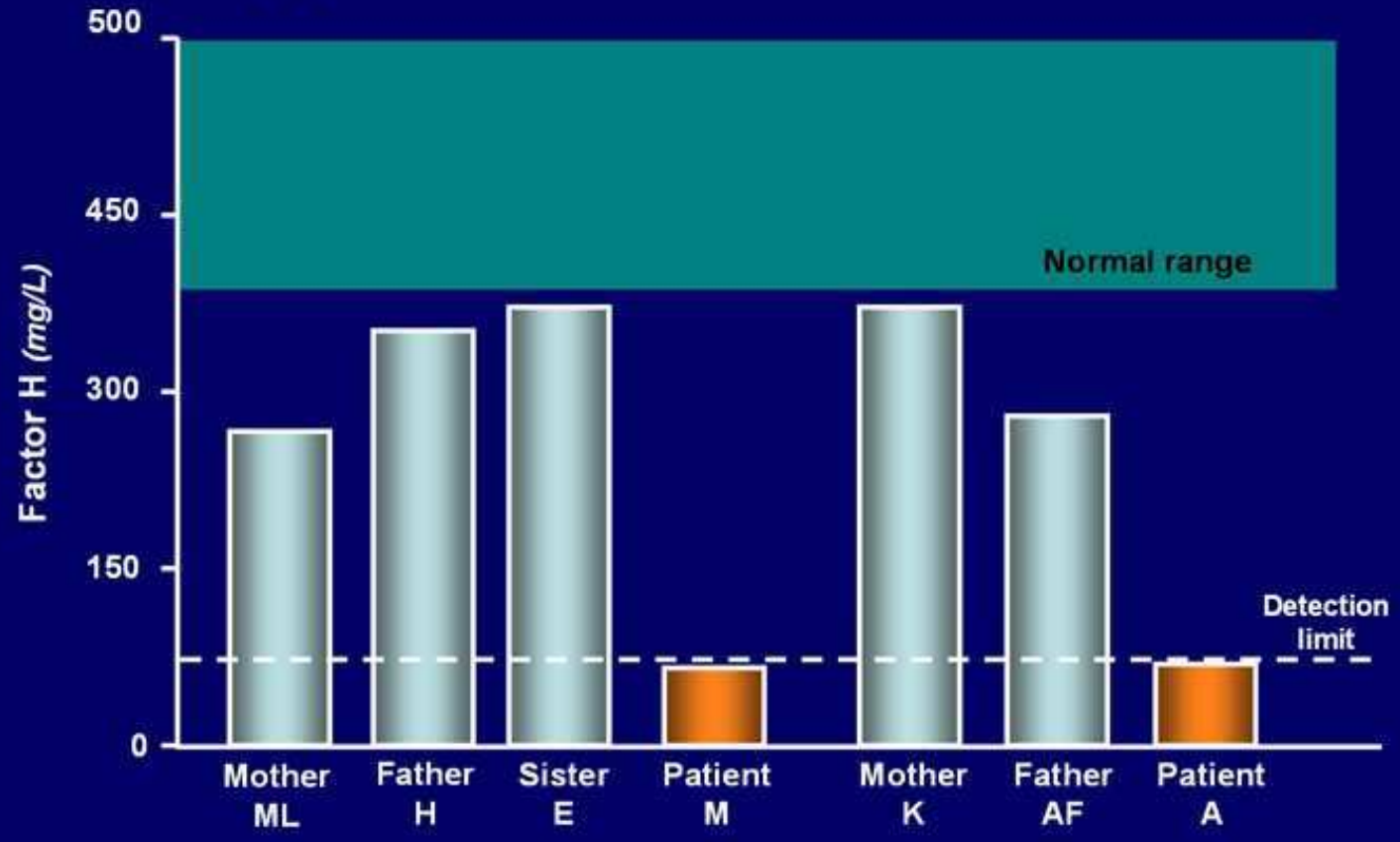
In three large families with HUS and area on chromosome 1q32, where factor H gene is mapped, segregated with the disease

Warwicker and Goodship, Kidney Int, 1998

FAMILIAL HUS IN A BEDUIN FAMILY

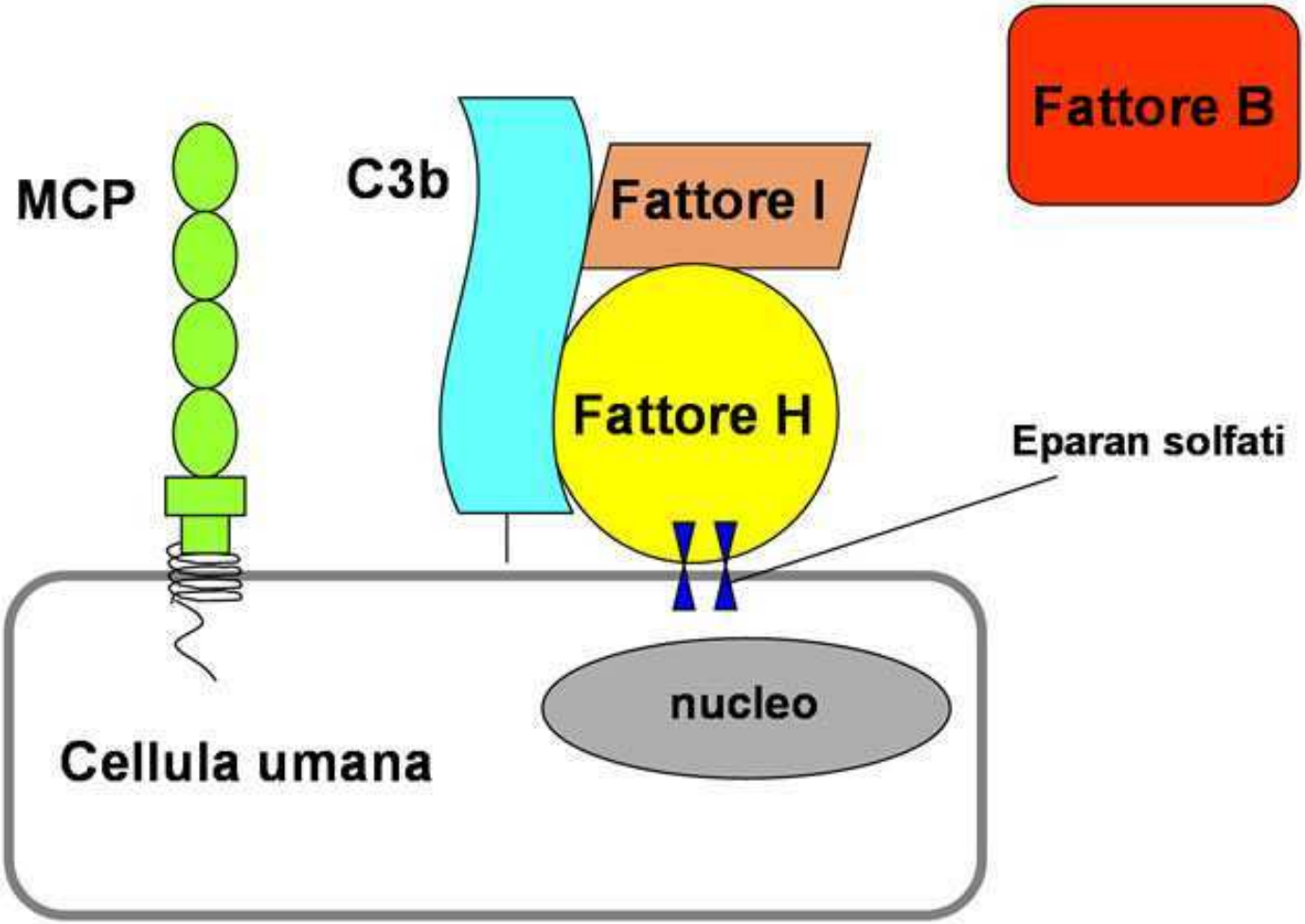
Factor H below the limit of detection in two patients, 50 % of normal in healthy relatives

Families of *Italian Registry of Familial HUS/TTP*



* Low C3 (< 15 mg/dl)

