

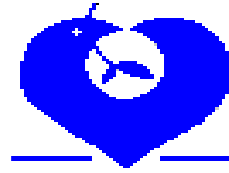
Genetic Factors Governing Susceptibilities to Severe Infections

GSK-Chair of Infectious Diseases

Pr Jean-Paul MIRA



Inserm



GENETIC PREDISPOSITION TO SEVERE SEPSIS

**Université catholique de Louvain
Cliniques universitaires Saint-Luc
17 février 2005**

Pr. Jean-Paul MIRA

Medical ICU & Dept. of Cell Biology

Cochin University Hospital & Cochin Institute, Paris, F

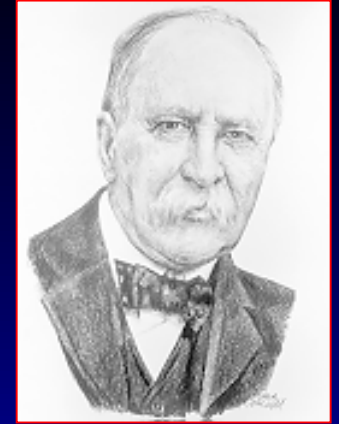
Recurrent Purpura Fulminans

2002/01: 15 yo girl admitted in ICU

- Temperature 40°C; HR 125; BP 74/45; RR 38
- Meningitis with purpura fulminans
- MOF (Shock, ARDS, ARF, DIC, Lactic acidosis)
- Meningococcus type N in the skin biopsy
- Survival with multiple finger amputations and skin grafting
- 6 month hospitalization

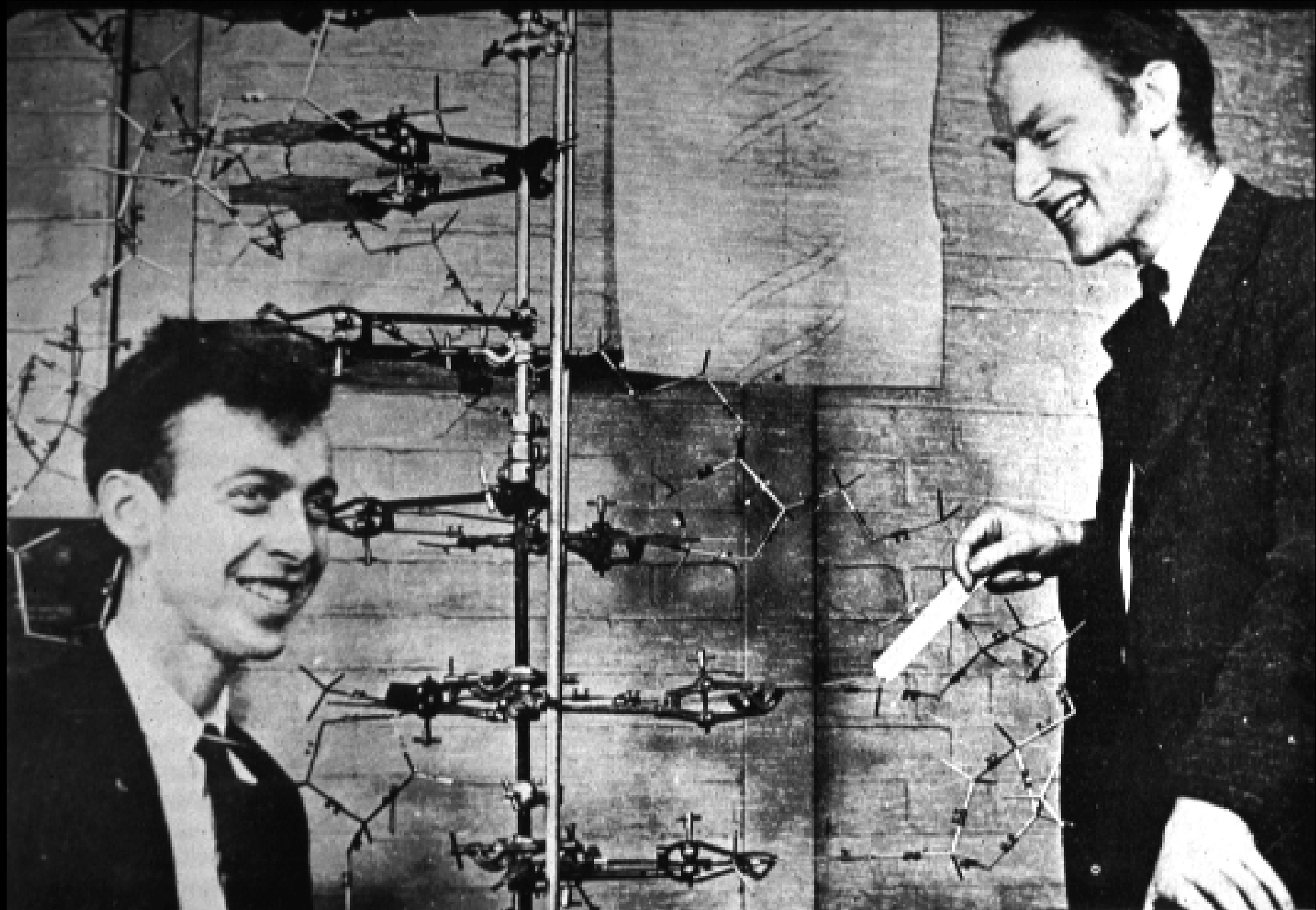
2003/02:

- Temperature 39°C; HR 125; BP 83/48; RR: 33
- Meningitis with purpura fulminans
- Lumbar puncture → meningococcus type Y
- Shock and DIC
- Survival (Xigris) with new skin grafting
- 3 month hospitalization



**« If it were not for the great variability among individuals
medicine might as well be a science and not an art »**

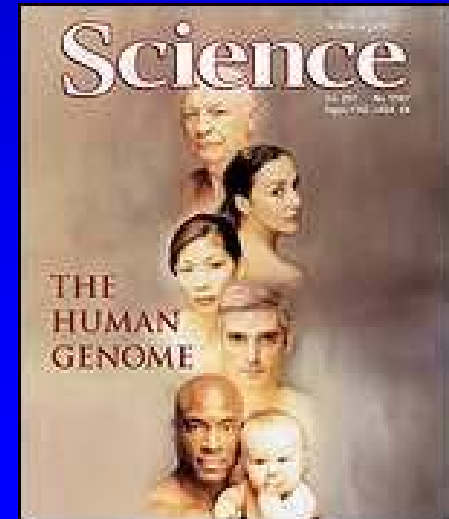
Sir William Osler, 1892



Watson JD, Crick FHC. Nature 1953;171:737

From Watson and Crick to Human Genome

- 1953 Watson and Crick: double helical structure of DNA
- 1960s Role of RNA and Genetic Code
- 1970s Recombinant DNA technology
- 1977 Sanger and Gilbert: DNA sequencing
- 1983 Mapping of disorders by linkage (Huntington disease)
- 1986 Polymerase Chain Reaction
- 1990 Human Genome Project
- 1995 *Haemophilus influenzae* genome
- 2003 Mice and Human genome sequence
Human SNP Map



**WE ALL, AS HUMANS, SHARE
THE SAME BASIC GENES**

BUT...

Small differences in genotype make big differences to phenotype

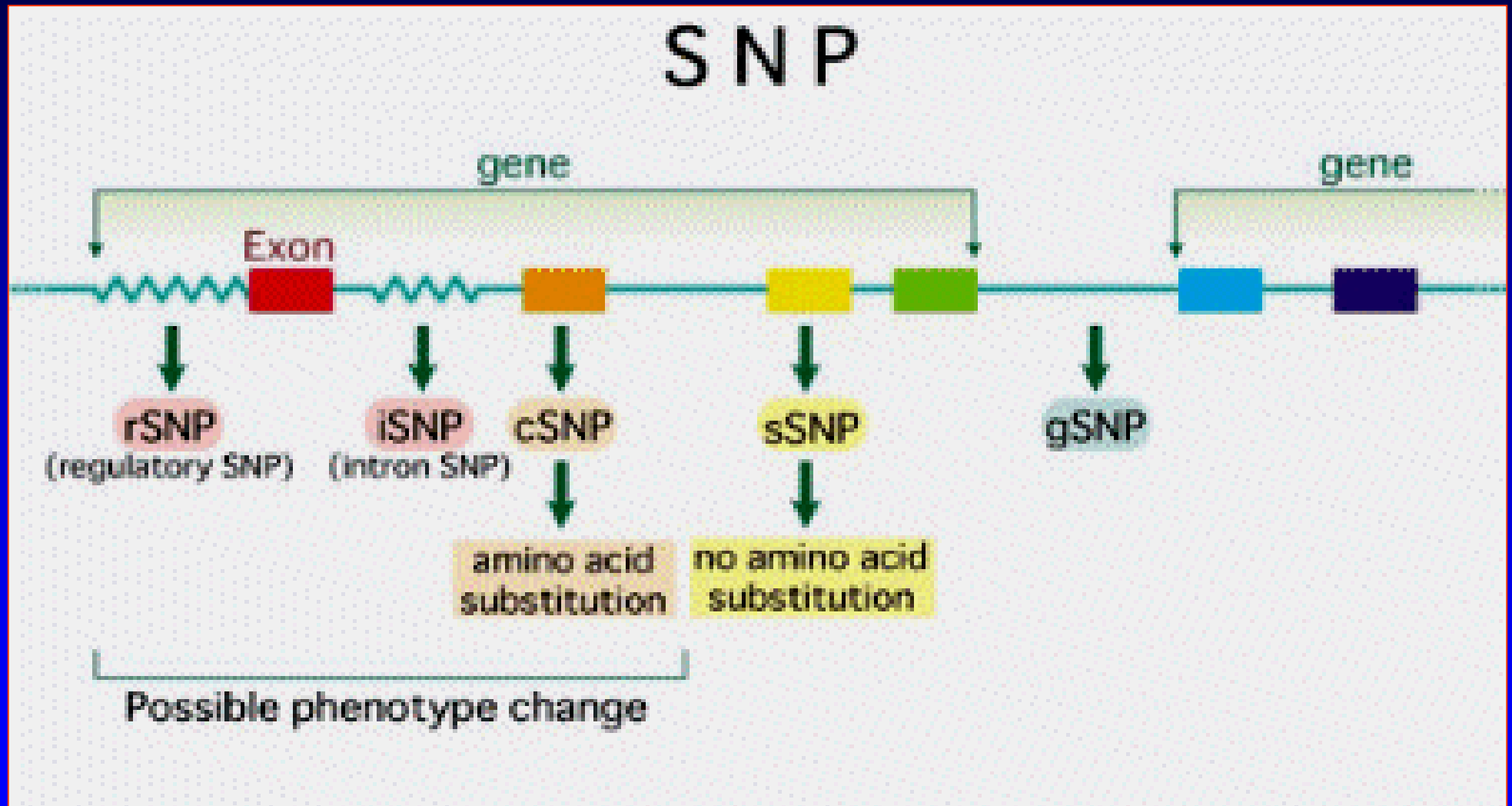


Genetic Polymorphisms



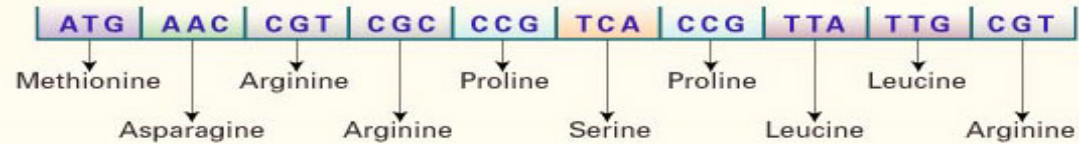
→ Human SNP Map

Genetic Polymorphisms



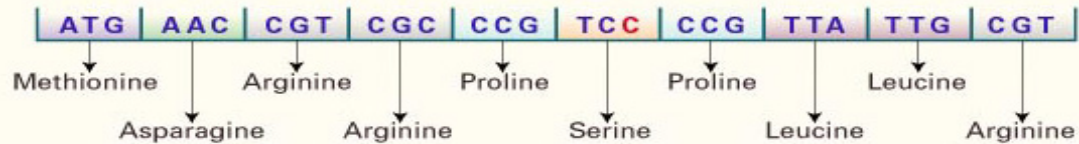
A

Normal sequence



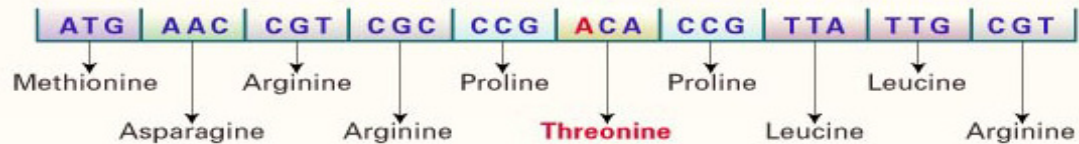
B

Silent mutation



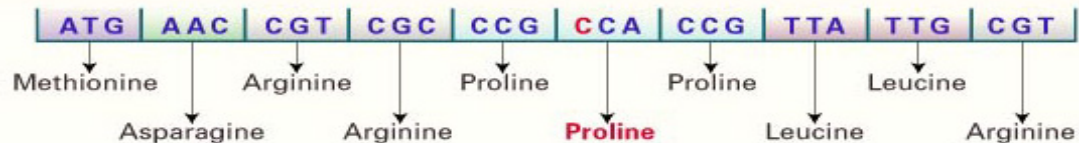
C

Conservative missense mutation



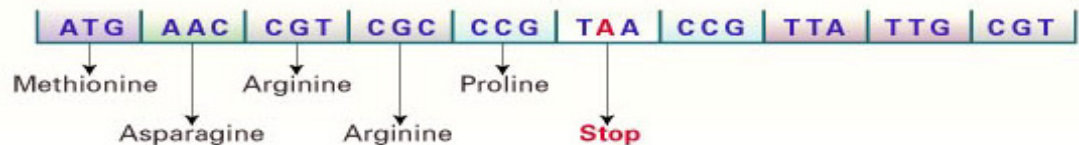
D

Nonconservative missense mutation



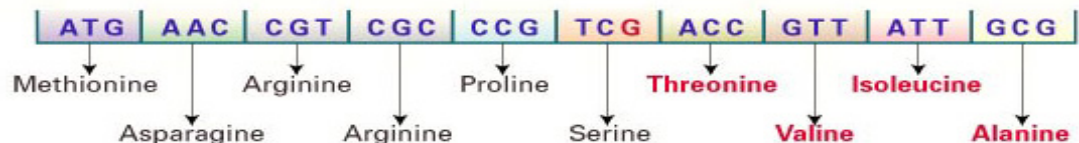
E

Nonsense mutation



F

Frame-shift mutation

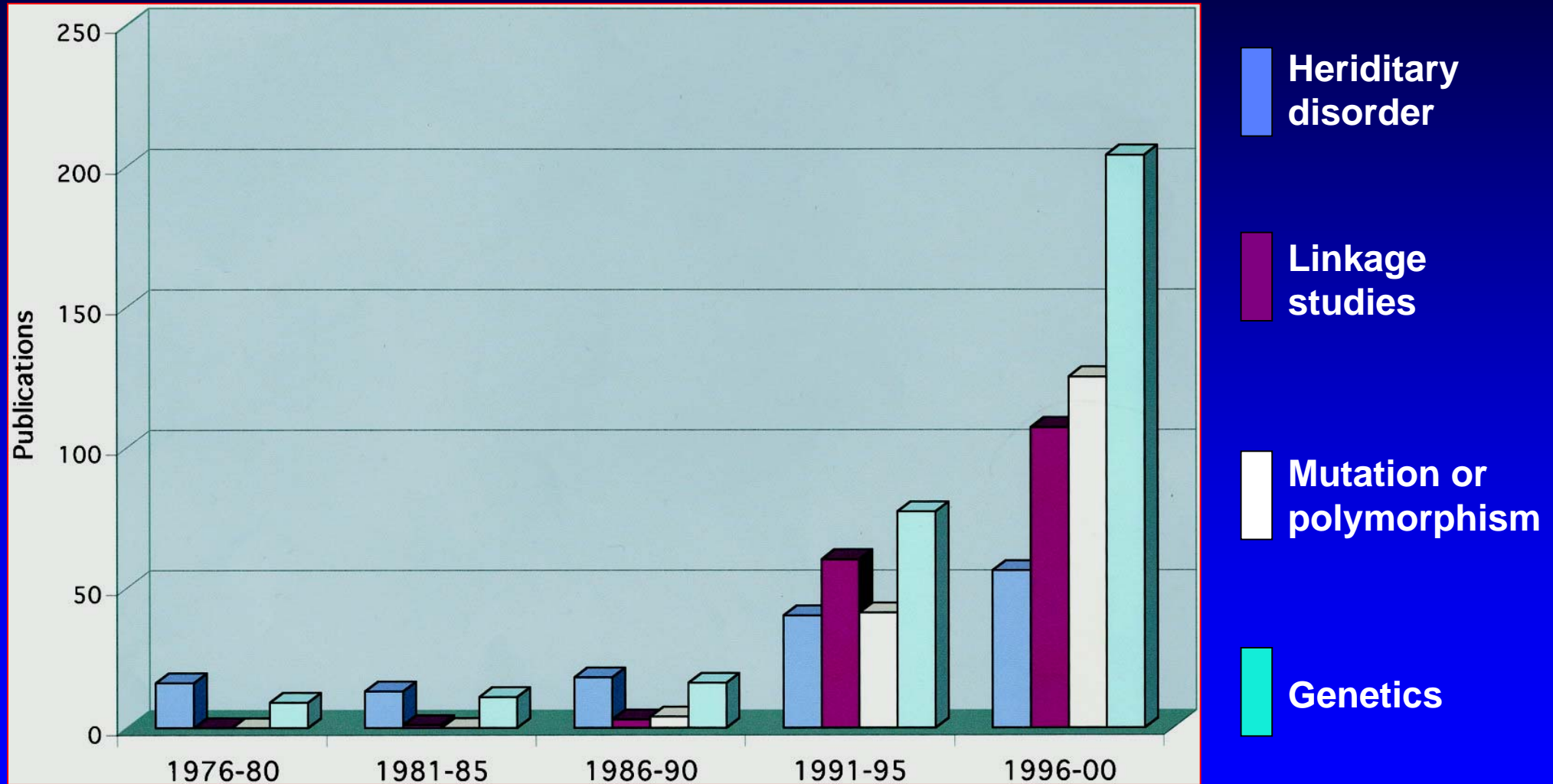






DNA
EVIDENCE

Molecular Genetic Research



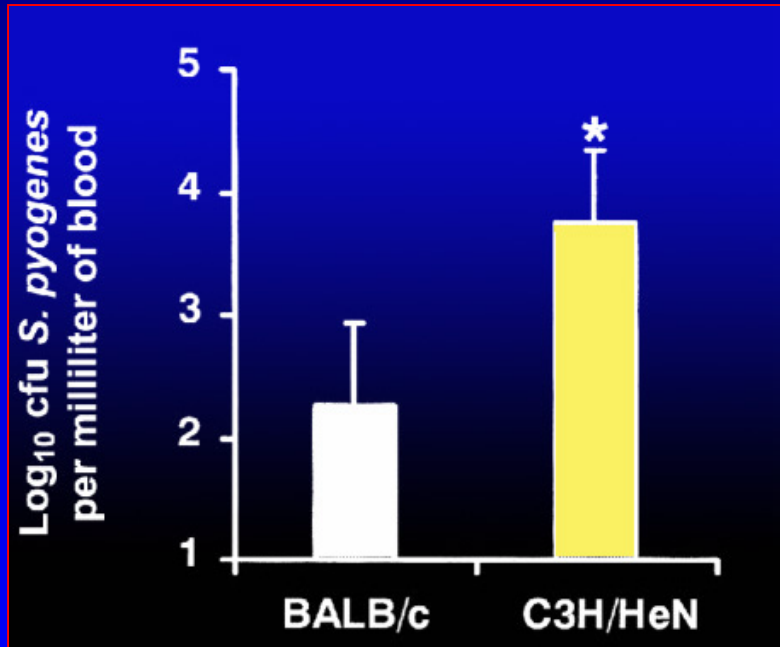
Evidences for a genetic component to sepsis

Animal Studies

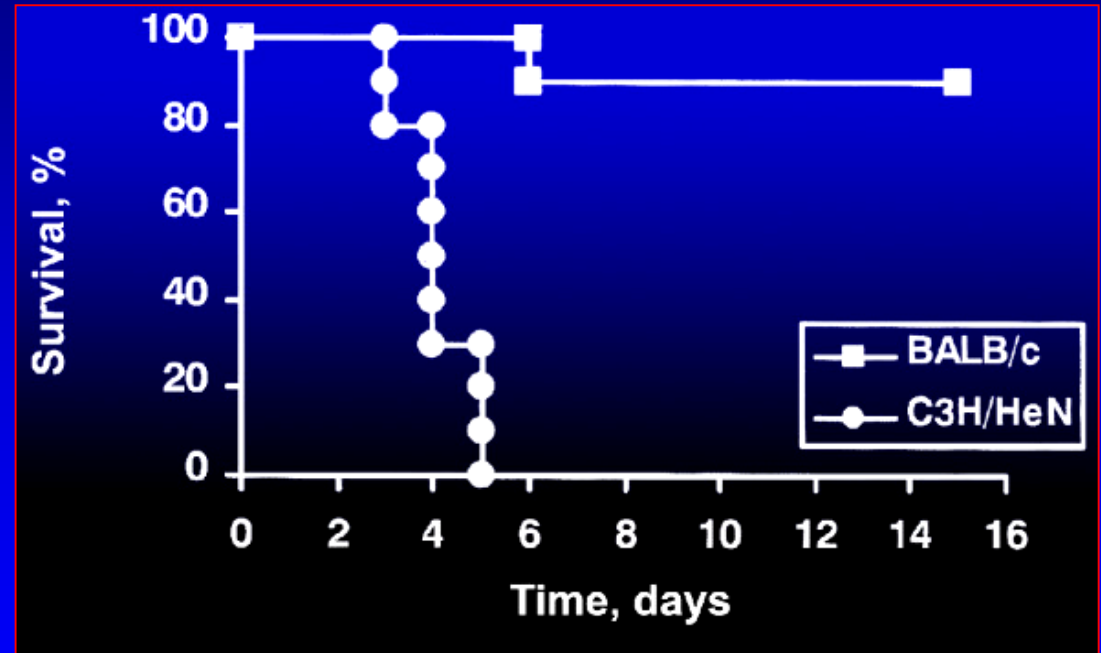
- Susceptibility/resistance to certain infection in mice
- Susceptibility/resistance phenotypes of knock-out mice

