Genetic polymorphisms that modulate cardiovascular injury & function

It's your genes ….. Or is it?

Meredith Allen
Consultant Intensive Care
Royal Brompton Hospital, London
&
Critical Care Group, Institute of Child Health, London

m.allen@rbht.nhs.uk

Why look at genes?

Small variations in genotype can make big differences to phenotype (1.24%)

Why look at genes?

(i) We think we know what biological mediators are playing a role in cardiac dysfunction – *use genetics to confirm this*

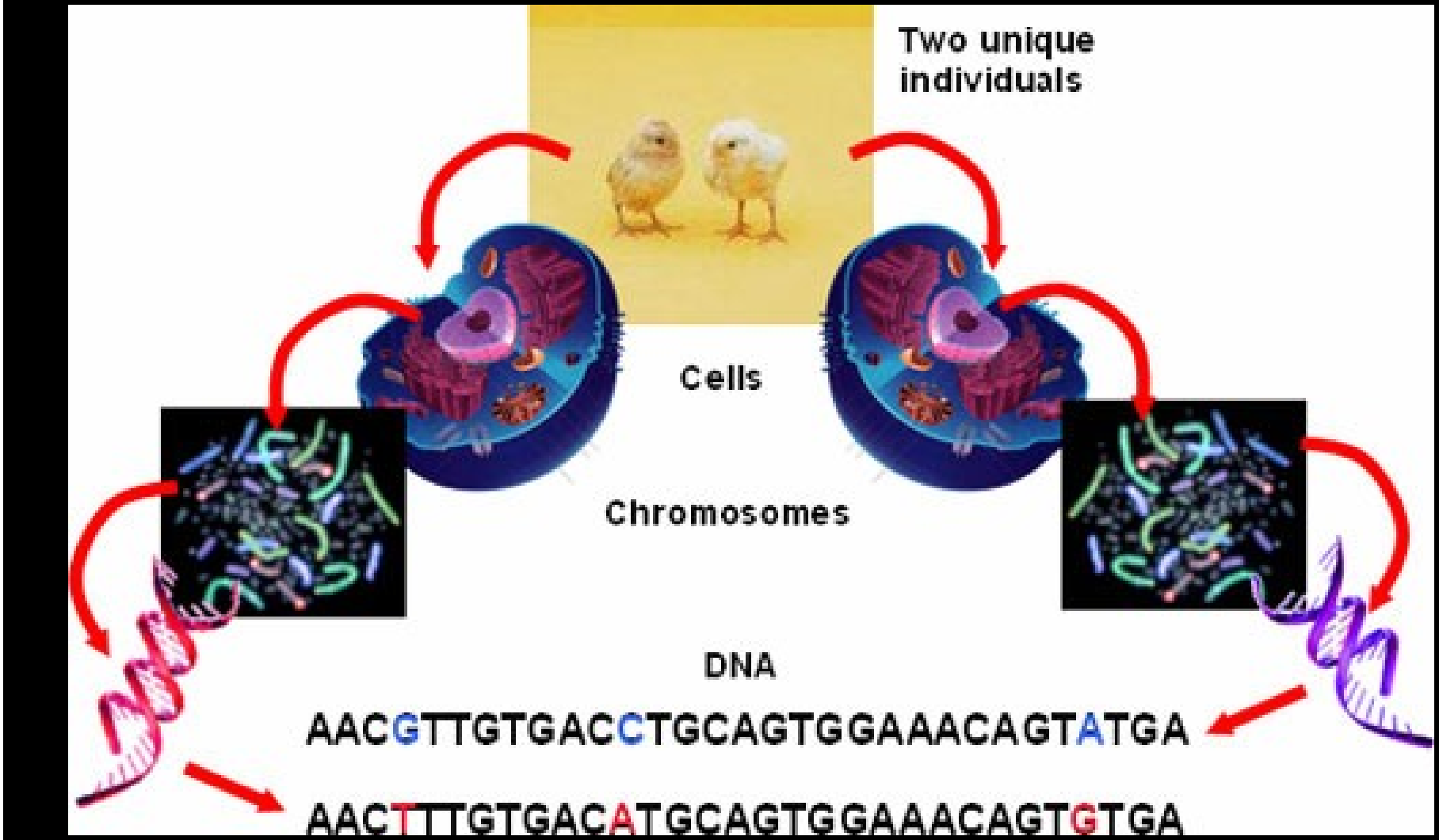
Single Nucleotide Polymorphisms

(ii) We don't really understand what's going on & we want help as to where to look – *use genetics to give us clues*

Genome wide association studies


AP

Using SNP's to help confirm the important biological mediators


Two unique individuals
Cells
Chromosomes
DNA
AACGTTGTGACCTGCAGTGGAAACAGTATGA
AACTTTGTGACATGCAGTGGAAACAGTGTGA

HapMap Project

Why look at genes?

- unchanged through life
- not altered by disease itself or body's response to the disease
- enable more accurate determination of an individual's risk than a clinical measure (not susceptible to biological fluctuations or measurement error)

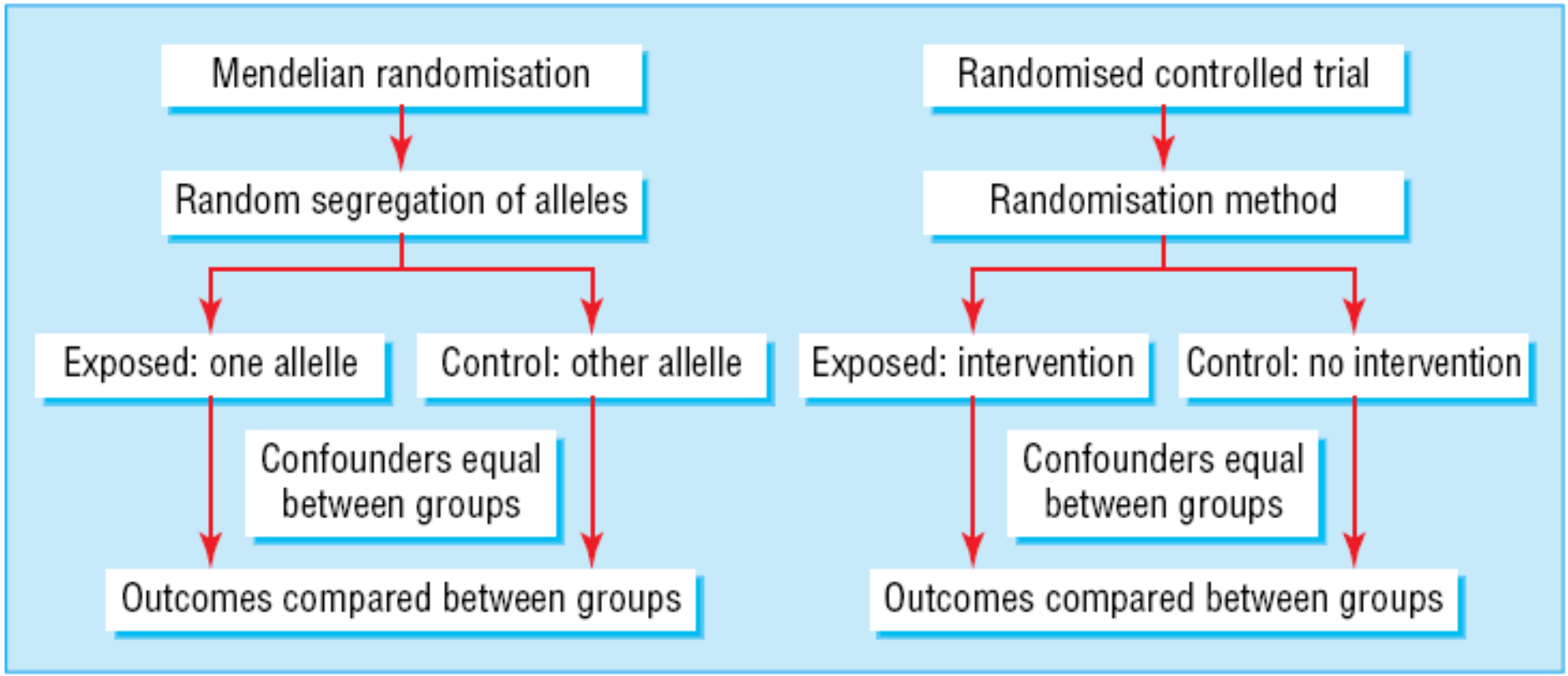
If we think we know what biological processes are involved in cardiac injury / dysfunction