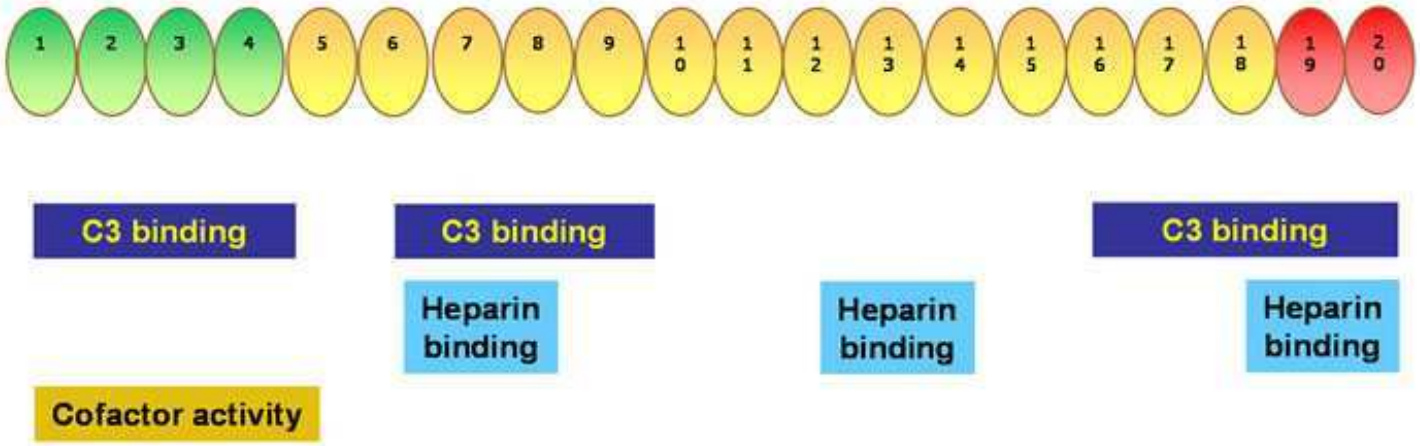
Noris et al., *J Am Soc Nephrol*, 1999


Fattore B
MCP
C3b
Fattore I
Fattore H
Eparan solfati
nucleo
Cellula umana

STRUCTURE AND FUNCTIONAL DOMAINS OF FACTOR H

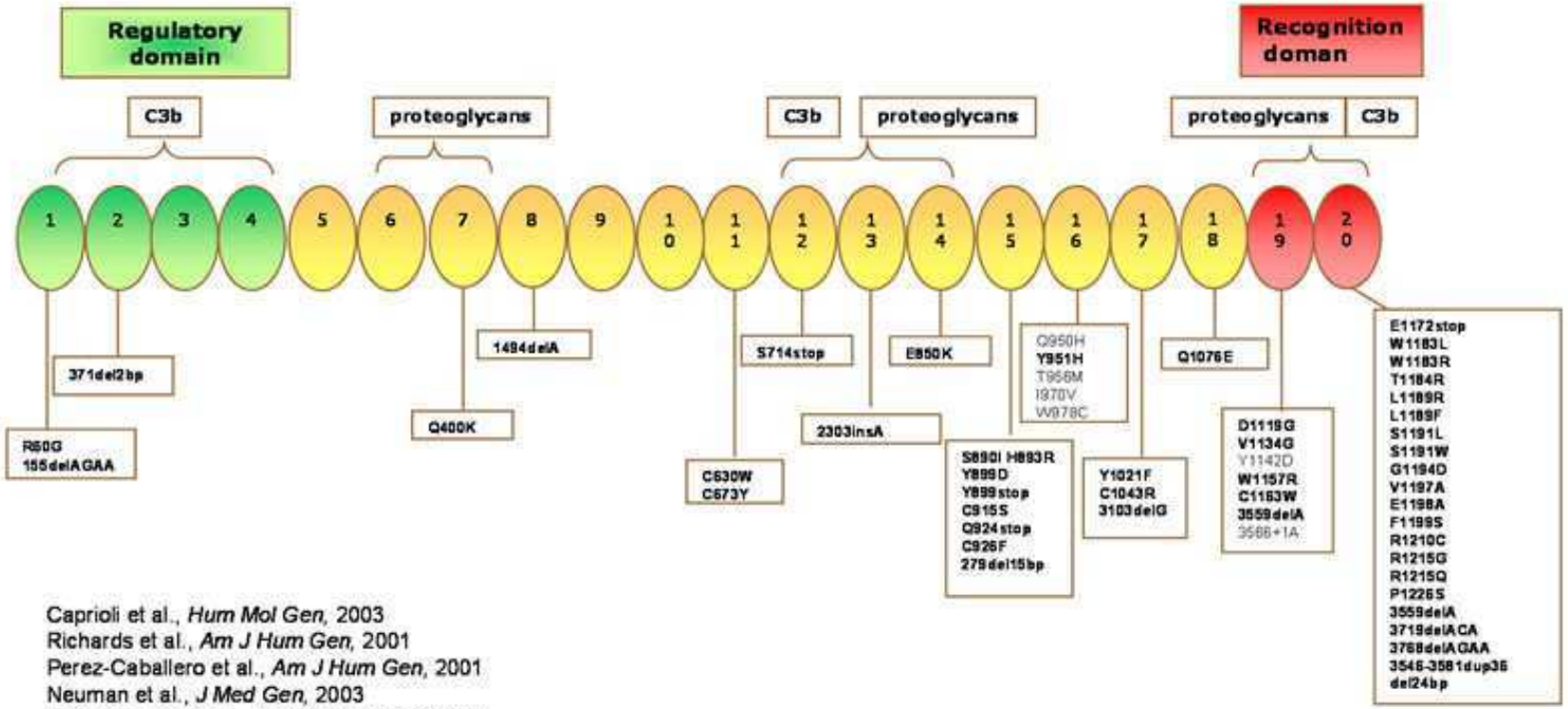
- Factor H plays a pivotal role in the regulation of the alternative pathway of complement activation.

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



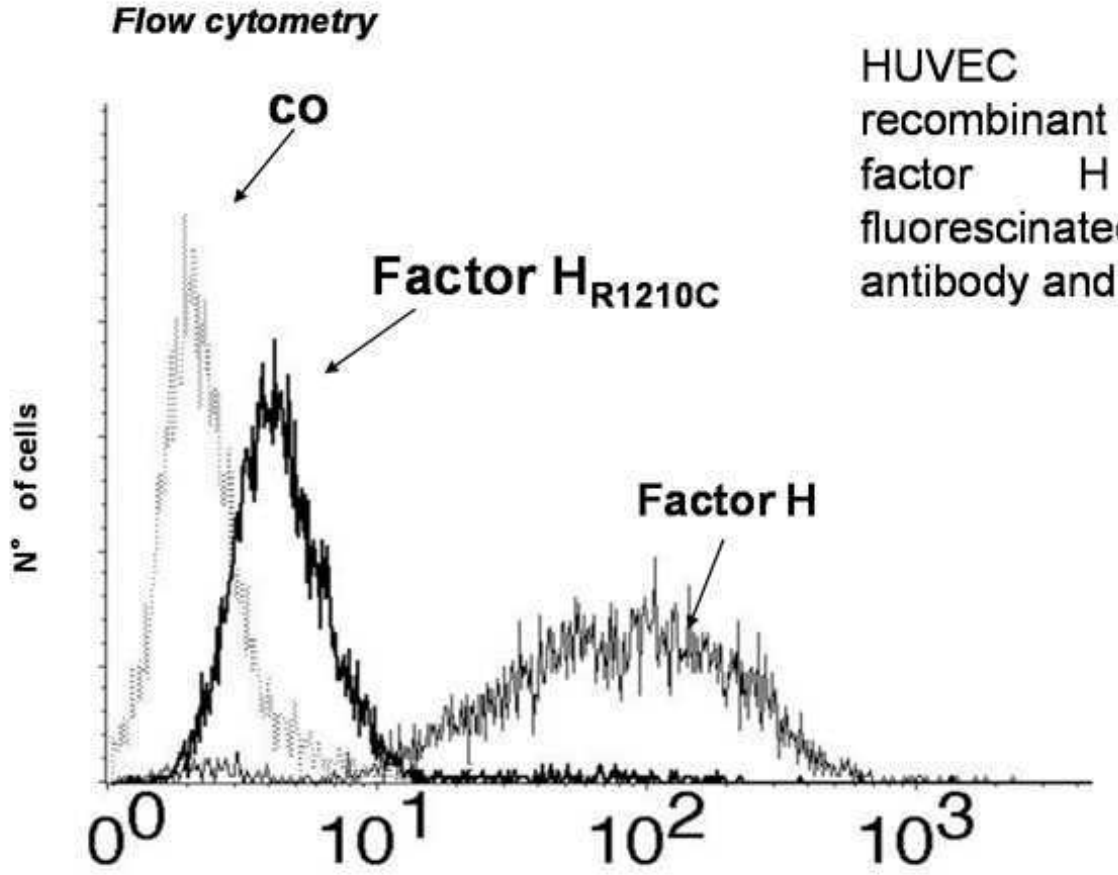
PUBLISHED DATA ON CFH MUTATIONS IN aHUS

- 80 different mutations identified in 120 patients (54 familial, 66 sporadic)
- Mutation frequency: familial forms 40 %
  sporadic forms 13-17 %
- 90% are heterozygous
- Most clustering in the C-terminus domain



Caprioli et al., *Hum Mol Gen*, 2003
Richards et al., *Am J Hum Gen*, 2001
Perez-Caballero et al., *Am J Hum Gen*, 2001
Neuman et al., *J Med Gen*, 2003
Dragon-Durey et al., *J Am Soc Nephrol*, 2004
Caprioli et al., *Blood*, 2006

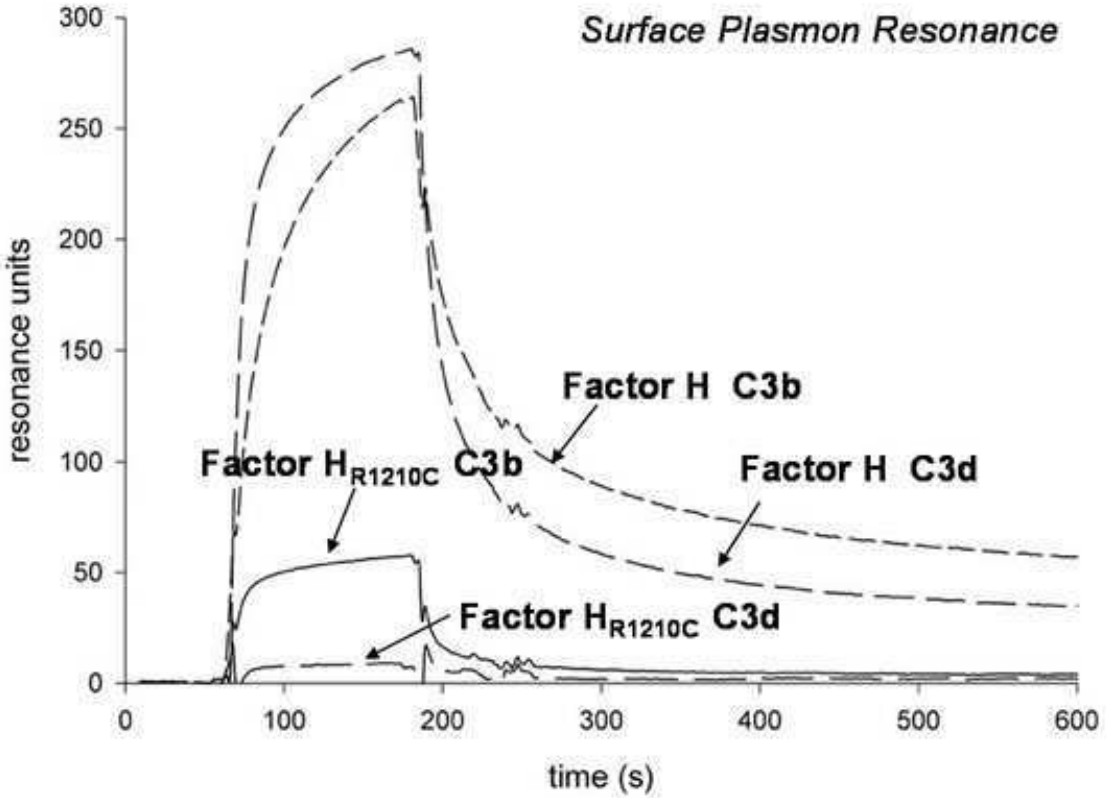
SINGLE MUTATION CHANGES IN SCR 20 OF FACTOR H AFFECT ENDOTHELIAL CELL BINDING



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

Manuelian et al., *J Clin Invest,* 2003

SINGLE MUTATION CHANGES IN SCR 20 OF FACTOR H AFFECT C3b BINDING



In contrast SCR20 mutants have a normal co-factor activity in fluid phase

Manuelian et al., *J Clin Invest,* 2003

Mutations in SCR20, by reducing the capacity of factor H to bind proteoglycans and C3b, would favor the occurrence of microvascular endothelial damage in the presence of complement activating trigger

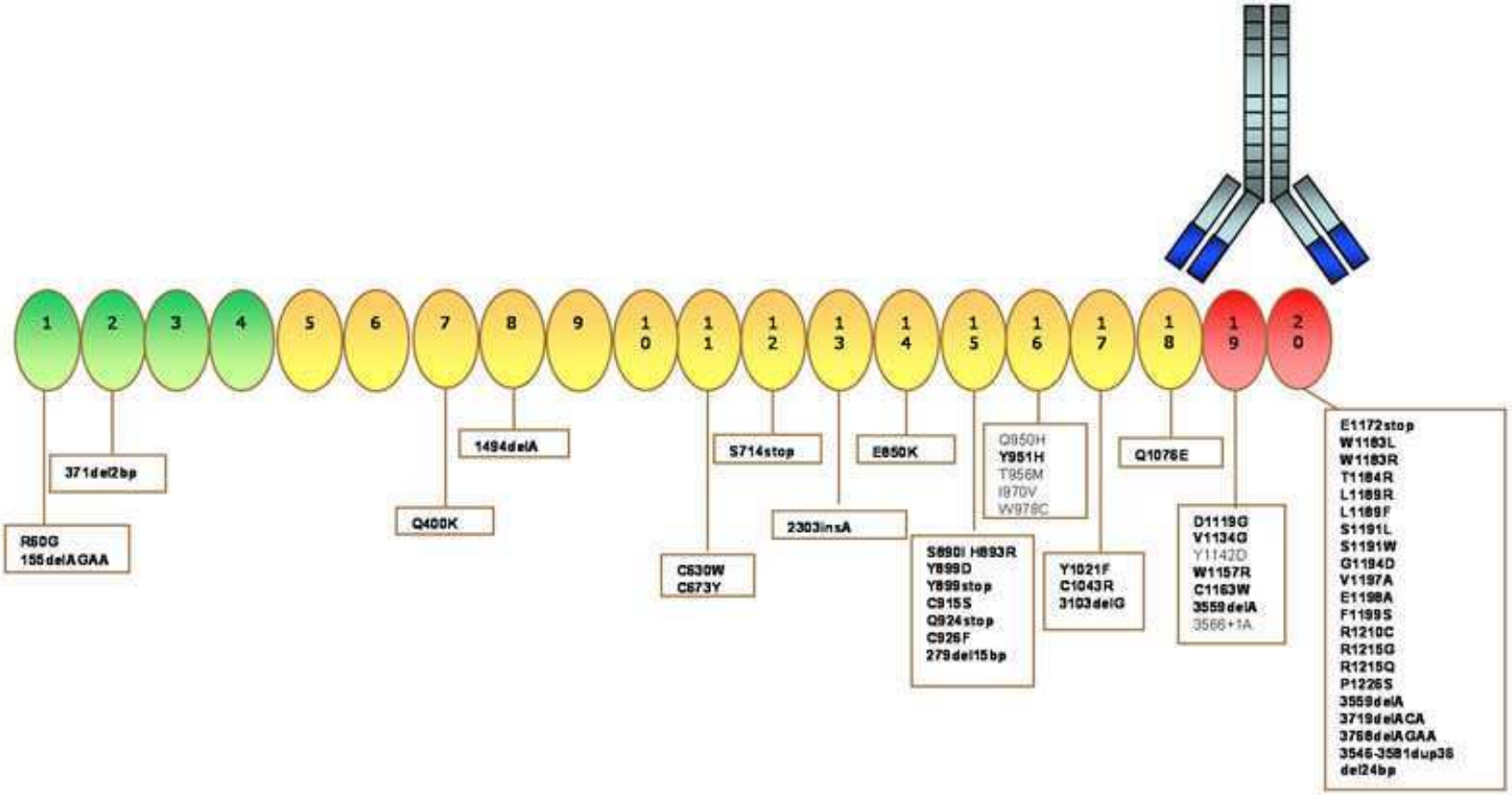
DO THE SAME MUTATION TRANSLATES TO THE SAME PHENOTYPE?

