6e JNI

Génétique et Sepsis

9 Juin 2005

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« 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

1953

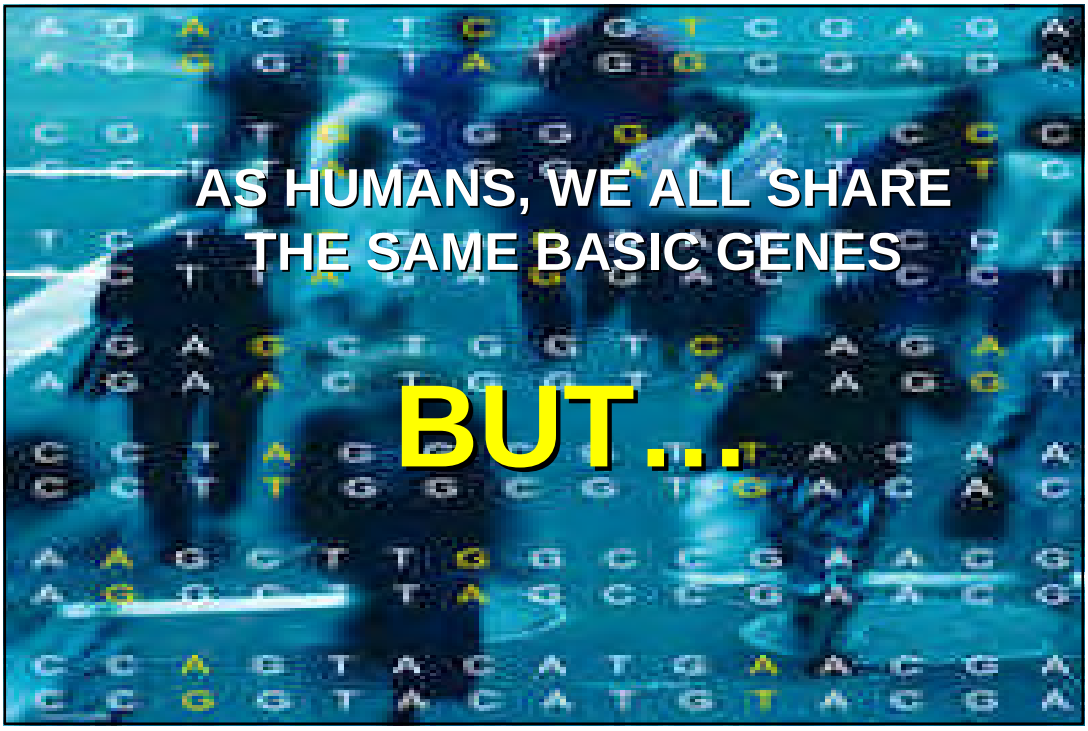
2001-2003

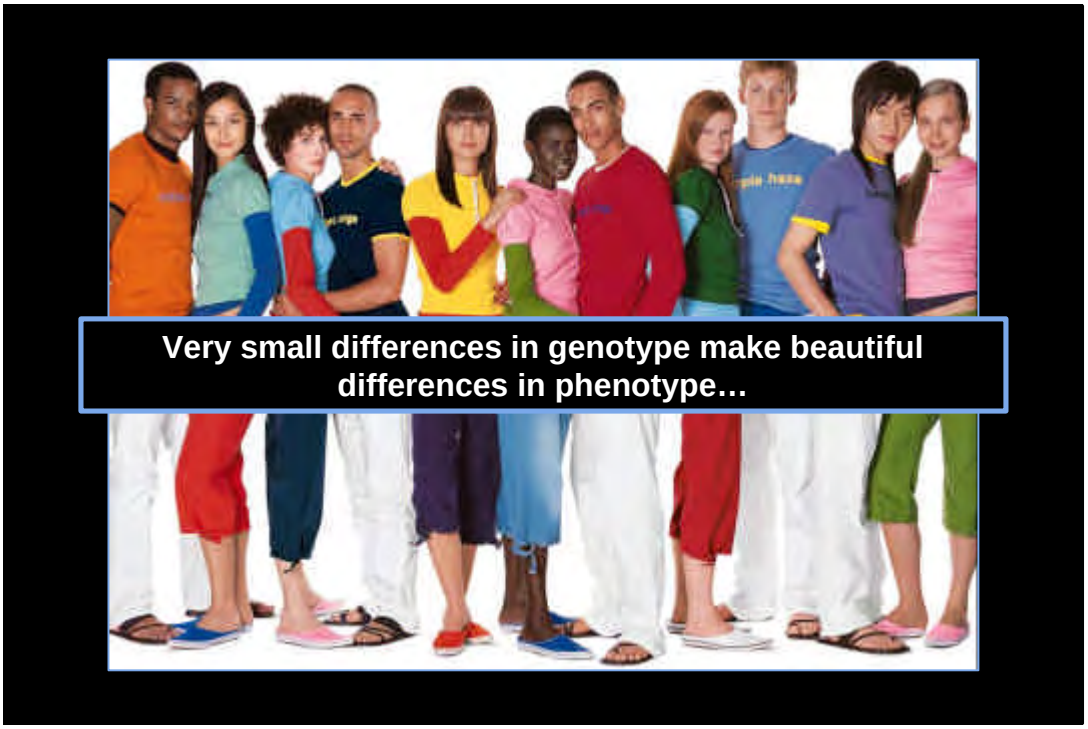
« Today we are learning the language in which God created life. It will revolutionize the diagnosis, prevention and treatment of most, if not all human diseases. »

William J. Clinton, June 26, 2000

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

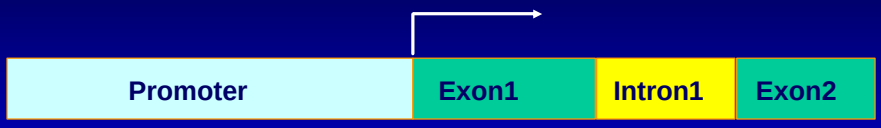

AS HUMANS, WE ALL SHARE
THE SAME BASIC GENES
BUT…


Very small differences in genotype make beautiful differences in phenotype…

Genetic Polymorphisms

→ Human SNP Map

How single nucleotide polymorphisms influence human biology



Quantitative changes

Pathogen recognition (CD14; MBL)
Cytokines (TNF; IL-1;...)
Coagulation (PAI-1; VII;...)
Drug metabolizing enzymes (CYP)
...