Mice Susceptibility to Infection with Group A Streptococci

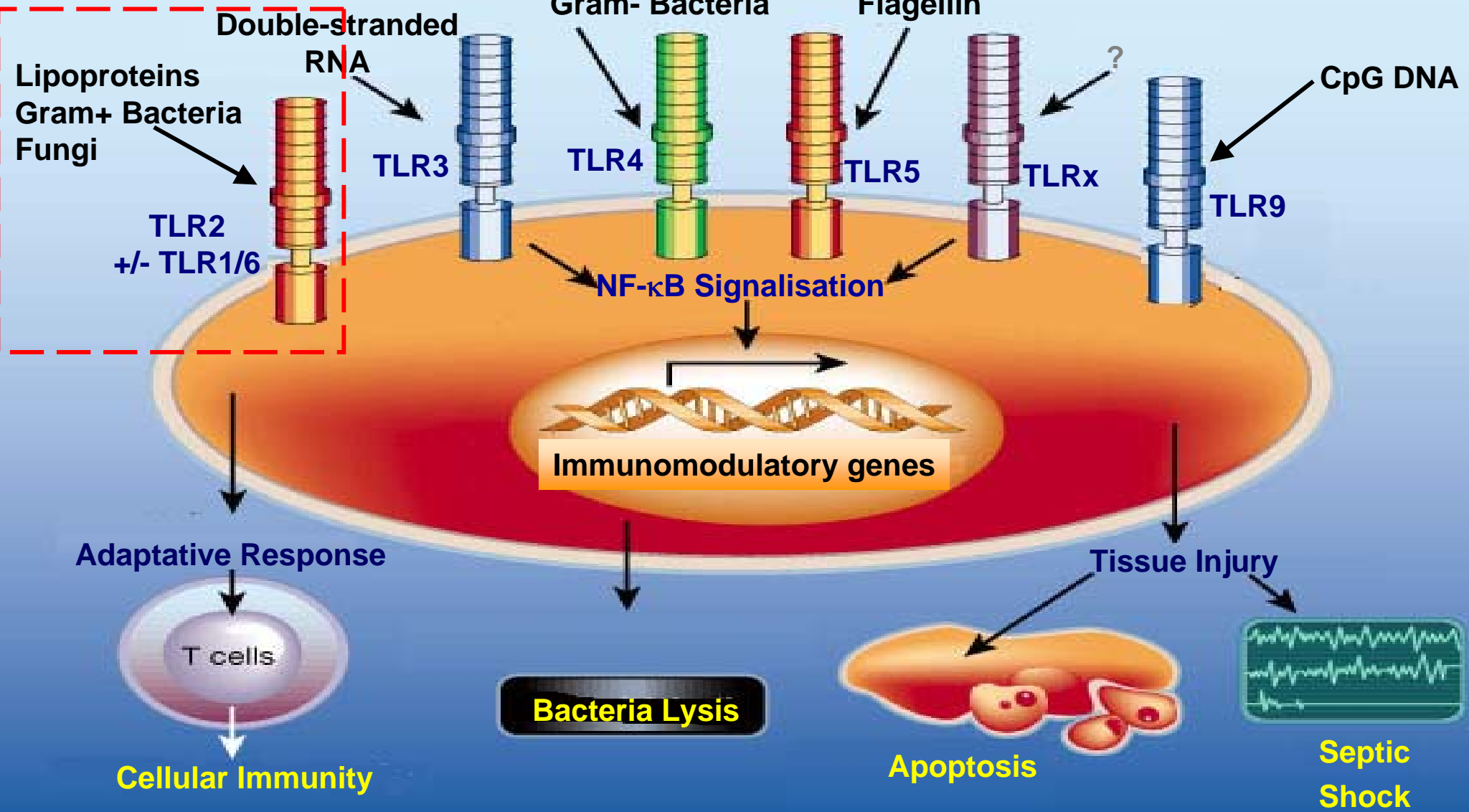
10^3 cfu *Strepto* Subcutaneous



d2

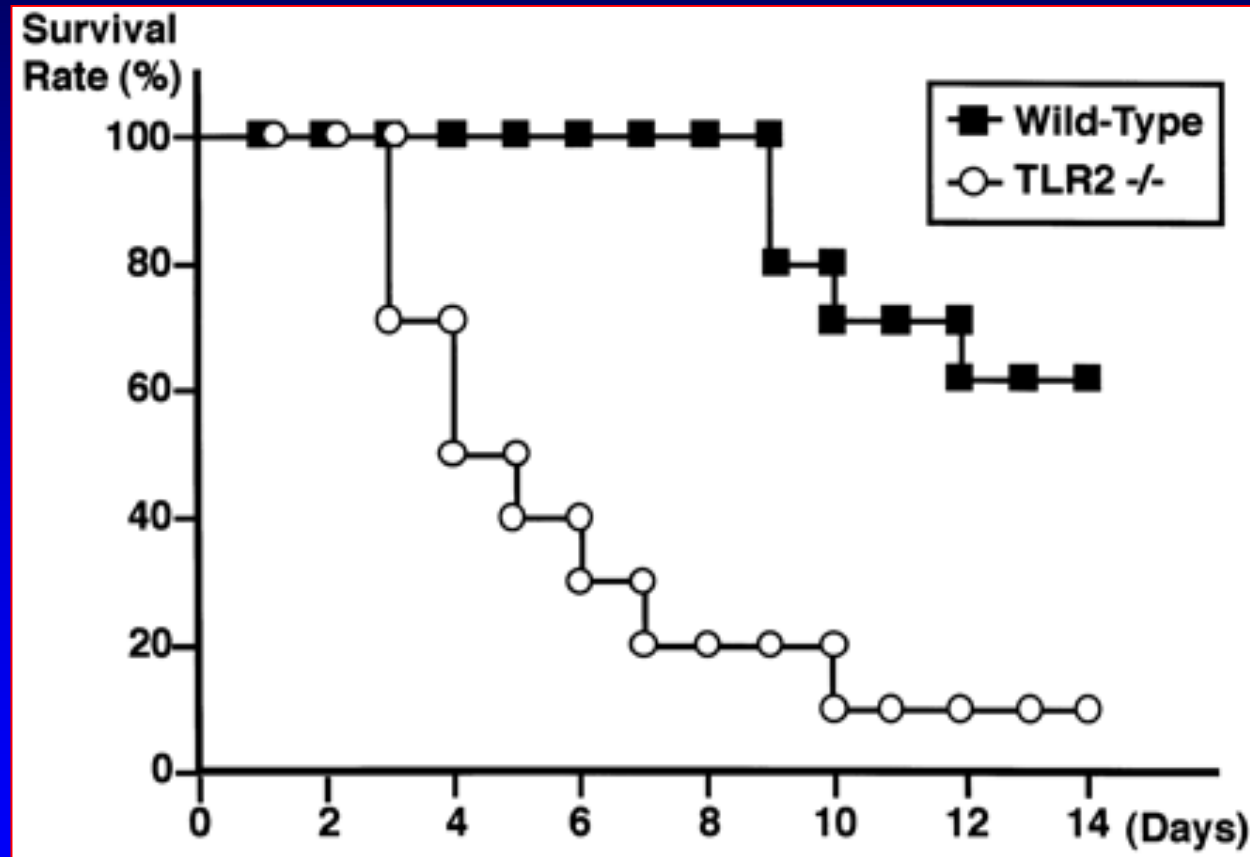


Monocyte or Dendritic cell



TLR2-KO Mice and Response to Gram Positive Bacteria

Intravenous infusion of *Staphylococcus aureus*



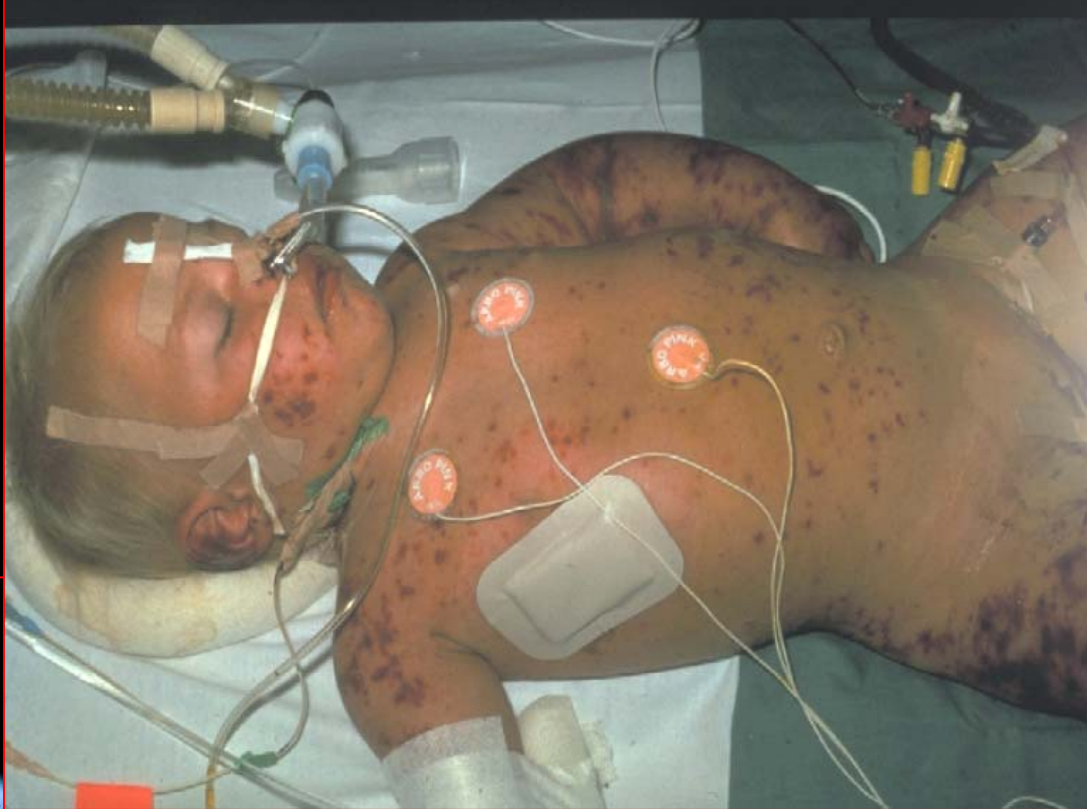
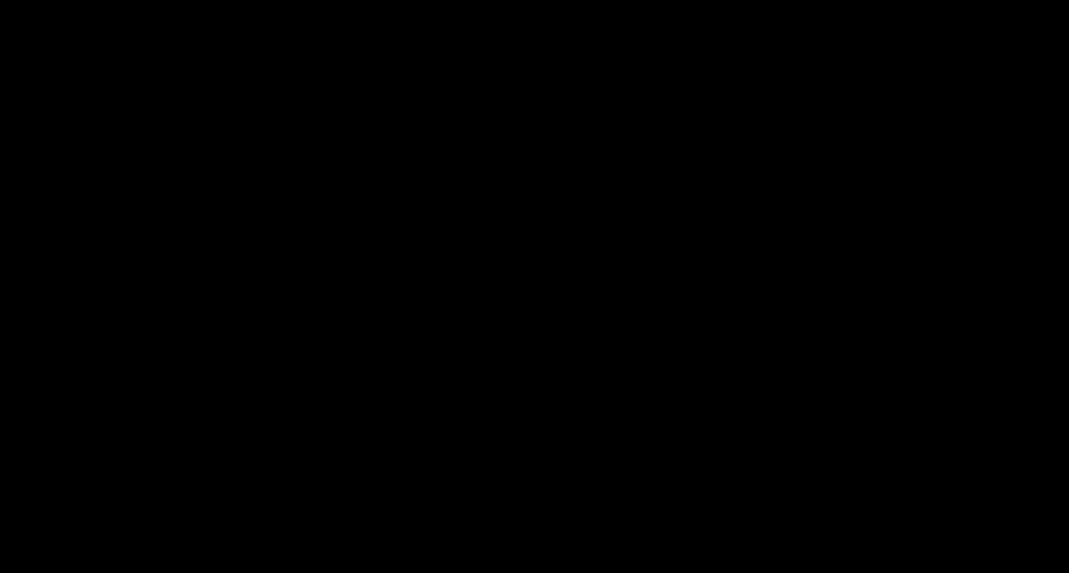
Evidences for a genetic component to sepsis

Animal Studies

- Susceptibility/resistance to certain infection in mice
- Susceptibility/resistance phenotypes of knockout mice

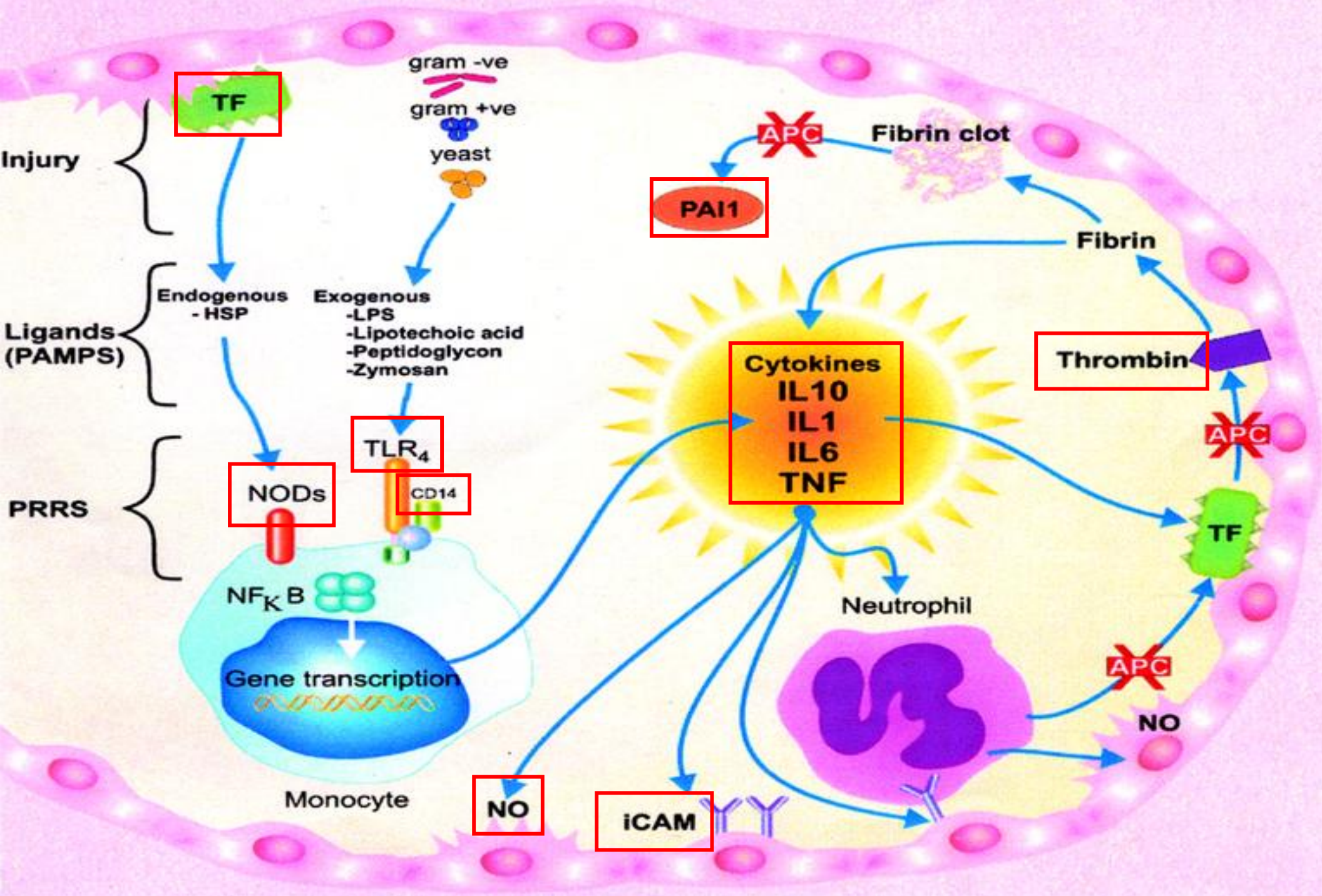
Human Studies

- Clinical Evidences
- Ethnic Differences
- Twin Studies
- Adoptee Studies



Genetic and environmental influences on premature death in adult adoptees

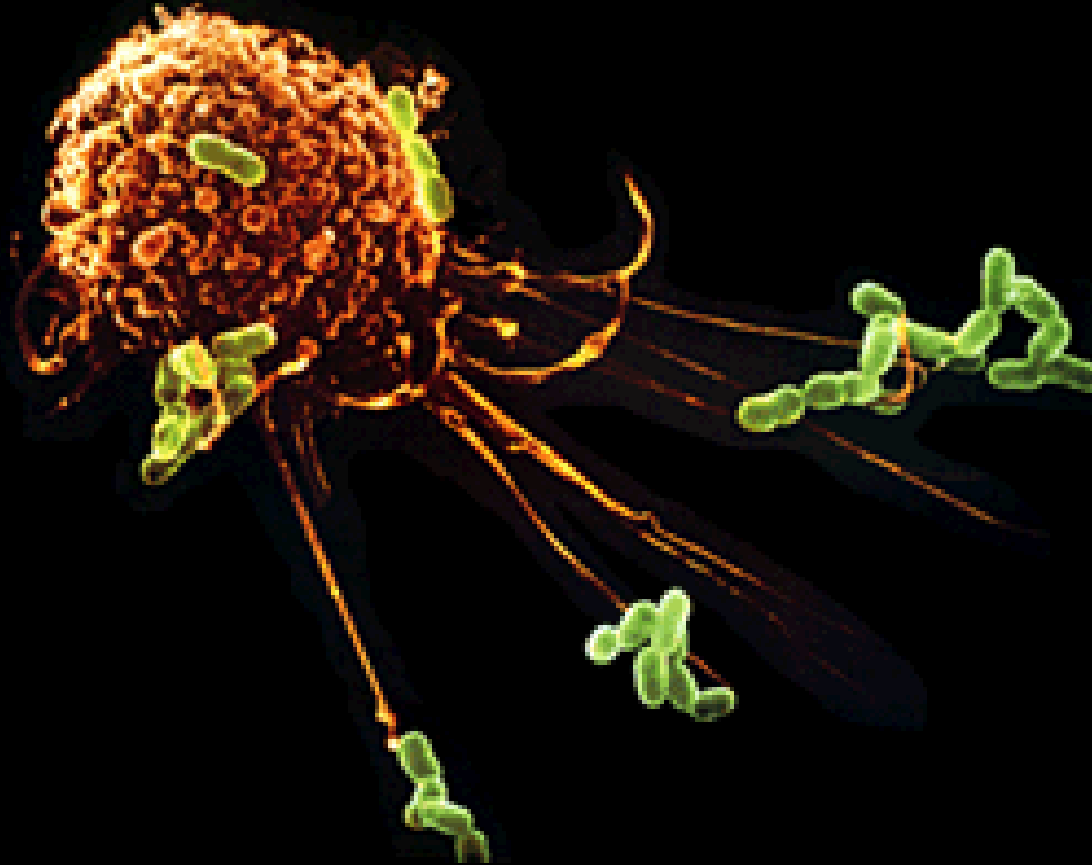
Cause of Death (Parent Dead before the age of 50)	Relative risk for the adoptee to die from the same cause
All causes	
Biologic	1.71
Adoptive	0.71
Infection	
Biologic	5.8
Adoptive	0.73
Vascular	
Biologic	4.5
Adoptive	3.1

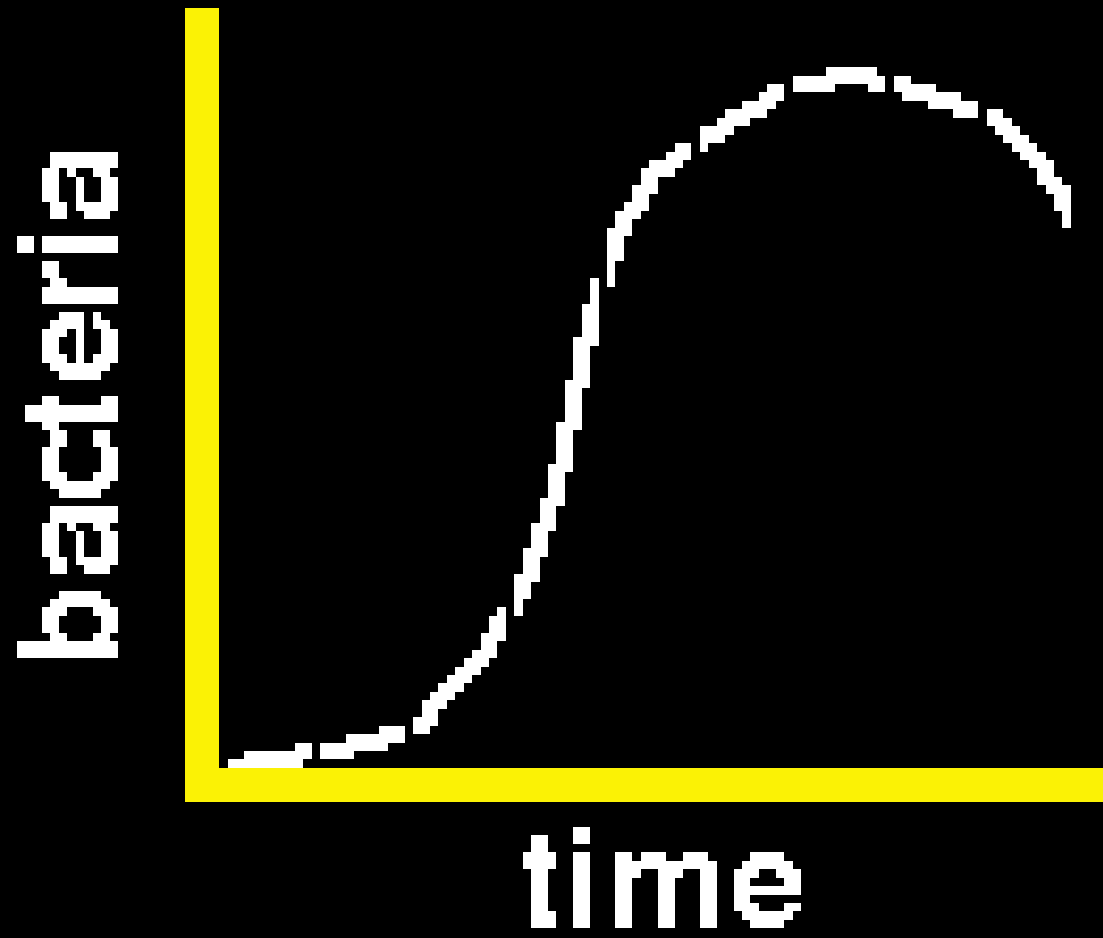


Genetic Polymorphisms and Severe Sepsis

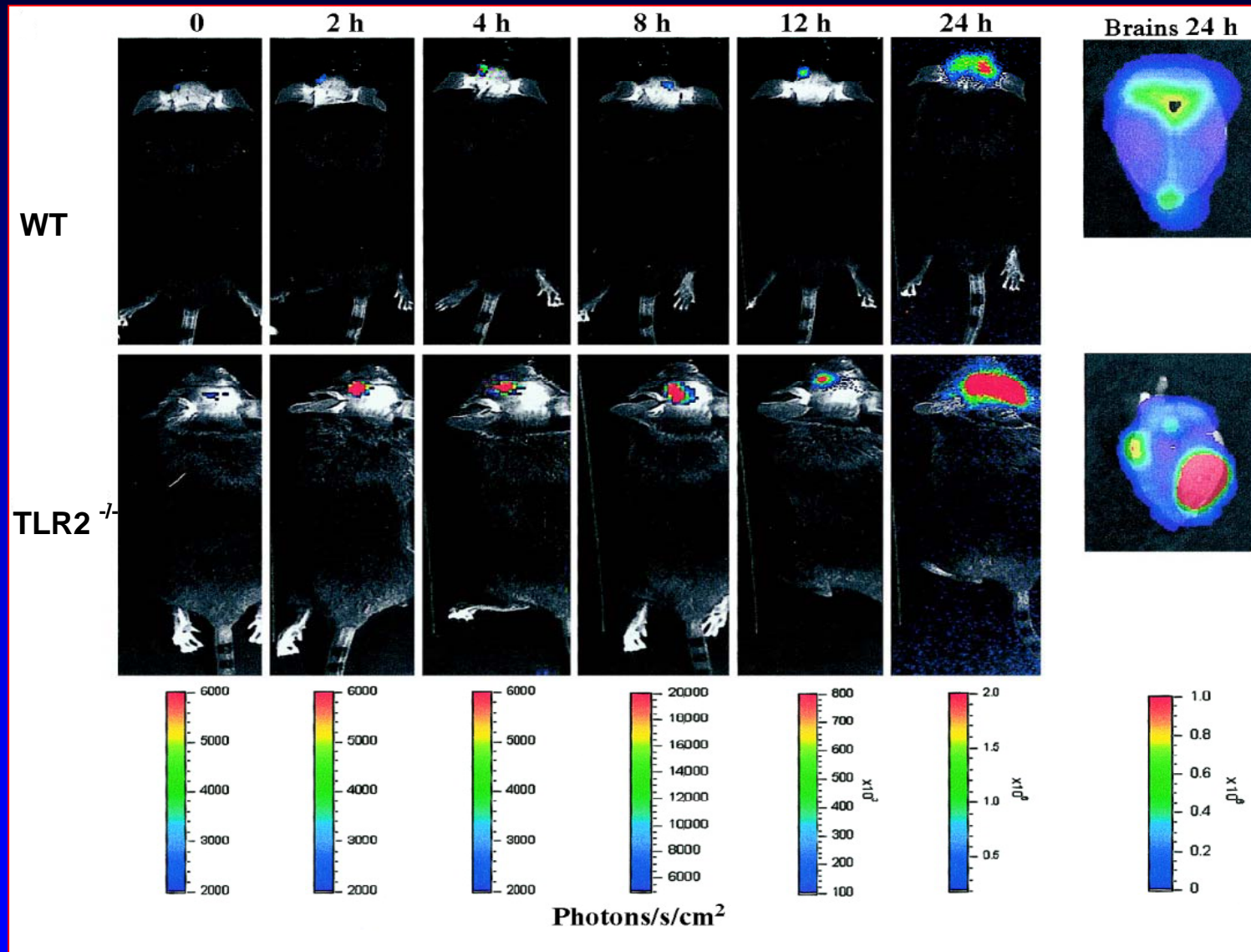
Gene	Susceptibility and/or Outcome
Mannose Binding Lectin	Meningococemia, Pneumococemia Severe sepsis
Toll-Like Receptor 4/2	Gram negative/positive Septic Shock
Toll-Like Receptor 5	Legionnaire's Disease
CD14	Septic Shock
FC γ RII Receptor	Meningococemia; Pneumococemia
TNF locus	Meningococemia Septic Shock; Cerebral Malaria
IL-18	Severe Sepsis
IL-10	Severe Sepsis, Meningococemia
IL-6	Severe sepsis
IL-1 locus	Severe Sepsis
IL-4	Viral Pneumonia
Caspase 12	Severe Sepsis
PAI-1	Meningococemia; Severe sepsis
Factor V Leiden	Meningococemia; Severe sepsis