THE R1210C CFH MUTATION: ONSET AND OUTCOME

	DD*	DG*	D9*	LU	SE	GS	ZM	BV	BM
Onset (years)	8	3	6	31	31	35	0.5	43	53
Outcome (1st episode)									
complete rem	●				●		●		
partial rem		●		●					
ESRD						●		●	●
death			●						
Recurrencies	●	●			●		●		
Long term outcome									
complete rem	●								
partial rem									
ESRD		●		●	●	●	●	●	●
death			●						
Kidney transplant									
ok						●			
failure				●					

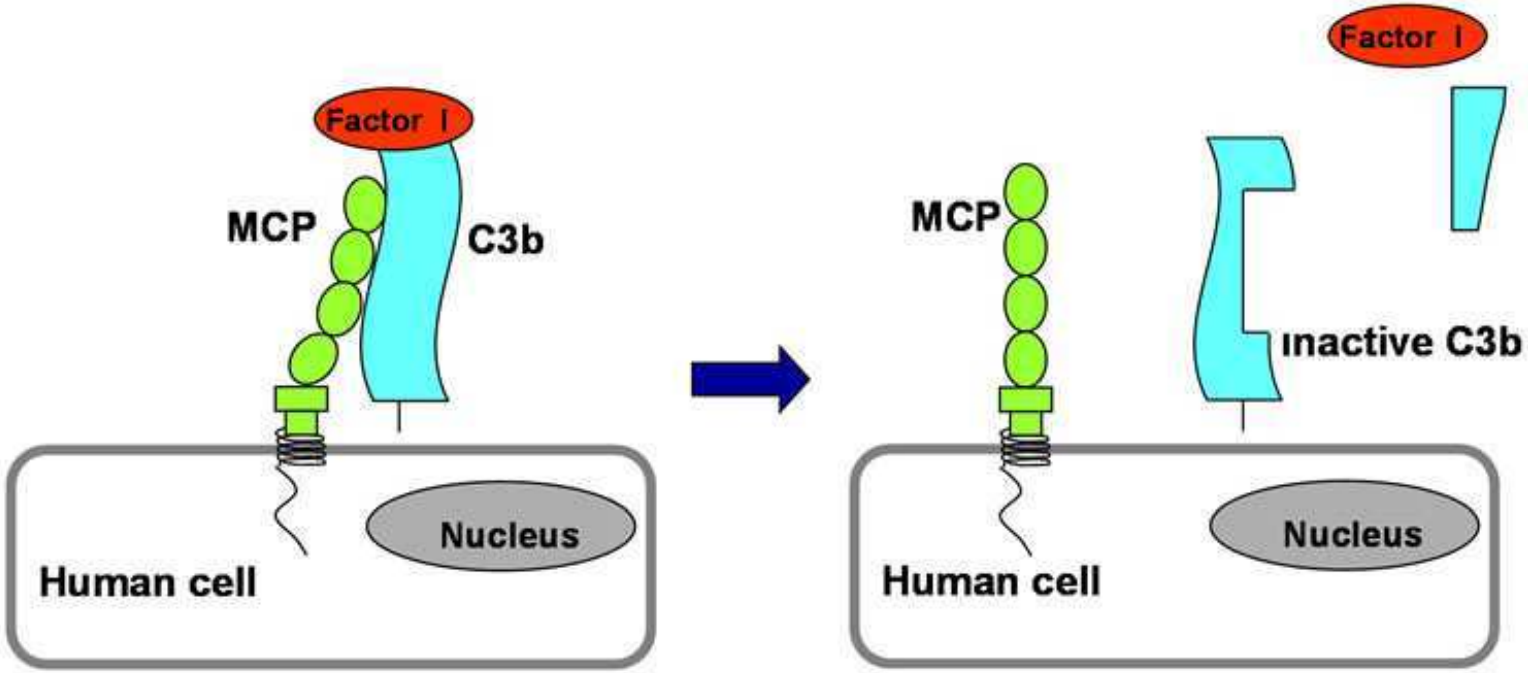
*these patients also carry mutations in MCP

Caprioli J. et al., 2006



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



MCP acts as a cofactor for factor I-mediated cleavage of C3b to inactive C3b

PNAS

Mutations in human complement regulator, membrane cofactor protein (CD46), predispose to development of familial hemolytic uremic syndrome

Anna Richards*†, Elizabeth J. Kemp*, M. Kathryn Liszewski‡, Judith A. Goodship*, Anne K. Lampe*, Ronny Decorte§, M. Hamza Müslümanoğlu¶, Salih Kavukcu‖, Guido Filler**, Yves Pirson††, Leana S. Wen‡, John P. Atkinson‡, and Timothy H. J. Goodship*†‡‡

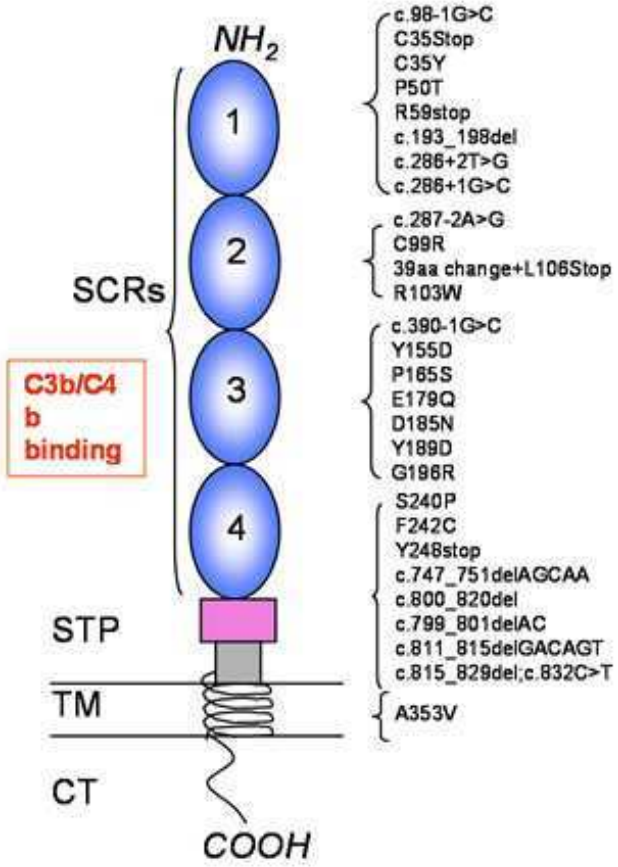
October 28, 2003

Mechanisms of disease

Familial haemolytic uraemic syndrome and an *MCP* mutation

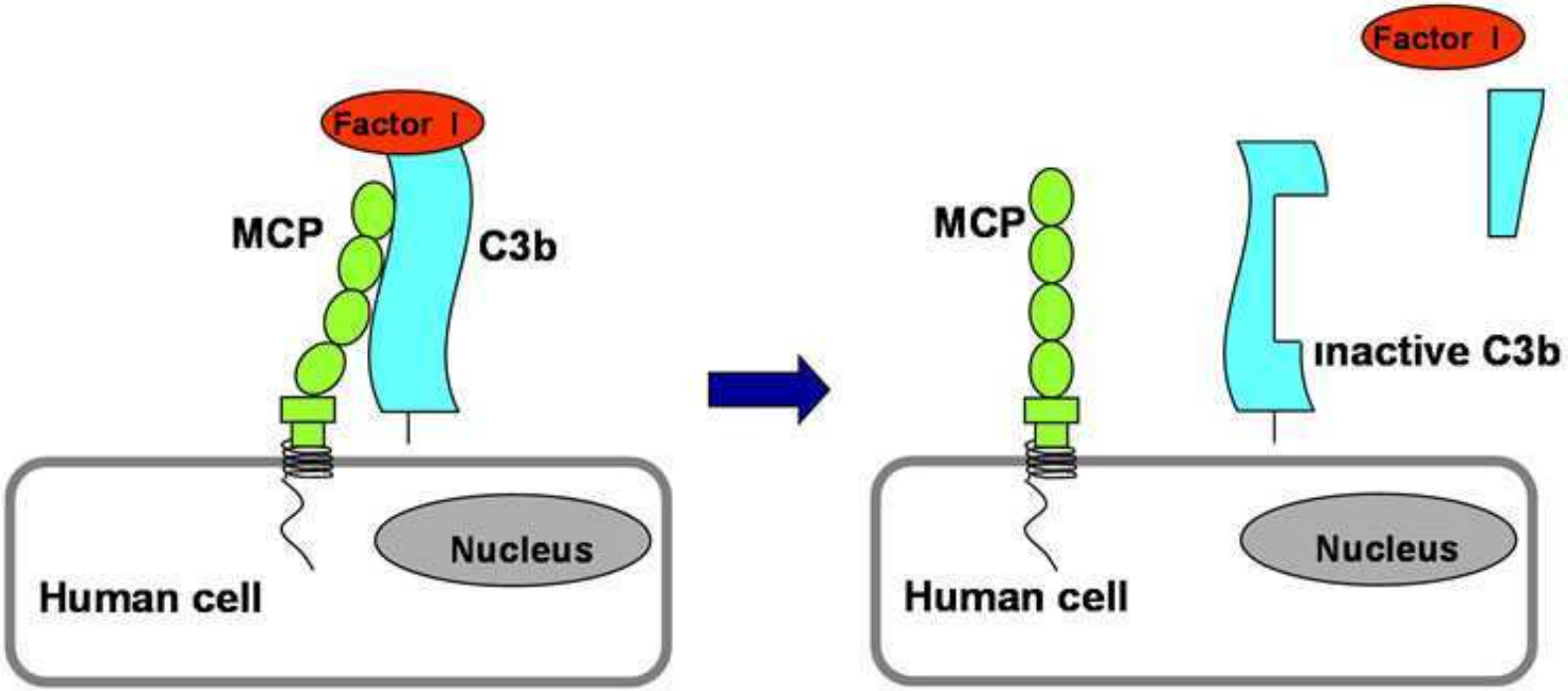
*Marina Noris, Simona Brioschi, Jessica Caprioli, Marta Todeschini, Elena Bresin, Francesca Porrati, Sara Gamba, Giuseppe Remuzzi for the International Registry of Recurrent and Familial HUS/TTP**