Qualitative changes

Pathogen recognition (CCR5; TLR; MBL, FcgRII)
Coagulation (Leyden;...)
Drug metabolizing enzymes
...

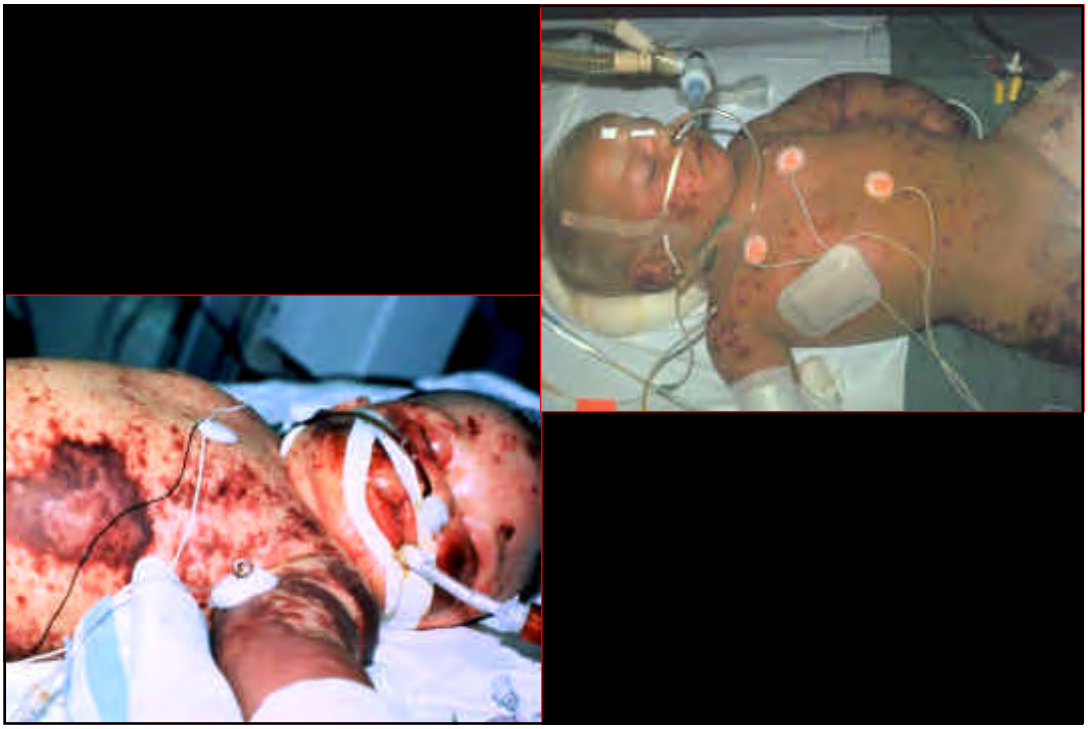
Evidences for a genetic component to sepsis

Animal Studies

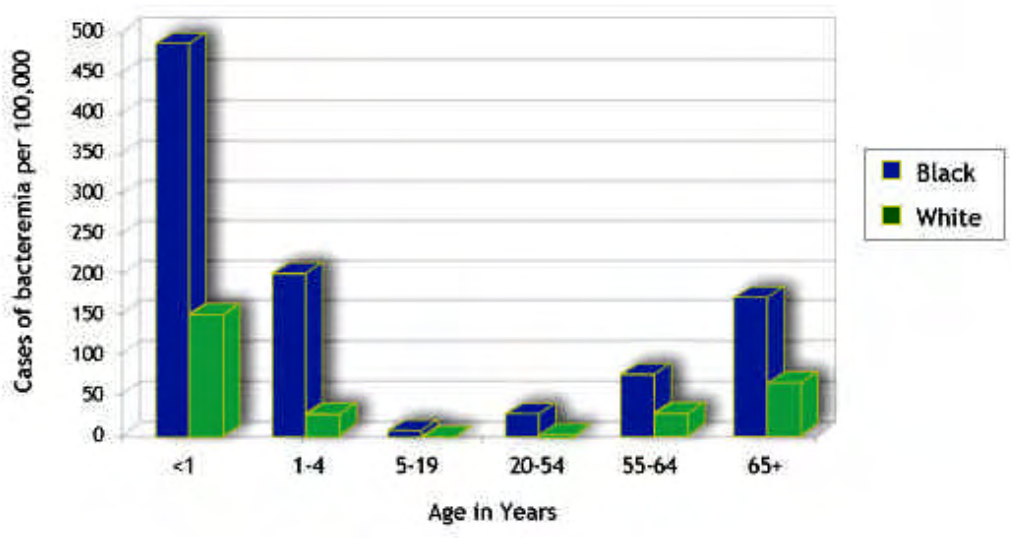
- Susceptibility/resistance to certain infection in mice
- Susceptibility/resistance phenotypes of knockout mice

