C-reactive protein and Coronary Heart Disease

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London School of Hygiene and Tropical Medicine

University College London
Structure of the lecture

Cholesterol and CHD - illustrative example

Inflammation [CRP] and CHD

Integration of genetics and blood-markers for aetiology and drug-target validation
Uses of biomarkers in coronary heart disease

1. Aetiology
2. Drug-target validation
3. Risk prediction
1. Aetiology
2. Drug-target validation
3. Risk prediction
From aetiology to risk modification: Simplified steps

1. Distal exposure
2. Identify a biomarker
3. Search for a drug-target that affect the biomarker
4. Randomised trial to evaluate effect on outcome
Structure of the lecture

Cholesterol and CHD - illustrative example

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Integration of genetics and blood-markers for aetiology and drug-target validation
Average diet and serum total cholesterol concentration in clinically healthy men selected from 21 population groups.
International studies: comparing rates of ischaemic heart disease between communities with varying mean cholesterol concentrations
Biomarkers and clinical event
Meta-analysis of prospective cohort studies

Cholesterol and CHD

Ischaemic Heart Disease
Hazard ratio (95%CI)

Total cholesterol (mmol/L)

Lancet 2005
IHD mortality (3020 deaths) versus usual non-HDL cholesterol

Age at risk:
- 40-59: 43% ↓ risk
- 60-69: 34% ↓ risk
- 70-89: 27% ↓ risk

Usual non-HDL cholesterol (mmol/L)

Hazard ratio (floating absolute risks & 95% CI)

1 mmol/L ↓ non-HDL
- 27% ↓ risk
- 34% ↓ risk
- 43% ↓ risk
“The cholesterol myth”
Identification of a “drug-target”
(e.g. HMG-CoA reductase)

Drug-target discovery

Endo A, Kuroda M, Tanzawa K.
Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity.

FEBS Lett. 1976
The final arbiter of the validity of the therapeutic target is the RCT in humans.

- **Drug**
  - Statins
    - LDL-C decreased
      - CHD decreased
    - LDL-C unmodified
      - CHD unchanged
  - Placebo

- **Comparator**

Use randomisation to balance confounders.
Validation of a “drug-target”
(e.g. HMG-CoA reductase)

Drug-target validation

Cholesterol Treatment Trialists’ collaborators
*Lancet 2005.*
Cholesterol, HMG-CoA reductase and CHD: A successful story

Dietary cholesterol

LDL-cholesterol

HMG-CoA reductase inhibitors

Randomised trials to evaluate effect on CHD
Structure of the lecture

Cholesterol and CHD illustrative example

Inflammation [CRP] and CHD

Integration of genetics and blood-markers for aetiology and drug-target validation
INTERHEART study: 9 risk factors explain ~90% of CHD

Smoking
Blood pressure
Cholesterol
Diabetes
Obesity
Fruit & vegetables
Physical activity
Alcohol consumption
Psychosocial factors
Risk factors with effective known interventions that reverse risk of CHD

Smoking

Blood pressure

Cholesterol

Diabetes

Obesity

Fruit & vegetables

Physical activity

Alcohol consumption

Psychosocial factors
Evidence of a role of Inflammation in CHD

• Experiments *in vitro* and *in vivo* in animals

• Associations between infections and CVD

• Associations between inflammatory diseases and CVD

• Associations of CV risk factors and the inflammatory response

• Involvement of inflammatory cells and products in atherosclerotic plaques

• Associations between circulating agents/markers of the inflammatory response and CVD
A model linking infection and inflammation with atherosclerosis and its acute complications

- Acute infection
- Chronic low-grade Infection/ inflammation
- Systemic inflammatory response
- Cytokines APRs
- Endothelium
- Coagulation pathways
- Local inflammation
- Atherosclerosis
- Atherothrombosis
- Infarction
- Local inflammation
Atherosclerosis timeline

Endothelial dysfunction

Genetic predisposition
Risk factor exposure

Early lesion

Complex lesion
Clinical events

Ischaemia and infarction

Atherosclerosis

Atherothrombosis
Biology of C-reactive protein

Tissue injury
Infection
Inflammation
Adverse non-physiological “stress”

Regulators
- IL-1
- IL-6
- TNF-alpha

Liver

CRP
- phosphocholine

Opsonisation

Complement fixation

Clearance (constant half-life 19h)

Bacterial cell wall
Fungi, parasites
Apoptotic cells
Modified lipids

Hirschfield and Pepys, JCI 2003
CRP as a useful biomarker to evaluate the role of inflammation on Coronary Heart Disease

CRP was discovered in 1930

Technology: ~ mid-1990s Immunoassays methods available

CRP levels were associated with endothelial dysfunction

CRP levels were correlated with higher levels of atherosclerosis (C-IMT)

CRP levels were associated with incident CHD in several prospective studies
Observation: CRP is associated with risk CHD

Systematic Review of 31 prospective studies

C-reactive protein and CHD: cause, consequence or confounding

Smoking, Alcohol, physical inactivity, LDL, excess of adiposity ...........