Pathogen Detection

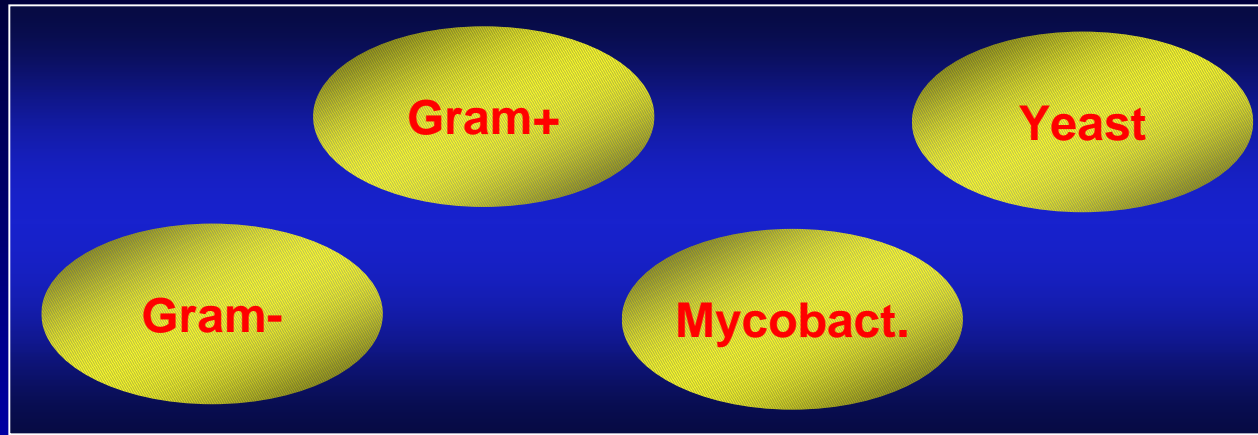




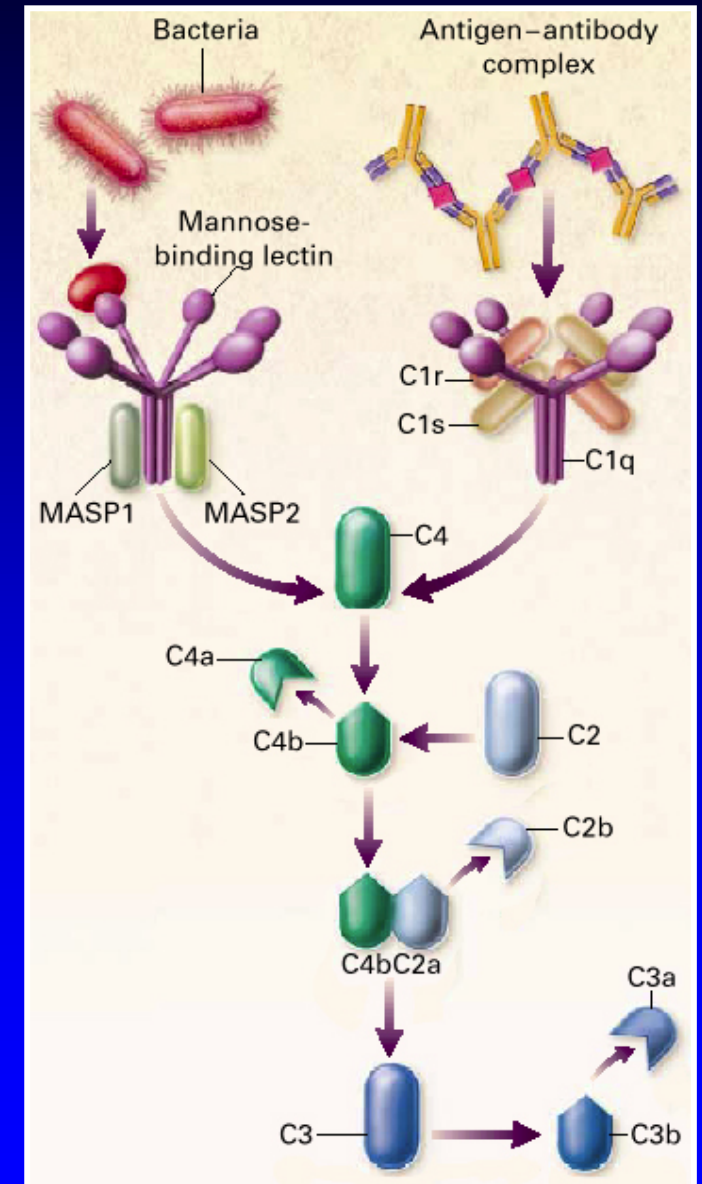
TLR2 and *Streptococcus pneumoniae* meningitis



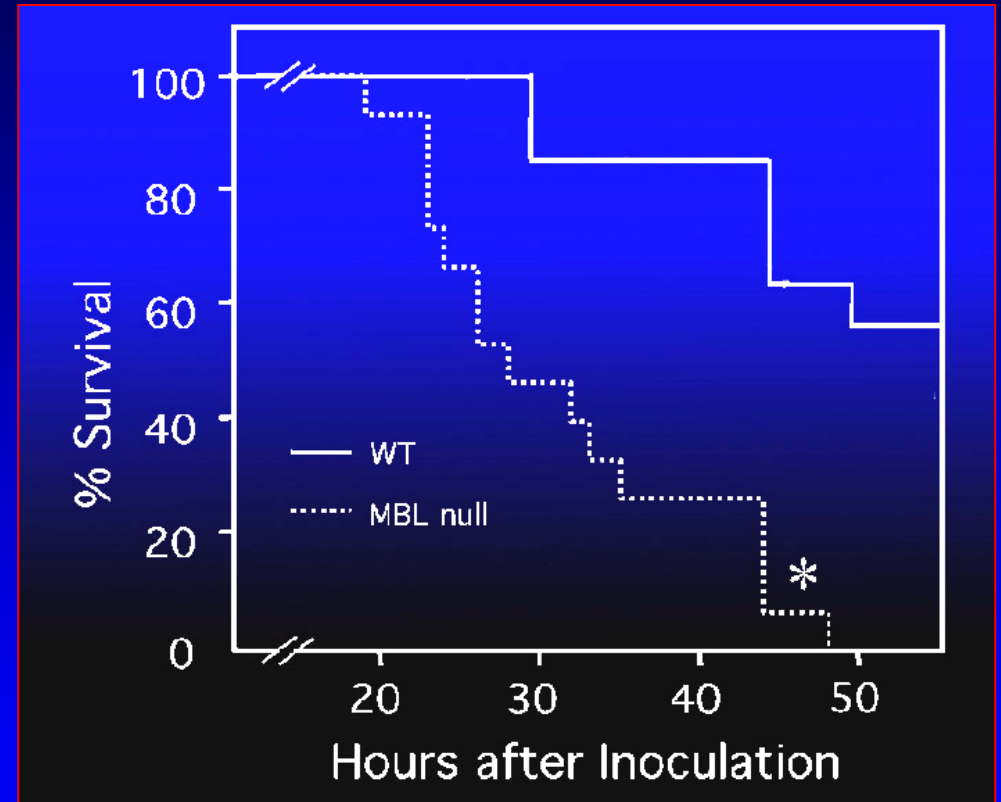
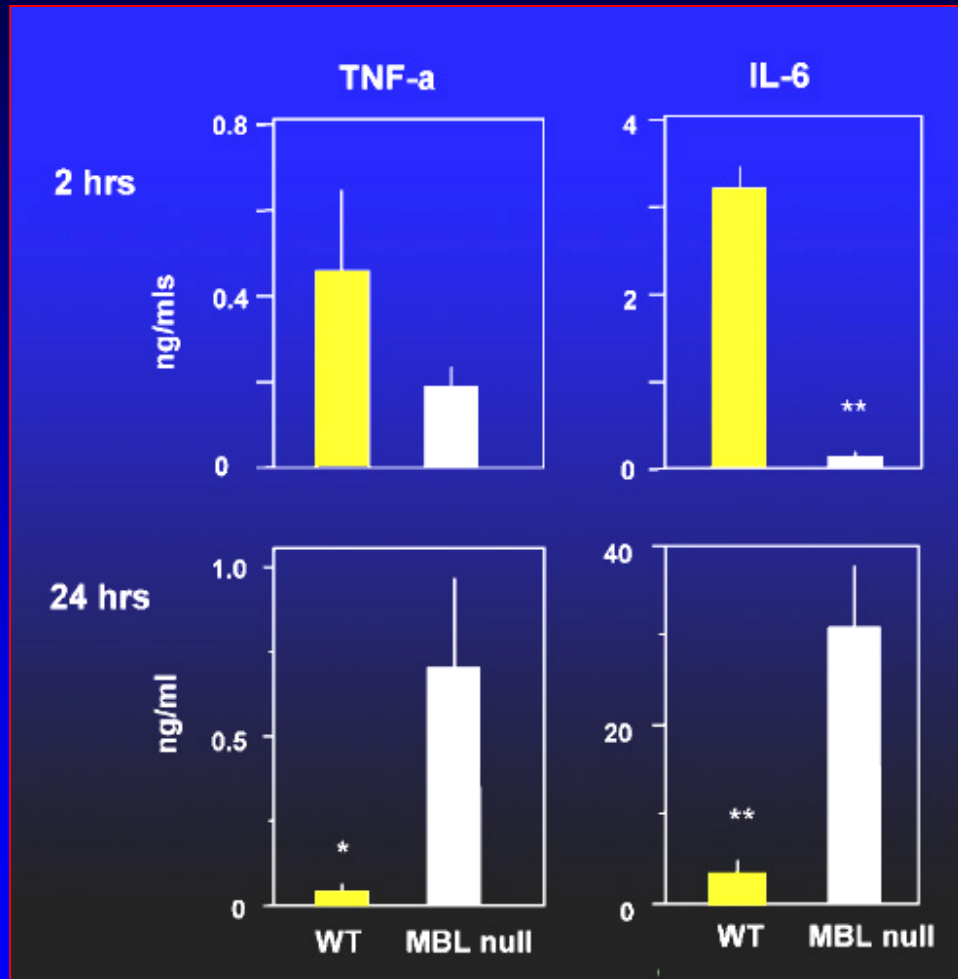
Mannose-Binding Lectin



- Collectin
- Structural homology with C1q
- Associated to 2 serine proteases
- Variability:
 - Point mutations codons 52, 54, 57
 - Polymorphisms in the promoter



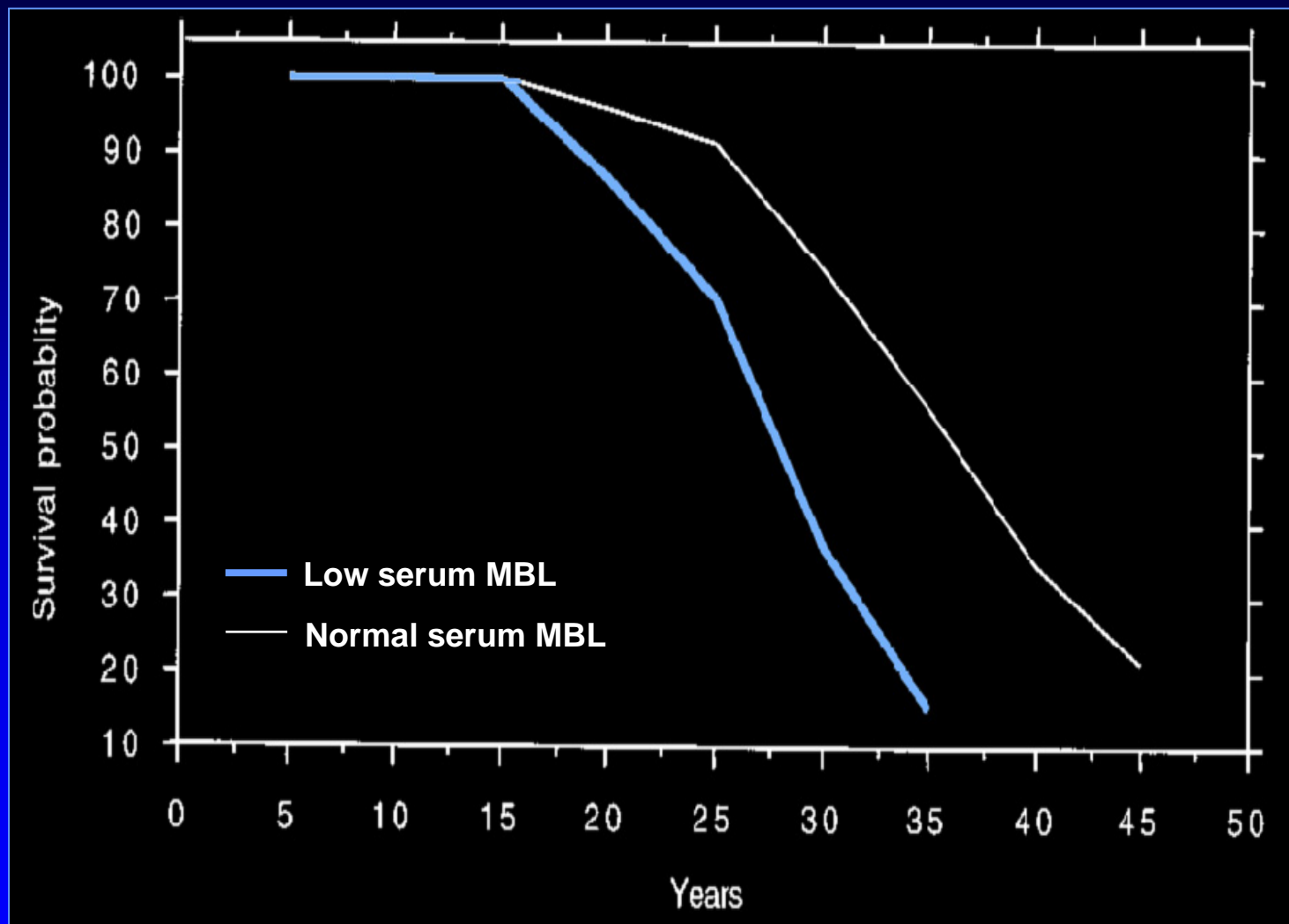
MBL-Deficient Mice and Staphylococcus Infection



Mannose-binding Lectin Polymorphisms & The Risk of Infections

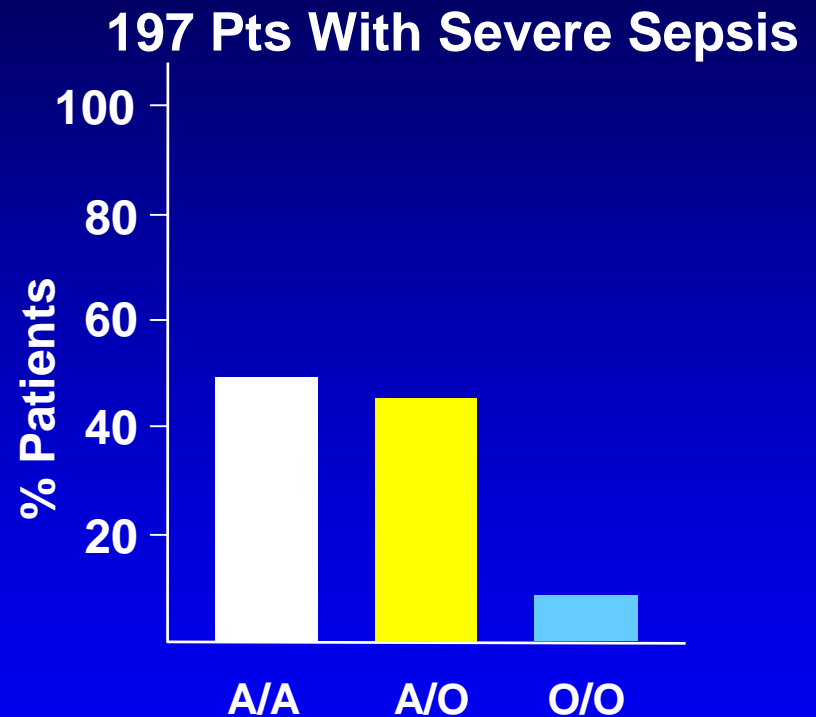
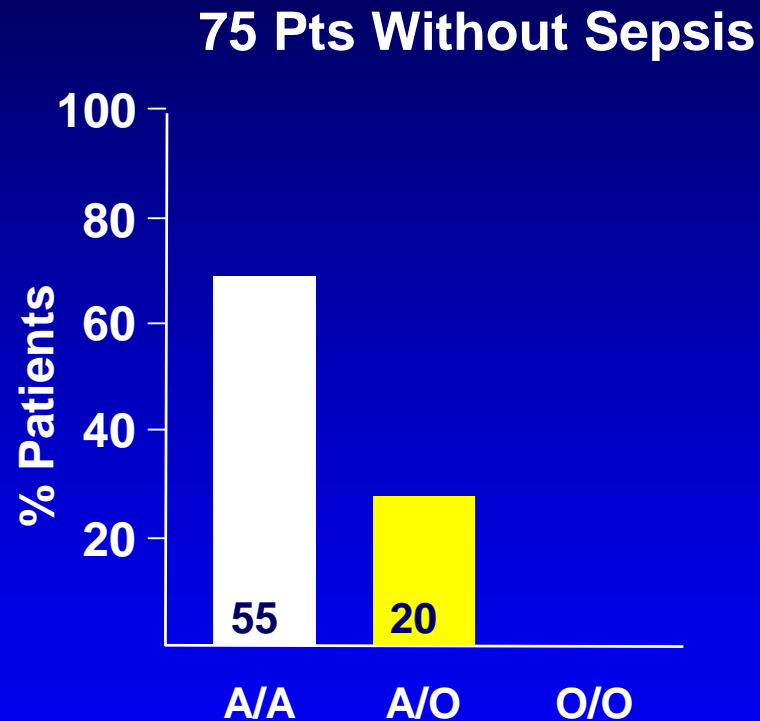
- **Repeated bacterial and fungal infections**
 - Sumiya et al., Lancet 1991
 - Summerfeld et al., Lancet 1995
 - Garred et al., Lancet 1995
 - Summerfeld et al., BMJ 1997
- **Infections after chemotherapy**
 - Neth et al., Lancet 2001
 - Peterslund et al., Lancet 2001
- **Increased severity of lung disease and low survival in cystic fibrosis**
 - Garred et al., J. Clin. Invest. 1999
- **Meningococcal disease**
 - Hibberd et al., Lancet 1999

Association of mannose-binding lectin gene heterogeneity with severity of lung disease and survival in cystic fibrosis