Human Studies

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



Pneumococcal Bacteremia by Ethnie and Age



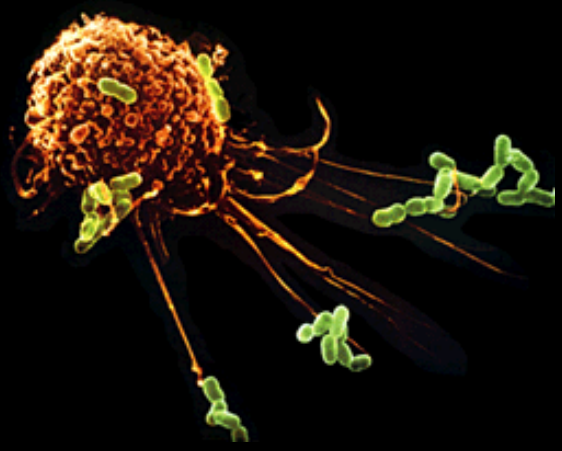
Monroe County, New York, 1985-1989

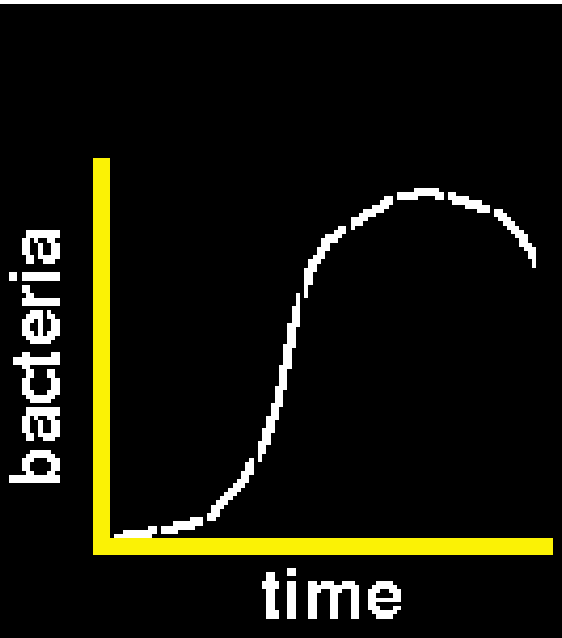
Bennet NM; Am J Public Health 1992;82:1513

Genetic Polymorphisms and Severe Sepsis

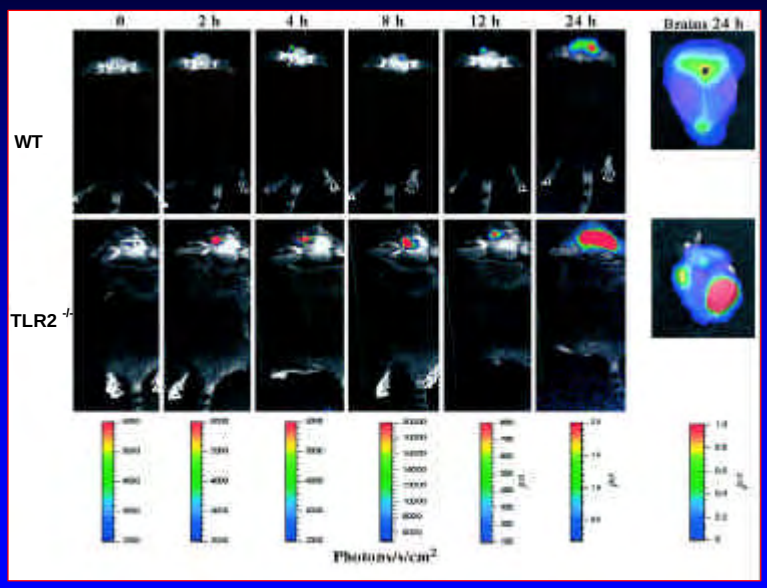
Gene	Susceptibility and/or Outcome
Mannose Binding Lectin	Meningococcemia, Pneumococcemia Severe sepsis
Toll-Like Receptor 4/2	Gram negative/positive Septic Shock
Toll-Like Receptor 5	Legionnaire's Disease
CD14	Septic Shock
FCgRII Receptor	Meningococcemia; Pneumococcemia
TNF locus	Meningococcemia Septic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

Pathogen Detection



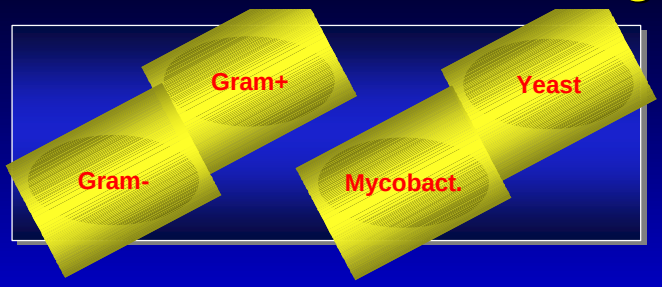


TLR2 and *Streptococcus pneumoniae* meningitis

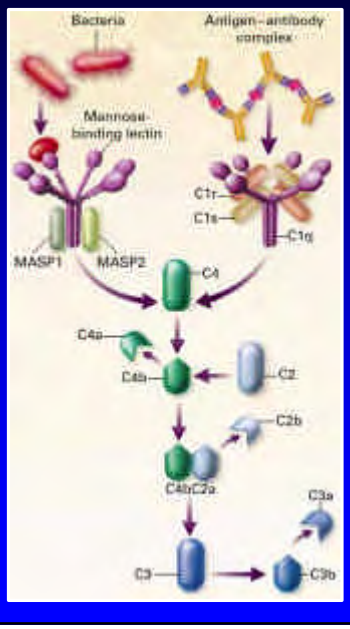


Echchannaoui H et al. JID 2002;186:798

Mannose-Binding Lectin



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



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

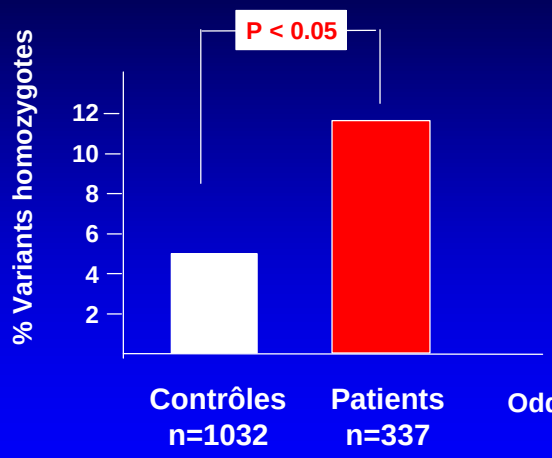
- Increased severity of lung disease and low survival in cystic fibrosis