known gene variant that is associated with high or low producers

comparing outcomes between genetic groups

Identify biological processes that are truly important / worth targeting

Mendelian Randomisation



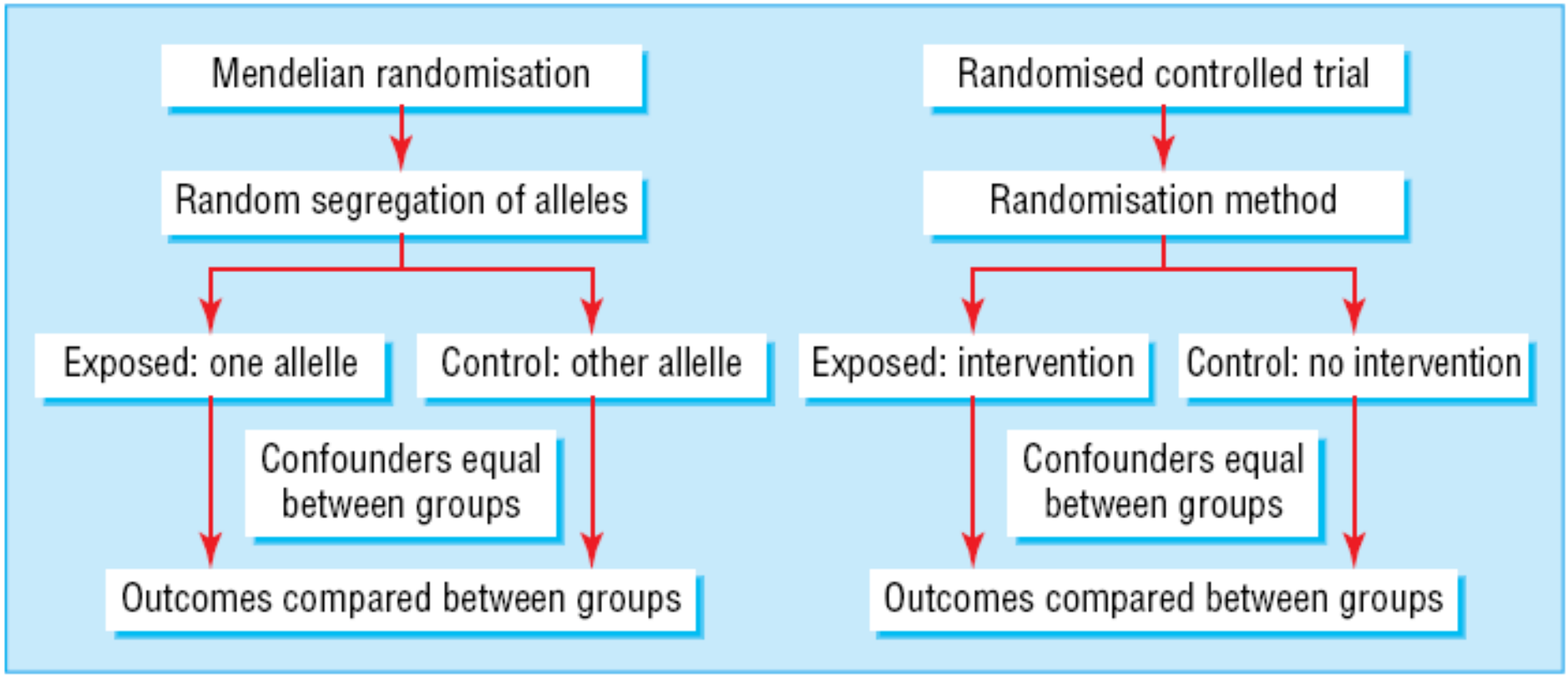
Davey Smith et al. BMJ 2005

Mendelian Randomisation

Example

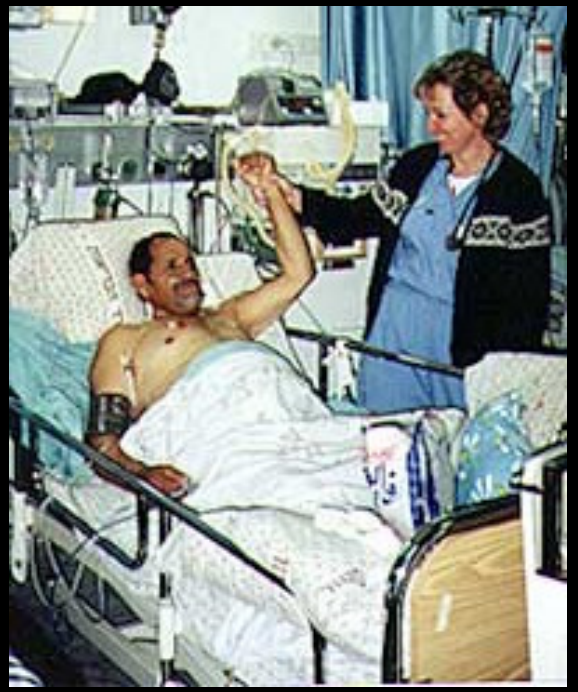
Does cholesterol play a role in coronary artery disease?

Mendelian Randomisation

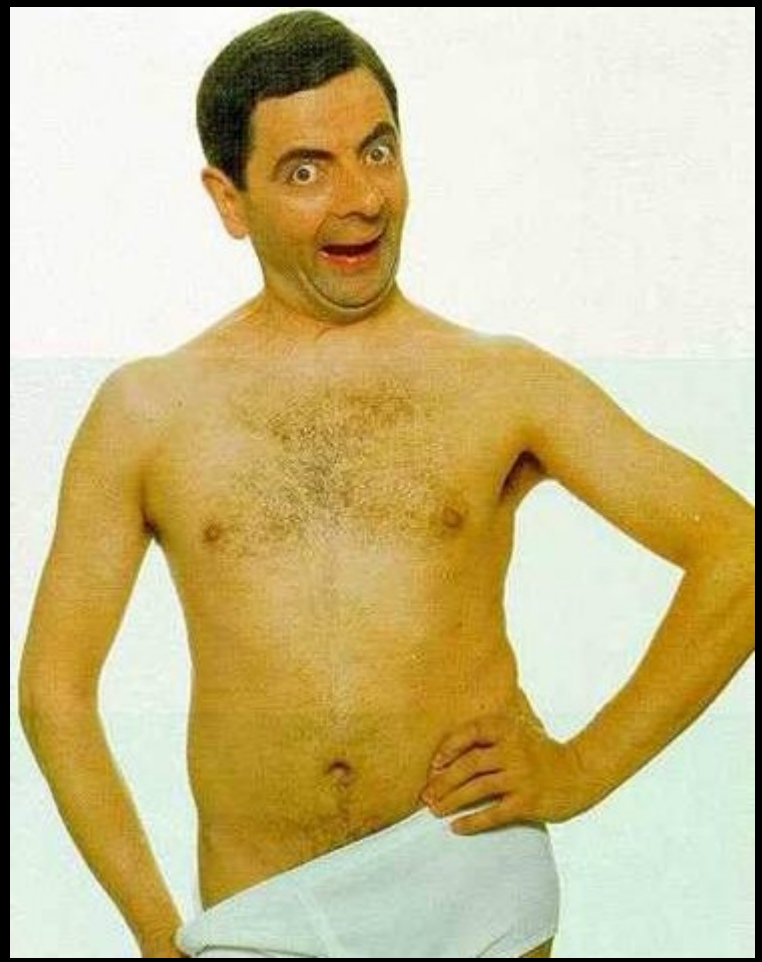


Davey Smith et al. BMJ 2005

What do we know?