MBL Polymorphisms, SIRS, and Sepsis

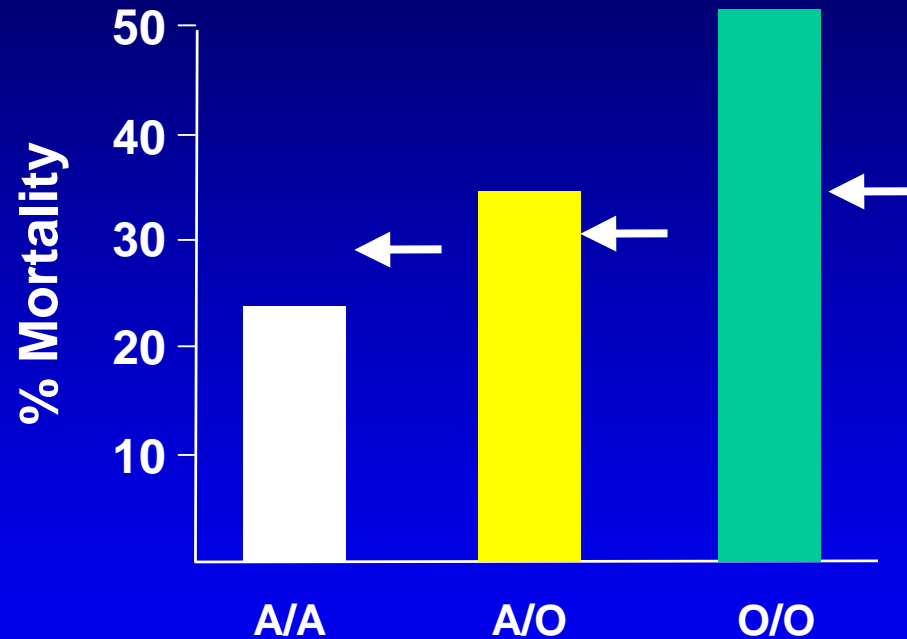
272 ICU Pts with SIRS



A: Wild Type

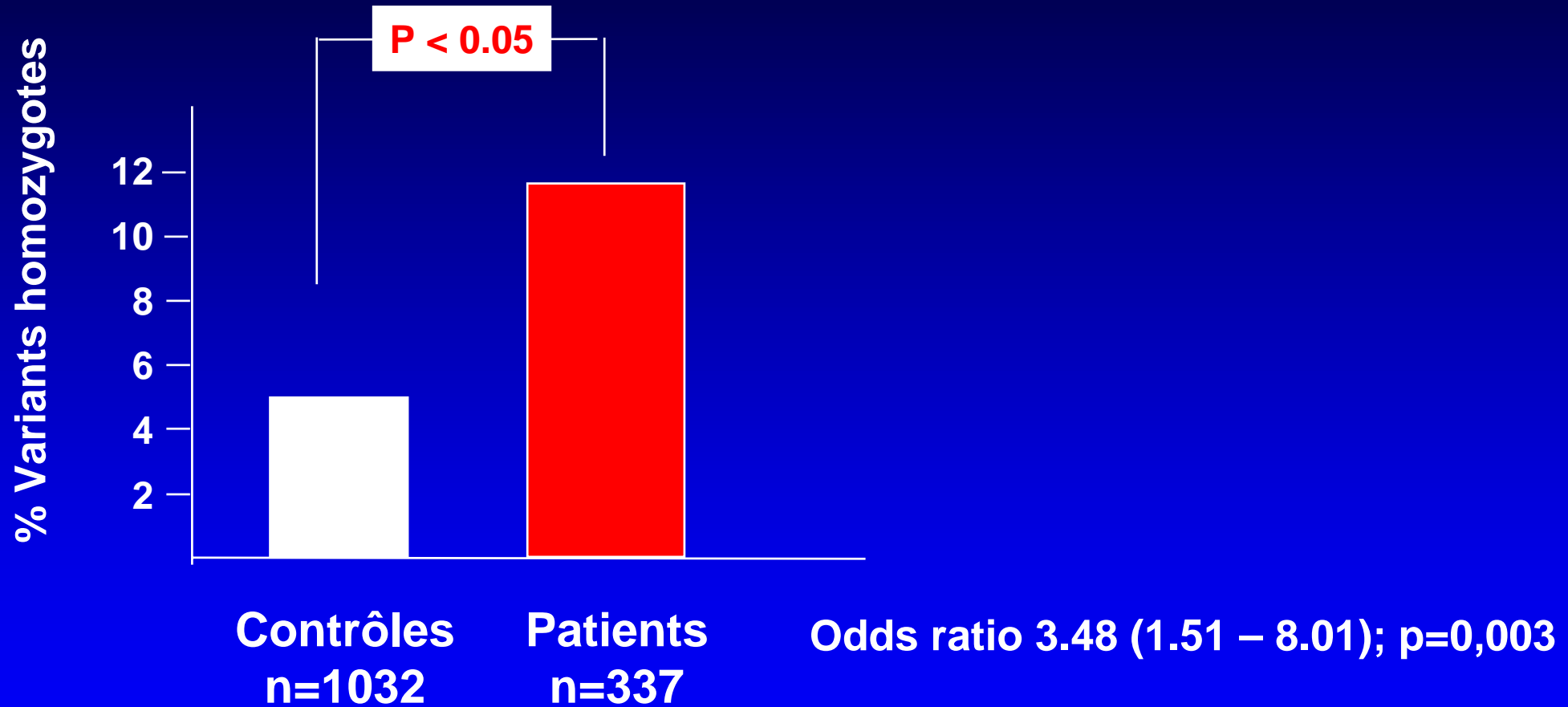
O: Structural polymorphism associated with low MBL levels

MBL Polymorphisms, SIRS, and Sepsis

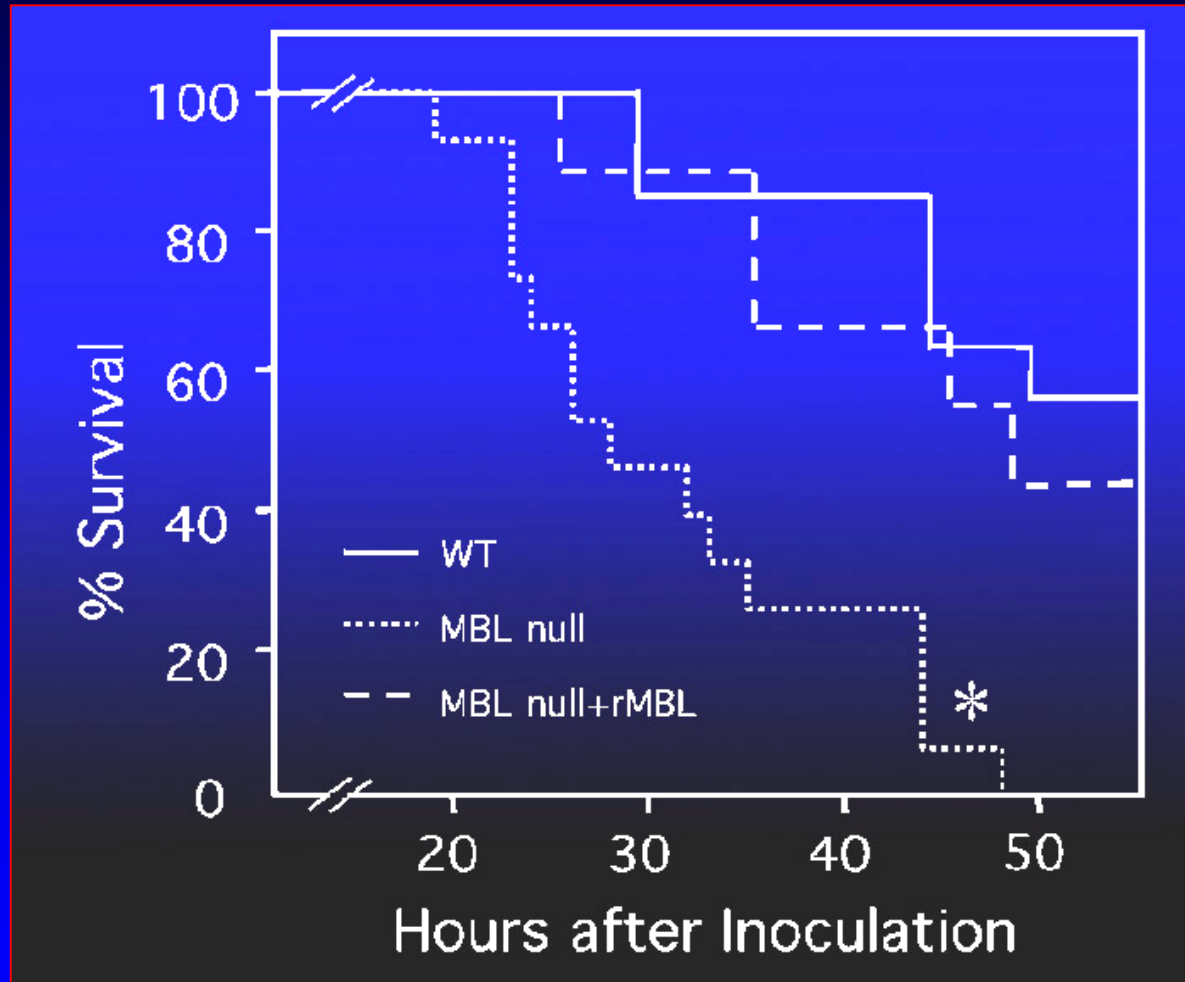


← : Predicted Mortality

MBL genotype and risk of invasive pneumococcal disease



MBL-Deficient Mice and Staphylococcus Infection

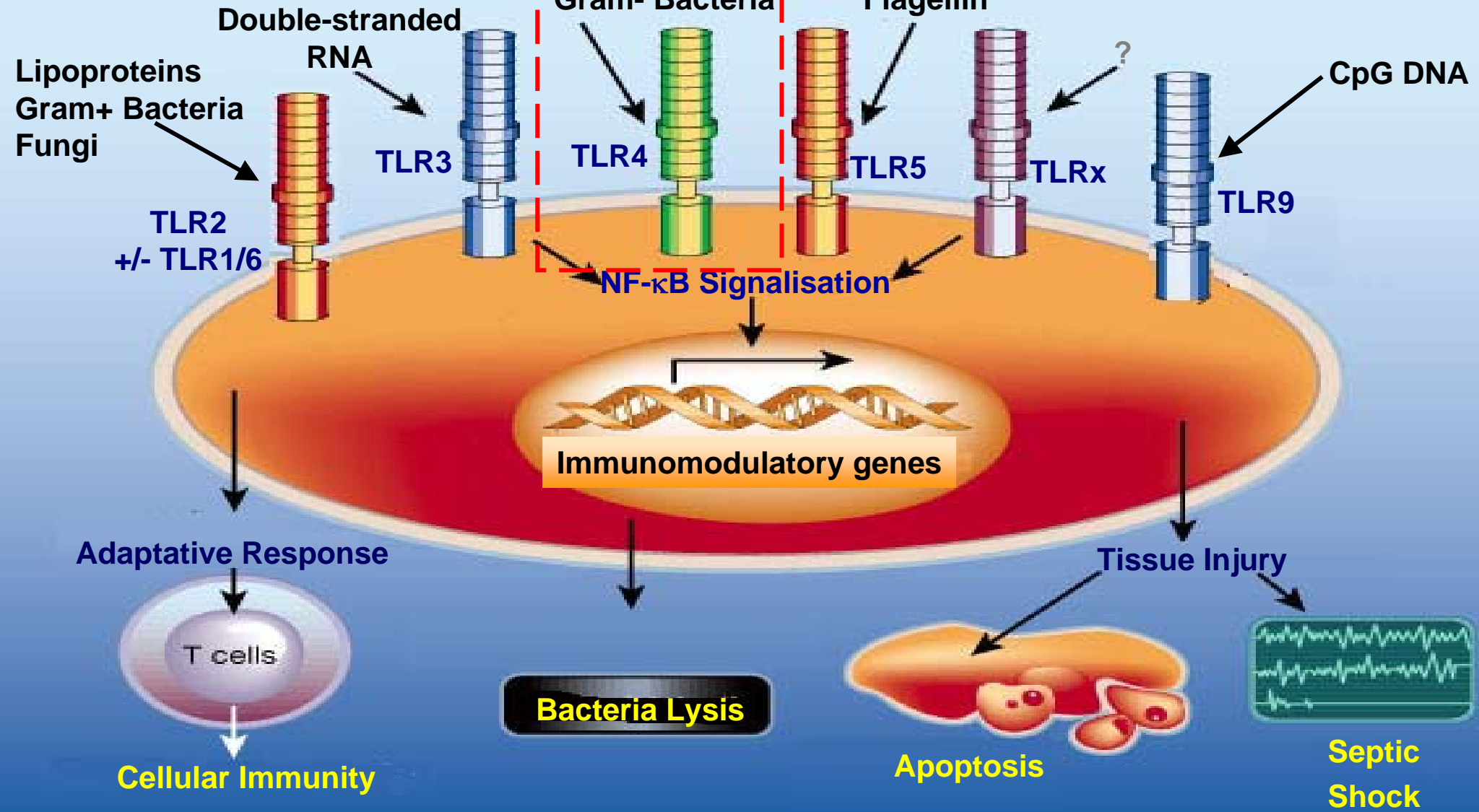


Clinical potential of mannose-binding lectin-replacement therapy

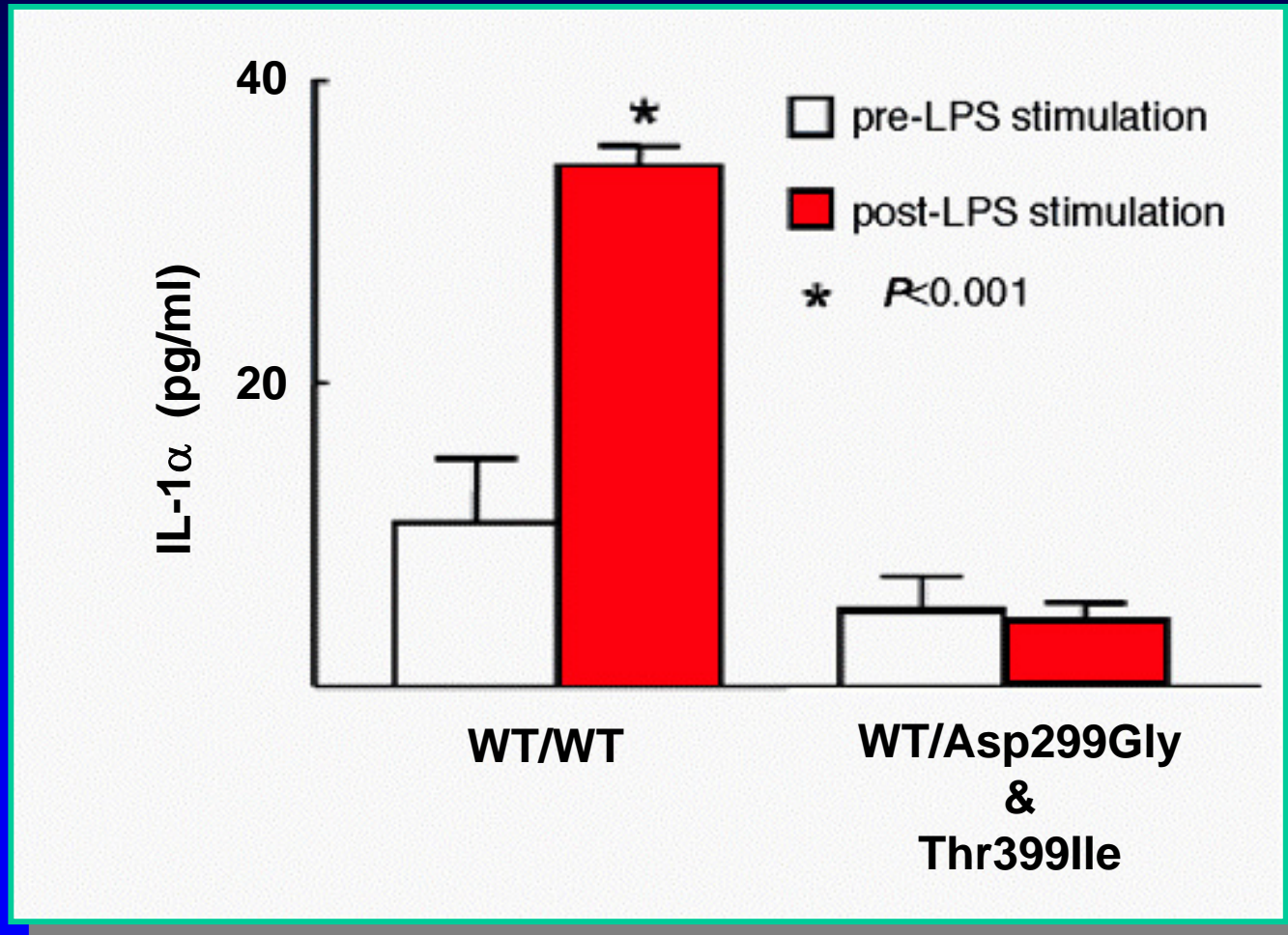
J.A. Summerfield¹

Division of Medicine, Faculty of Medicine, Imperial College London, St Mary's Campus, London W2 1NY, U.K.