  Garred et al., J. Clin. Invest. 1999

- Meningococcal disease

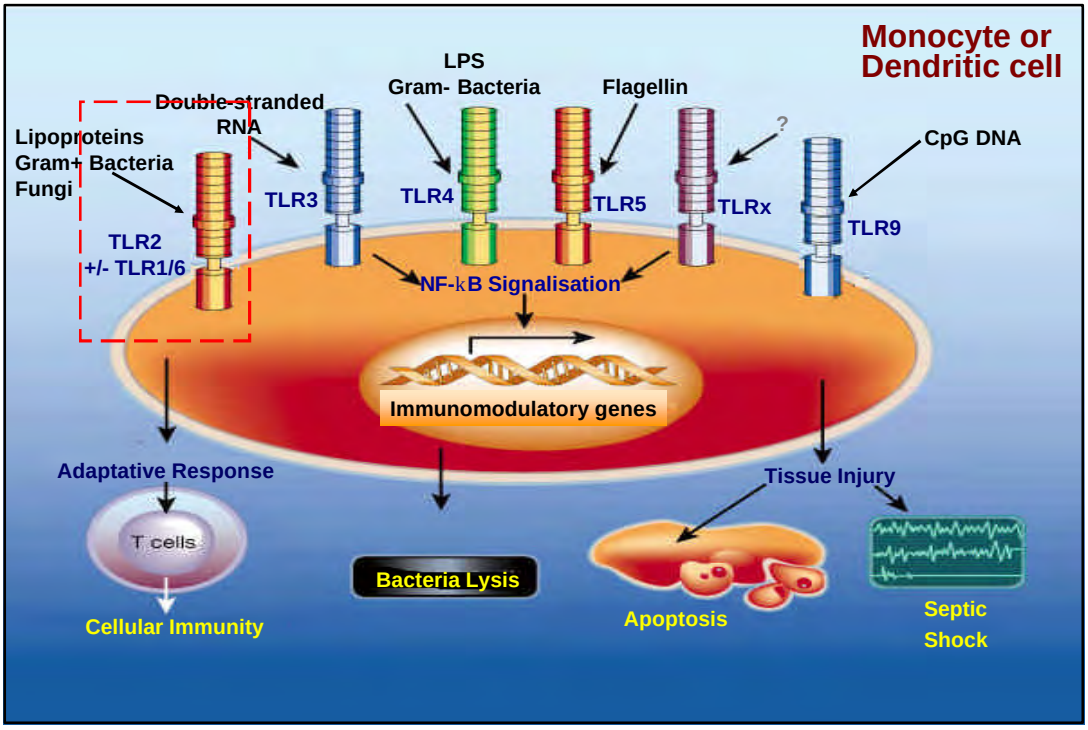
  Hibberd et al., Lancet 1999

MBL genotype and risk of invasive pneumococcal disease



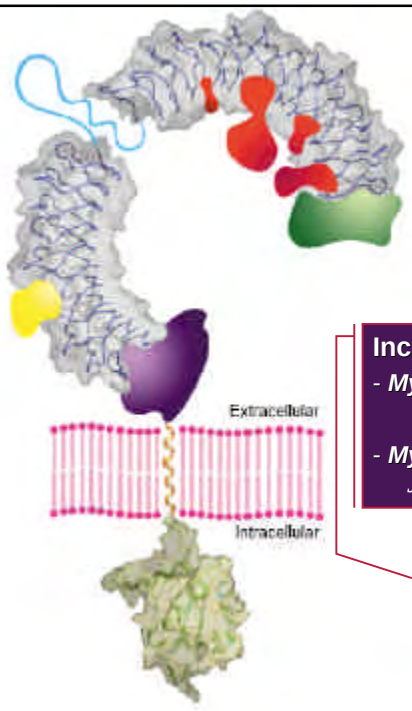
Odds ratio 3.48 (1.51 – 8.01); p=0,003

Roy et al. Lancet 2002; 359: 1569-1573



Synonymous

Position	rs number
35	5743697
199	3804099
213	5743698
450	3804100
541	5743700
542	5743701
707	IIPGA-TLR2-31410
781	5743709



Non-synonymous

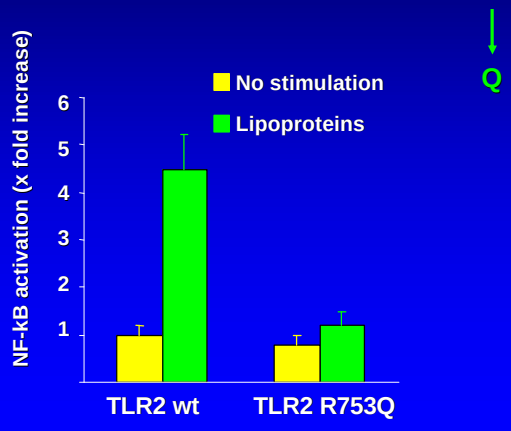
Position	rs number
411(T/I)	5743699
556 (I/T)	5743702
579 (R/H)	5743703
631 (P/H)	5743704
677 (R/W)	5743706
715 (Y/N)	5743707
715 (Y/Amb)	
753 (R/Q)	5743708

Increased susceptibility to

- *Mycobacterium tuberculosis*
  Cytokine 2002;20(2):56-62
- *Mycobacterium leprae*
  J Immunol 2003;170(7):3451-4

R753Q Polymorphism in the TLR2 gene

	743																		765
Human TLR2	K	A	I	P	Q	R	P	C	K	L	R	K	I	M	N	T	K	T	Y	L	E	W	P
Mouse TLR2	K	A	I	P	Q	R	P	C	K	L	R	K	I	M	N	T	K	T	Y	L	E	W	P