Gender differences



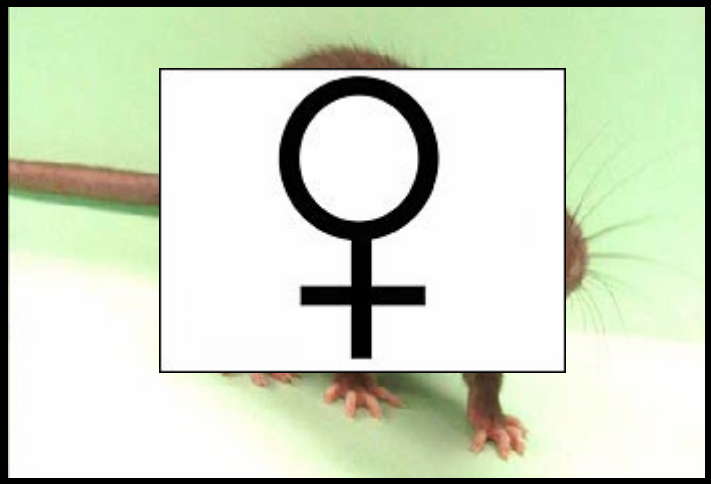
Lifetime Risk of CHD From Framingham Heart Study

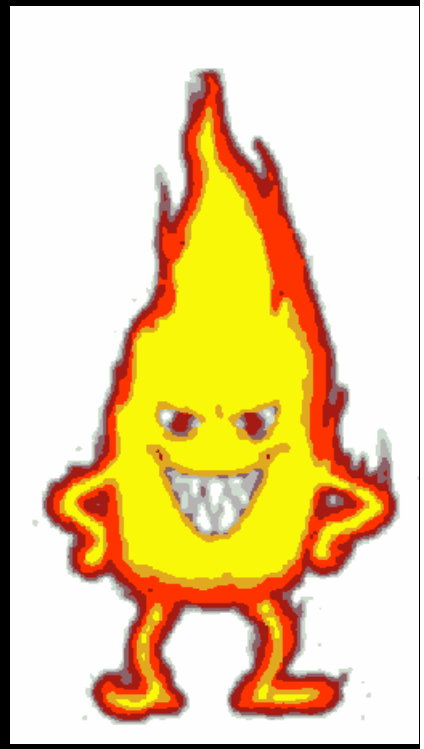
The lifetime risk of developing CHD for 40-year-olds was
1 in 2 for men (RR 48.6% 95% CI 45.8%-51.3%)
and 1 in 3 for women (RR 31.7% 95% CI 29.2%-34.2%)

? Oestrogen Effect

oestrogen

Protected from I/R injury

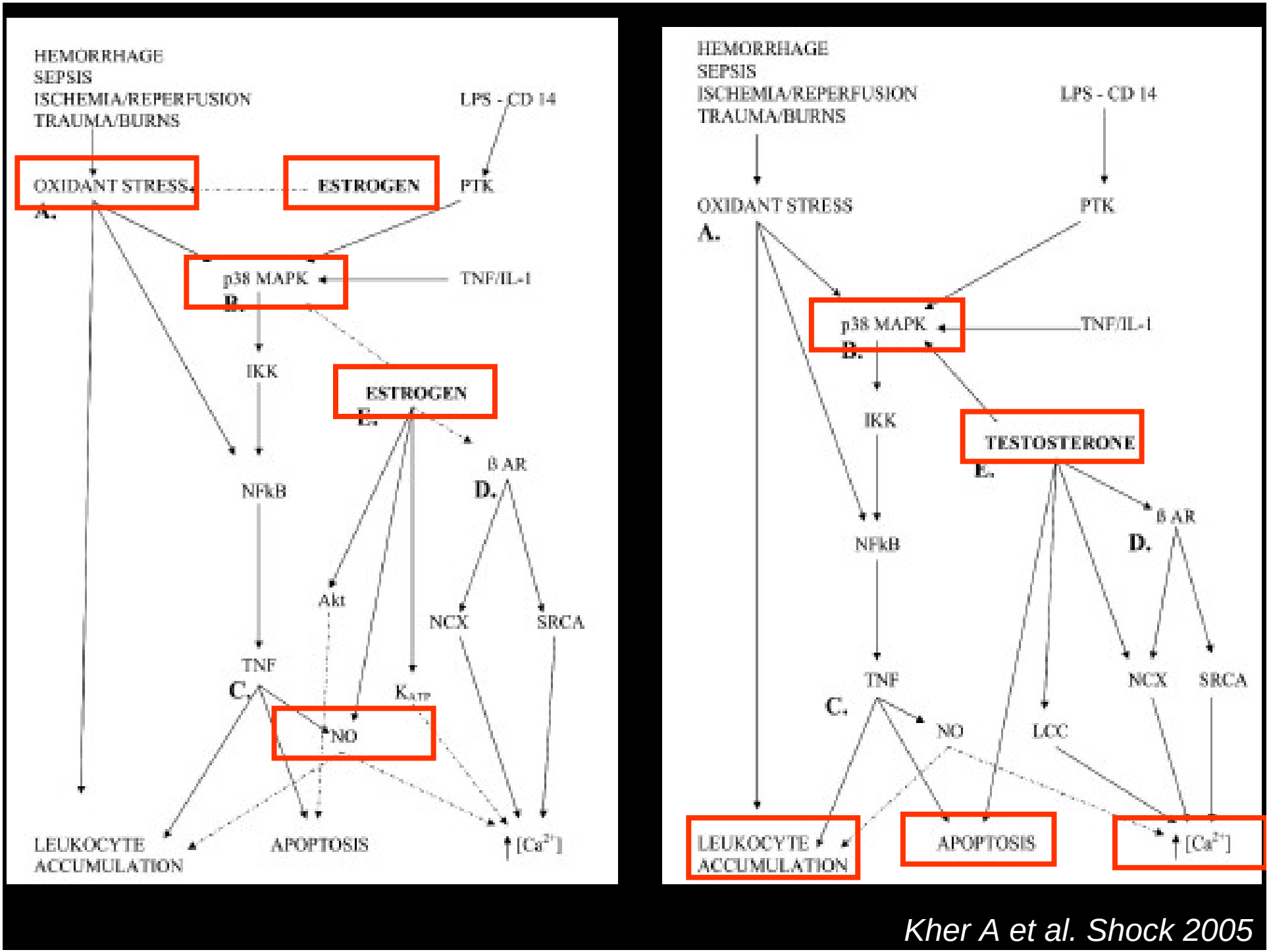




? cytokine production injury

? cardiac dysfunction

protected against acute injury



Kher A et al. Shock 2005

Editorials

Gender differences in pediatric cardiac surgery: The surgeon's perspective

Anthony Azakie, MD, CM[a]
Isobel A. Russell, MD, PhD, FACC[b]

cardiologist's perspective

Wanda C. Miller-Hance, MD[a]
Theresa A. Tacy, MD[b]