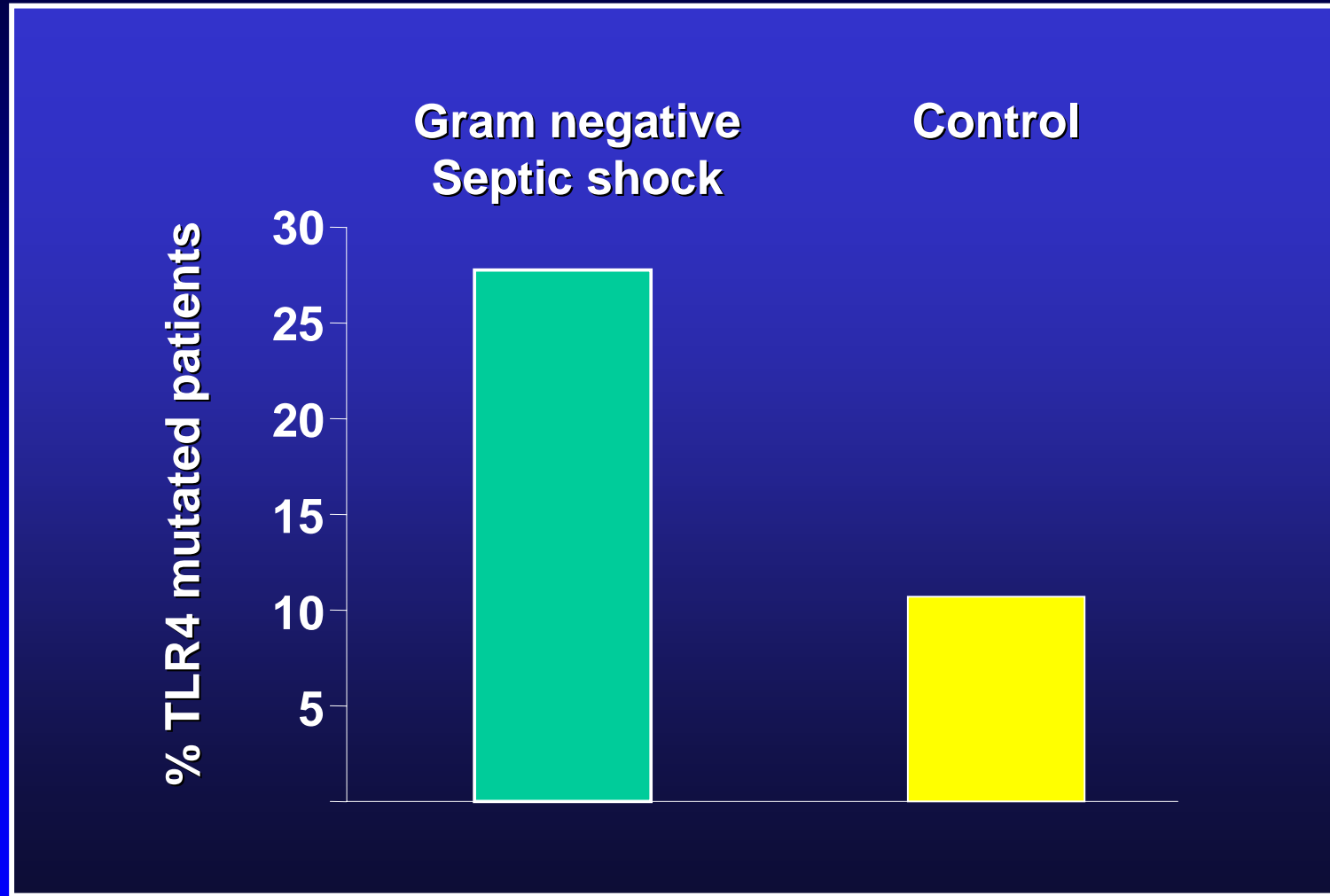
Monocyte or Dendritic cell



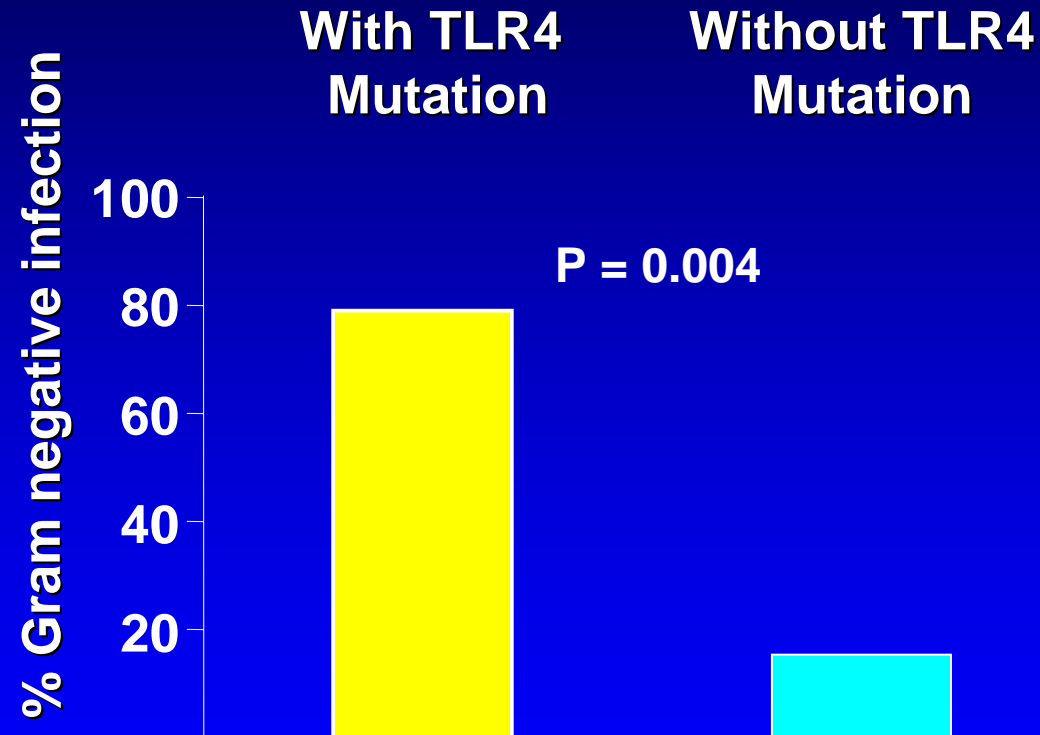
TLR4 mutation and LPS responsiveness



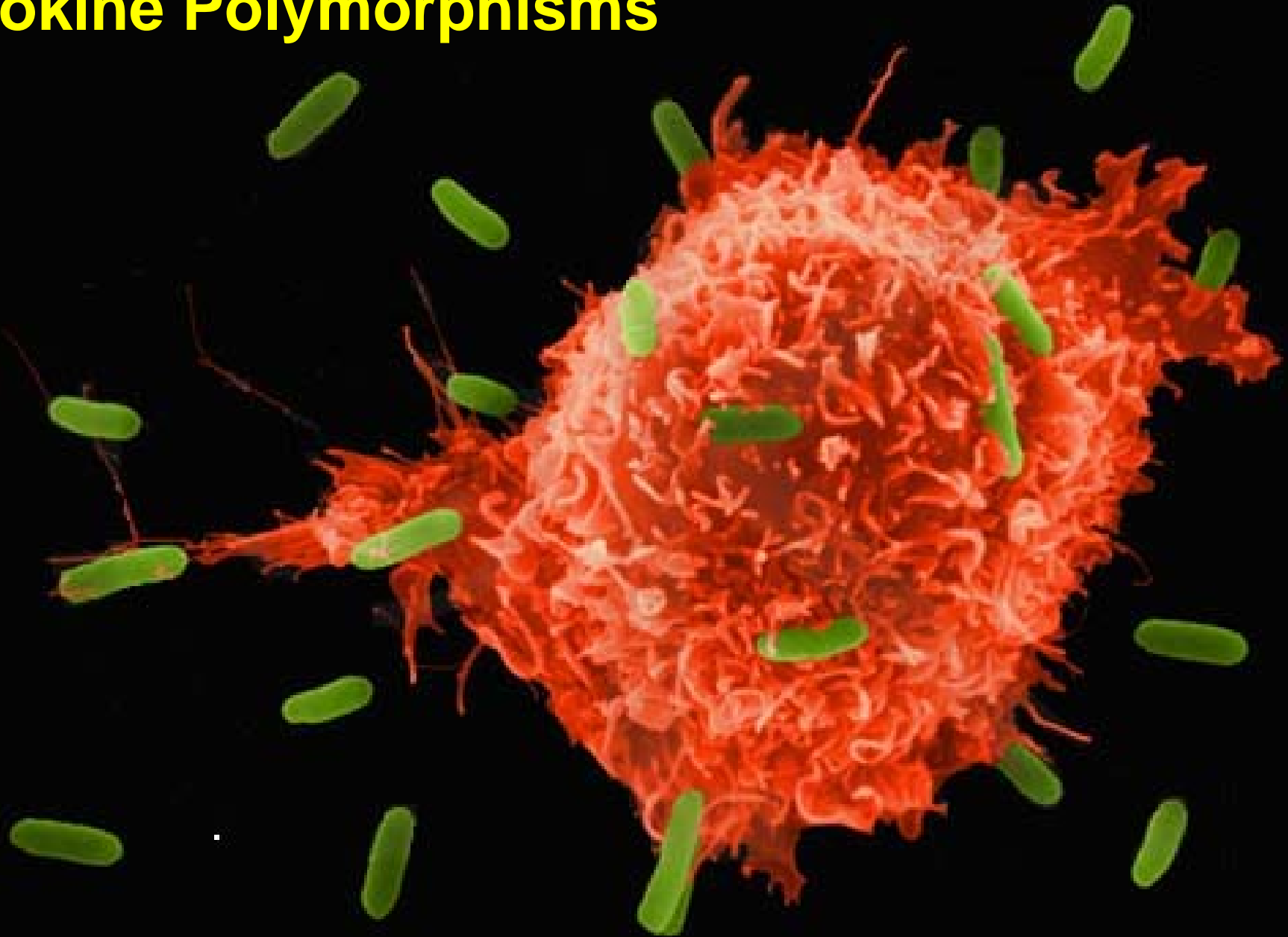
TLR4 Polymorphisms and Septic Shock



TLR4 Variants and Predisposition to Gram Negative Sepsis



Cytokine Polymorphisms



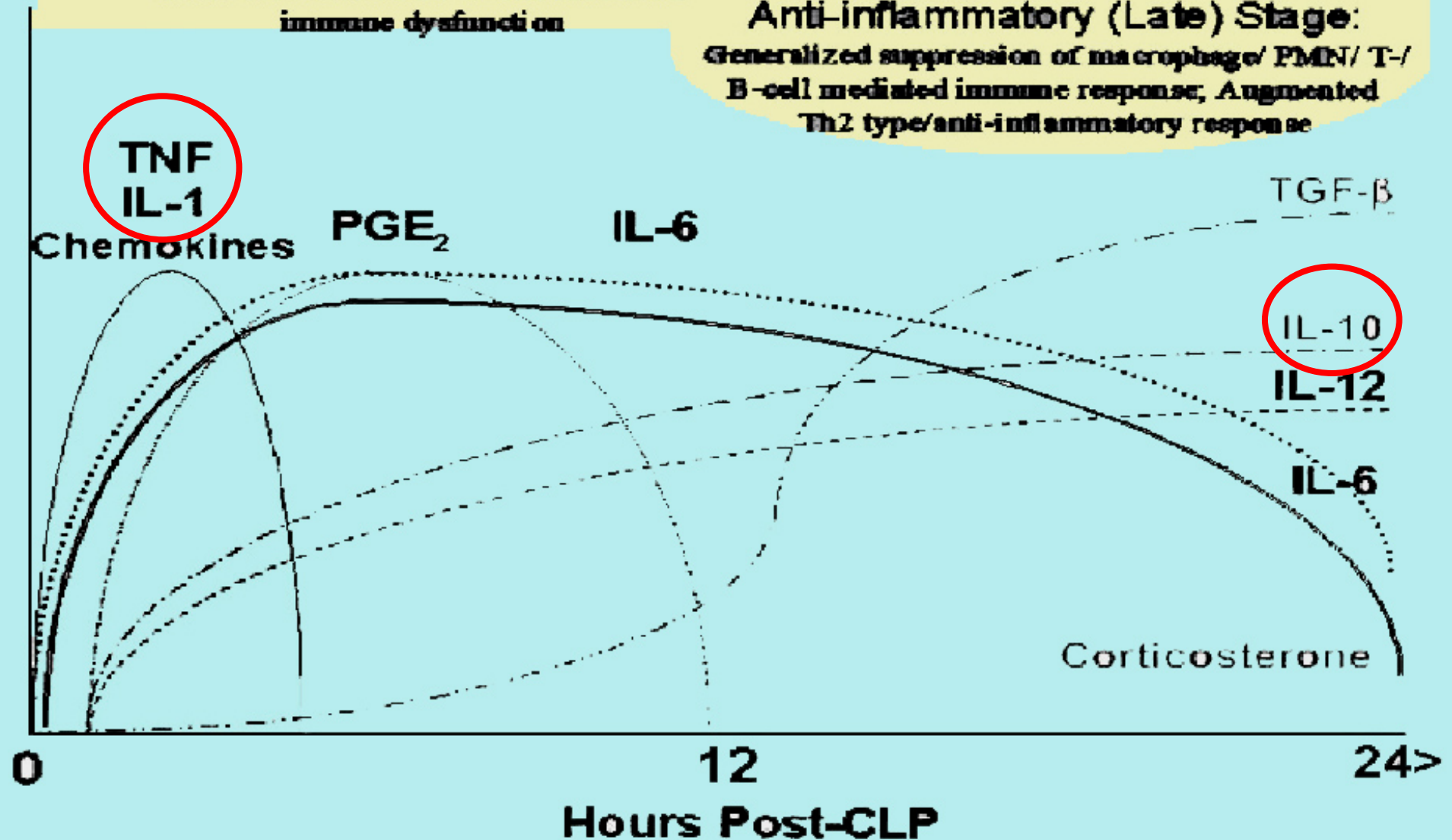
**Hyper-dynamic/metabolic
Pro-inflammatory (Early) Stage:**

Macrophage/ PMN activation;
Little evidence of T-/ B-cell mediated
immune dysfunction

**Hypo-dynamic/metabolic
Anti-inflammatory (Late) Stage:**

Generalized suppression of macrophage/ PMN/ T-/
B-cell mediated immune response; Augmented
Th2 type/anti-inflammatory response

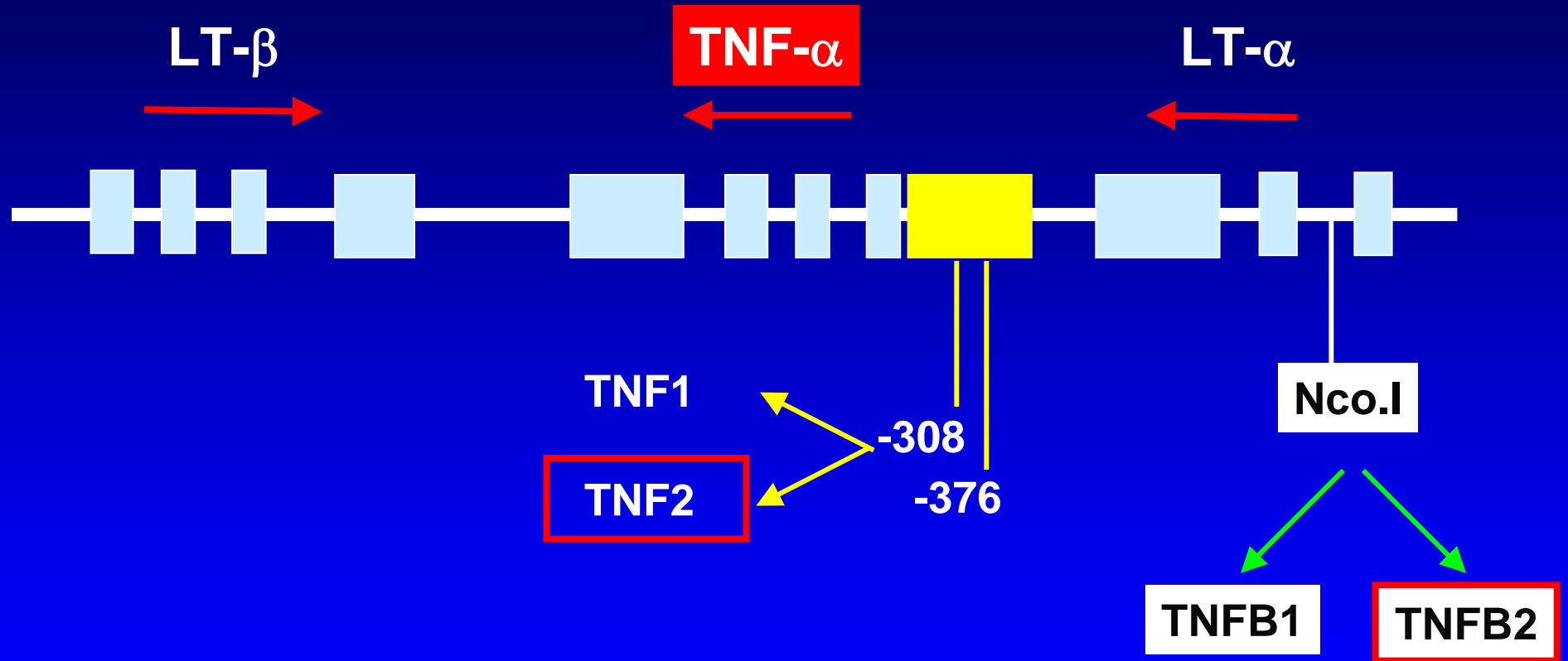
Arbitrary Blood Cytokine Levels



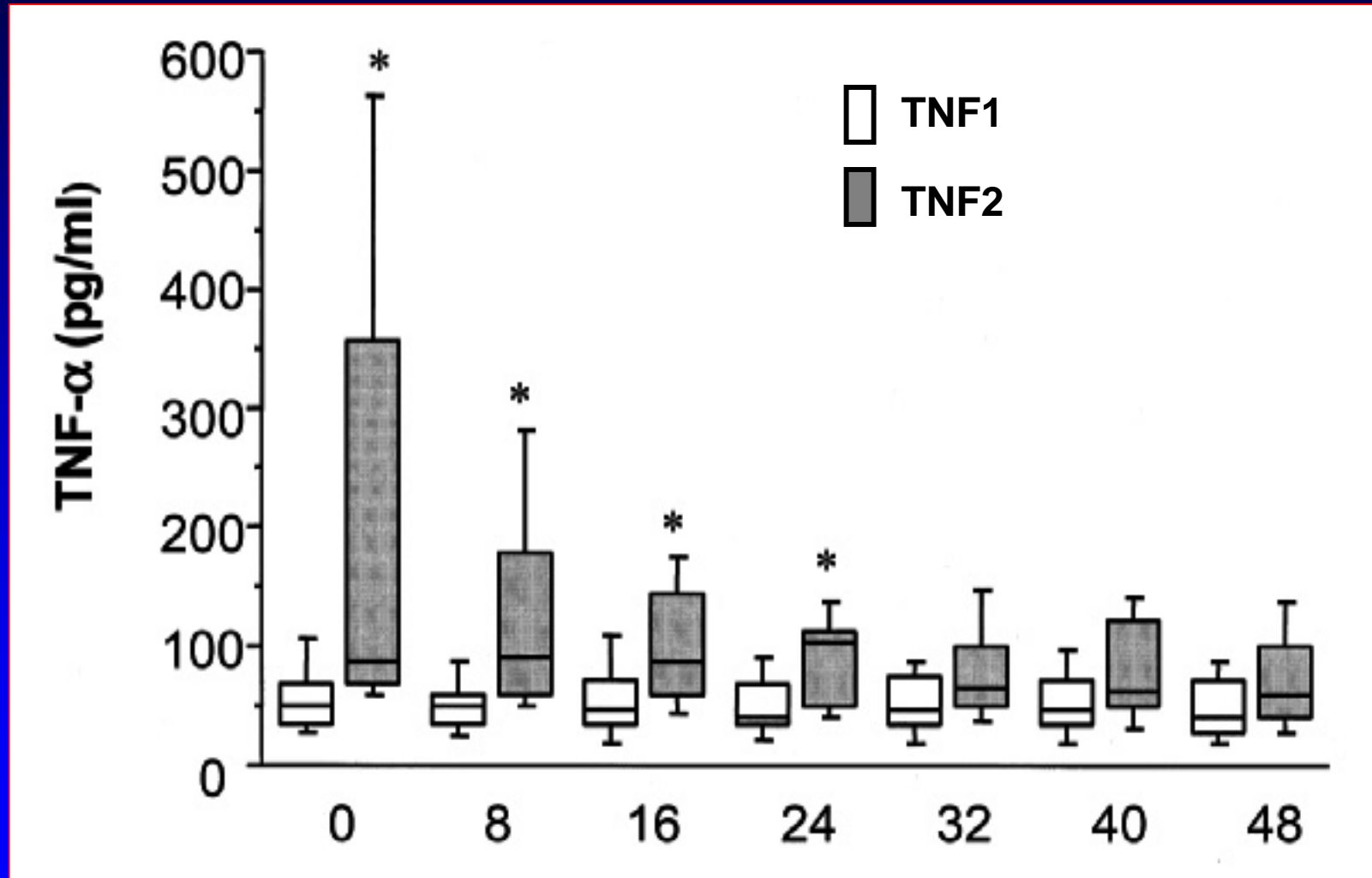
Cytokine Polymorphisms and Meningococccemia

Gene	Polymorphism	Csqqs	Pts	Su	Severity	Outcome	Ref
ACE	DD (deletion)	↑ ACE	110			↑14% Death [OR]= 2.8	<i>Harding D.</i> 2002
TNF	- 308 (TNF2)	↑ TNF α	98			[OR] =2.5 [CI]: 1.1-5.7	<i>Nadel S.</i> 1996
IL-6	-174 (G→C)	↑ IL-6	85		[OR]= 3.06	[OR] = 2.64 [CI]: 1.1- 6.2	<i>Balding J.</i> 2001
IL-1B ILRN	-511 (1+) +2018 (2+)	→ IL-1β ↓ IL -1β	1106			[OR] = 0.61 [CI] 0.38-0.98	<i>Read RC.</i> 2003

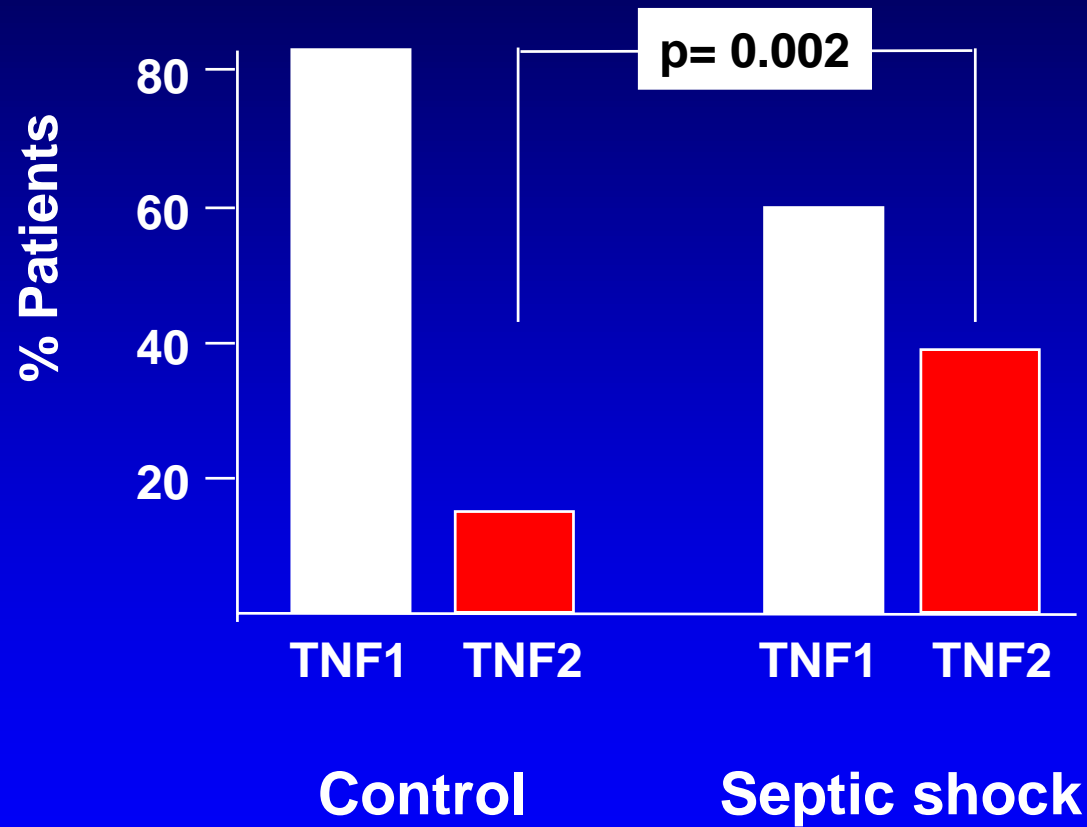
TNF locus



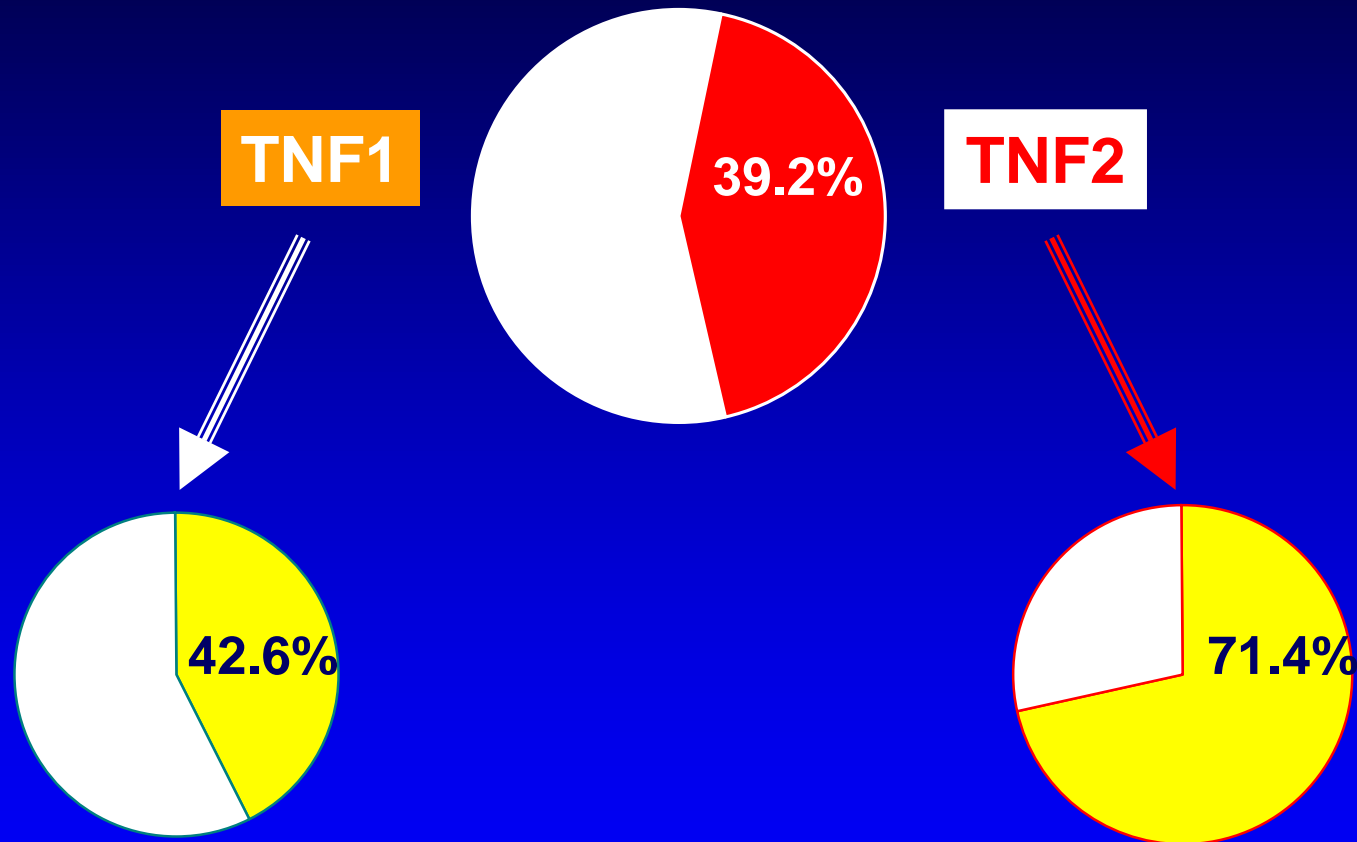
Association of TNF2 with TNF levels in Septic Shock