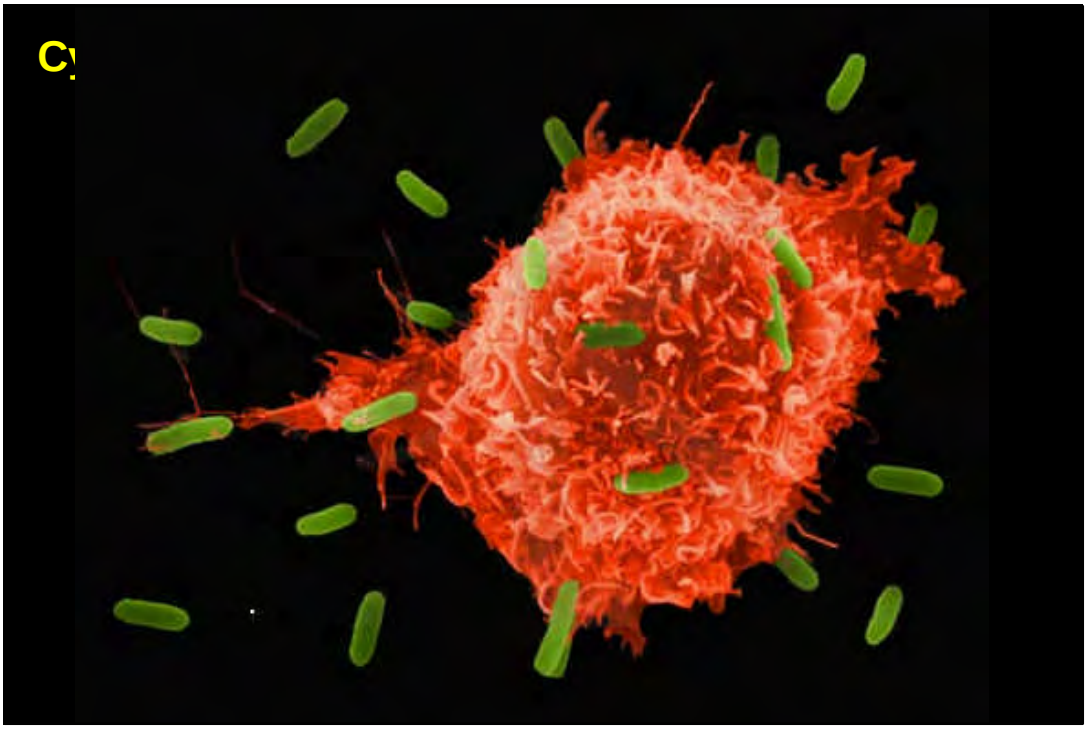
Lorenz, Infect. Immun. 2OOO

$TLR2_{R753Q}$ in MICU

1103 Caucasian ICU Pts (322 SS) → 28 Pts $TLR2_{R753Q}$ (2,5%)

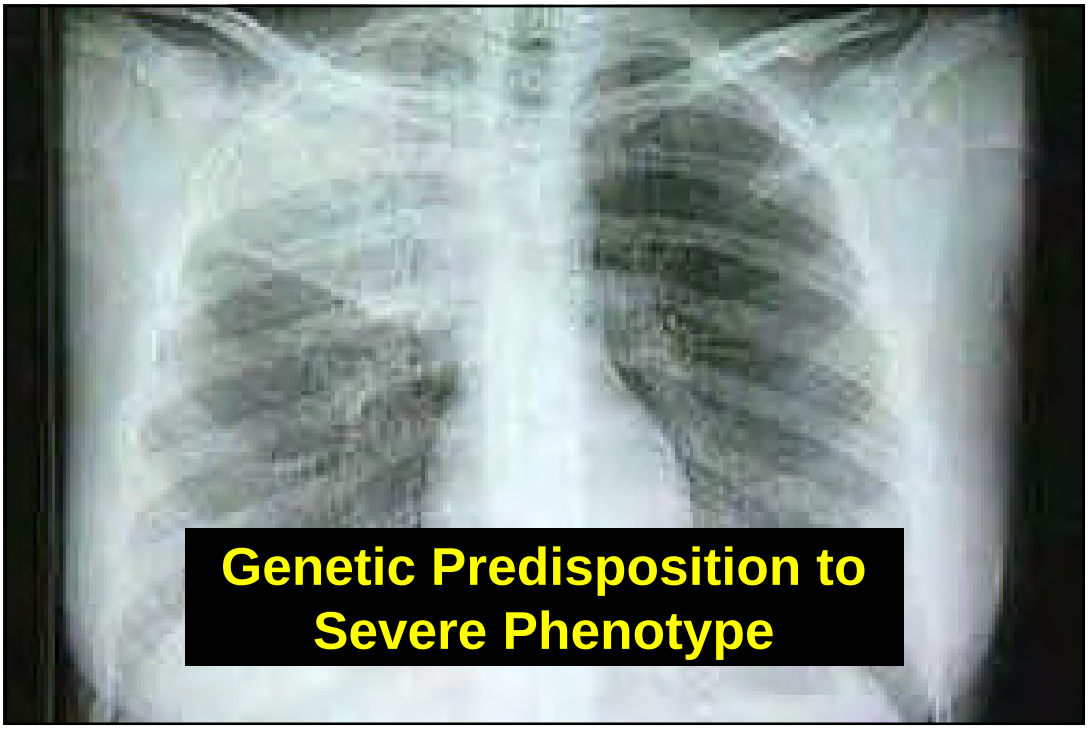
- Age 47,2 ± 18
- SAPS 2 40,5 ± 19
- Survivors 23/28
- Infections 16/28 → 16 Septic Shock (5.1% of SS group)
- Origin of Infections
  - Pneumonia 10
  - Meningitis 4
  - Septicemia 5
  - Others 4
- Microorganisms
  - *S. pneumoniae* 8
  - *S. aureus* 5
  - *Candida sp.* 2
  - *Aspergillus sp.* 2
  - Others 6