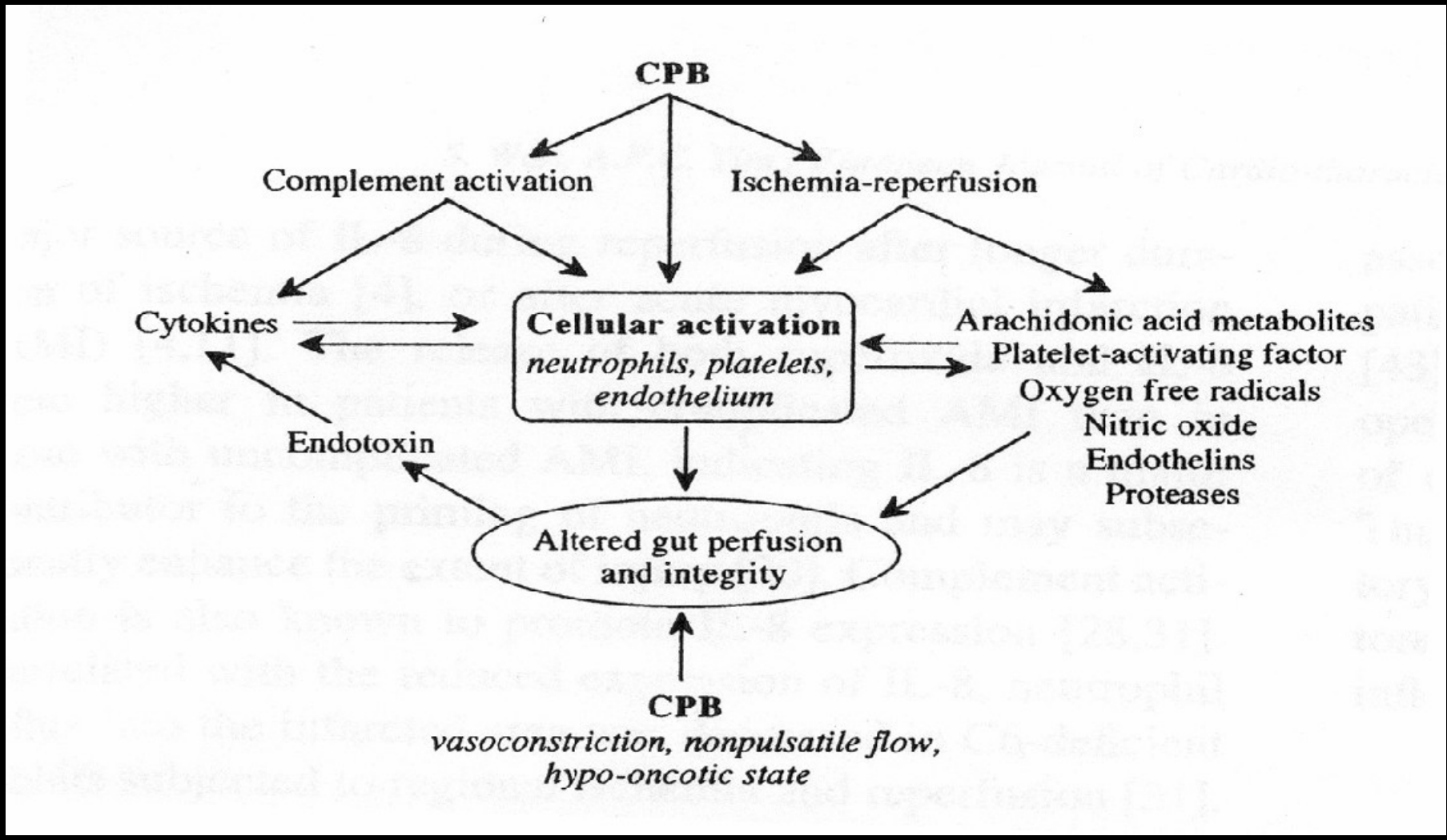
regression analysis demonstrated that female patients had a significantly higher odds ratio for mortality than male patients (odds ratio, 1.51; $P < .01$).

JTCS 2004

Where do we look?

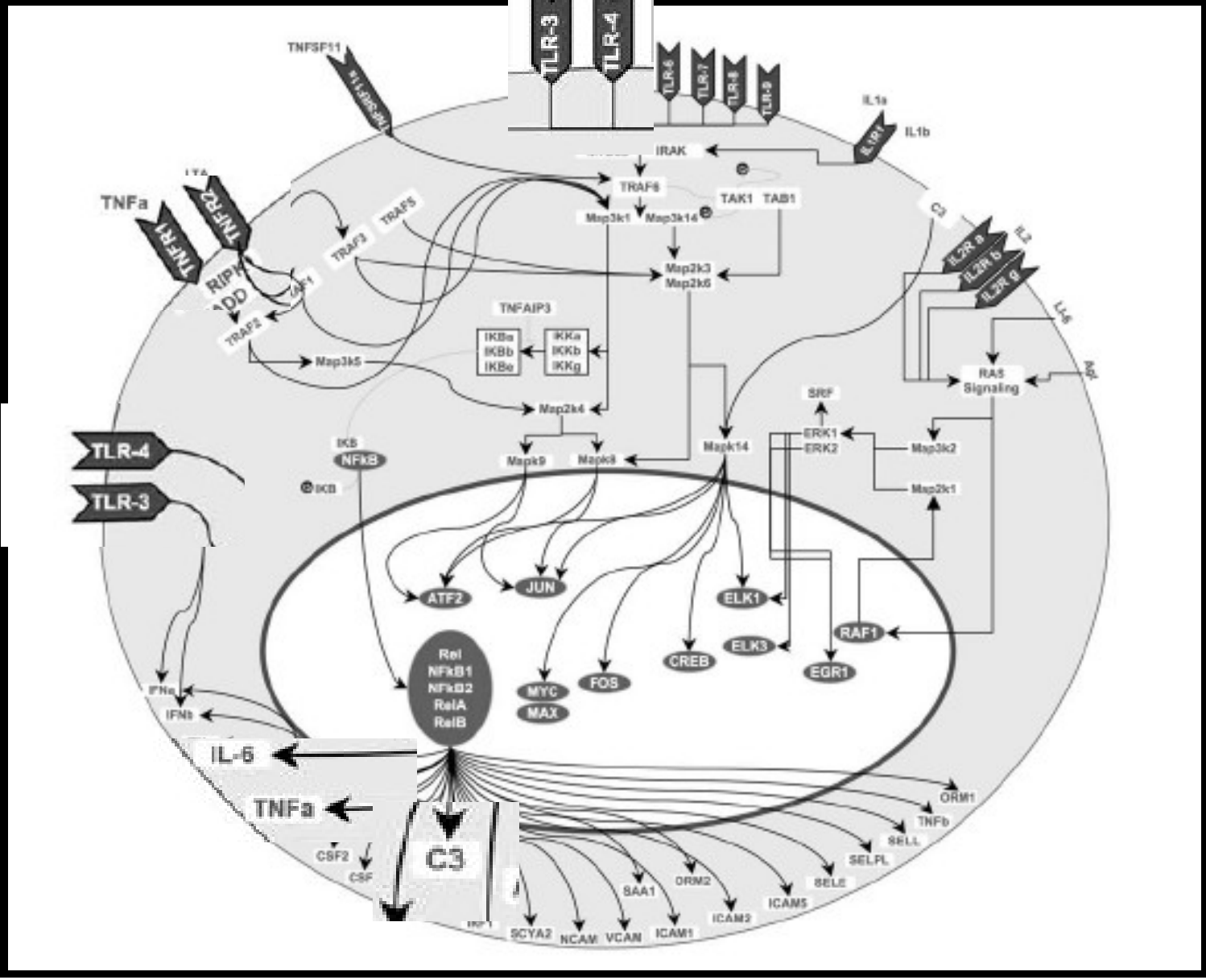
Under '*extreme stress*' what problems do our patients face?