TNF2 polymorphism and septic shock susceptibility

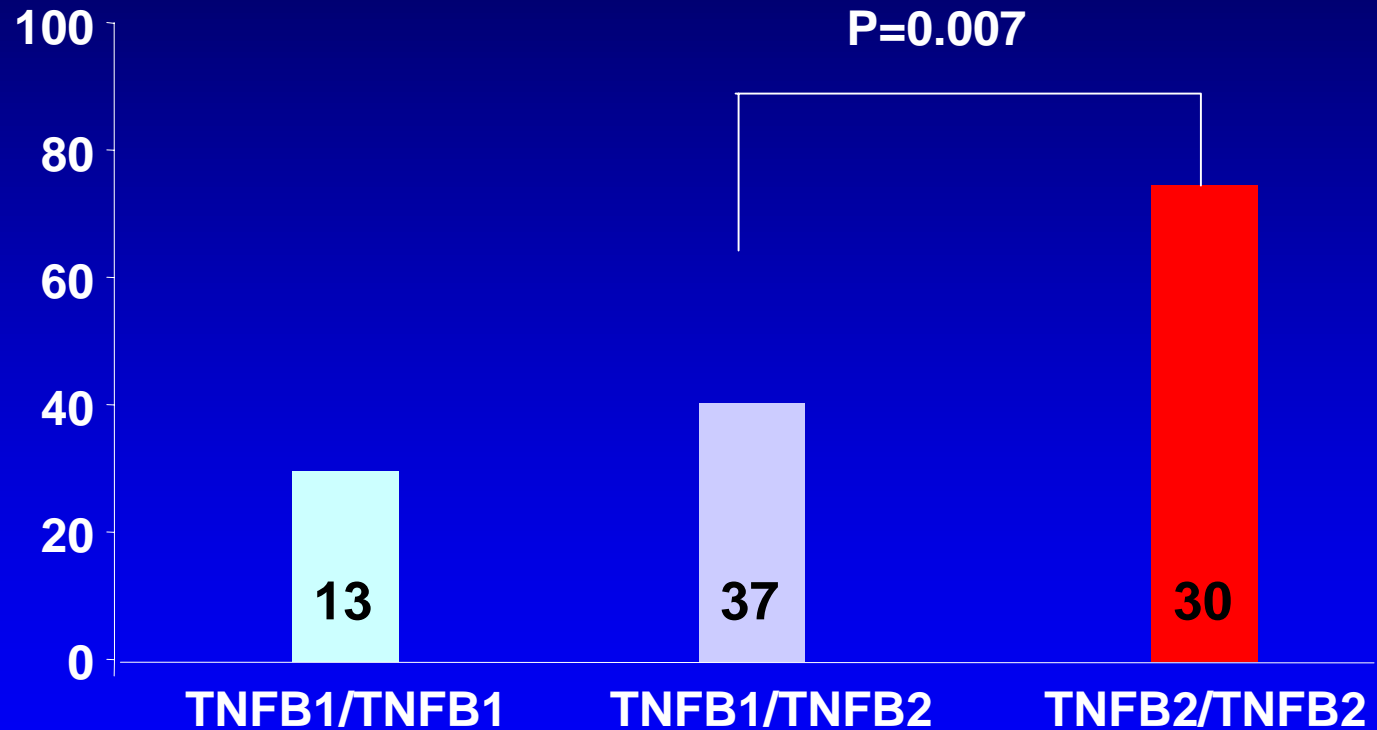


TNF2 polymorphism and septic shock outcome



TNFB2 in *LT-α* and severe sepsis outcome

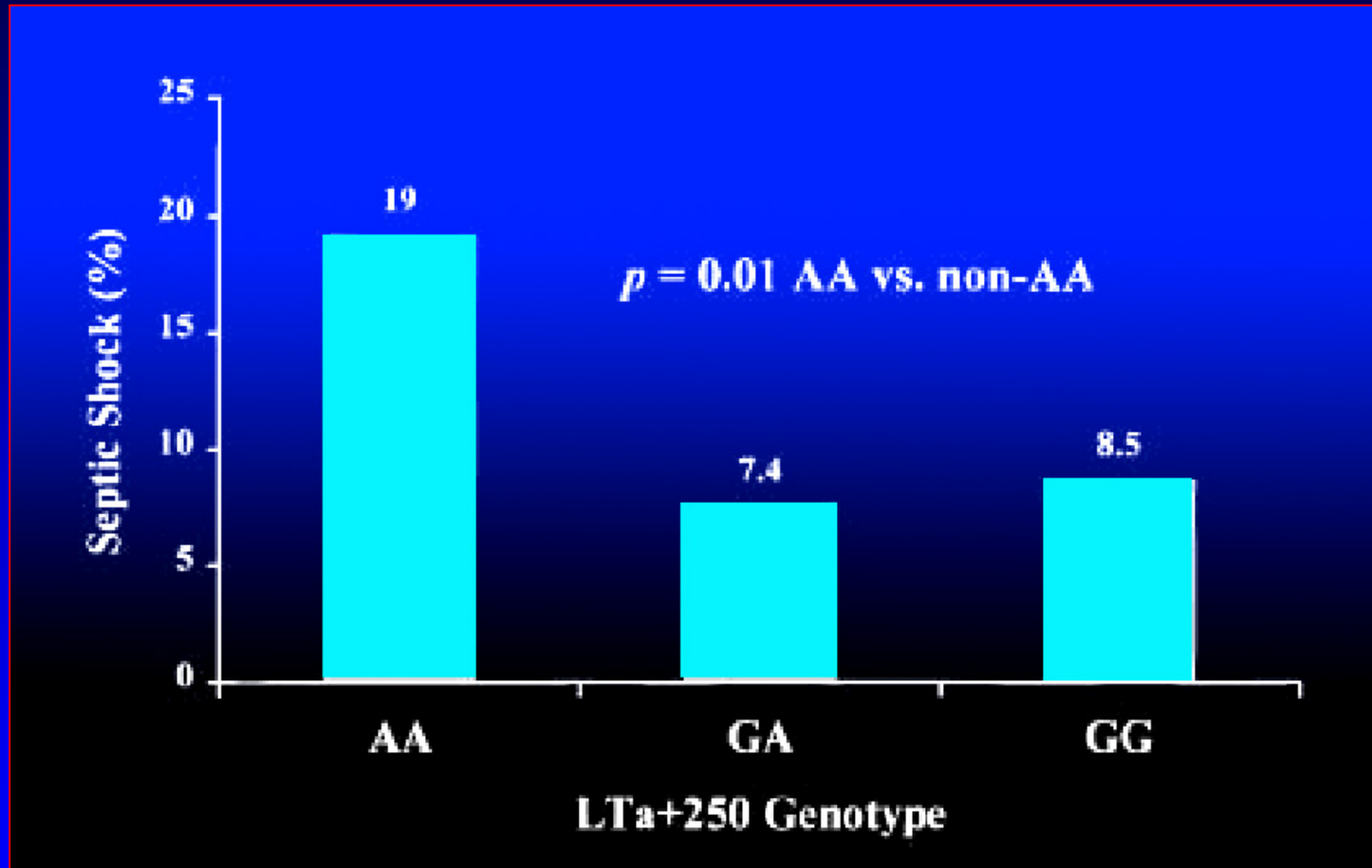
Mortality (%)



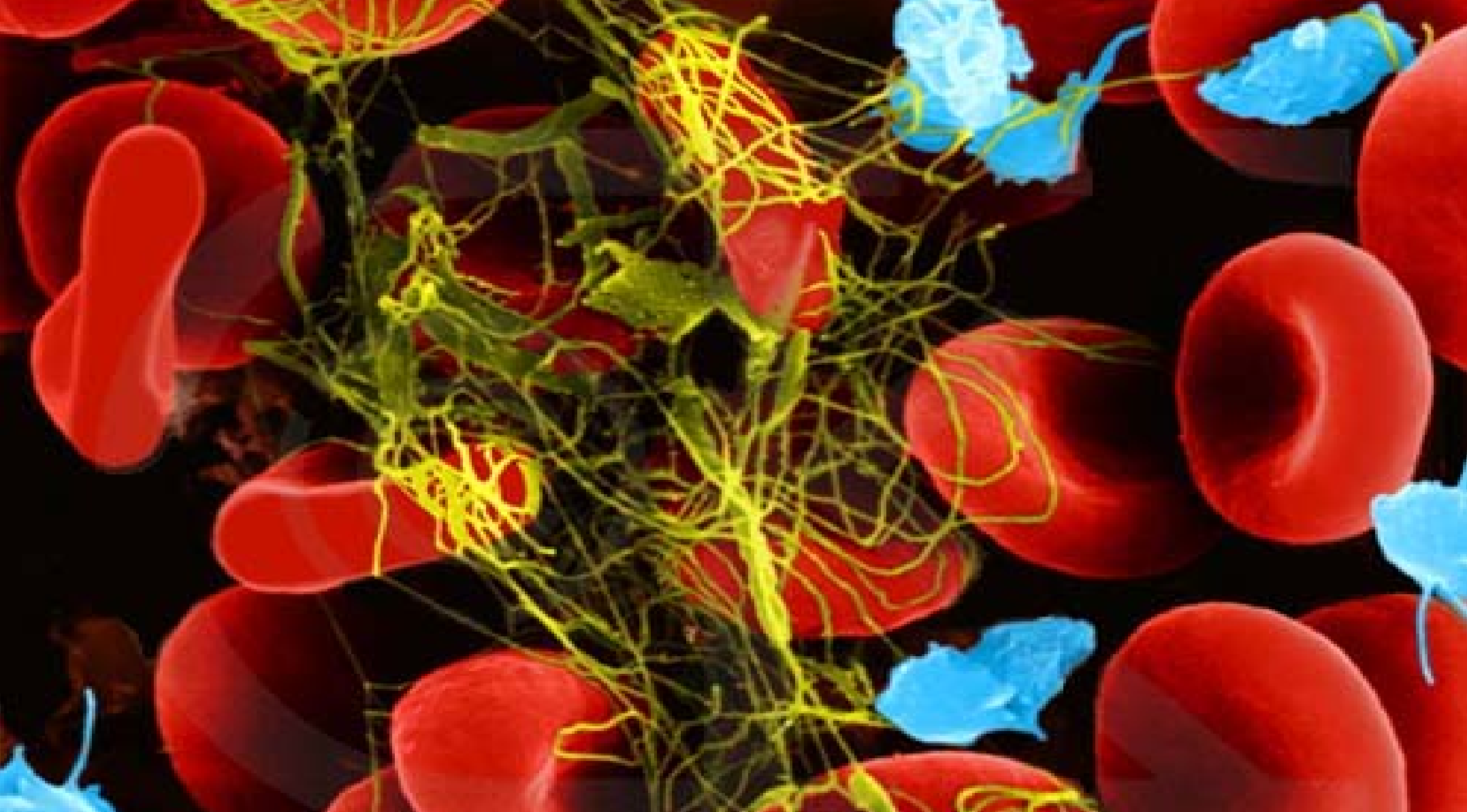
Community-Acquired Pneumonia and TNF polymorphisms

280 CAP

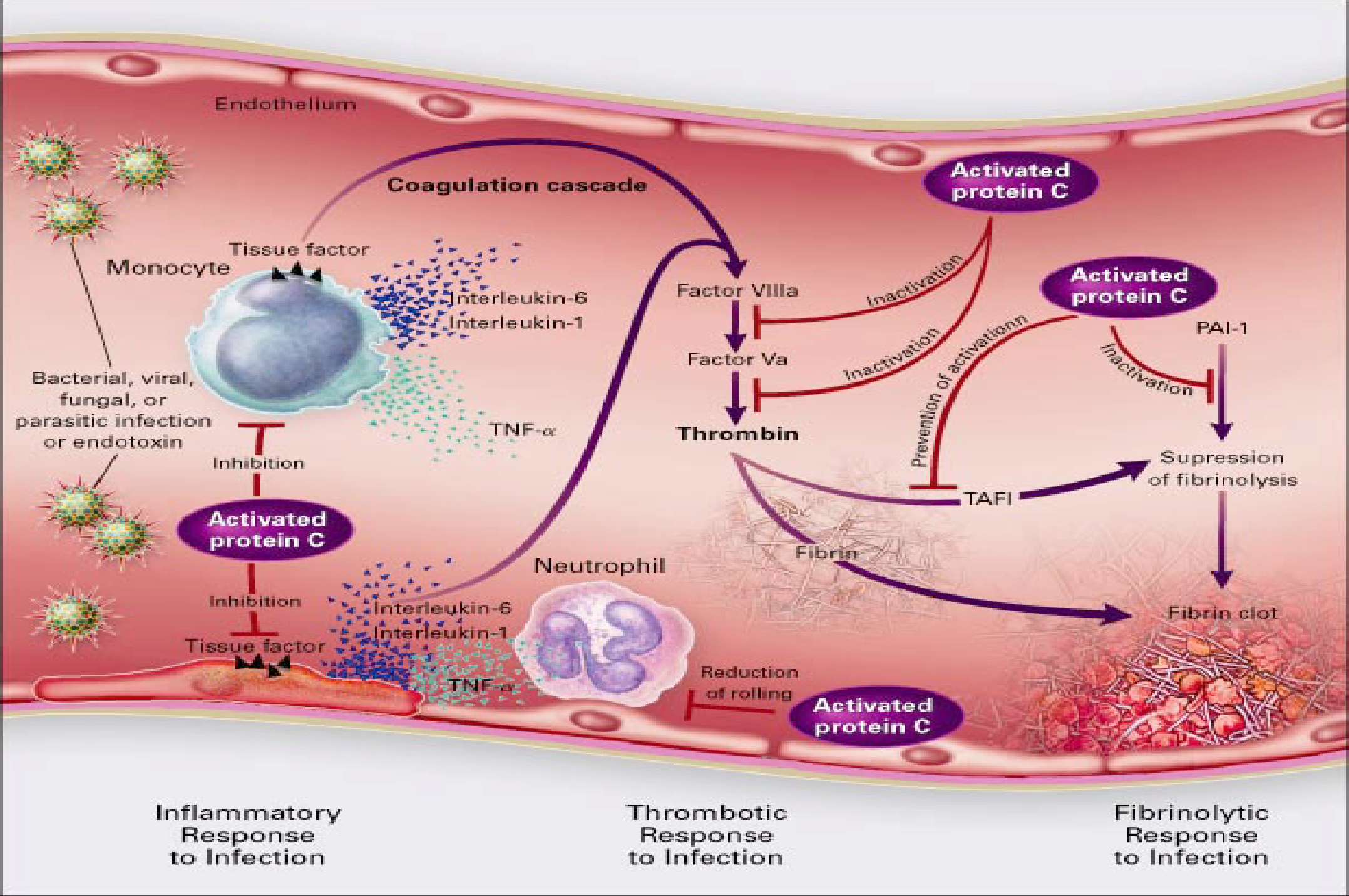
No association with mortality rate



LTα+250 AA genotype RR= 2.48 (1.28 – 4.78), Age-adjusted RR = 3.64 (1.28 – 10.66)



Coagulation Polymorphisms



Endothelium

Coagulation cascade

Monocyte

Tissue factor

Interleukin-6

Interleukin-1

TNF- α

Bacterial, viral, fungal, or parasitic infection or endotoxin

Inhibition

Activated protein C

Inhibition

Tissue factor

Interleukin-6

Interleukin-1

TNF- α

Neutrophil

Factor VIIIa

Factor Va

Thrombin

Inactivation

Inactivation

Prevention of activation

Fibrin

TAFI

Suppression of fibrinolysis

PAI-1

Fibrin clot

Reduction of rolling

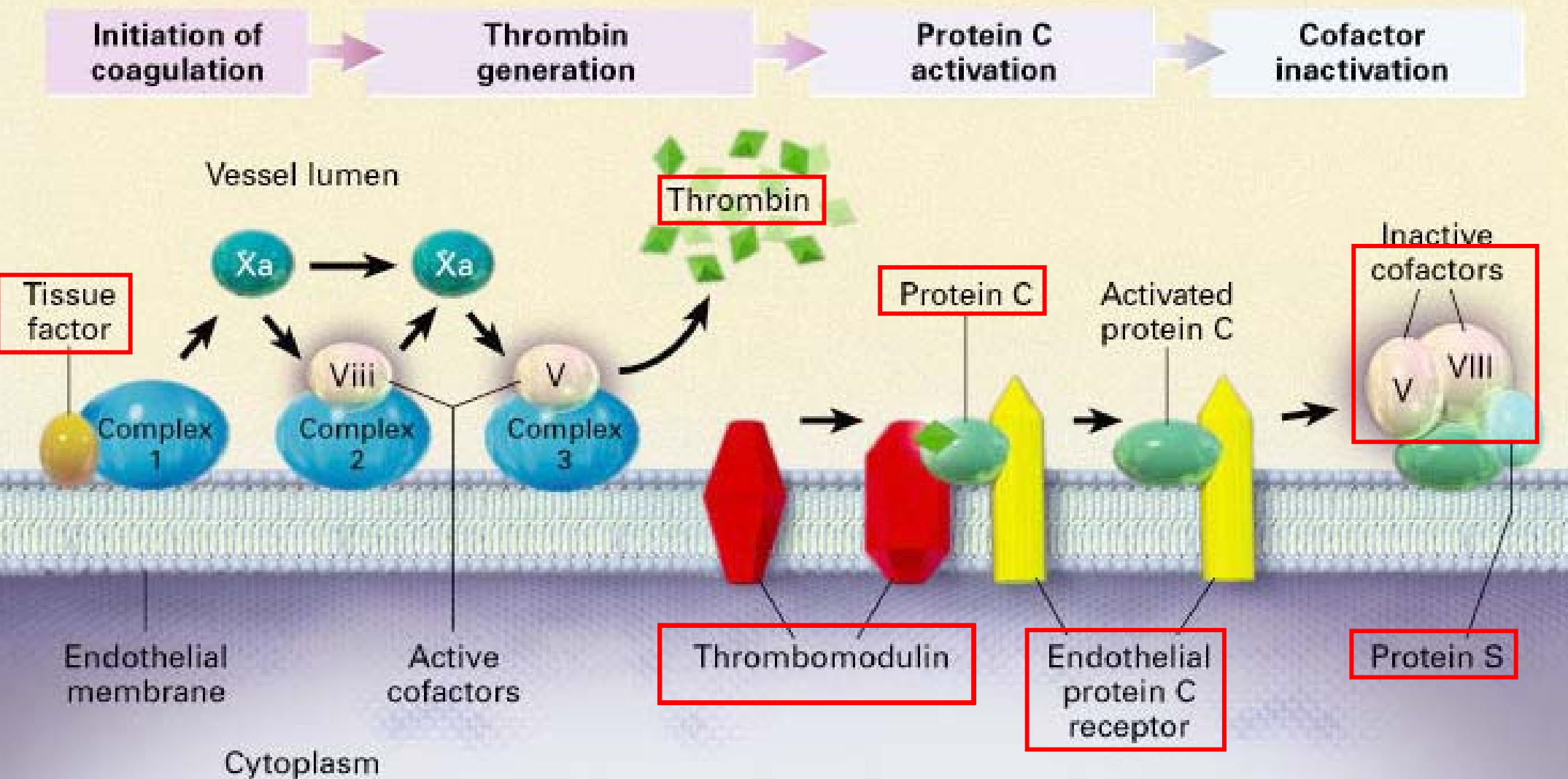
Activated protein C

Inflammatory Response to Infection

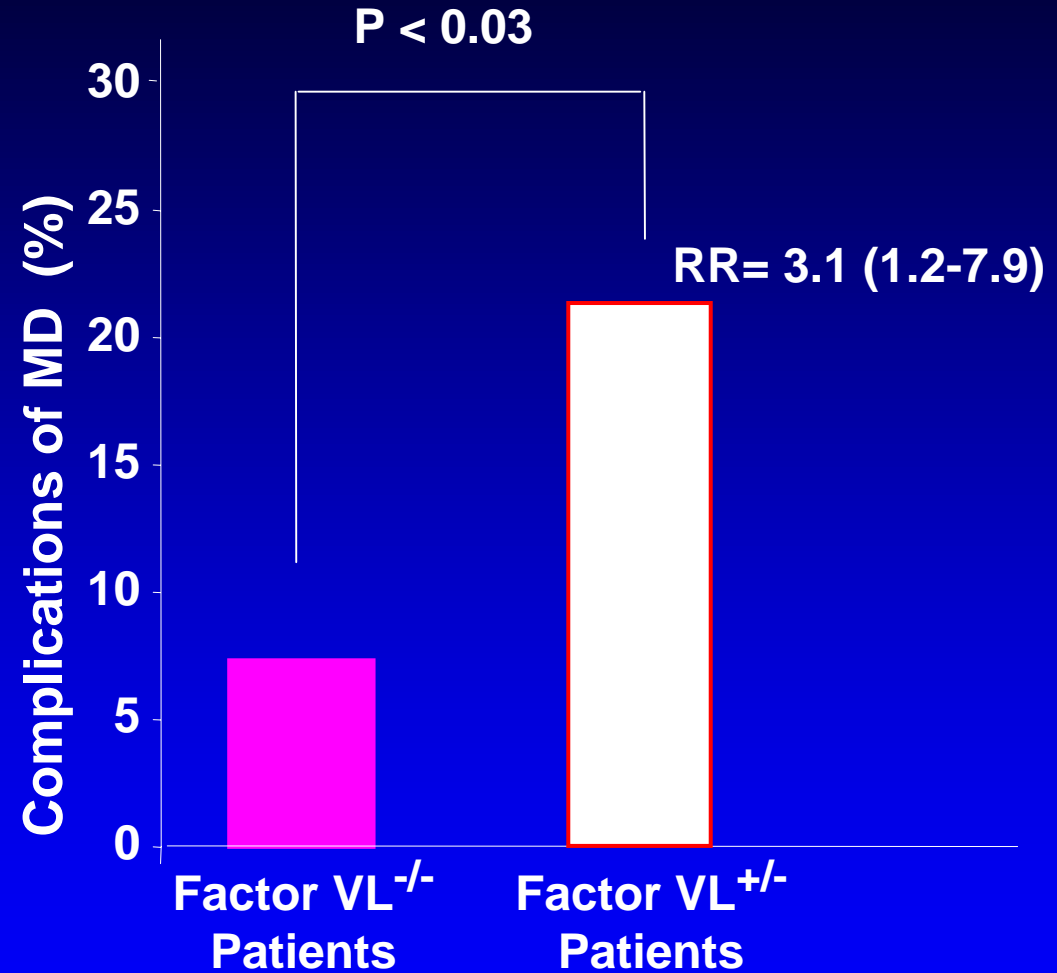
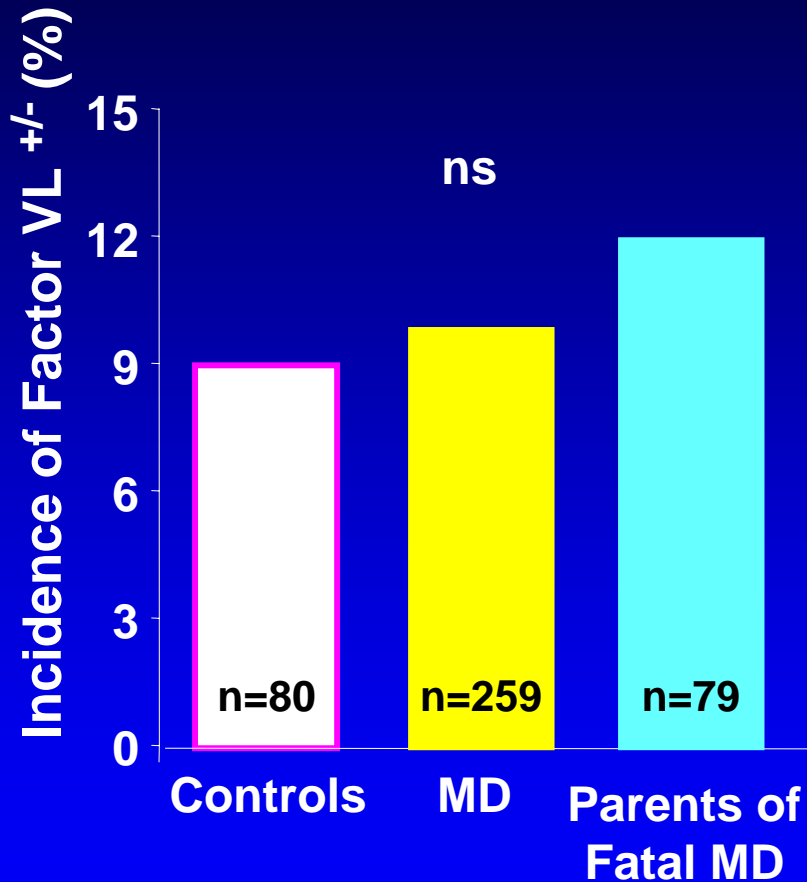
Thrombotic Response to Infection