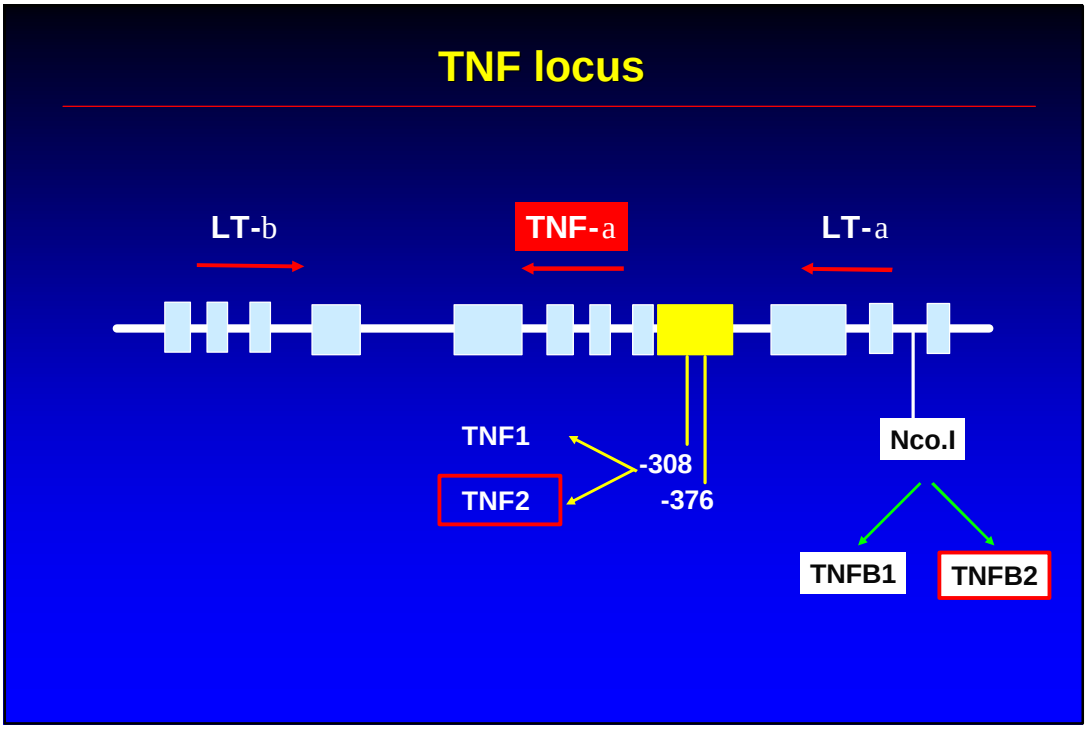
→ 11.1% of Gram positive SS group



Cytokine Polymorphisms and Meningococcemia

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

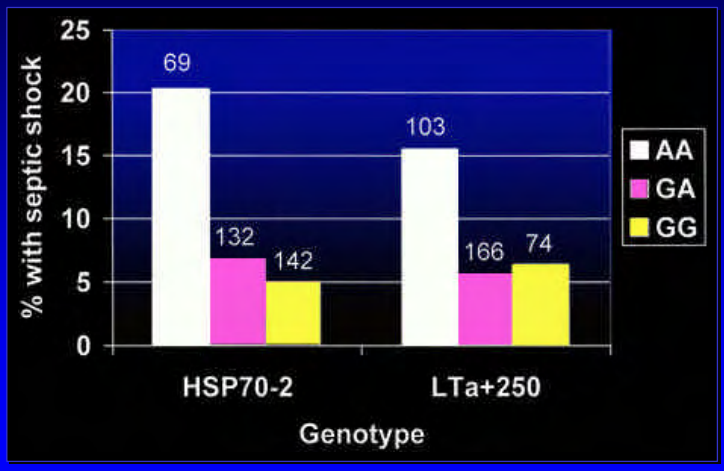

Genetic Predisposition to
Severe Phenotype


TNF locus
LT-b
TNF-a
LT-a
TNF1
TNF2
-308
-376
Nco.I
TNFB1
TNFB2

Severity of Community-acquired Pneumonia, LTa & HSP70-2 Polymorphisms

343 Community-acquired pneumonias ; 30 SS

No link with mortality



HSP70-2 +1267AA: OR = 3.5 (1.8–6.8); LTa +250 AA genotype OR= 2.7 (1.4 – 5.3)

Waterer GW. Crit Care Med 2003; 31: 1367

Perspectives and Conclusions

⇒ Screening of a high number of polymorphisms in large cohorte

⇒ 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

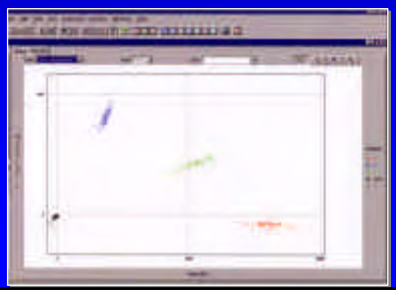
- Screening of a high number of polymorphisms in large cohorts
  - ⇒ UK: 1000 Patients – Peritonitis
  - ⇒ UK: 2000 Patients – Community-Acquired Pneumonia
  - ⇒ USA: 2000 Patients – Severe Sepsis
  - ⇒ USA: 1500 Patients – Severe Sepsis
  - ⇒ France: 3500 Patients – Nosocomial Pneumonia
  - ⇒ France: 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 punction → meningococcus type Y
- Shock and DIC
- Survival (Xigris) with new skin grafting
- 3 month hospitalization

Bohé J. Clin Infect Dis 2005

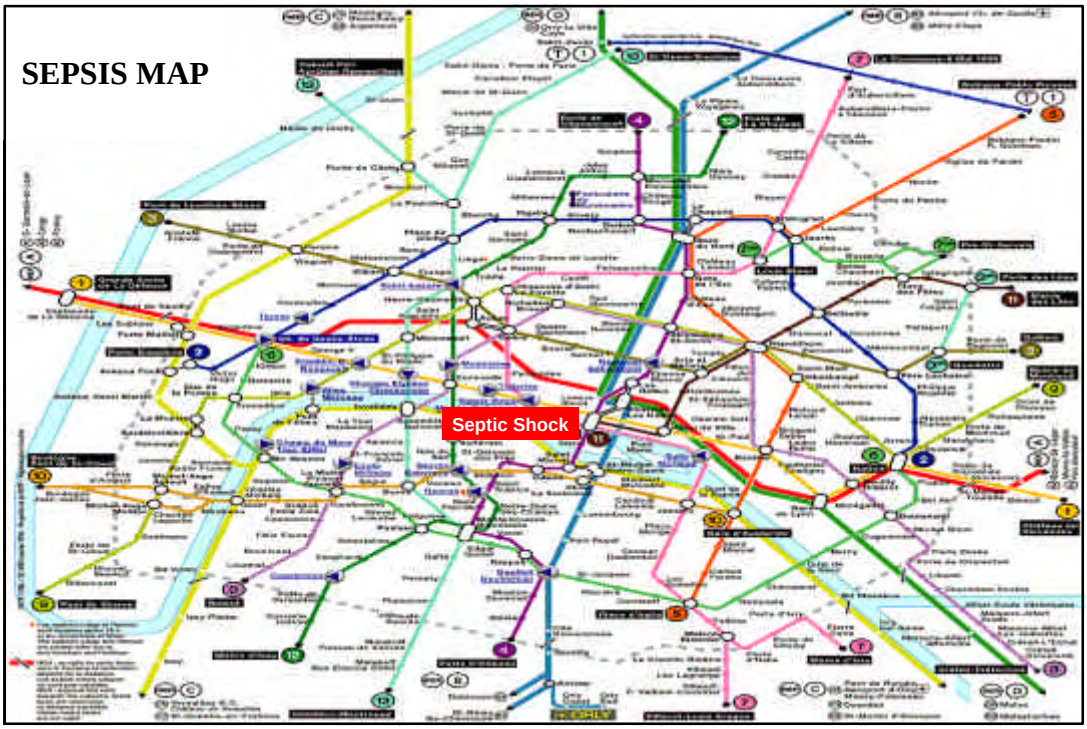
Recurrent Purpura Fulminans

Genetic predisposition?