MCP



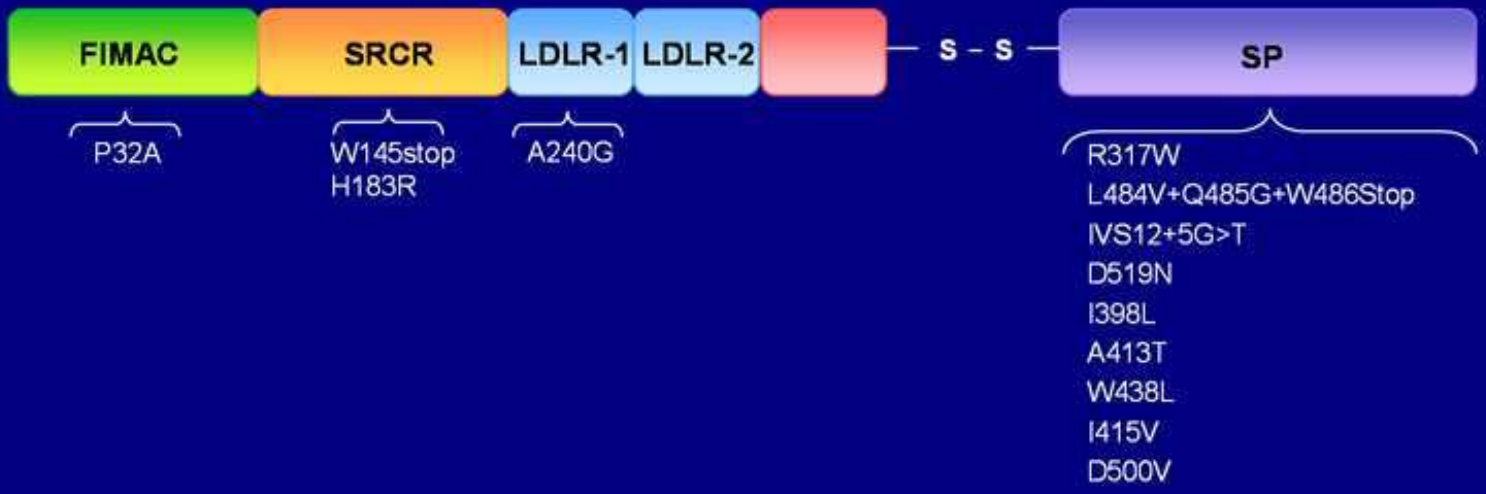
- Mutations frequency: 12,9 %
-Mutations cause either reduced protein expression on the cell membrane or defective complement inhibitory activity

Noris et al, *CJASN,* 2010



Factor I is a serine protease that cleaves C3b to inactive C3b

PUBLISHED DATA ON CFI MUTATIONS IN aHUS PATIENTS

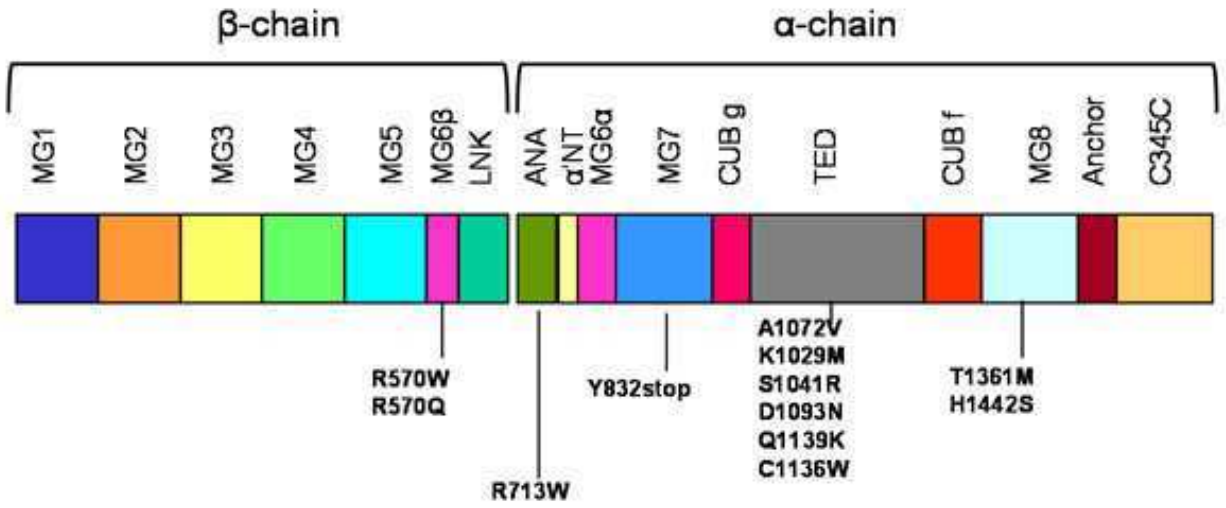


- 22 mutations identified in 32 patients (mutation frequency: 4-10 %)

50% of mutations result in low CFI levels, the others cause impaired C3b inctivation

Caprioli et al., *Blood*, 2006
Fremeaux Bacchi et al., 2007
Kavanagh et al., *JASN*, 2005
Noris et al., *CJASN*, 2010

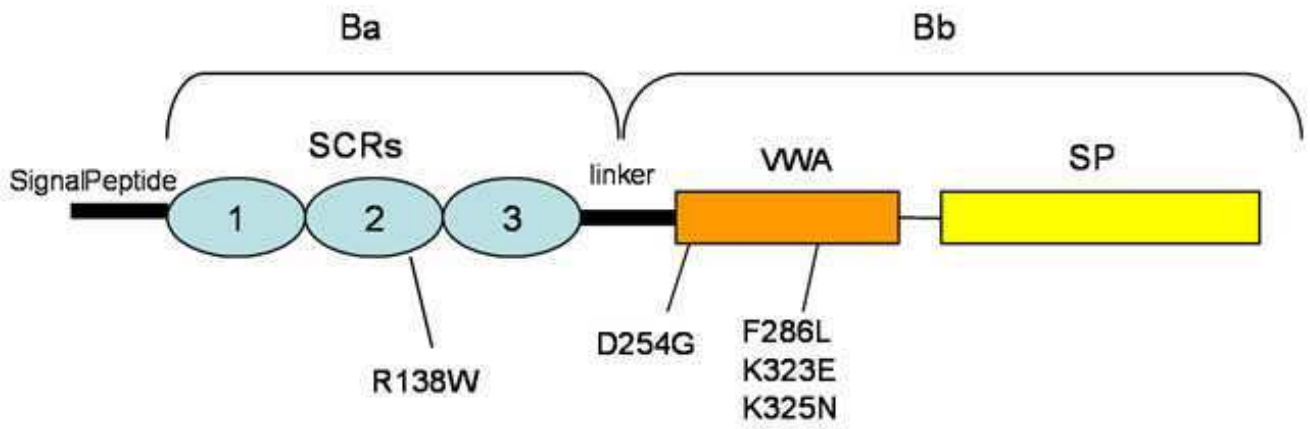
PUBLISHED DATA ON C3 MUTATIONS IN aHUS PATIENTS



- Mutations frequency: 5-8 %
- All heterozygous
- Cluster in the TED domain
- Most mutations reduce C3b binding to CFH and MCP and severely impair degradation of mutant C3b

Fremeaux-Bacchi et al., *Blood,* 2008
Noris et al., *CJASN,* 2010

PUBLISHED DATA ON CFB MUTATIONS IN aHUS PATIENTS



- Mutations frequency: 1-2 %
- All heterozygous
- Cluster in the Bb chain
- Mutants form an hyperactive C3 convertase that enhance C3b formation

Goicoechea et al., *PNAS*, 2007
Roumenina et al., *Blood*, 2009
Noris et al., *CJASN*, 2010

Atypical HUS

Clinically very severe

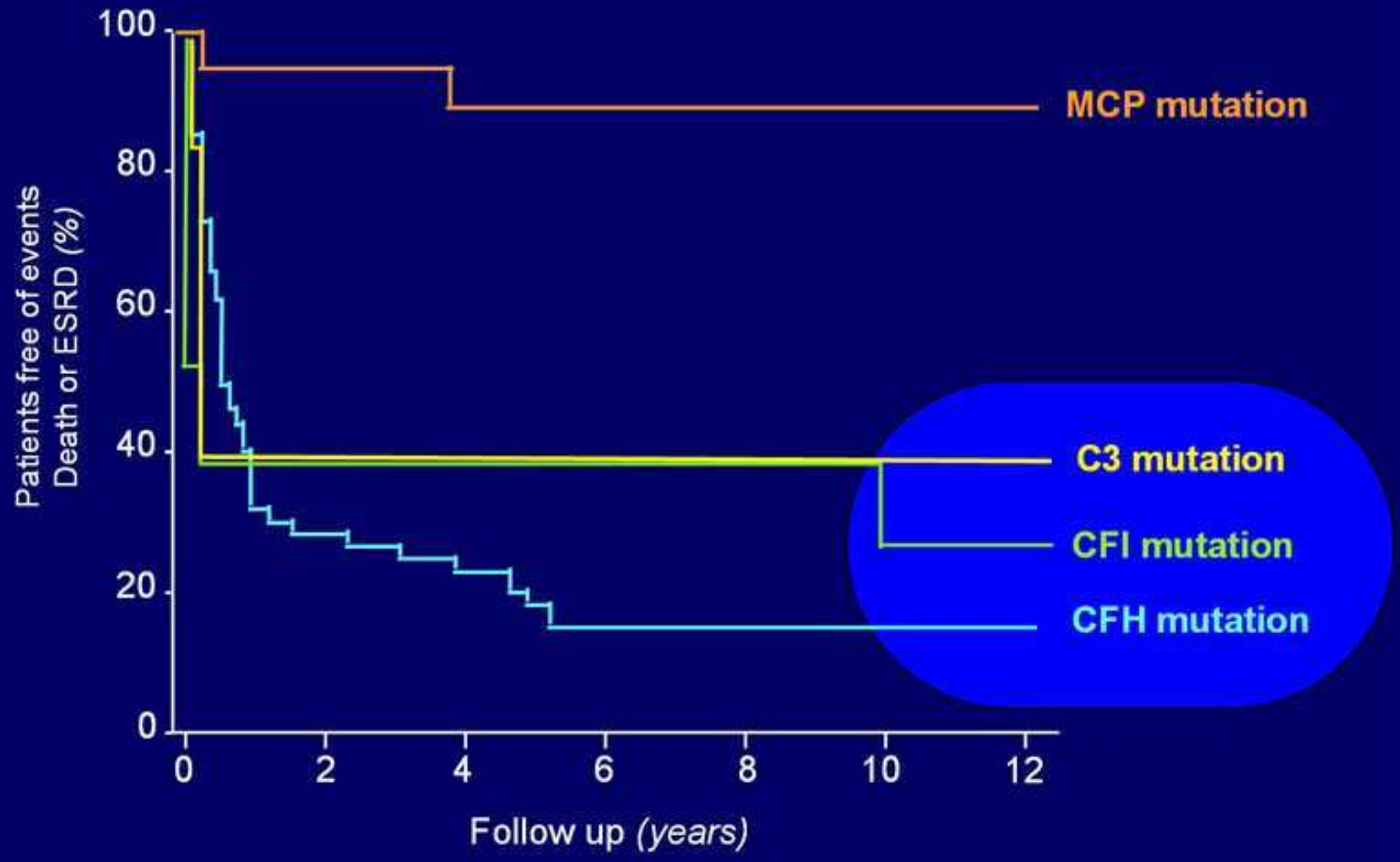
15% died
25% ESRD
15% renal insufficiency
} **60% major sequelae**

1/3 recover without significant renal disease
most (75%) of these had a single episode
few (25%) of these had recurrent aHUS

(a pediatric series)

Taylor et al Ped Neph 2004

LONG TERM OUTCOME OF aHUS PATIENTS



TRANSPLANTATION OUTCOMES

Kidney graft lost because aHUS recurrence

Patients with:

- CFH mutations	12 out 17
- anti-CFH Ab	0 out of 2
- CFI mutations	4 out 6
- C3 mutations	3 out 7