Fibrinolytic Response to Infection

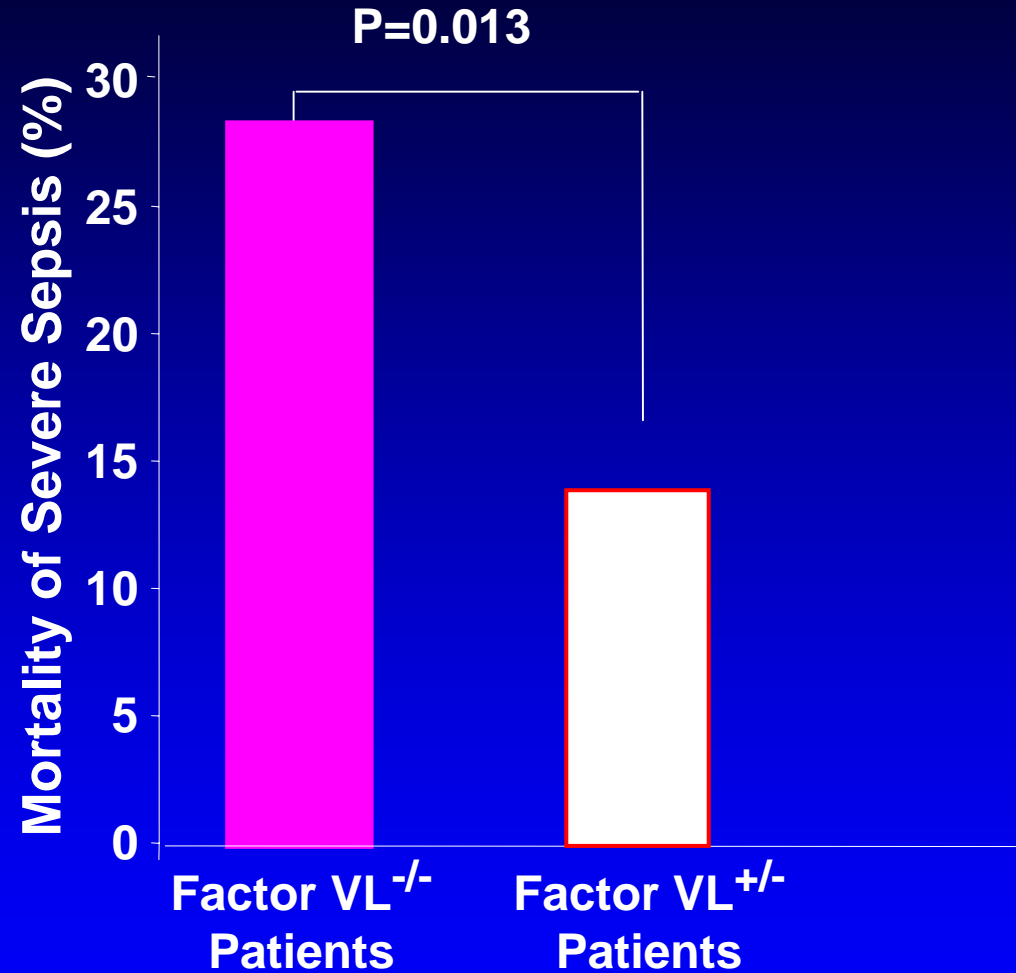
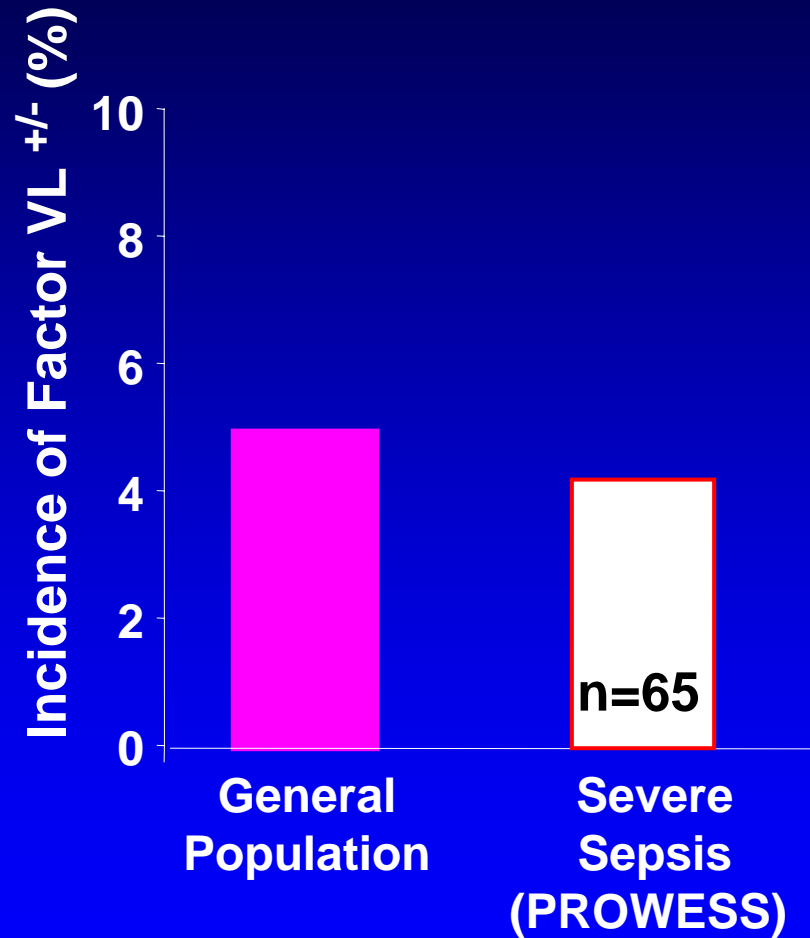
Protein C Pathway



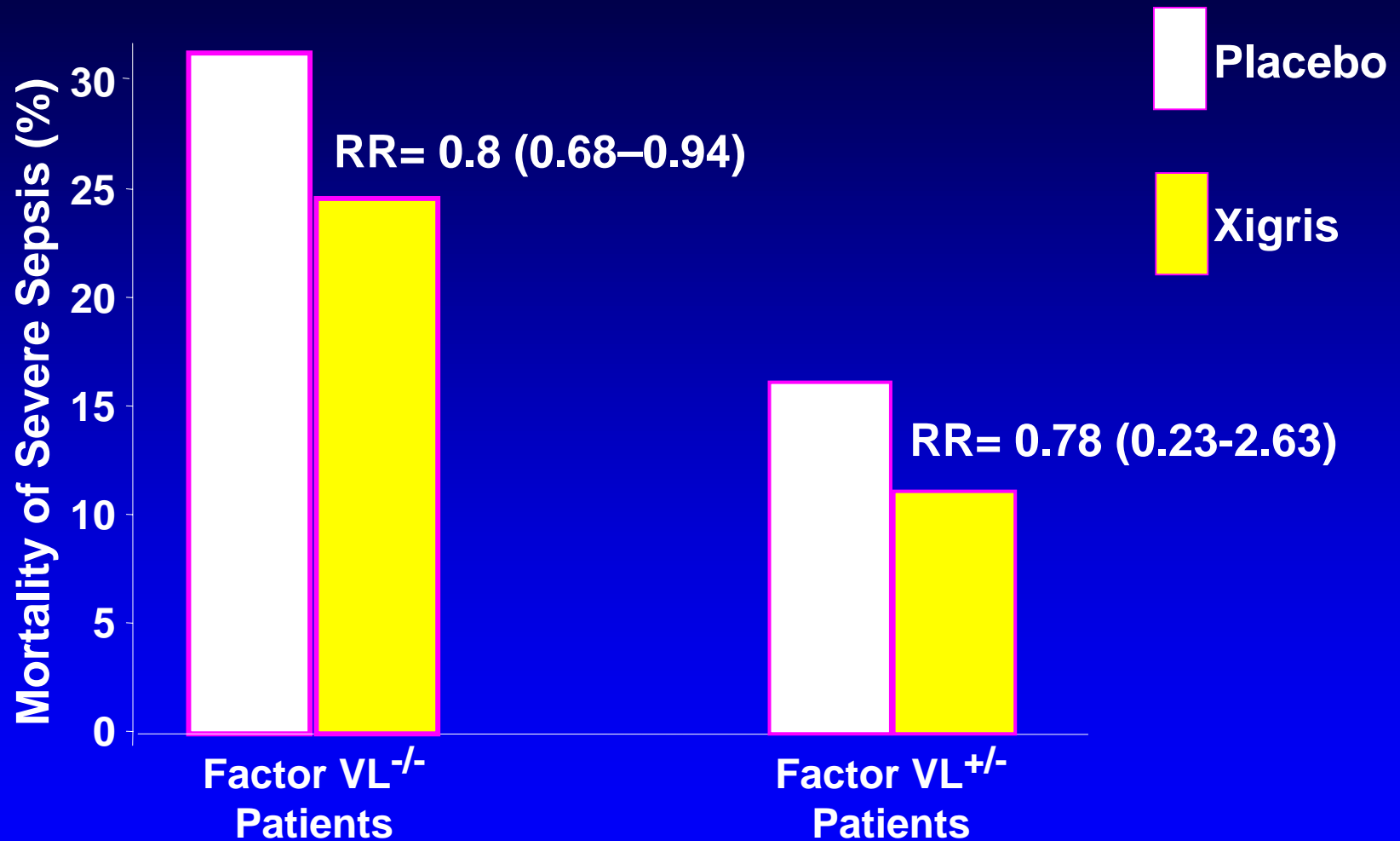
Factor V Leiden mutation and Meningococcal Disease



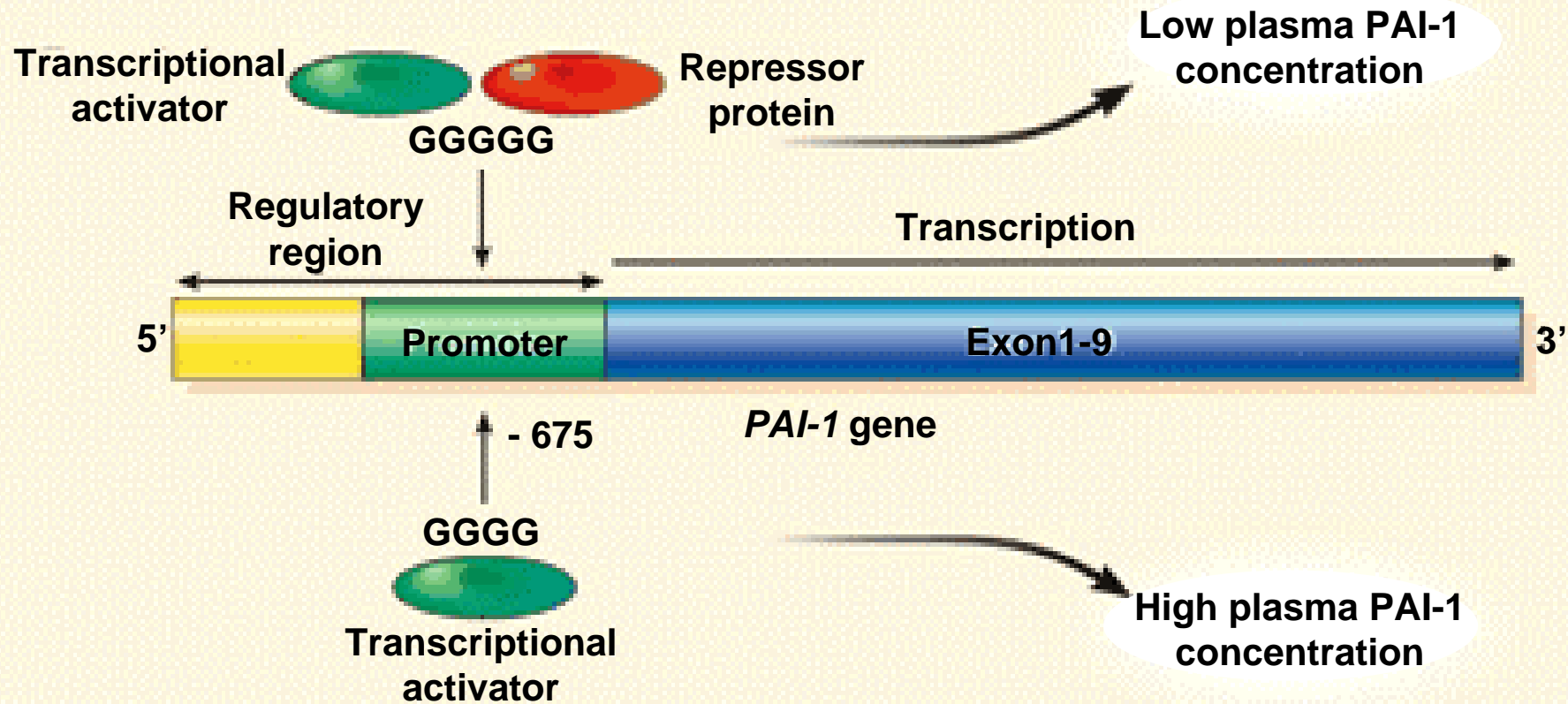
Factor V Leiden and Severe Sepsis



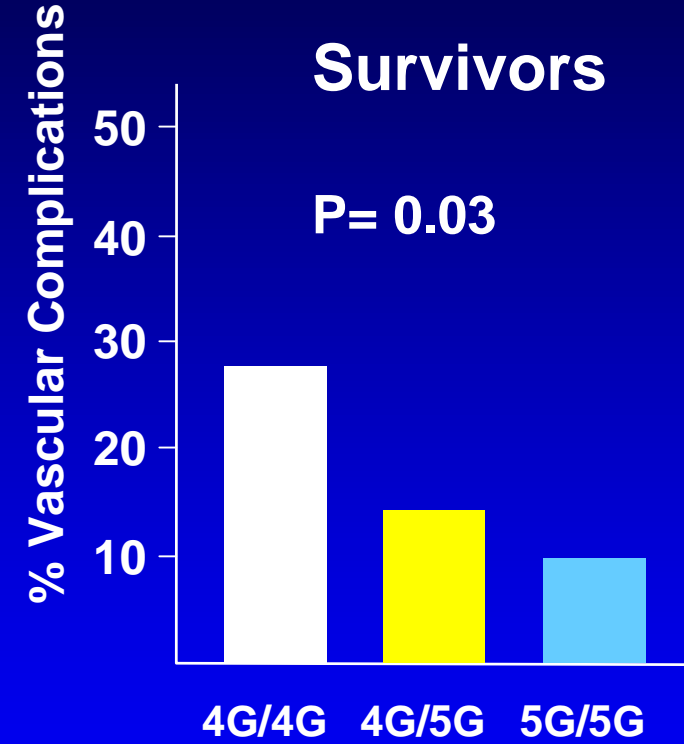
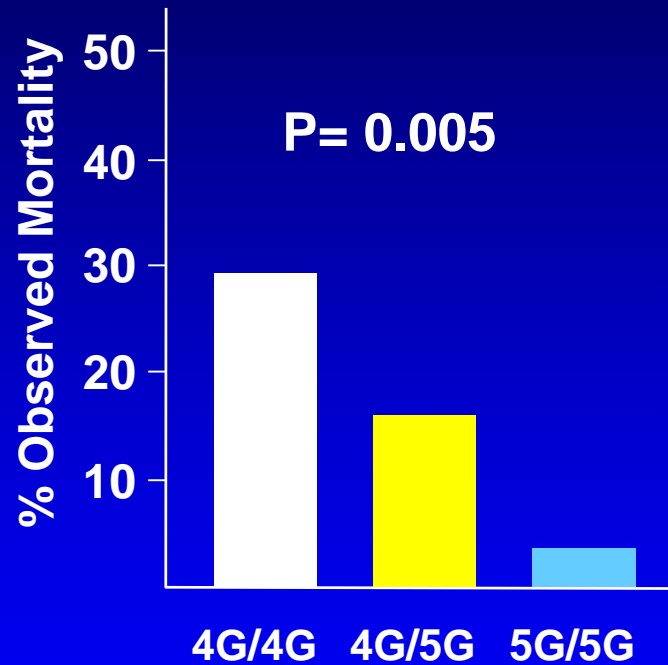
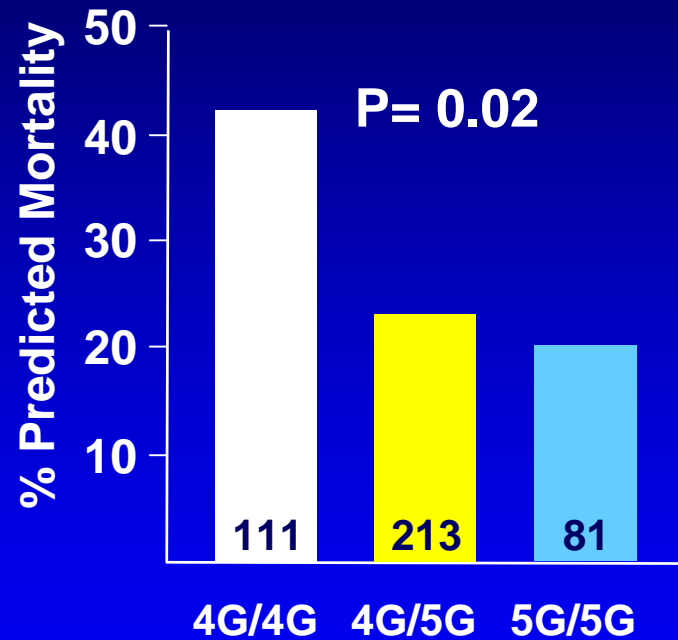
Factor V Leiden and APC Efficacy in Severe Sepsis



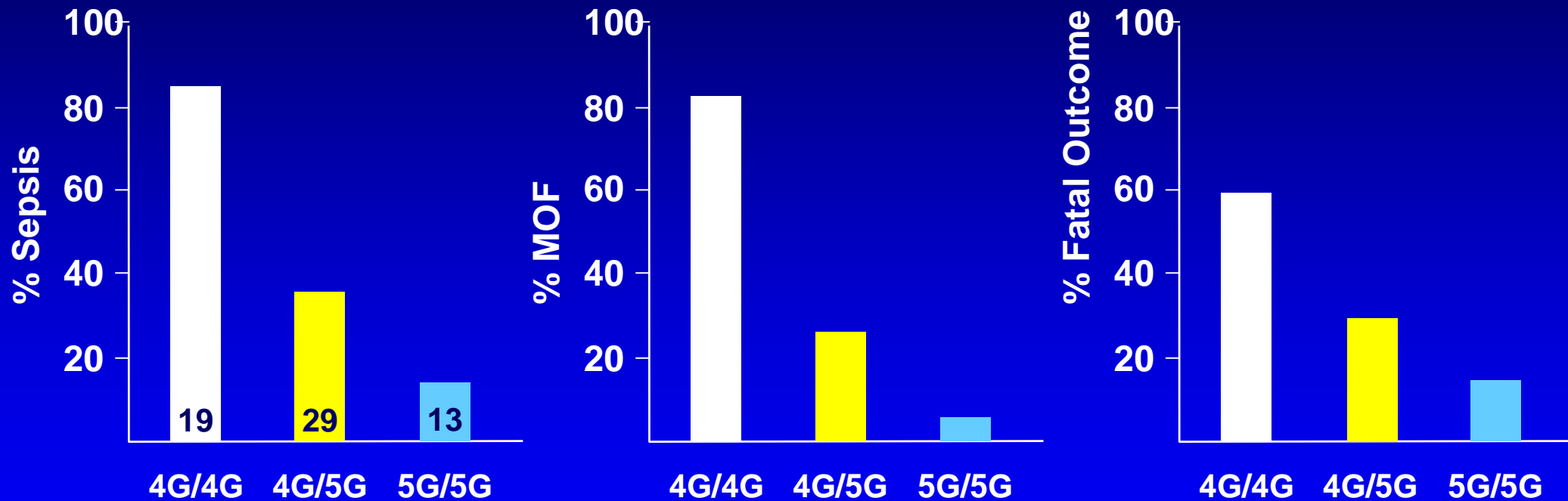
4G/5G PAI-1 Polymorphism



4G/5G PAI-1 Polymorphism and Meningococcal Disease



4G/5G promoter polymorphism in the PAI-1 gene and severe trauma patients





Perspectives and Conclusions

- ⇒ Screening of a high number of polymorphisms in large cohorts
 - ⇒ SNPs or haplotype
 - ⇒ Micro-arrays, Taqman, Mass Spectroscopy, ...

Yamada Y et al. *N Engl J Med* 2002; 347: 1916-23.

⇒ **2819 patients with myocardial infarction**

⇒ **2242 controls**

⇒ **112 polymorphisms of 71 candidate genes**

PAI-1, connexin 37, stromelysin

Perspectives et Conclusions

- **Screening of a high number of polymorphisms in large cohorts**
 - ⇒ **UK: 1000 Patients – Peritonitis**
 - ⇒ **UK: 2000 Patients – Community-Acquired Pneumonia**
 - ⇒ **USA: 2000 Patients – Severe Sepsis**
 - ⇒ **USA: 1500 Patients – Severe Sepsis**
 - ⇒ **France: 3500 Patients – Nosocomial Pneumonia**
 - ⇒ **France: 3500 Severe Trauma**
 - ⇒ **Australia ?**
 - ⇒ **Japan ?**

High Throughput Genotyping



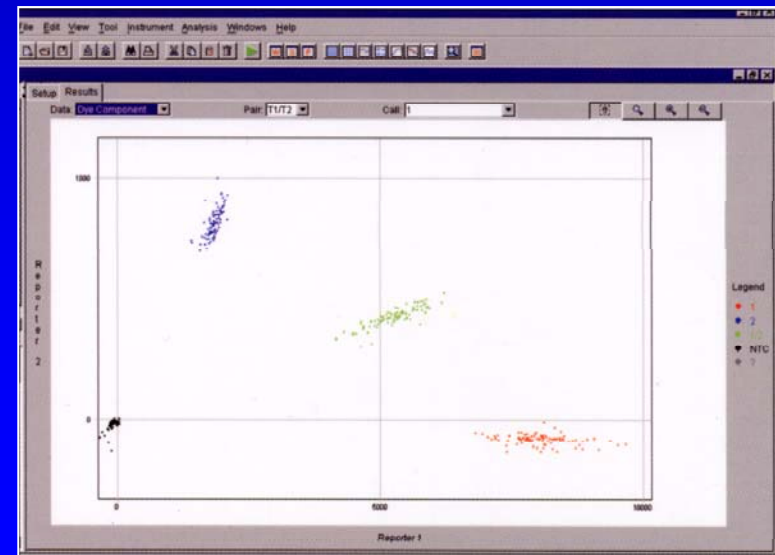
20'



30'



90'



Recurrent Purpura Fulminans

2002/01: 15 yo girl admitted in ICU

- Temperature 40°C; HR 125; BP 74/45; RR 38
- Meningitis with purpura fulminans
- MOF (Shock, ARDS, ARF, DIC, Lactic acidosis)
- Meningococcus type N in the skin biopsy
- Survival with multiple finger amputations and skin grafting
- 6 month hospitalization

2003/02:

- Temperature 39°C; HR 125; BP 83/48; RR: 33
- Meningitis with purpura fulminans
- Lumbar puncture → meningococcus type Y
- Shock and DIC
- Survival (Xigris) with new skin grafting
- 3 month hospitalization

Recurrent Purpura Fulminans

Genetic predisposition?