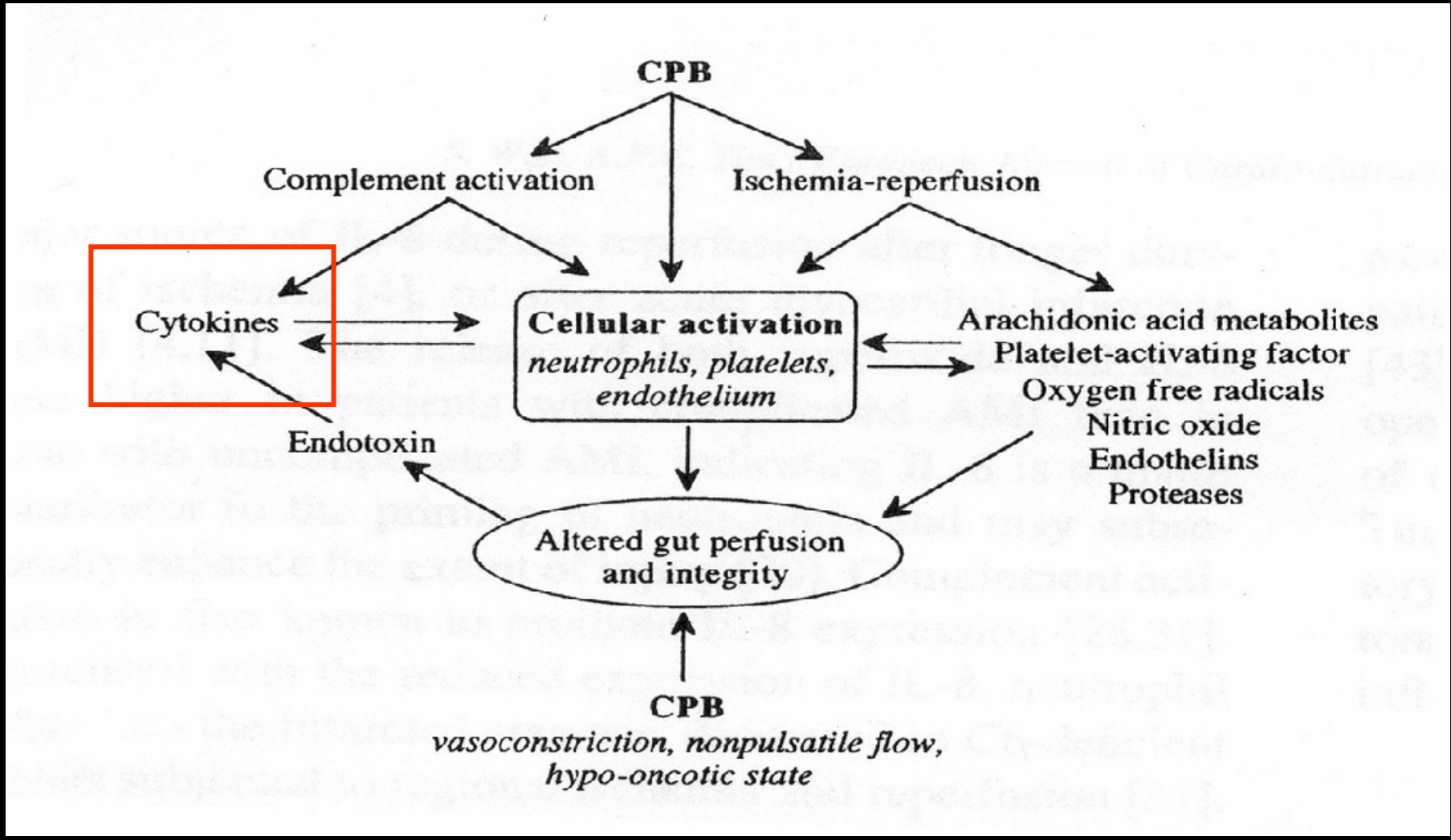
- LV dysfunction
- endothelial leak
- coagulation disturbances
- nosocomial infection