- Innate immunity
- Inflammation
- Coagulation

Innate Immunity	Inflammation	Coagulation
TLR4	TNFa	Tissue Factor
CD14	LTa	Prothrombin
FcgRIIa	IL-1	Factor V
FcgRIII	IL-6	Factor VII
MBL	IL-10	Factor XIII
Complement	ACE	PAI-1
C7 deficiency		

Bohé J. Clin Infect Dis 2005



Perspectives and Conclusions

⇒Screening of a high number of polymorphisms in large cohorte

➔ Effect of association of polymorphisms ?

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

➔ Genetic profiling → Individual risk assessment

→ Prevention, Vaccination

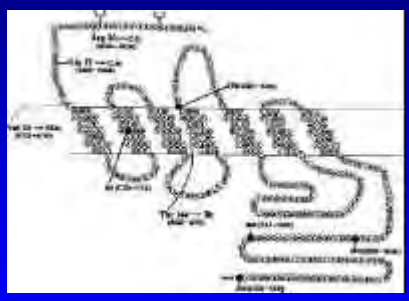
→ To tailor prescriptions to each patient

þ 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.

Perspectives and Conclusions

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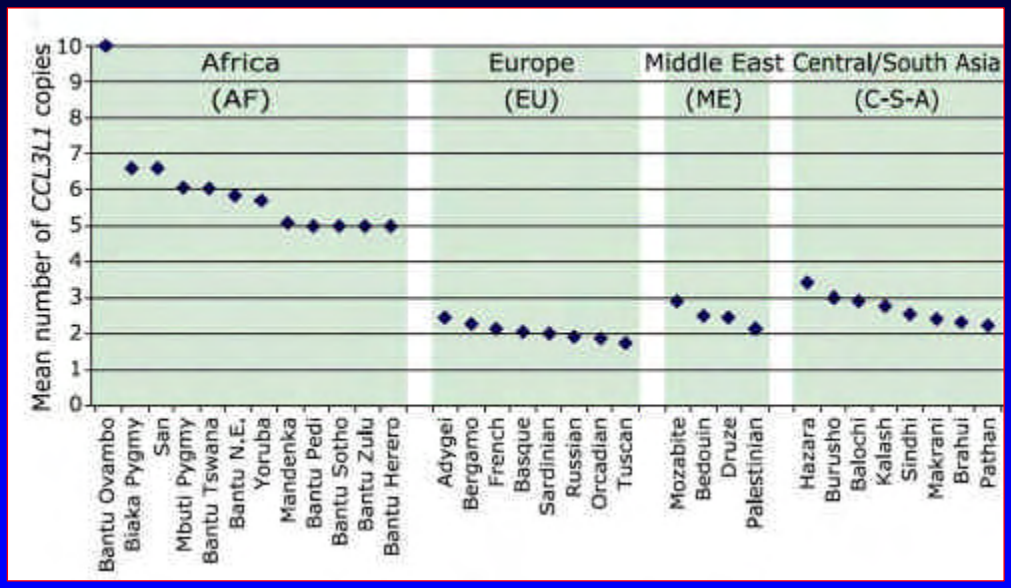
Þ **SNPs of Pathogen Recognition Receptors leading to Host advantage**

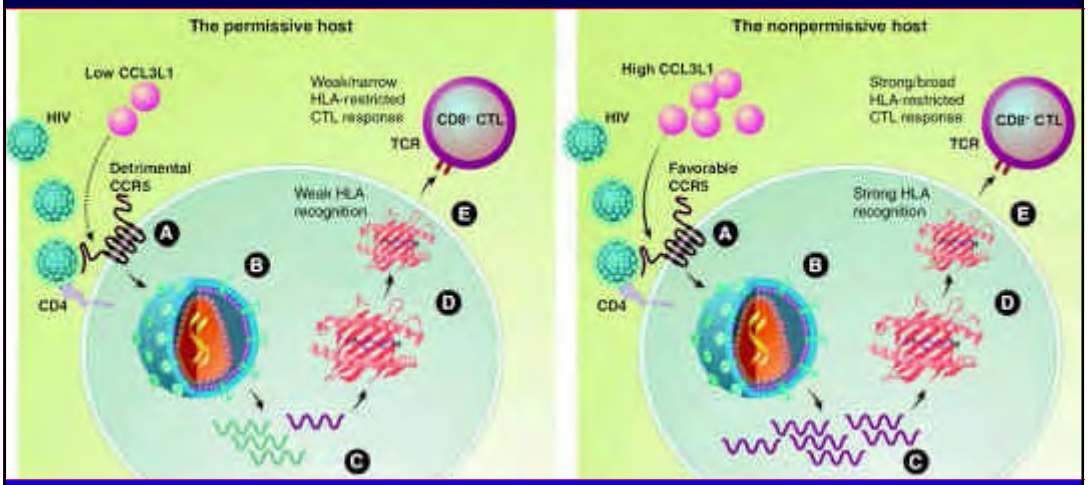
- **TLR4 D299G protects from Legionnaire's disease** (Hawn TR., PNAS 2005)
- **SNPs from TLR4, CD14, or MBL are associated with low atherosclerosis risk**
- **CCR5 and CCL3L1genotypes and HIV infection**

