Genetic polymorphisms that modulate cardiovascular injury & function

It’s your genes ..... Or is it?

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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*
Using SNP’s to help confirm the important biological mediators
Two unique individuals

Cells

Chromosomes

DNA

AACGTGTGACCTGCAGTGGAACACGATATGA

AACCTTTGTGACATGCAGTGGAACACGATGATGA
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

![Diagram showing Mendelian randomisation and randomised controlled trial](image)

Davey Smith et al. BMJ 2005
Mendelian Randomisation

Example

*Does cholesterol play a role in coronary artery disease?*
Mendelian Randomisation

Mendelian randomisation

Random segregation of alleles

Exposed: one allele
Control: other allele

Confounders equal between groups

Outcomes compared between groups

Randomised controlled trial

Randomisation method

Exposed: intervention
Control: no intervention

Confounders equal between groups

Outcomes compared between groups

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
Editorials

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

Anthony Azakie, MD, CMa
Isabel A. Russell, MD, PhD, FACCb

cardiologist’s perspective

Wanda C. Miller-Hance, MDa
Theresa A. Tacy, MDb

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

Diagram:

- CPB
  - Complement activation
  - Ischemia-reperfusion

- Cytokines
- Endotoxin

- Cellular activation
  - neutrophils, platelets, endothelium

- Arachidonic acid metabolites
  - Platelet-activating factor
  - Oxygen free radicals
  - Nitric oxide
  - Endothelins
  - Proteases

- Altered gut perfusion and integrity

- CPB
  - vasoconstriction, nonpulsatile flow, hypo-oncotic state
Plausible candidate genes
Wan S et al 1999

Diagram showing the relationships between CPB, complement activation, ischemia-reperfusion, cellular activation of neutrophils, platelets, and endothelium, and various factors like cytokines, endotoxin, arachidonic acid metabolites, platelet-activating factor, oxygen free radicals, nitric oxide, endothelins, proteases, altered gut perfusion and integrity, and CPB-related vasoconstriction, nonpulsatile flow, and hypo-oncotic state.
<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Biological effects</th>
<th>Hypothesis</th>
<th>Study design</th>
<th>Outcome association</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-? (-308 G/A) (TGFB2 allele)</td>
<td>Inflammation, Post-pump syndrome, Myocardial dysfunction</td>
<td>Increased risk of MI or CAD</td>
<td># Pop assoc adult studies &gt;2000</td>
<td>LV dysfn [OR 3.84]</td>
</tr>
<tr>
<td>IL-6 (-174 G/C) (-572 G/C)</td>
<td>Inflammation, Myocardial dysfunction</td>
<td>Increased inflammation</td>
<td># Pop assoc adult studies &gt;2000</td>
<td>No assoc with CV risk factors, CV fn, or MI (Lieb W et al 2004)</td>
</tr>
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</table>

**No effect** LOS or mortality (Tomasdottir et al 2006)

**Ventilation time** (Yende et al 2003)

Prolonged hospital stay (Burzotta et al 2001)

Risk of postop MI (Podgoreanu et al 2006)

Early coronary disease in Tx pts
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<tr>
<td><strong>IL-10</strong></td>
<td>- inflammatory</td>
<td>Low producers ?</td>
<td>148 Tx donors &amp; recipients</td>
<td>Not assoc with cardiac transplant rejection</td>
</tr>
<tr>
<td>(-1082 G/A),</td>
<td>supresses pro-inflammation</td>
<td>pro-inflammation unchecked</td>
<td></td>
<td>(Plaza et al 2003, Densem et al 2003, Bijlsma et al 2001)</td>
</tr>
<tr>
<td>(-819 C/T),</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-592 A/C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TGF-β</strong></td>
<td>Endothelium, collagen, profibrotic, ?-inflammatory</td>
<td>Candidate gene for arterial</td>
<td>&gt;3500 adult population based study</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stiffness</td>
<td></td>
<td>(Sie et al 2007)</td>
</tr>
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Wan S et al 1999