Noris et al., *CJASN*, 2010

Complement and Atypical HUS

Risk of recurrence after "unmodified" kidney transplant

Protein	Gene	Source	Location	Recurrence Rate
Factor H	*CFH*	Liver	circulates	**> 80%**
Factor I	*CFI*	Liver	circulates	**> ~ 80%**
MCP	*MCP*	Widespread	Membrane bound	**~ 20%**
Factor B	*CFB*	Liver, ?	circulates	**?**
C3	*C3*	Liver, ?	circulates	**?**
Anti-FH-Ab	*CFHR1/ CFHR3*	Lymphocyte	circulates	**?**
No known mutation				**30%**

Loirat, C et al. Pediatric Transplantation 2008, Saland et al. JASN 2009

Complement and Atypical HUS

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No known mutation				**30%**

Loirat, C et al. Pediatric Transplantation 2008, Saland et al. JASN 2009

Lancet 2002; **359:** 1671–72

Combined kidney and liver transplantation for familial haemolytic uraemic syndrome

Giuseppe Remuzzi, Piero Ruggenenti, Daniela Codazzi, Marina Noris, Jessica Caprioli, Giuseppe Locatelli, Bruno Gridelli

American Journal of Transplantation 2005; 5: 1146–1150

Hemolytic Uremic Syndrome: A Fatal Outcome after Kidney and Liver Transplantation Performed to Correct Factor H Gene Mutation

Giuseppe Remuzzi[a,b,*], Piero Ruggenenti[a,b], Michele Colledan[a], Bruno Gridelli[b], Alessandro Bertani[a], Paola Bettinaglio[b], Sara Bucchioni[b], Aurelio Sonzogni[a], Ezio Bonanomi[d], Valter Sonzogni[a], Jeffrey L. Platt[c], Norberto Perico[b] and Marina Noris[b]

SIX MORE CASES

	Age	*Factor H mutations*
#1 Male	4 months	V1197A and C973Y
#2 Male	1 year	R1215Q
#3 Female	16 years	R1215Q
#4 Male	6 years	S1191L
#5 Male	21 years	R1215Q

- Extensive plasma exchange pre and peri transplant the surgery
- #1 #2 #3 #4 #5 Good renal and liver function at follow-up
- One additional unpublished combined transplant: good outcome

Saland et al., Am J Transplantation 2006
Jalanko et al, Am J Transplantation 2008
Koskinen et al , Mol Immunol 2008

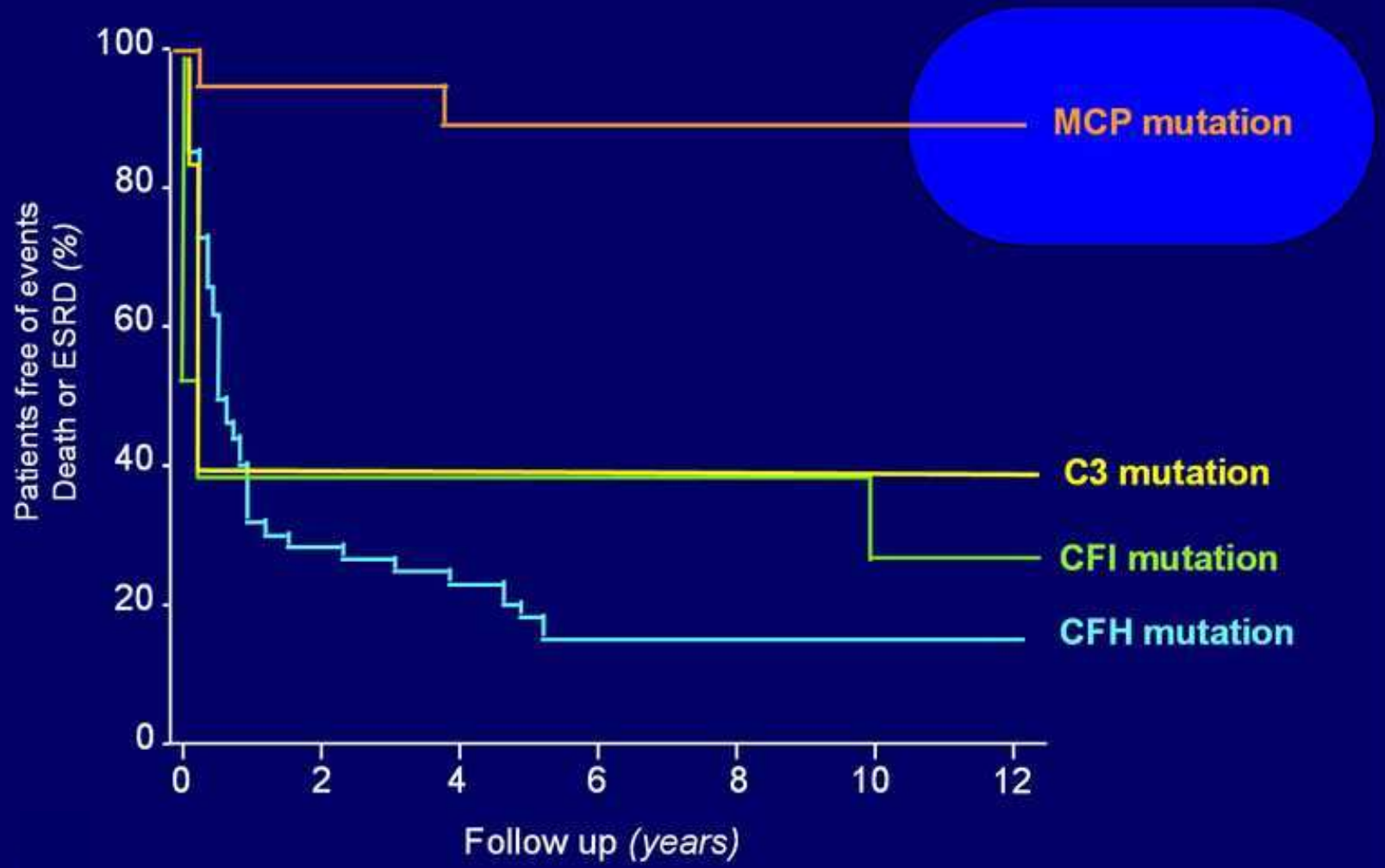
ANOTHER FATAL CASE OF LIVER/KIDNEY TRANSPLANTATION

- Severe hemodynamic instability upon portal vein clamping
- Additional plasma exchange
- Hepatic artery thrombosis
  Fatal hepatic encephalopathy
- Liver biopsy: No TMA, No rejection
  Kidney biopsy: No TMA, ATN

Saland et al., *JASN*, 2009

Despite intensified plasma therapy, liver or liver-kidney transplantation for Factor H associated HUS remains a potentially fatal procedure (death risk 15 to 30%)