Wan S et al 1999

Plausible candidate genes

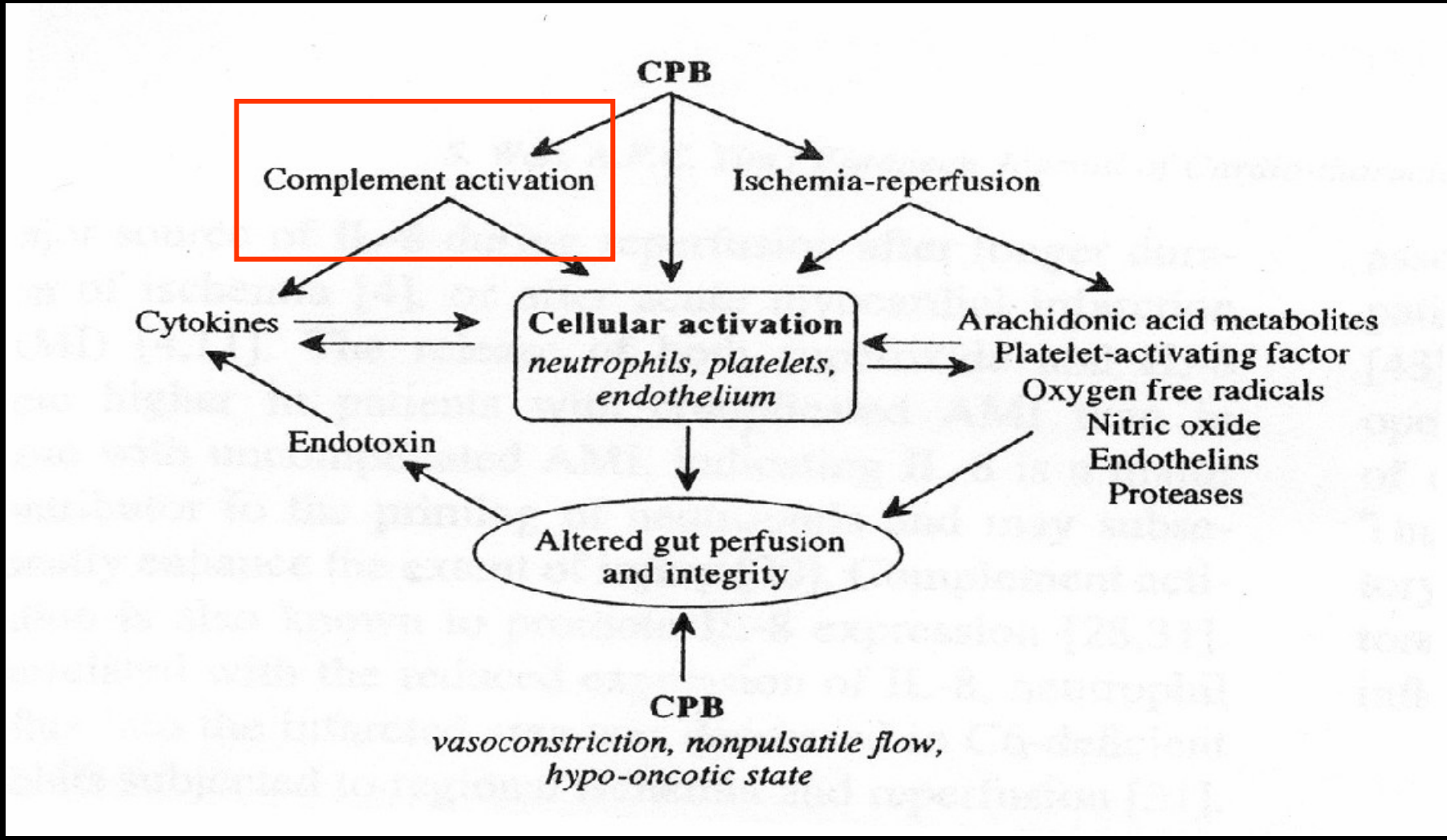




Wan S et al 1999

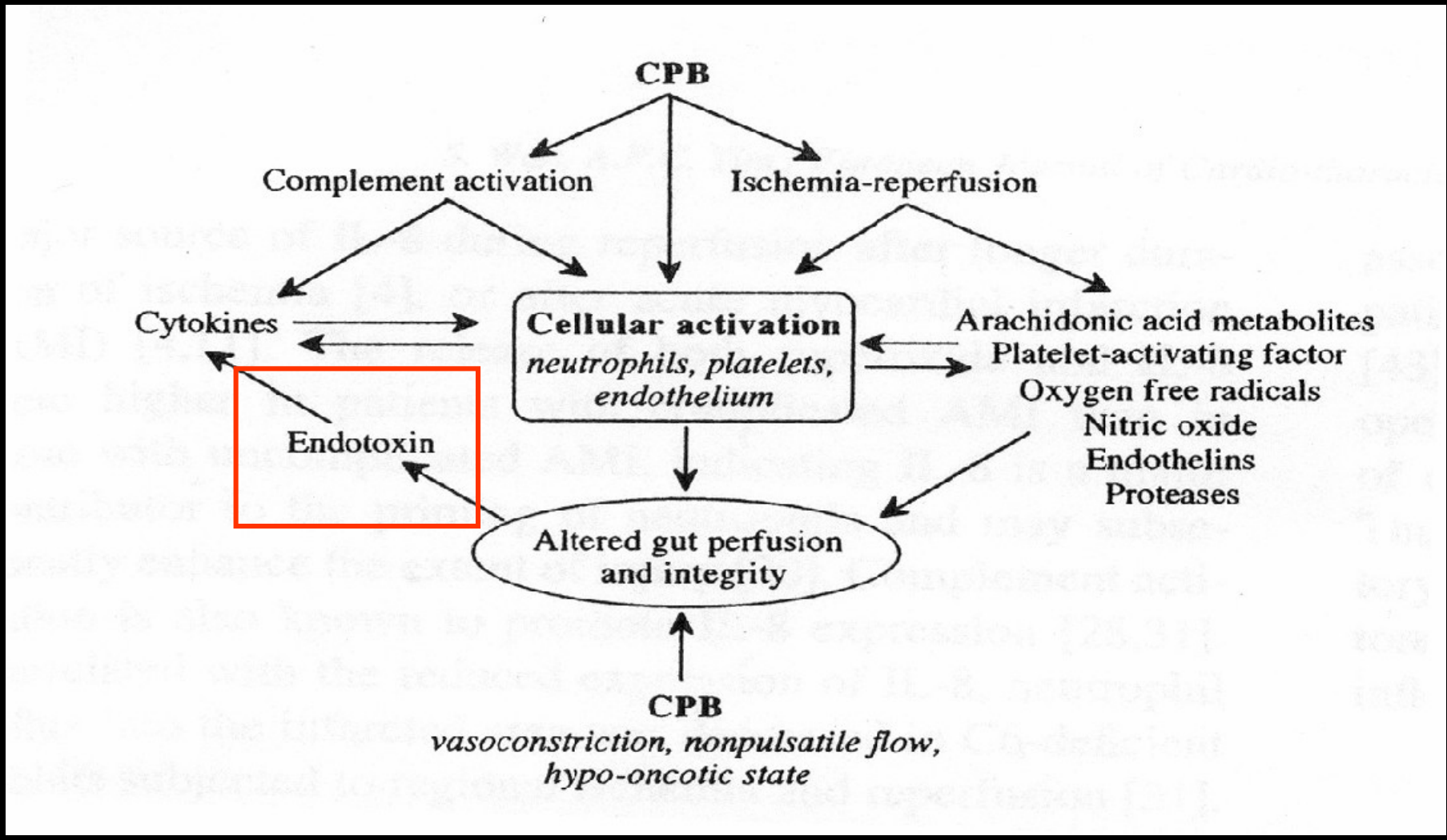
Cytokine	Biological effects	Hypothesis	Study design	Outcome association
TNF-? (-308 G/A) (TGFB2 allele)	Inflammation, Post-pump syndrome, Myocardial dysfunction	Increased risk of MI or CAD	# Pop assoc adult studies >2000	? LV dysfn [OR 3.84] **No effect** LOS or mortality *(Tomasdottir et al 2006)* *?***ventilation time** *(Yende et al 2003)*
IL-6 (-174 G/C) (-572 G/C)	Inflammation, Myocardial dysfunction	Increased inflammat^{n}	# Pop assoc adult studies >2000	No assoc with CV risk factors, CV fn, or MI *(Lieb W et al 2004)* Prolonged hospital stay *(Burzotta et al 2001)* Risk of postop MI *(Podgoreanu et al 2006)* Early coronary disease in Tx pts

Cytokine	Biological effects	Hypothesis	Study design	Outcome association
IL-10 (-1082 G/A), (-819 C/T), (-592 A/C)	?-inflammatory suppresses pro-inflammation	Low producers ? pro-inflam^{n} unchecked	148 Tx donors & recipients	Not assoc with cardiac transplant rejection *(Plaza et al 2003, Densem et al 2003, Bijlsma et al 2001)*
TGF-ß	Endothelium, collagen, profibrotic, ?-inflammatory	Candidate gene for arterial stiffness	>3500 adult population based study	Nil *(Sie et al 2007)*



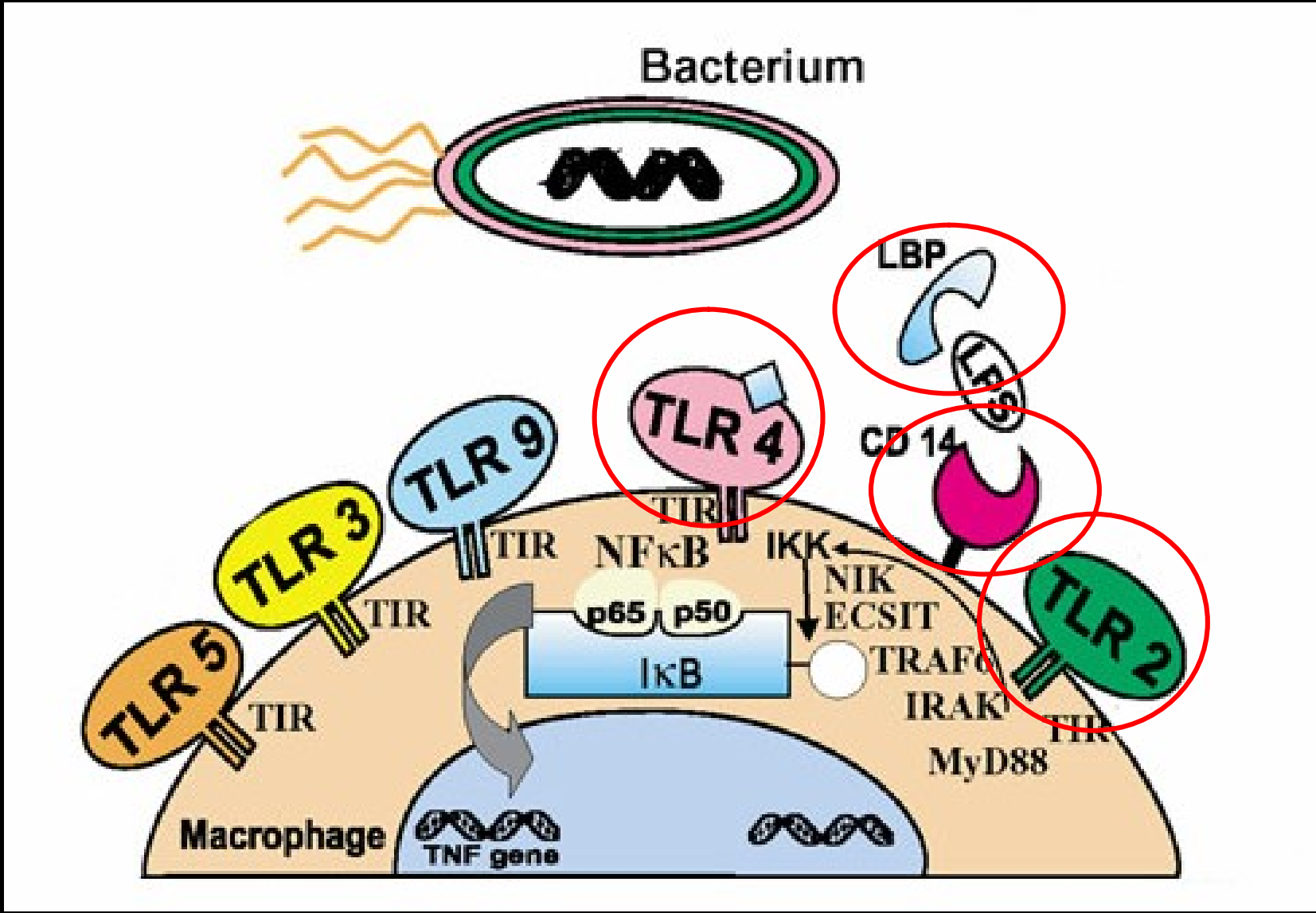
Wan S et al 1999

C' component	Biological effects	Hypothesis	Study design	Outcome association
C4 (C4A isotype)	Activated by classic & lectin pathway Null allele ? ? circ levels of C4a & C3a	Increased risk of SIRS	156 paed CPB pts 116 C4A def pts	Homozygous C4A null allele (n=7) **?capillary leak** (p<0.01) *(Zhang S et al 2004)* RCT of C4A def children to C4A-rich plasma in CPB prime ? SIRS (biochem & clinical) *(Zhang Lancet 2005)*
MBL	MBL def assoc with ? infect risk	Infection plays a role in atherosclerosis ? MBL def assoc ?atherosclerosis	434 adults CAD	MBL def haplotype assoc with OR 3.2 [1.5-7] after adjusting for other risk factors *(Best et al 2004)* Prob co-factor in development of atherosclerosis