Complement activation

Ischemia-reperfusion

Cytokines

Endotoxin

Cellular activation
neutrophils, platelets, endothelium

Arachidonic acid metabolites
Platelet-activating factor
Oxygen free radicals
Nitric oxide
Endothelins
Proteases

Altered gut perfusion and integrity

CPB

vasoconstriction, nonpulsatile flow, hypo-oncotic state
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<tr>
<td><strong>C4</strong> (C4A isotype)</td>
<td>Activated by classic &amp; lectin pathway Null allele? ? circ levels of C4a &amp; C3a</td>
<td>Increased risk of SIRS</td>
<td>156 paed CPB pts 116 C4A def pts</td>
<td>Homozygous C4A null allele (n=7) ? capillary leak (p&lt;0.01) (Zhang S et al 2004) RCT of C4A def children to C4A-rich plasma in CPB prime ? SIRS (biochem &amp; clinical) (Zhang Lancet 2005)</td>
</tr>
<tr>
<td><strong>MBL</strong></td>
<td>MBL def assoc with ? infect risk</td>
<td>Infection plays a role in atherosclerosis ? MBL def assoc ?atherosclerosis</td>
<td>434 adults CAD</td>
<td>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</td>
</tr>
</tbody>
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Pattern Recognition Receptors
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<th><strong>PRR Receptor</strong></th>
<th><strong>Biological effects</strong></th>
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<tr>
<td><strong>CD14</strong></td>
<td>Co-receptor with Toll’s for bact products</td>
<td>Inflammation &amp; infection play a role in atheroma, Endothelial &amp; muscle cells activated by solb CD14</td>
<td>&gt;3000 adult pts in total</td>
<td>Knock-out mouse has ? LV dysfn following I-RP (Favre et al 2007)</td>
</tr>
<tr>
<td><strong>TLR</strong></td>
<td>Important in innate immune response to pathogens</td>
<td>May mediate inflammation in non-infectious injury</td>
<td>Mice, 657 men</td>
<td>Toll 4 (-299 A/G) not assoc with atherosclerosis (Hernesniemi et al 2007)</td>
</tr>
<tr>
<td><strong>LBP</strong> (-326 T/C)</td>
<td>Endotoxin binding protein Polymorphism assoc with variable circ levels &amp; sepsis</td>
<td>Endotoxin peaks 4-24hrs post CPB may play a role in myocardial injury</td>
<td>adult</td>
<td>No ? incidence of freq’ in MI pts (Hubacek et al 2002) may be associated with post CPB morbidity</td>
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Wan S et al 1999

CPB

Complement activation

Ischemia-reperfusion

Cytokines

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neutrophils, platelets, endothelium

Arachidonic acid metabolites
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Altered gut perfusion
and integrity

CPB

vasoconstriction, nonpulsatile flow,
hypo-oncotic state

Wan S et al 1999
## Other Polymorphisms

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<tr>
<td><strong>eNOS</strong> (-894 G/T)</td>
<td>Constitutively expressed</td>
<td>Exaggerated vasoconstrictive response</td>
<td>105 adult CABG pts</td>
<td>No difference in CI, SVRI, PVRI (Liakopoulos et al 2006)</td>
</tr>
<tr>
<td><strong>ACE</strong></td>
<td>ability of mitochondria to fn in anaerobic conditions</td>
<td>Improved outcome in critical illness</td>
<td>2711 healthy males over 15yrs</td>
<td>Currently under debate. No clear assoc with CHD. Probably a modifier gene (Muthumala et al 2007)</td>
</tr>
<tr>
<td><strong>CRP</strong></td>
<td>Pro-inflammatory effect Pro-atherosclerotic</td>
<td>High CRP producers will be proatherogenic? infact size</td>
<td>Mendelian randomisation 4659 men</td>
<td>High producers not assoc with risk. May be a co-factor (Casas JP et al 2006)</td>
</tr>
</tbody>
</table>
Thus far....

genetic association studies plagued by:
inconsistency
lack of reproducibility
generally small sample sizes
Potential study population 1600 infants & children over 54 mth period

Consented 763

Phenotypic & genotypic data on 588

Short vs Long stay / ventilation
Sepsis – early vs late
SIRS in the immediate post-operative period

Unfortunately no direct measure of cardiac function
What does predict outcome in our patients?