LONG TERM OUTCOME OF aHUS PATIENTS



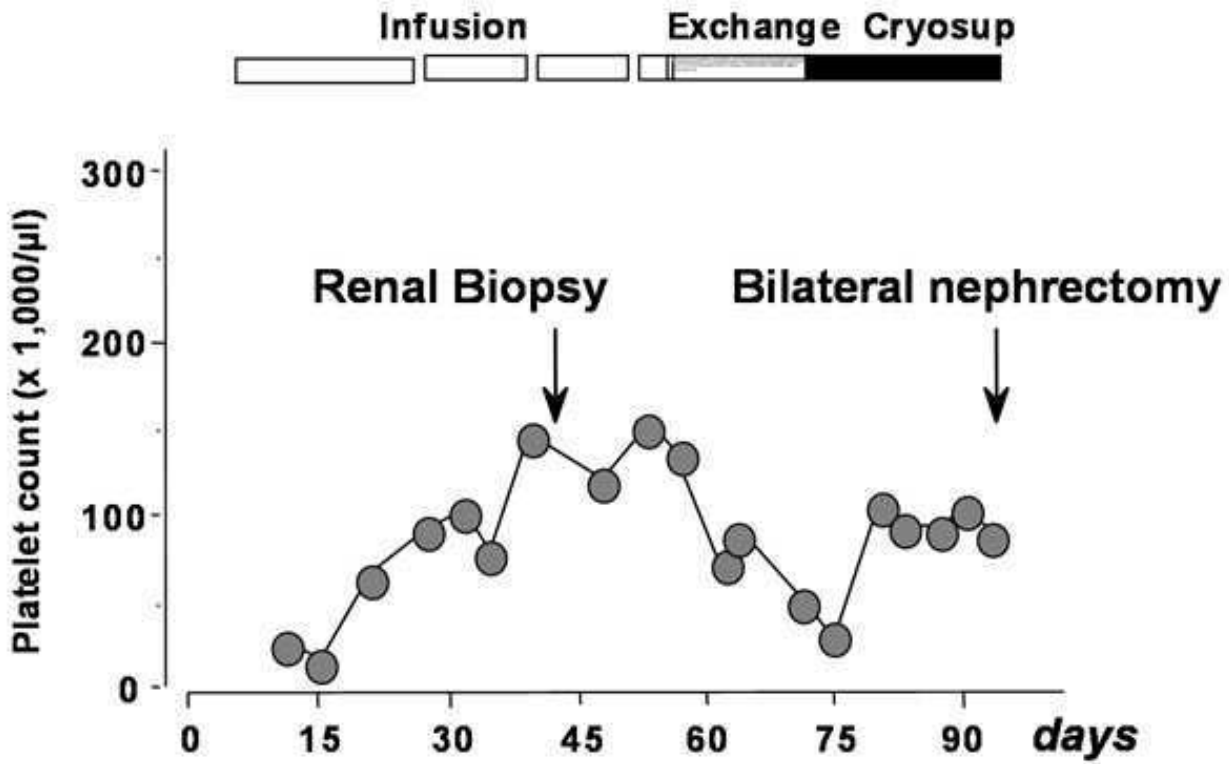
THE FIRST CASE

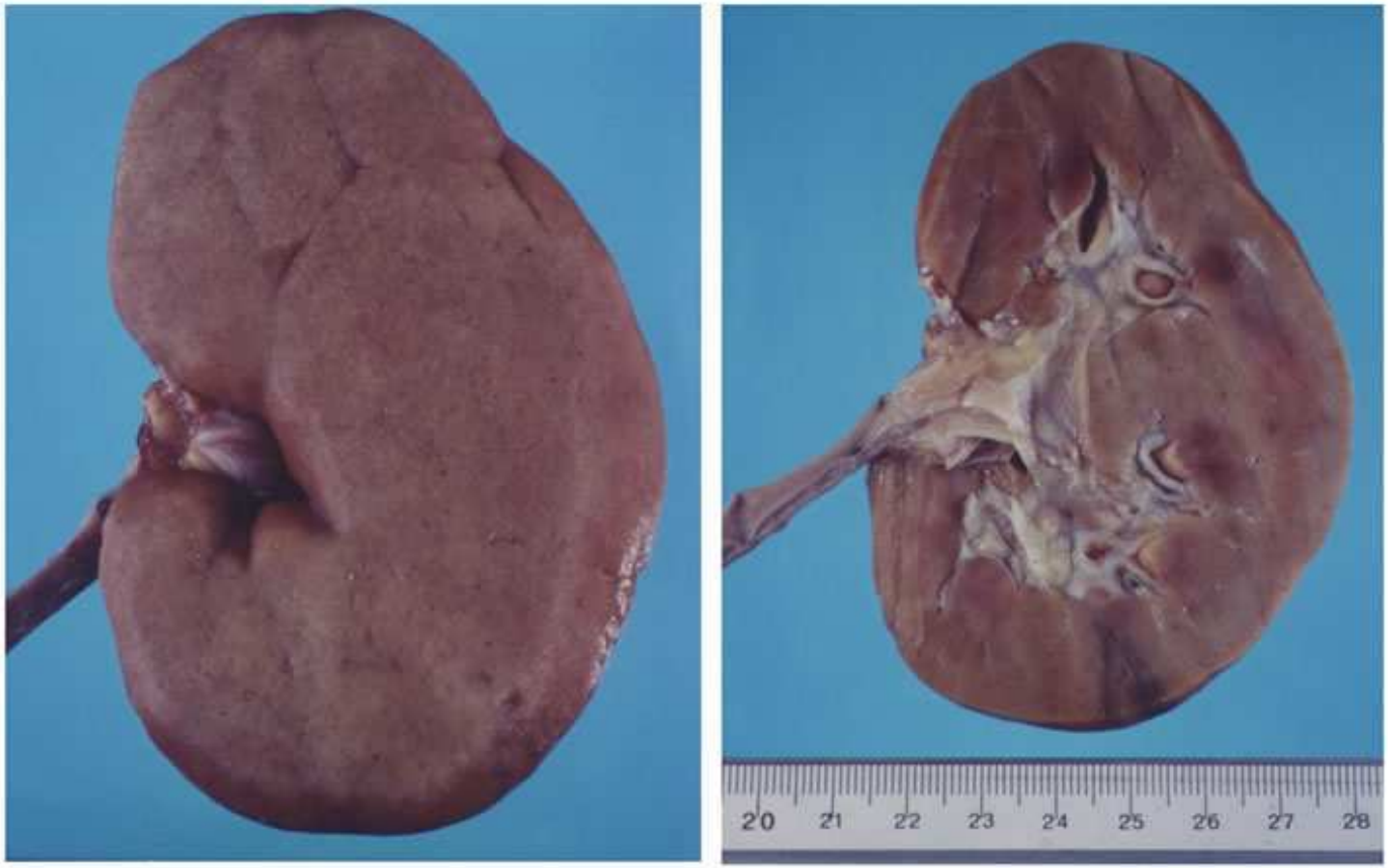
November 22, 1991

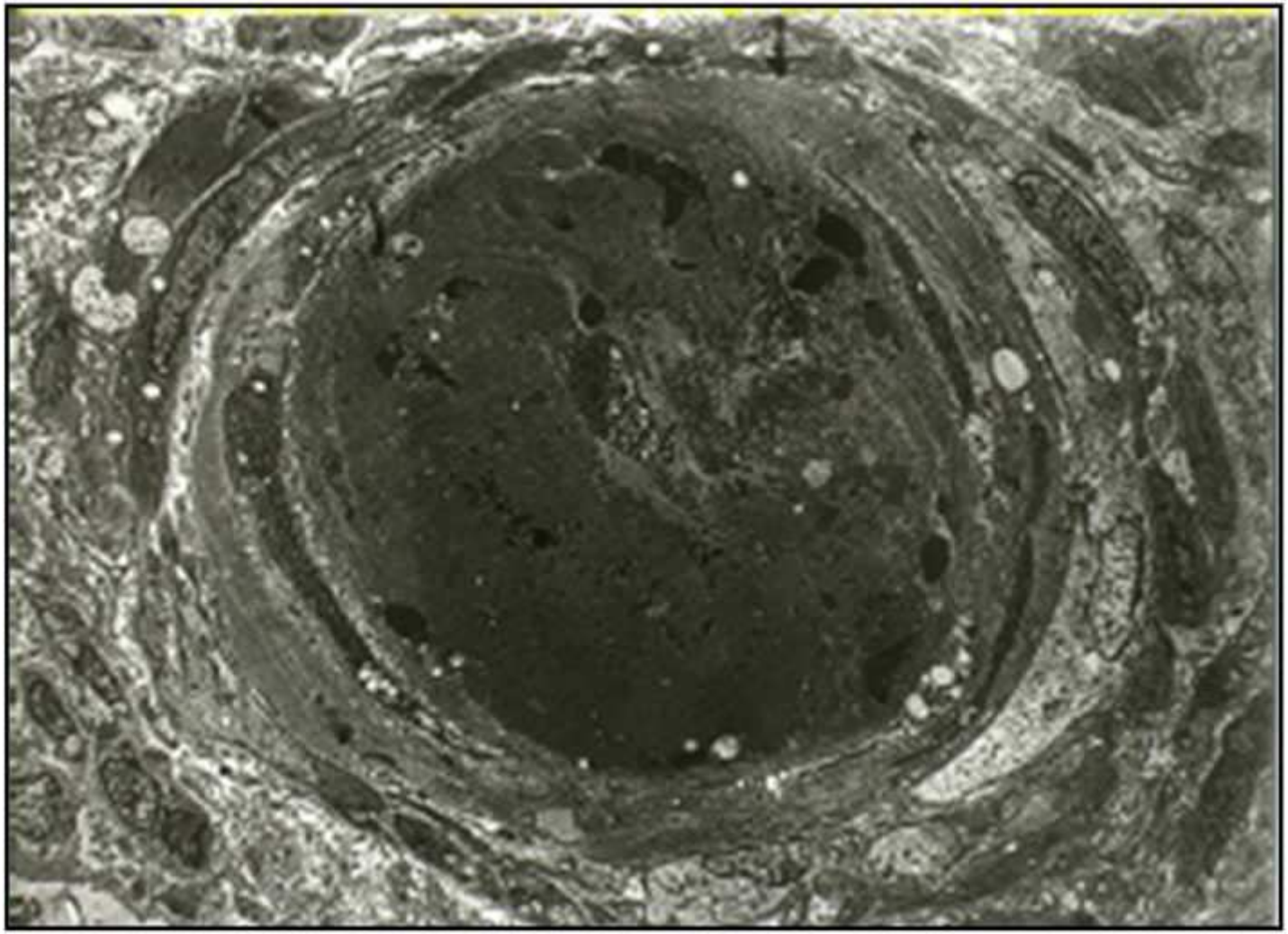
- 26-year-old woman with syncope, vomiting and diarrhea

Main finding at admission

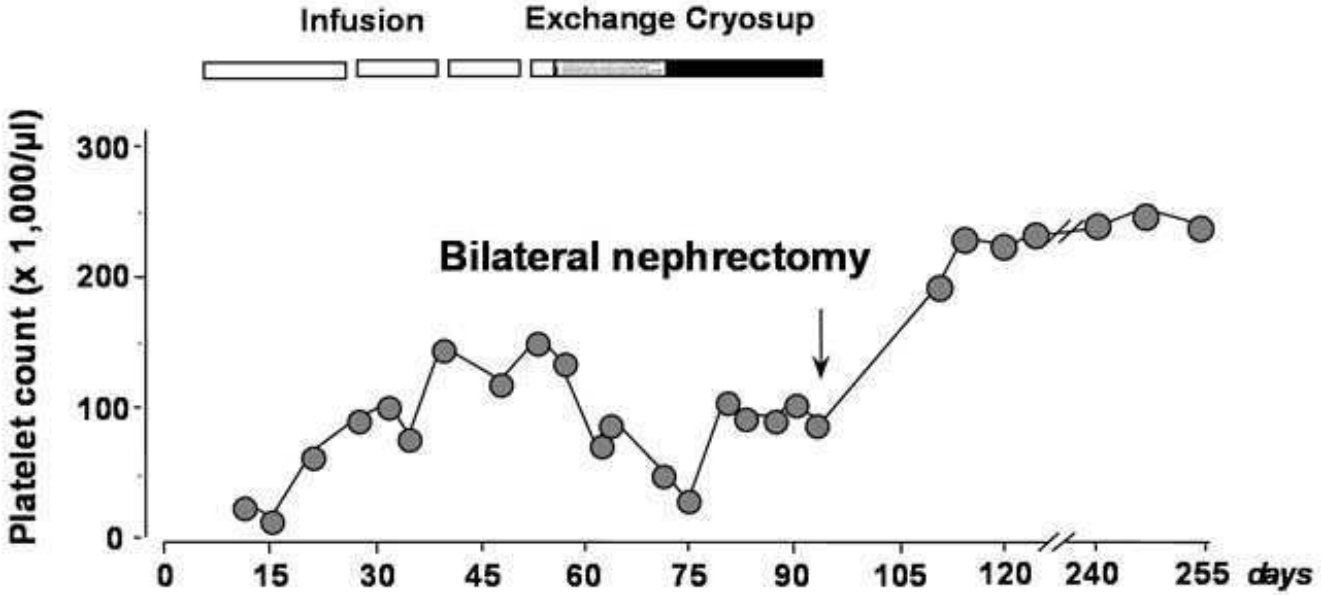
- Blood pressure: - 220/120 mmHg
- Funduscopy: - narrow arteries and hemorrhages
- Laboratory tests:
  - hemoglobin: 8.1 g/dl
  - LDH: 2,200 I.U./L
  - blood smear: schysocytosis
  - **platelets: 27,000/μL**
  - serum creatinine: 2 mg/dl


Infusion
Exchange
Cryosup
Platelet count (x 1,000/µl)
300
200
100
0
Renal Biopsy
Bilateral nephrectomy
0
15
30
45
60
75
90
days


20
21
22
23
24
25
26
27
28



vWF fragmentation	+++	++++	+
LDH *(IU/liter)*	1800	2200	250



Kidney transplants performed in 3 patients with MCP mutations have good graft function at 16, 6, and 3 years post-transplantation

Noris et al, CJASN 2010

- *A consensus protocol for transplantation in aHUS*

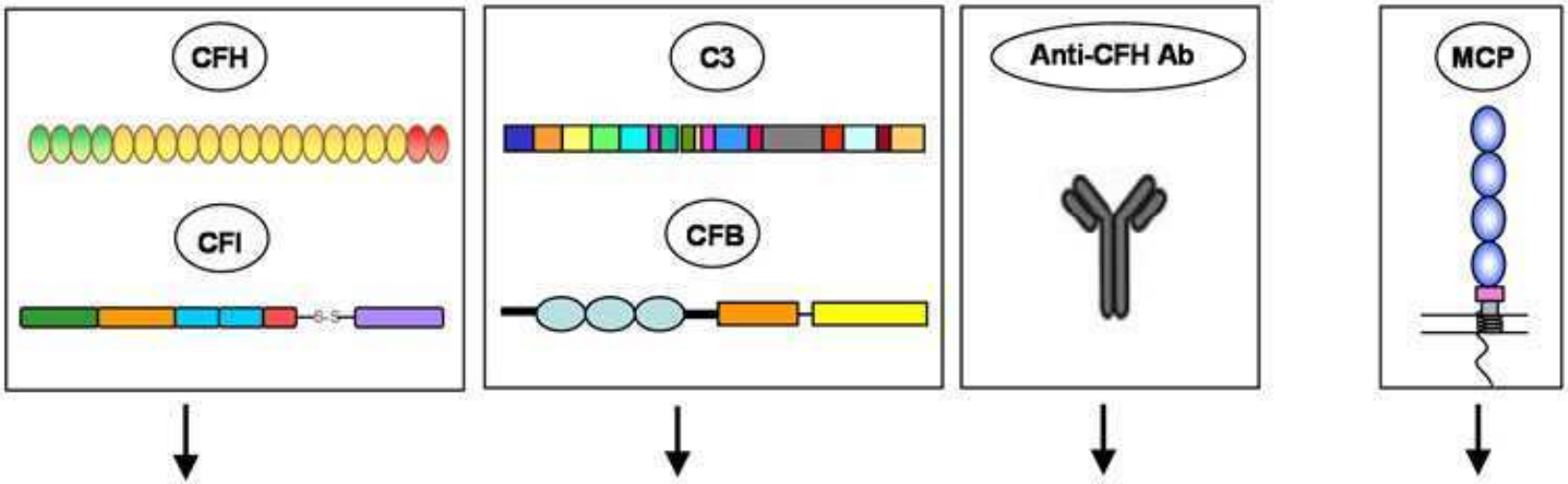
  - kidney and liver CFH/CFI
    CFB/C3 ?