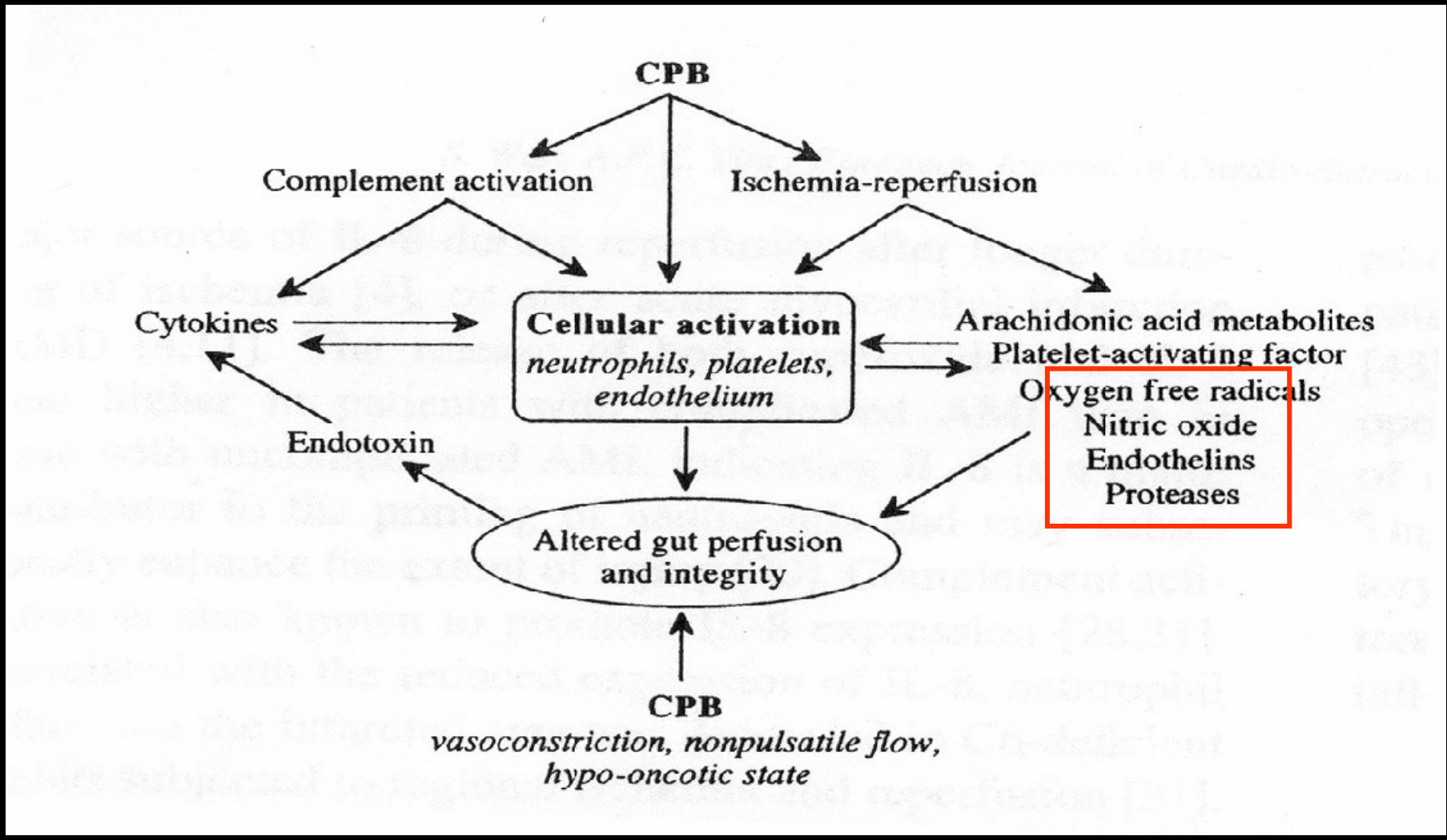


Wan S et al 1999

Pattern Recognition Receptors



PRR Receptor	Biological effects	Hypothesis	Study design	Outcome association
CD14	Co-receptor with Toll's for bact products	Inflam^{n} & infection play a role in atheroma Endothelial & sm muscle cells activated by sol^{b} CD14	>3000 adult pts in total	Nil *(Unckelbach et al 1999, Zee et al 2001)* ? proinflammatory gene expression in plaque *(Giacconi et al 2006)* ? risk of acute cardiovascular event *(Andreotti et al 2002, Arroyo-Espliguero et al 2005)*
TLR	Important in innate immune response to pathogens	May mediate inflammation in non-infectious injury	Mice 657 men	Knock-out mouse has ? LV dysfn following I-RP *(Favre et al 2007)* Toll 4 (-299 A/G) not assoc with atherosclerosis *(Hernesniemi et al 2007)*
LBP (-326 T/C)	Endotoxin binding protein Polymorphism assoc with variable circ levels & sepsis	endotoxin peaks 4-24hrs post CPB may play a role in myocardial injury	adult	No ? incidence of freq^{y} in MI pts *(Hubacek et al 2002)* may be associated with post CPB morbidity



Wan S et al 1999

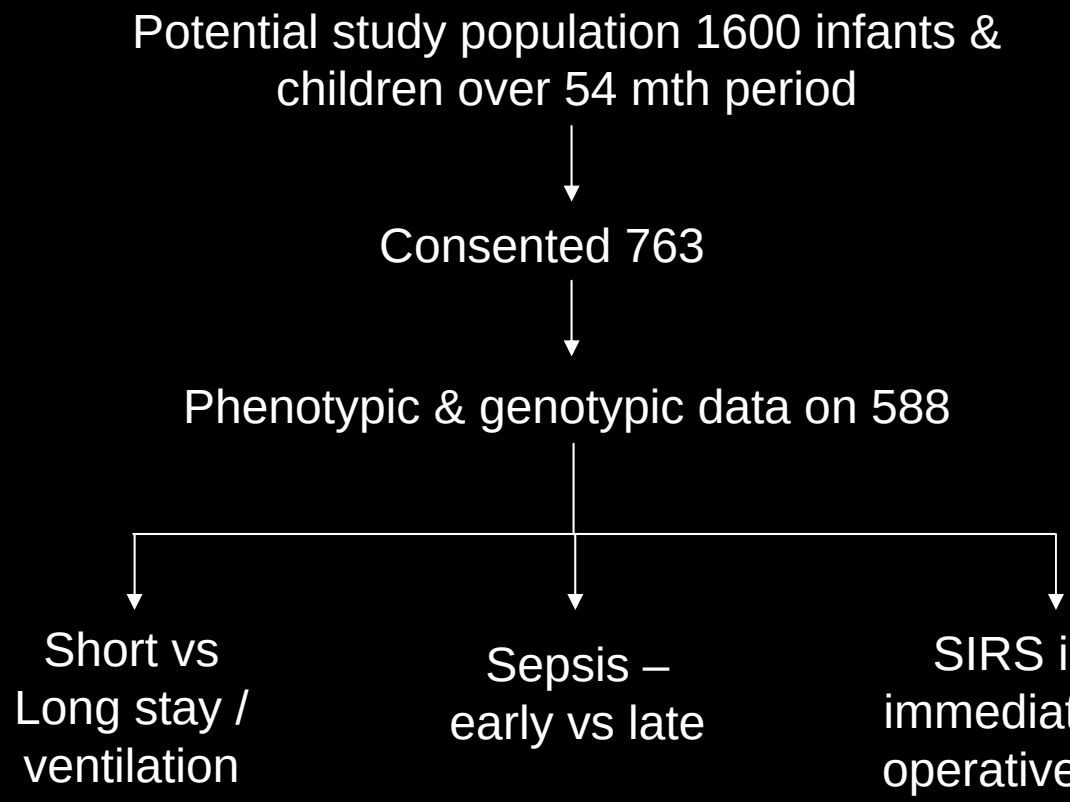
Other Polymorphisms

	Biological effects	Hypothesis	Study design	Outcome association
eNOS (-894 G/T)	Constitutively expressed	Exaggerated vasoconstrictive response	105 adult CABG pts	No difference in CI, SVRI, PVRI *(Liakopoulos et al 2006)*
ACE	ability of mitochondria to fn in anaerobic conditions	Improved outcome in critical illness	2711 healthy males over 15yrs	Currently under debate No clear assoc with CHD Probably a modifier gene *(Muthumala et al 2007)*
CRP	Pro-inflammaty effect Pro-atherosclerotic	High CRP producers will be pro-atherogenic ? infact size	Mendelian randomisation 4659 men	High producers not assocn with risk May be a co-factor *(Casas JP et al 2006)*

Thus far….

genetic association studies plagued by:

- inconsistency
- lack of reproducibility
- generally small sample sizes



Unfortunately no direct measure of cardiac function

What does predict outcome in our patients?