<table>
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<tr>
<th></th>
<th>Length of Stay (correlation co-efficient [95%CI])</th>
<th>Sepsis (OR [95%CI])</th>
<th>SIRS (in the 1st 72 hours) (OR [95%CI])</th>
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<td>Age</td>
<td>-0.10 [-0.13 - -0.08] (p&lt;0.001)</td>
<td>0.82 [0.71 – 0.95] (p=0.008)</td>
<td>1.11 [1.06 – 1.16] (p&lt;0.001)</td>
</tr>
<tr>
<td>Male</td>
<td>0.15 [-0.06 - 0.35] (p&lt;0.16)</td>
<td>0.86 [0.46 – 1.597] (p=0.62)</td>
<td>1.27 [0.84 – 1.94] (p=0.26)</td>
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<tr>
<td>CPB time</td>
<td>0.01 [0.007 - 0.01] (p&lt;0.001)</td>
<td>1.01 [1.00 – 1.015] (p&lt;0.001)</td>
<td>1.0 [1.000 – 1.007] (p=0.049)</td>
</tr>
<tr>
<td>X-clamp time</td>
<td>0.01 [0.01 – 0.014] (p&lt;0.001)</td>
<td>1.01 [1.00 – 1.015] (p=0.02)</td>
<td>1.0 [0.99 – 1.005] (p=0.91)</td>
</tr>
<tr>
<td>RACHS-1 classification</td>
<td>0.56 [0.48 - 0.65] (p&lt;0.001)</td>
<td>1.56 [1.22 – 2.01] (p&lt;0.001)</td>
<td>0.90 [0.74 – 1.11] (p=0.33)</td>
</tr>
<tr>
<td>PIM II score (risk of mortality)</td>
<td>0.04 [0.03 - 0.05] (p&lt;0.001)</td>
<td>1.00 [0.98 – 1.04] (p=0.57)</td>
<td>0.97 [0.93 – 1.01] (p=0.17)</td>
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<tr>
<td>Genetic profile</td>
<td>?</td>
<td>?</td>
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<td>High TNF-? or IL-6 producers</td>
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<td><strong>High TNF-? or IL-6 producers (TNF-?)</strong></td>
<td>no effect 0.78 [0.5-1.2] (p=0.16)</td>
<td>no effect 0.63 [0.3-1.3] (p=0.15)</td>
<td>no effect 0.76 [0.4-1.2] (p=0.16)</td>
</tr>
<tr>
<td><strong>High TNF-? / Low IL-10 producers (n=109 vs 80)</strong></td>
<td>no effect</td>
<td>no effect</td>
<td>no effect</td>
</tr>
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<td><strong>Complement variants (MBL)</strong></td>
<td>no effect 0.98 [0.66-1.45] (p=0.50)</td>
<td>no effect 1.55 [0.84 – 2.87] (p=0.106)</td>
<td>no effect 0.68 [0.44-1.07] (p=0.06)</td>
</tr>
<tr>
<td><strong>PRR haplotype variants (Toll 4)</strong></td>
<td>no effect 0.77 [0.4-1.5] (p=0.26)</td>
<td>no effect 0.78 [0.3-2.3] (p=0.44)</td>
<td>no effect 0.85 [0.4-1.7] (p=0.39)</td>
</tr>
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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.
Host response to the insult of CPB is too complex to expect any single gene polymorphism to have a significant influence on outcome.
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
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’