Innate immunity

Inflammation

Coagulation

Innate Immunity

TLR4

CD14

FcγRIIIa

FcγRIII

MBL

Complement

C7 deficiency

Inflammation

TNF α

LT α

IL-1

IL-6

IL-10

ACE

Coagulation

Tissue Factor

Prothrombin

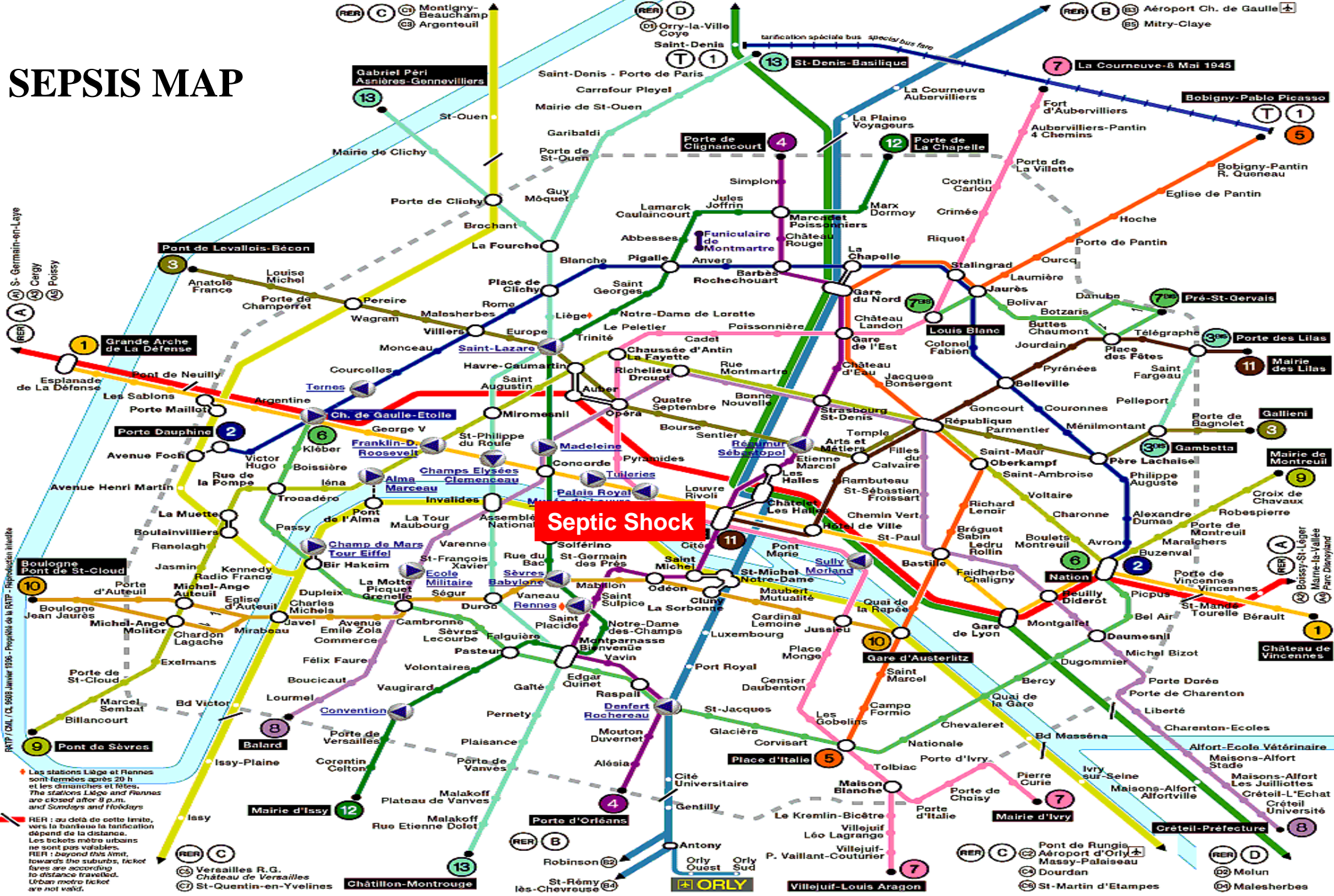
Factor V

Factor VII

Factor XIII

PAI-1

SEPSIS MAP



Perspectives and Conclusions

⇒ Screening of a high number of polymorphisms in large cohorts

→ Effect of association of polymorphisms ?

⇒ Identify potential markers of susceptibility, severity, and clinical outcome

→ Genetic profiling

→ Individual risk assessment

→ Prevention, Vaccination

→ To tailor prescriptions to each patient

Perspectives and Conclusions

⇒ Screening of a high number of polymorphisms in large cohorts

→ Effect of association of polymorphisms ?

⇒ Identify potential markers of susceptibility, severity, and clinical outcome

→ Genetic profiling

→ Individual risk assessment

→ Prevention, Vaccination

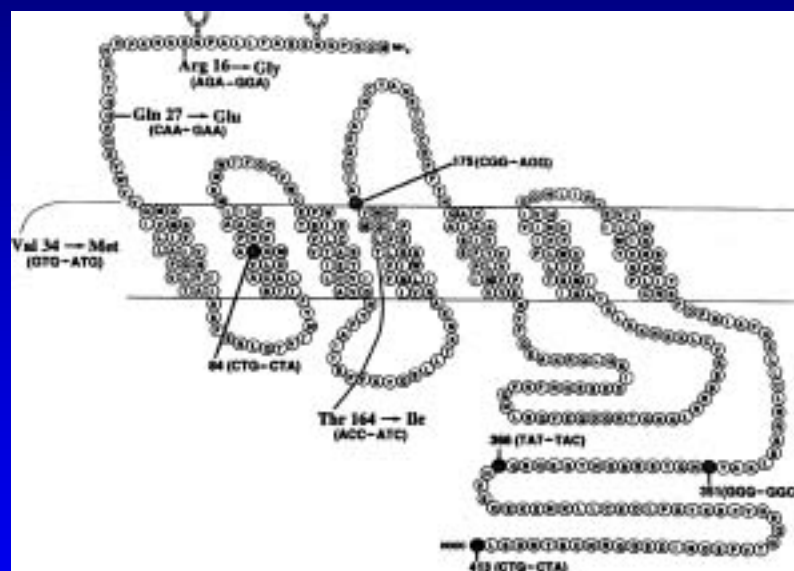
→ To tailor prescriptions to each patient

⇒ Stratification of patients by genotype in the design of treatment trials

→ Identify potential markers for responders vs non-responders

Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial

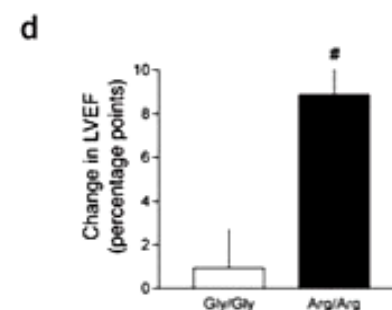
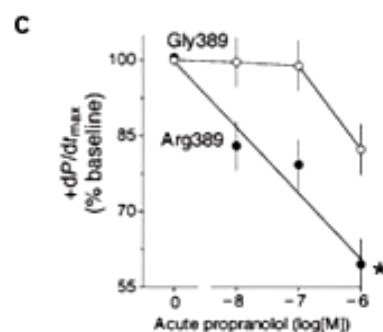
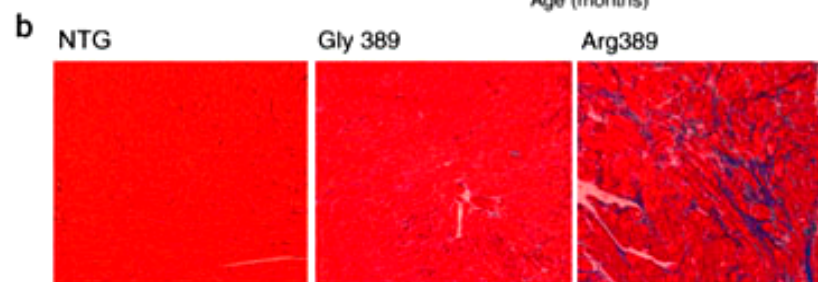
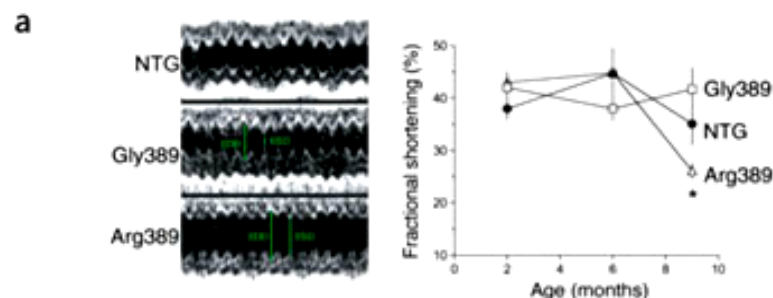
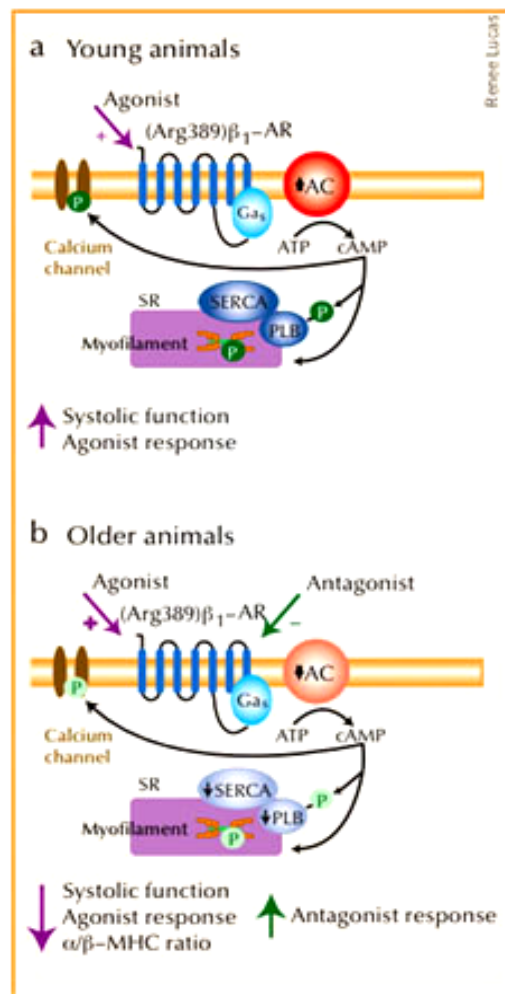
Elliot Israel, Vernon M Chinchilli, Jean G Ford, Homer A Boushey, Reuben Cherniack, Timothy J Craig, Aaron Deykin, Joanne K Fagan, John V Fahy, *Lancet* 2004; 364: 1505-12



Interpretation Genotype at the 16th aminoacid residue of the β_2 -adrenergic receptor affects the long-term response to albuterol use. Bronchodilator treatments avoiding albuterol may be appropriate for patients with the Arg/Arg genotype.

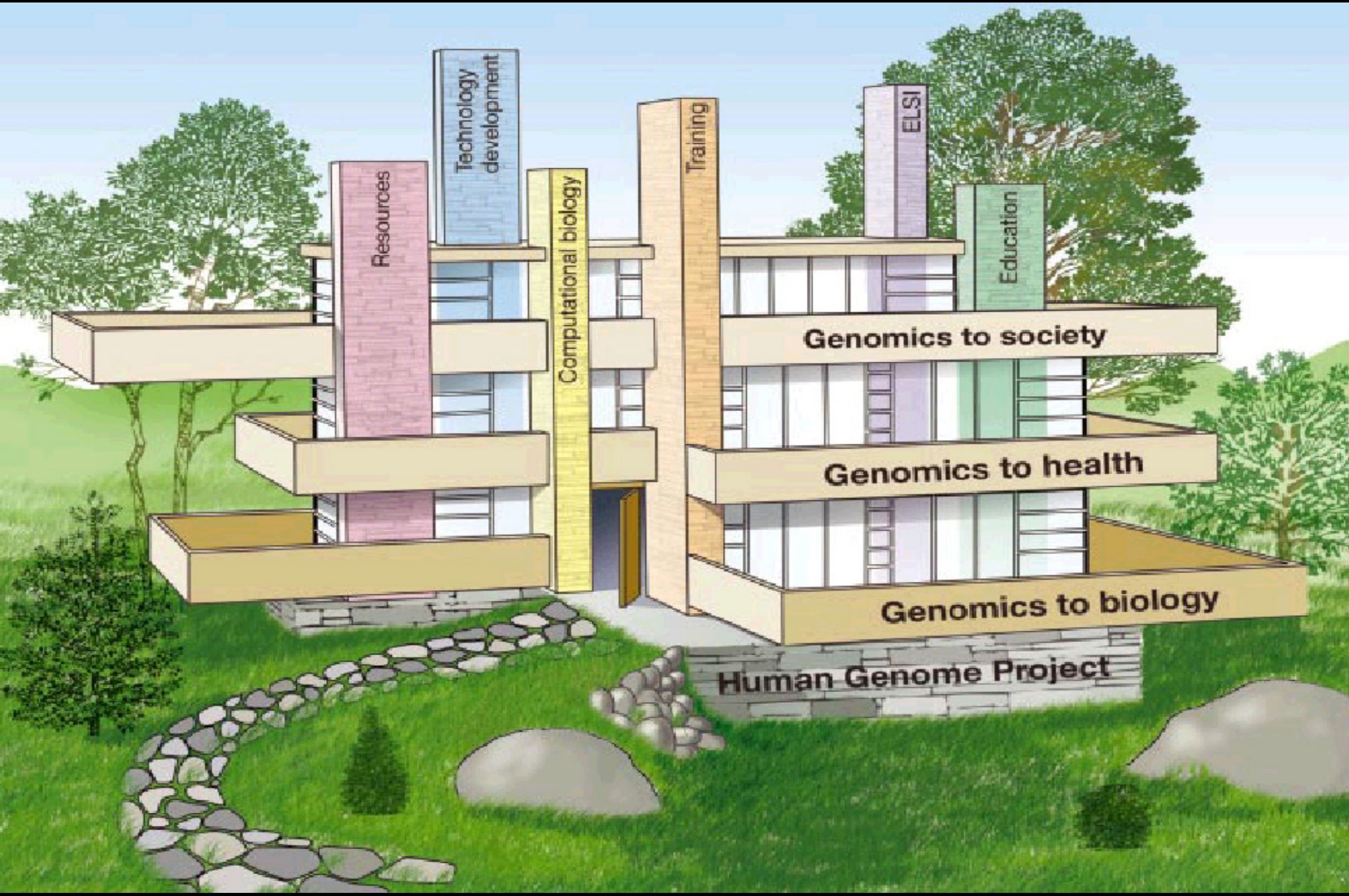
β_1 -adrenergic receptor polymorphisms confer differential function and predisposition to heart failure

Jeanne Mialet Perez^{1,4}, Deborah A Rathz^{2,4}, Natalia N Petrashevskaya³, Harvey S Hahn¹, Lynne E Wagoner¹, Arnold Schwartz³, Gerald W Dorn II^{1,2} & Stephen B Liggett^{1,2}



PUTTING SCIENCE
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Resources

Technology development

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Genomics to biology

Human Genome Project

OCTOBER 25, 2004

IRAQ'S POWER BROKER
PIXAR'S INCREDIBLE NEW MOVIE

TIME

THE
GOD
GENE

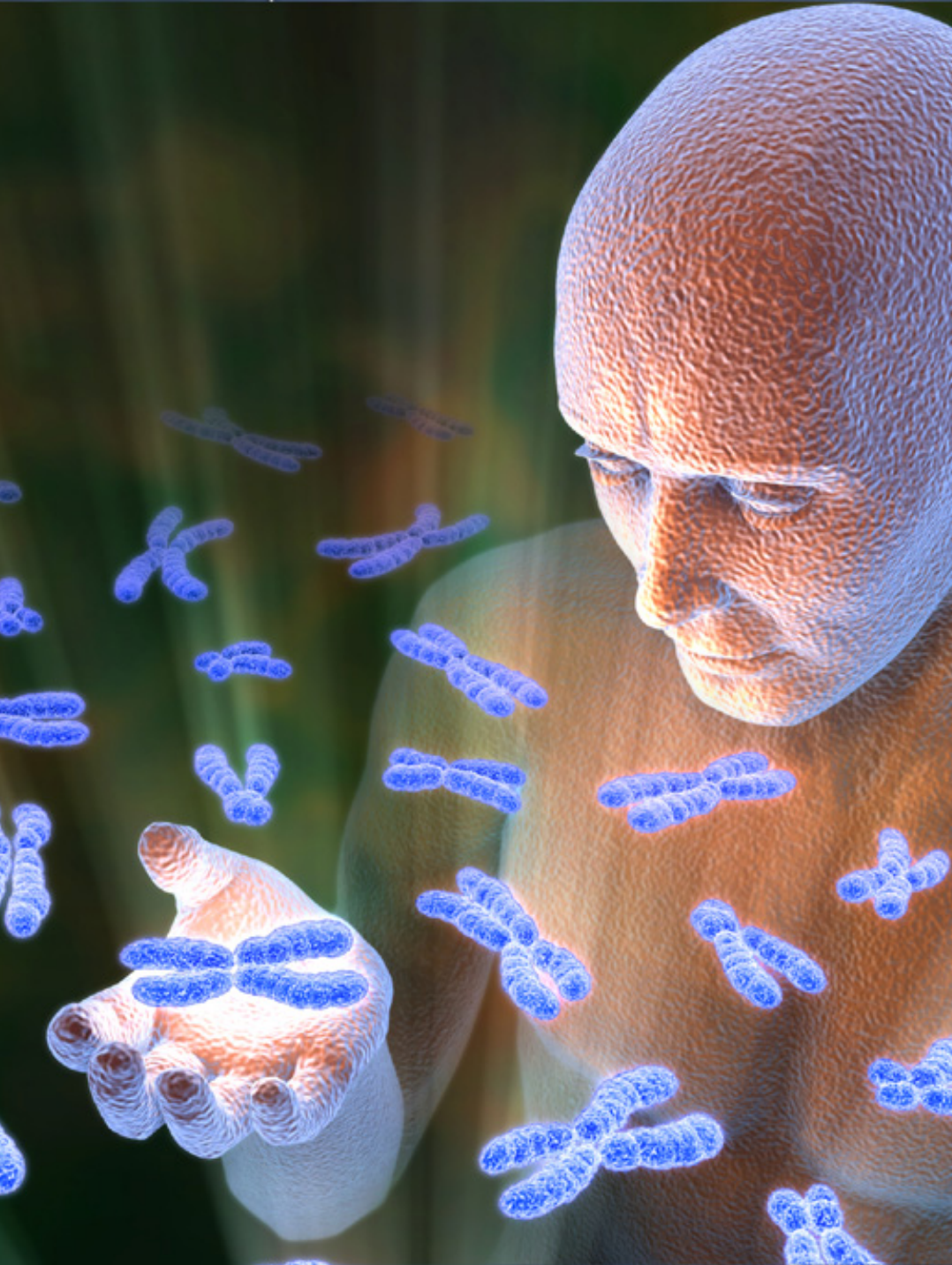
Does our DNA
compel us to seek
a higher power?
Believe it or not,
some scientists
say yes

PLUS: A quiz—
How spiritual
are you?

The « challenge »

From functional genomics to rationale
therapeutics



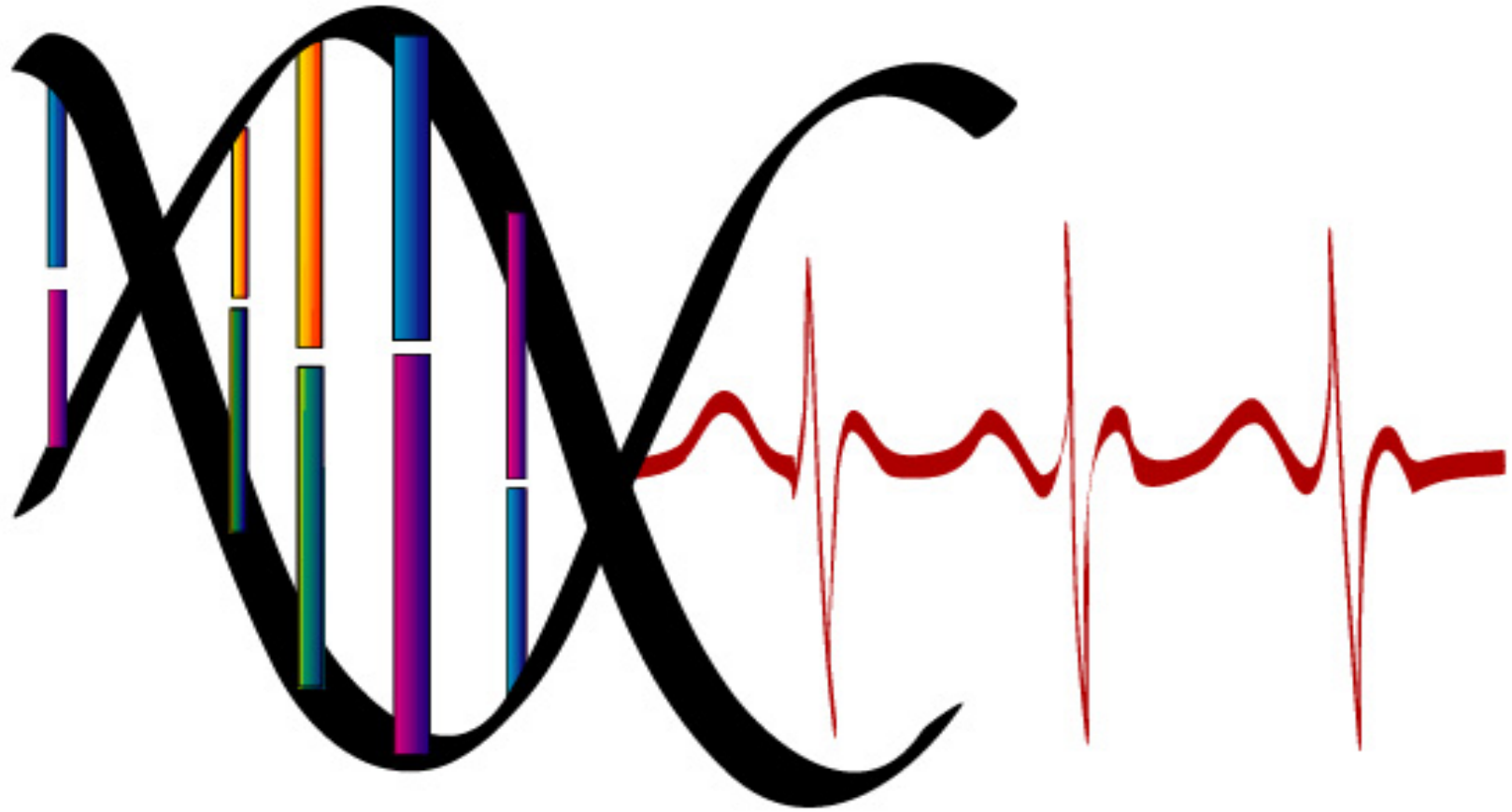


« Man is a tool-using animal....
Without tools he is nothing,
with tools he is all .»

Thomas Carlyle

19th-century

FUNCTIONAL GENOMICS OF



CRITICAL ILLNESS AND INJURY