CCL3L1 Genotypes and Ethnies



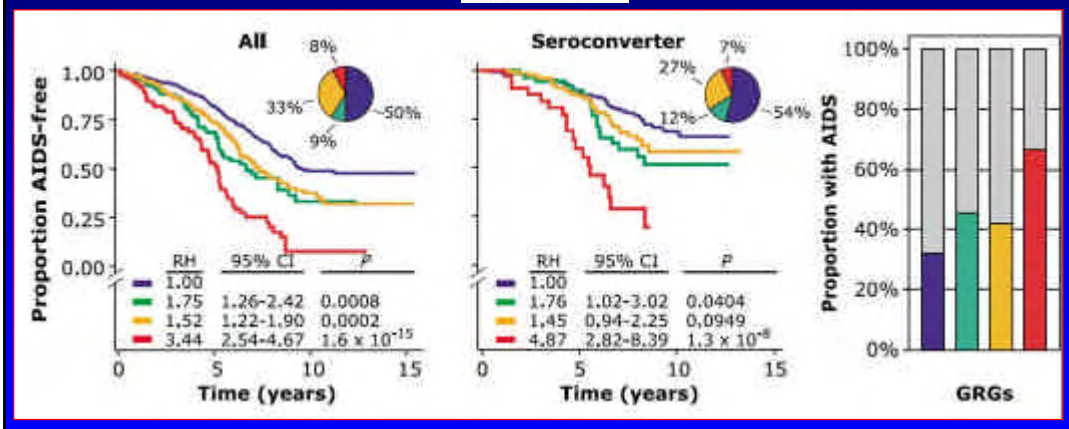
Gonzales E, Science 2005;307:1434

Permissive and Non-Permissive Human Hosts for HIV

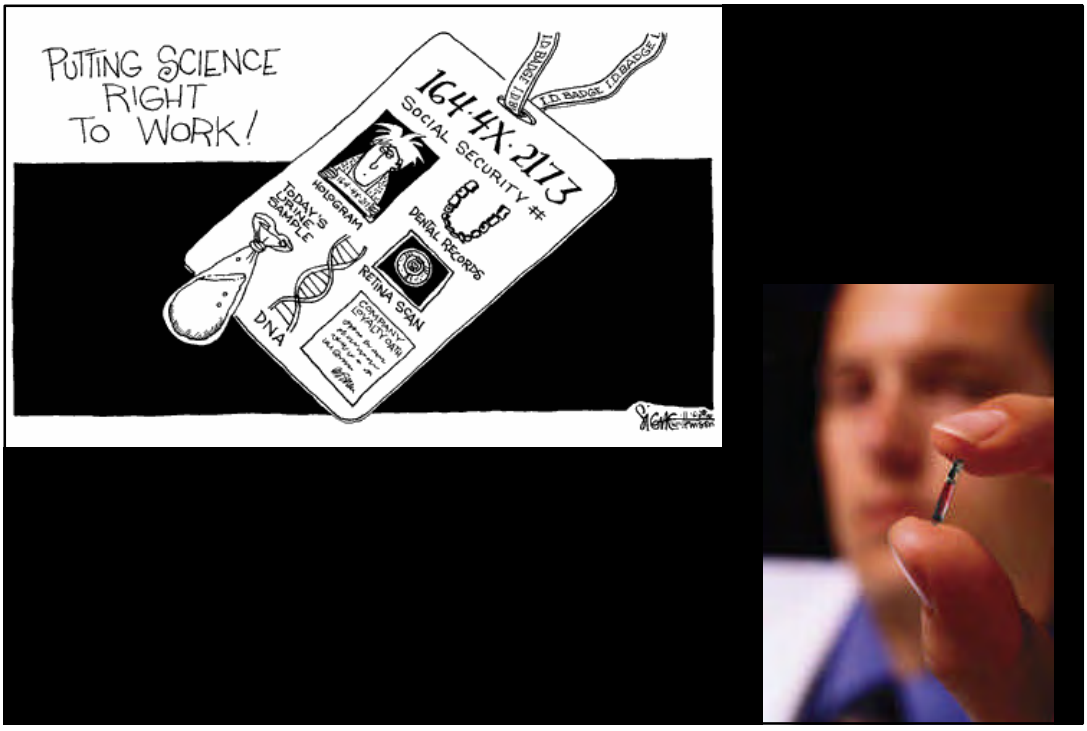


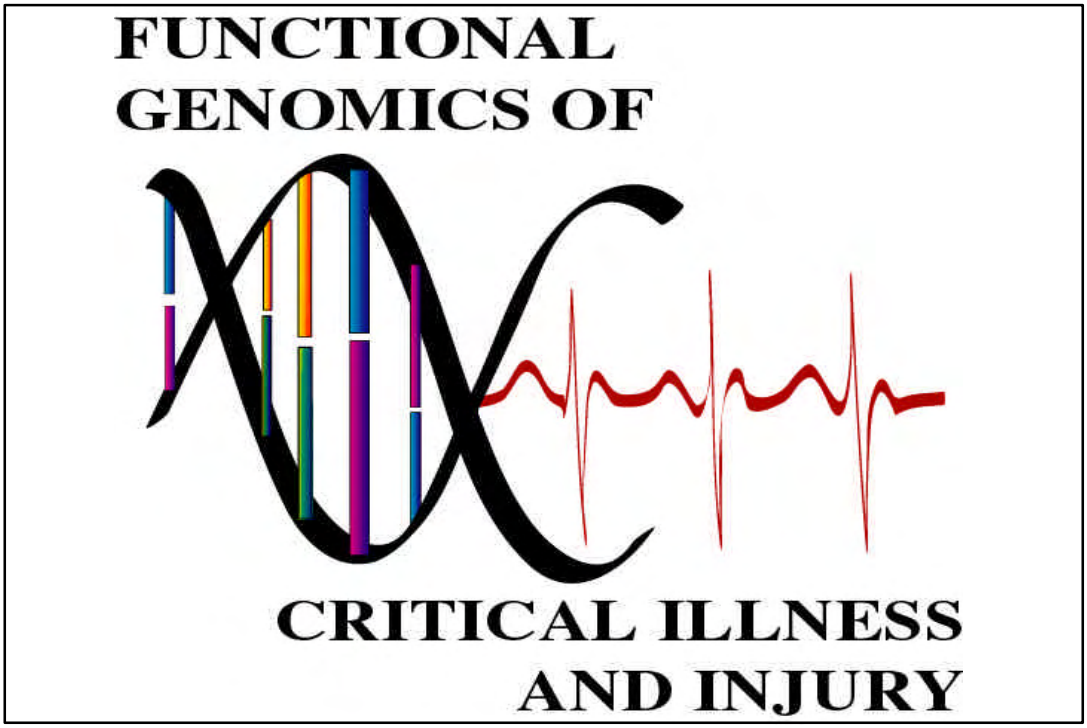
Nolan D., Science 2005;307:1422

CCL3L1 and CCR5 Phenotypes and HIV Risk



Gonzales E, Science 2005;307:1434




FUNCTIONAL
GENOMICS OF
CRITICAL ILLNESS
AND INJURY