  - kidney alone MCP

- *Perioperative management*

  - 1.5 vol. FFP exchanged before surgery
  - 10-20 ml/kg FFP infused after liver explant
  - low mol. weight heparin
  - aspirin up to 3 mo

Saland, Ruggenenti and Remuzzi, *JASN*, 2009



Kidney transplant
Liver transplant
Plasmapheresis
Eculizumab

Kidney transplant
Plasmapheresis
Eculizumab

Kidney transplant
Plasmapheresis
Rituximab

Kidney transplant

Noris and Remuzzi, Am J Transplant 2010

Recommended Initial Evaluation of HUS

- Because infections trigger both typical and atypical HUS, initial evaluation should encompass both
- Testing should include C3 level as well as classic evaluation (stool culture, LDH, smear, etc.)
- ADAMSTS13 / auto-Ab analysis if TTP not ruled out
- Save some plasma for later analysis (*CFH*, *CFI*, *MCP*, *C3*, *CFB*, anti-FH-Ab – *CFHR1/CFHR3;* more likely to be added)
- Contacting one of the major registries is prudent

Empiric Plasma Therapy

Fluid phase complement proteins reside in plasma and are therefore subject to plasma therapy

Caprioli, et al. Blood. 2006

Empiric Plasma Therapy

Fluid phase complement proteins reside in plasma and are therefore subject to plasma therapy

Plasma Infusion:

- **Repletes but does not remove mutant protein**

Plasma Exchange:

- **Removes mutant protein and repletes**

Caprioli, et al. Blood. 2006

Empiric Plasma Therapy

Fluid phase complement proteins reside in plasma and are therefore subject to plasma therapy

Plasma Infusion:

- Repletes but does not remove mutant protein

Plasma Exchange:

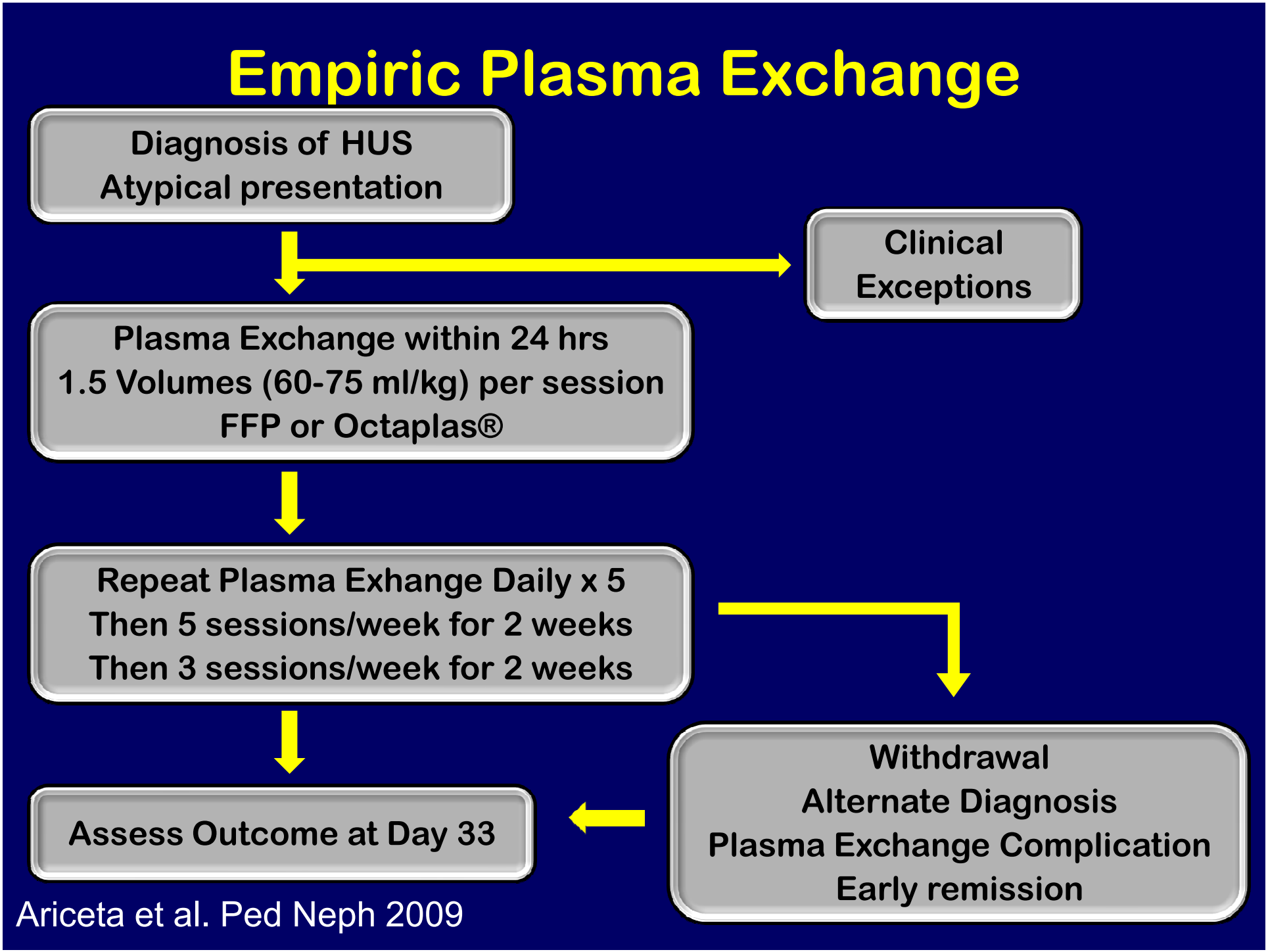
- Removes mutant protein and repletes

There are MANY anecdotes of prolonged preservation of kidney function in patients with *CFH* mutation, though most eventually suffer ESRD.

Benefit is not clear for *MCP* mutations– most (single) episodes seem to recover with or without exchange

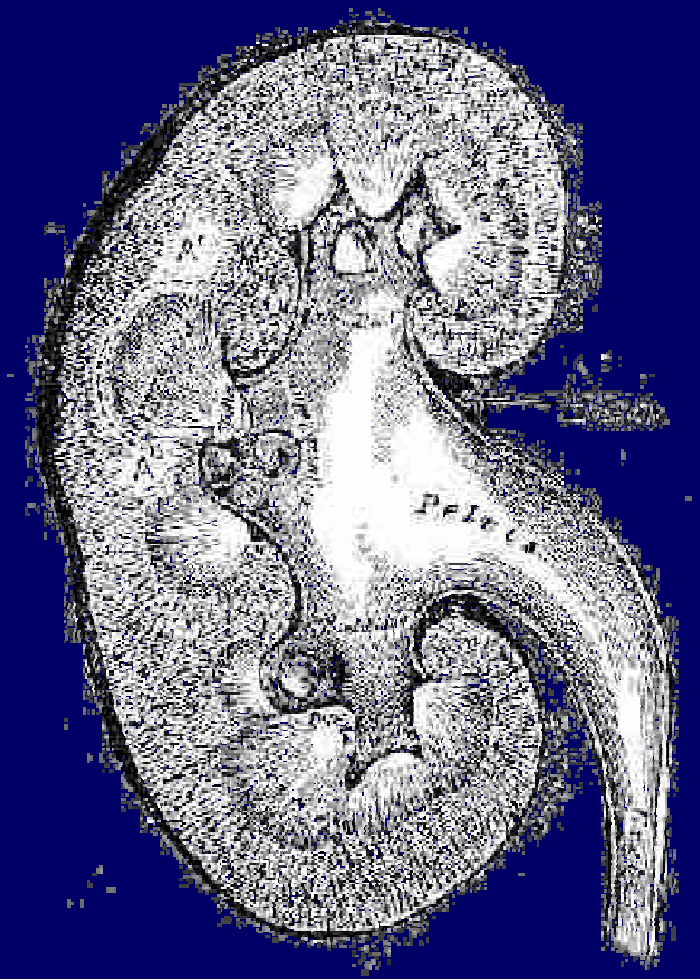
Caprioli, et al. Blood. 2006

Empiric Plasma Exchange



Ariceta et al. Ped Neph 2009

Transplant Considerations



Gray, Henry. *Anatomy of the Human Body.* Philadelphia: Lea & Febiger, 1918; Bartleby.com, 2000. www.bartleby.com/107/.

Post-Transplant HUS Recurrence

Most are within 1 month

Plasma responsiveness of the underlying defect is often retained.

If untreated, most result in graft loss

Chronic plasmapheresis may be required

Seitz, B et al. Transplantation Proceedings 2007

Options for Transplantation

Kidney transplantation

Options for Transplantation

Kidney transplantation

Combined liver-kidney transplantation

- Surgery is a trigger for complement activation

- Preparative plasma exchange before transplant followed by serial plasma exchange is recommended

Combined Liver Kidney Transplant For aHUS Secondary to CFH Mutation

First 3 Experiences not Encouraging

Auxiliary liver, several month function followed by acute decompensation, death

Hepatic graft failure* with neurological deficits, 2nd liver transplant at 1 month

Primary hepatic non-function*, death

*** Complement mediated injury to liver vasculature**

Cheong HI. (Abstract) ASN/ISN World Congress 2001, Remuzzi G, et al. Lancet 2002, Remuzzi G, et al. AJT 2005, Cheong HI et al. Pediatr Nephrol 2004

Liver-Kidney Transplant Protocol Modified by Plasma Exchange

- Hemodialysis (if needed) session no heparin
- Plasma exchange with FFP (minimum 1.5 volumes)
- < 6 hours of surgery
- 10- 20 ml/kg FFP intraoperatively
- Additional FFP if clinically indicated
- Post-operative LMW heparin prophylaxis
- Low dose aspirin prophylaxis

Plasma exchange removes mutant FH, replaces normal
LMW heparin used empirically
Hold anticoagulation for bleeding or coagulopathy

Saland, J et al. JASN 2009

Options for Transplantation

Kidney transplantation

Combined liver-kidney transplantation

Kidney transplantation

followed by chronic plasma exchange prophylaxis

Options for Transplantation

Kidney transplantation*
Combined liver-kidney transplantation*
Kidney transplantation*
  followed by chronic plasma exchange prophylaxis

Not yet …
followed by chronic anti-complement therapy
followed by specific factor replacement (eg. FH)