	Length of Stay (correlation co-efficient [95%CI])	**Sepsis** (OR [95%CI]	**SIRS (in the 1st 72 hours)** (OR [95%CI]
Age	-0.10 [-0.13 - -0.08] *p*<0.001	0.82 [0.71 – 0.95] *p*=0.008	1.11 [1.06 – 1.16] *p*<0.001
Male	0.15 [-0.06 - 0.35] *p*<0.16	0.86 [0.46 – 1.597] *p*=0.62	1.27 [0.84 – 1.94] *p*=0.26
CPB time	0.01 [0.007 - 0.01] *p*<0.001	1.01 [1.00 – 1.013] *P*<0.001	1.0 [1.000 – 1.007] *P*=0.049
X-clamp time	0.01 [0.01 – 0.014] *p*<0.001	1.01 [1.00 – 1.015] *p*=0.02	1.0 [0.99 – 1.005] *p*=0.91
RACHS-1 classification	0.56 [0.48 - 0.65] *p*<0.001	1.56 [1.22 – 2.01] *p*<0.001	0.90 [0.74 – 1. 11] *p*=0.33
PIM II score (risk of mortality)	0.04 [0.03 - 0.05] *p*<0.001	1.00 [0.98 – 1.04] *p*=0.57	0.97 [0.93 – 1.01] *p*=0.17
Genetic profile	?	?	?

What does predict outcome in our patients?

	Length of Stay Duration of vent (OR [95%CI]	Sepsis (OR [95%CI]	SIRS (in the 1st 72 hours) (OR [95%CI]
High TNF-? or IL-6 producers (TNF-?)			

What does predict outcome in our patients?

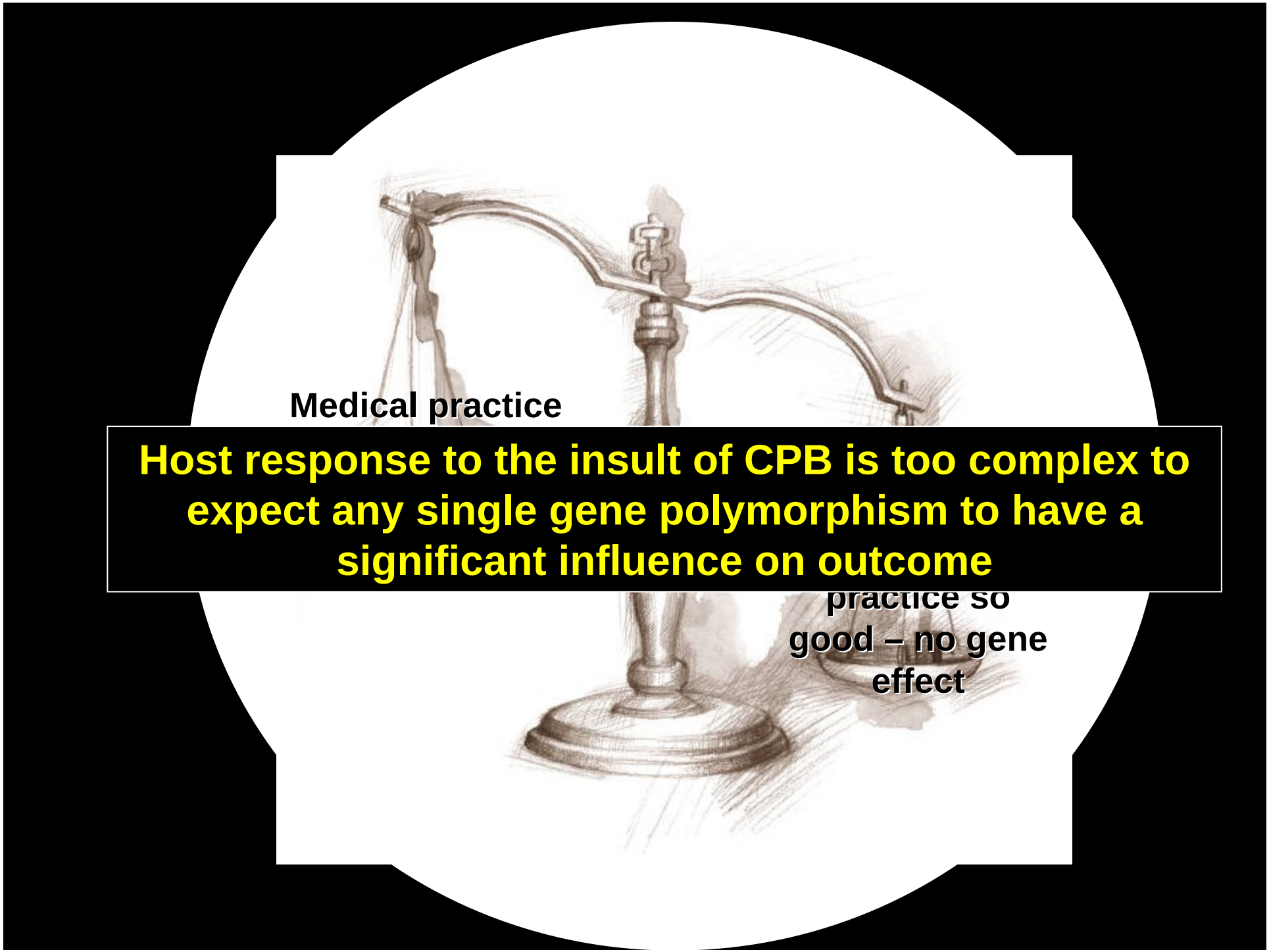
	Length of Stay (OR [95%CI]	Sepsis (OR [95%CI]	SIRS (in the 1st 72 hours) (OR [95%CI]
High TNF-? or IL-6 producers (TNF-?)	no effect 0.78 [0.5-1.2] *p*=0.16	no effect 0.63 [0.3-1.3] *p*=0.15	no effect 0.76 [0.4-1.2] *p*=0.16
High TNF-? / Low IL-10 producers (n=109 vs 80)	no effect	no effect	no effect
Complement variants (MBL)	no effect 0.98 [0.66-1.45] *p*=0.50	no effect 1.55 [0.84 – 2.87] *p*=0.106	no effect 0.68 [0.44-1.07] *p*=0.06
PRR haplotype variants (Toll 4)	no effect 0.77 [0.4-1.5] *p*=0.26	no effect 0.78 [0.3-2.3] *p*=0.44	no effect 0.85 [0.4-1.7] *p*=0.39

In the current era of PICU,

genetic effects, whilst inevitably present, are not detectable

Not useful for bedside risk stratification at present

'I'm sorry. You've tested positive for the naughty gene.'


Medical practice
Host response to the insult of CPB is too complex to expect any single gene polymorphism to have a significant influence on outcome
practice so
good – no gene
effect

Maybe we are looking at the wrong genes… or need to look at gene combinations

Role of genome wide association studies

Genome-wide association studies

High through-put testing involving looking at DNA blocks for variants associated with disease

"pattern recognition"

- requires '000's of pts & controls

Possible now because we have the statistical & bioinformatics infrastructure to support


GeneChip®
Human Mapping 50K Hind 240
GeneChip®
Human Mapping 50K Xba 240
For Research Use Only


deCODE genetics
PRODUCT PIPELINE
FROM GENES TO DRUGS
COMPANY
INVESTORS
SERVICES

Genome-wide association studies

May identify a region of interest but we will still need to understand & apply the biology ….

Conclusion

- Given complexity of biology – unlikely for single gene SNP to have *signif* effect
- SNP's may play a role in life-time risk models for CV disease
- Need to be aware of the genomic & proteomic research because success will rely on large collaborative projects

‘Your genes say ‘Man Flu’. But I’m not so sure’