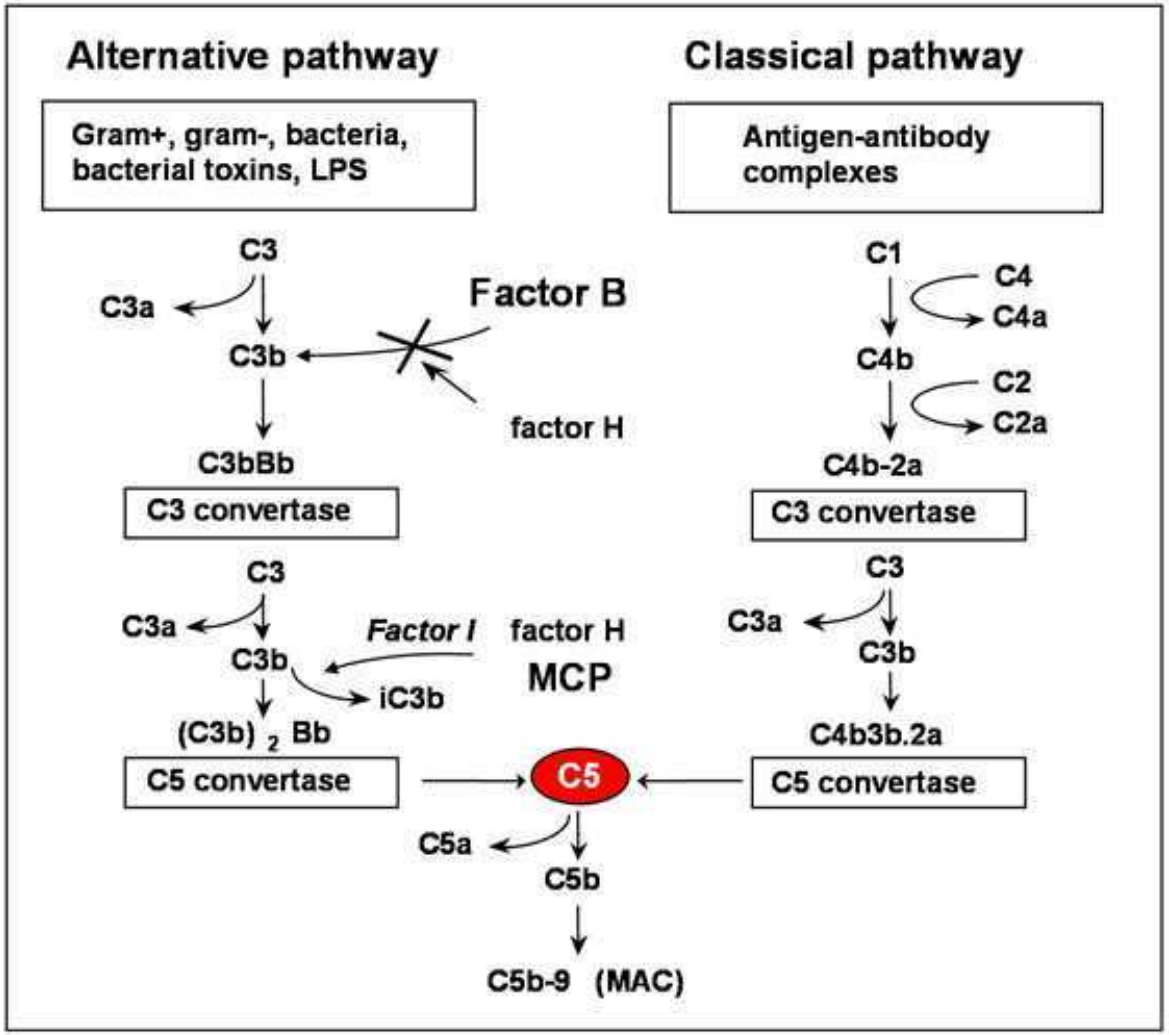
Complement and Atypical HUS

About 50%-60% of aHUS cases are associated with a mutation in a complement-related gene

Protein	Gene	Source	Location	% of aHUS
Factor H	*CFH*	Liver	circulates	~ 15-30%
Factor I	*CFI*	Liver	circulates	~ 5-10%
Membrane Cofactor Protein	*MCP*	Widespread	Membrane bound	~ 10-15%
Factor B	*CFB*	Liver, ?	circulates	<5%
C3	*C3*	Liver, ?	circulates	~ 5-10%
Anti-FH-Ab	*CFHR1/ CFHR3*	Lymphocyte	circulates	~ 10%
Unknown				~ 40-50%

Jozsi et al. Blood 2008, Frémeaux-Bacchi V et al. Blood 2008, Goicoechea de Jorge 2007, Caprioli, et al Blood 2006, Kavanagh Curr Opin Nephrol Hypertens, 2007

ROLE OF COMPLEMENT IN HUS



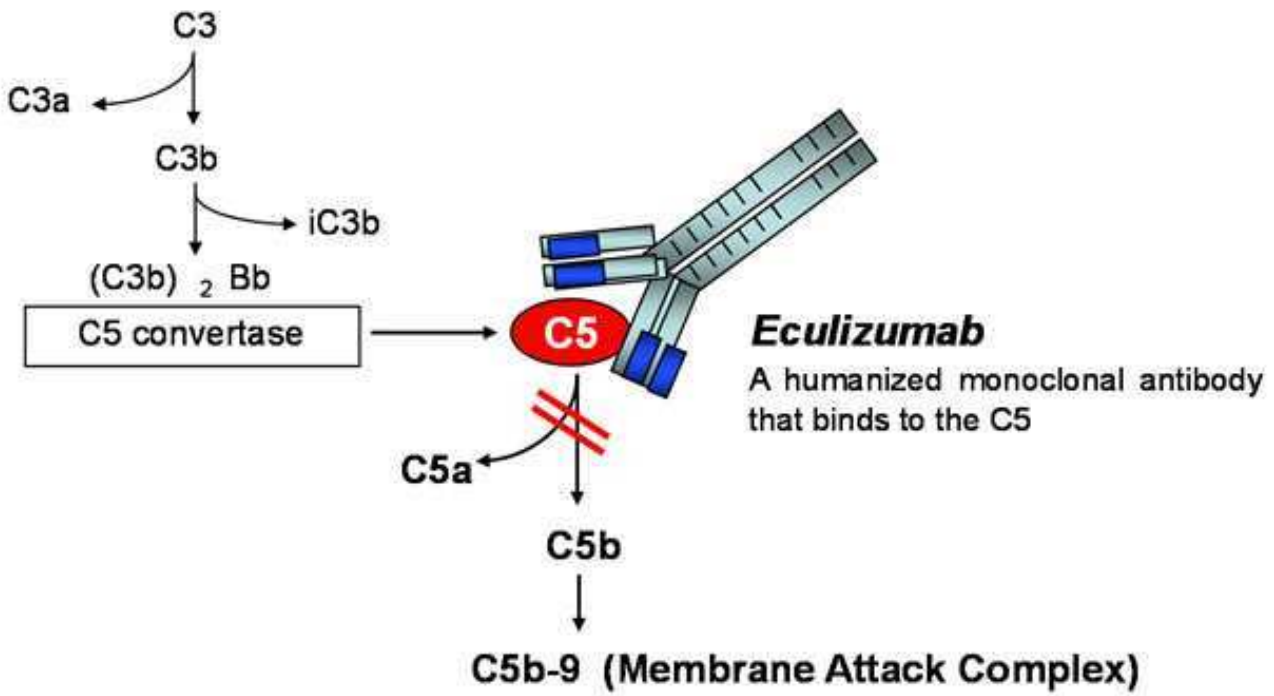
DELETION OF C5 PREVENTS SPONTANEOUS aHUS IN *Cfh*$^{-/-}$.FH1Δ6-20

- Inter-cross *Cfh*$^{-/-}$.FH1Δ6-20 mice with C5-deficient mice

- At 4 months:
  - ↓ mortality
  - ↑ renal function
  - ↑ renal histology

Pickering et al Mol immunol , 2010

Thus blockade of C5 could be effective therapeutic strategy in humans



- Eculizumab was safe and well tolerated in patients with Paroxistic Nocturnal Hemoglobimuria (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

Eculizumab for congenital Atypical Hemolytic-Uremic Syndrome

Gruppo et al, *N Engl J Med,* 2009

Eculizumab for Atypical Hemolytic-Uremic Syndrome

Nurnberger et al, *N Engl J Med,* 2009

Safety and Long-Term Efficacy of Eculizumab in a Renal Transplant Patient with Atypical Hemolytic-Uremic Syndrome

Chatelet et al., *Am J Kidney Dis,* 2009

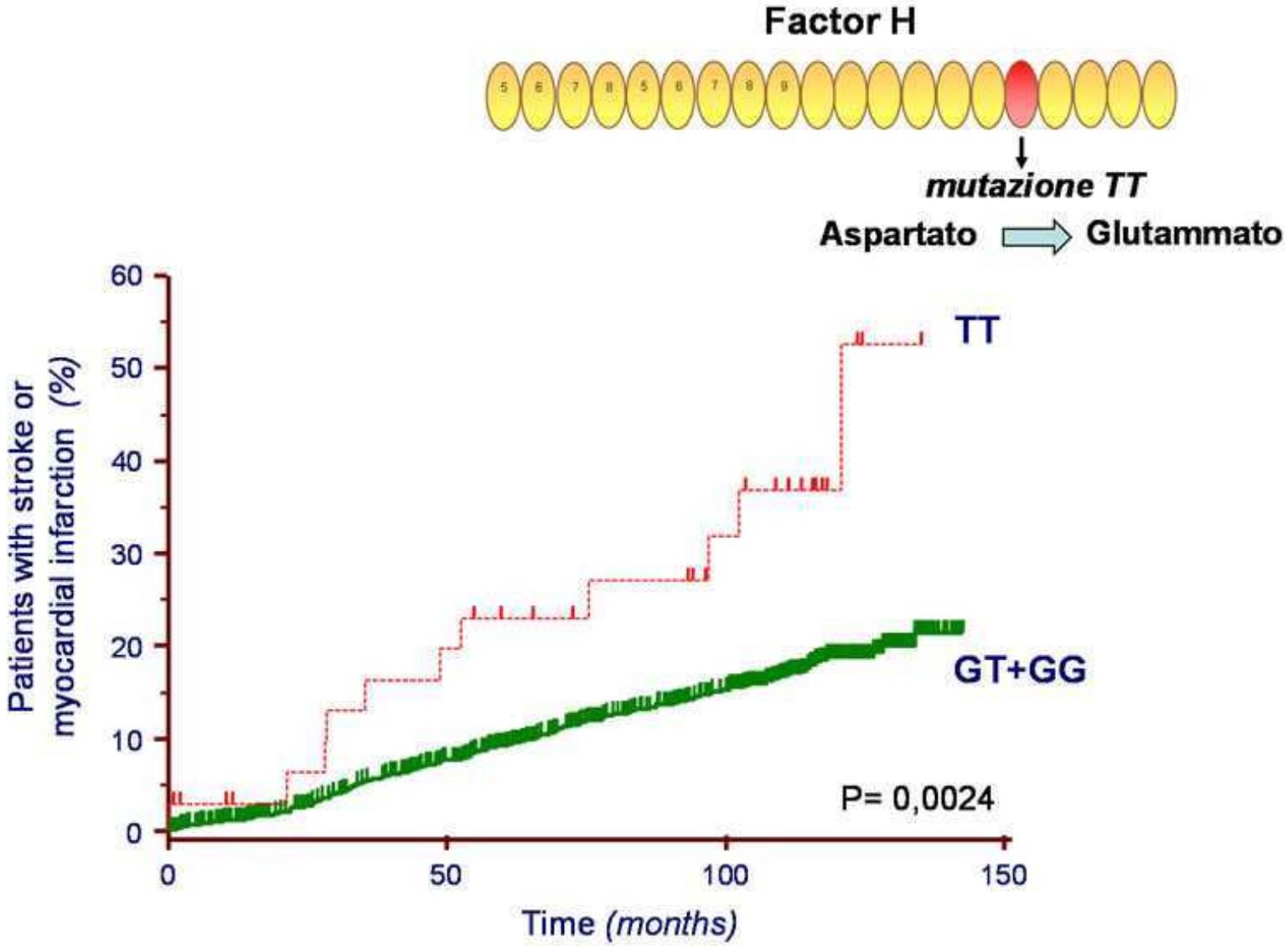
Eculizumab for aHUS: *Perspectives*

- *Four open-label, multicenter clinical trials to assess the efficacy of eculizumab in:*
  - Preventing recurrences in adults or adolescents with recurrent, plasma dependent aHUS
  - Achieving remission in adults or adolescents with plasma resistant aHUS
- *An academic, multicenter, prospective study to assess the efficacy of eculizumab in preventing aHUS after kidney transplantation*

FDA U.S. Food and Drug Administration U.S. Department of Health and Human Services

Eculizumab (Soliris)

On September 23, 2011, the U.S. Food and Drug Administration (FDA) granted accelerated approval for the use of eculizumab (Soliris®, Alexion, Inc.) for the treatment of pediatric and adult patients with atypical hemolytic uremic syndrome (aHUS).


Factor H
5
6
7
8
5
6
7
8
9
mutazione TT
Aspartato
Glutammato
60
50
40
30
20
10
0
Patients with stroke or myocardial infarction (%)
TT
GT+GG
P= 0,0024
0
50
100
150
Time (months)

JDILLA FOUNDATION

James Dewitt Yancey (February 7, 1974 – February 10, 2006)