CRP → CHD

CHD → CRP

CRP → CHD

CRP ← CHD
C-reactive protein and CHD: confounding

Effect of plasma CRP on 26 CV traits

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C-reactive protein and risk of CHD disease

A 48 studies, 10341 cases

B 31 studies, 5373 cases

C 20 studies, 3062 cases

Geometric mean usual CRP concentration (mg/L)

ERFC Lancet 2009
Randomisation to judge causality

Drug intervention

RCT

Sample

Randomisation

Intervention

Biomarker lower

CHD event rate lower

Control

Biomarker Unchanged

CHD event rate higher

Pepys MB et al. Nature 2006; 440: 1217-1221
Structure of the lecture

Cholesterol and CHD illustrative example

Inflammation [CRP] and CHD

Integration of genetics and blood-markers for aetiology and drug-target validation
Can genetics help us to obtain randomised - unbiased - evidence about environmental factors?
Mendel’s second law
– the law of independent assortment –

“the behaviour of each pair of differentiating characteristics in hybrid union is independent of the other differences between the two original plants, and, further, the hybrid produces just so many kinds of egg and pollen cells as there are possible constant combination forms”

Gregor Mendel, 1865.

Mendel in 1862
How can we capitalise on the Mendel second law for the search of causes?

- Minimise substantially confounding
- Abolish reverse causality
- Minimise / abolish regression dilution bias
Randomisation to judge CRP causality in CHD

Drug intervention

RCT

Sample

Randomisation

Intervention

CRP lower

CV event rate lower

Control

CRP higher

CV event rate higher

Genetics

Mendelian randomisation

Population

Random allocation of alleles

Genotype aa

CRP lower

CV event rate lower

Genotype AA

CRP higher

CV event rate higher
Nature’s *Life-time* randomised trials vs. Fixed-duration randomised trials

**Drug intervention**

Randomisation

Gamete formation

Middle-age

~ 5-years

Outcome

**Genetics**

Randomisation

Gamete formation

Middle-age

Outcome

Experiment take place
Mendelian randomisation analysis
“Instrumental variable” technique

Assumptions of IV technique for Mendelian randomisation:

1. The Instrumental variable (IV) $Z$ is associated with exposure of interest $X$

2. $Z$ is independent of the confounding factors $U$ (that confound $X$-$Y$ association)

3. Genotype is related to the outcome only via its association with the modifiable exposure
First step: Adequate selection of the most suitable “tools” to conduct a Mendelian randomisation experiment

15 SNPs in the CRP gene in individuals of European ancestors

SNPs: Flank, Intron, Synom, UTR

SNP location (in bases)
Maintenance of the random allocation of potential confounders among most common CRP haplotypes

**ii) CRP-haplotype & cardiovascular traits**

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Maintenance of the random allocation of potential confounders among most common CRP haplotypes

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Second step: Get the adequate sample size

CRP Coronary Disease Genetics Collaboration

The graph illustrates the relationship between the minimum odds ratio and the number of cases required for different prevalence rates (0.05 prevalence and 0.10 prevalence). The y-axis represents the minimum odds ratio, while the x-axis indicates the number of cases required. The graph shows that as the number of cases increases, the minimum odds ratio decreases, which is typical in genetic studies where larger sample sizes are needed to achieve statistically significant results.
Mendelian randomisation experiment to assess the causal relevance of CRP for coronary disease

Cross-sectional or prospective studies

Emerging risk factors collaboration

G \rightarrow \text{CRP} \rightarrow \text{CHD}

Case-control or Prospective studies

CRP Coronary Disease Genetic Collaboration (CCGC)
31 studies, 30,000 cases, 100,000 controls

Coordinators Centres: Cambridge, UCL and LSHTM
**CRP Coronary Disease Genetics Collaboration**

194,418 participants and 46,557 coronary heart disease events

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<th>Fibrinogen (μmol/l)</th>
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</table>

Standard deviation (95% CI) change in biomarker per-allele change in SNP

BMJ 2011
CRP Coronary Disease Genetics Collaboration

194,418 participants and 46,557 coronary heart disease events

<table>
<thead>
<tr>
<th>Single Nucleotide Polymorphism</th>
<th>Allele frequency</th>
<th>Per-allele difference (95% CI)</th>
<th>Per-allele Risk Ratio (95% CI)</th>
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</thead>
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<tr>
<td>rs3093077</td>
<td>0.05</td>
<td>0.19 (0.17, 0.22)</td>
<td>0.94 (0.88, 1.00)</td>
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<td>0.67</td>
<td>0.18 (0.16, 0.21)</td>
<td>1.00 (0.98, 1.03)</td>
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<tr>
<td>rs1130864</td>
<td>0.32</td>
<td>0.13 (0.11, 0.16)</td>
<td>0.98 (0.96, 1.00)</td>
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<tr>
<td>rs1800947</td>
<td>0.94</td>
<td>0.26 (0.19, 0.34)</td>
<td>0.98 (0.91, 1.04)</td>
</tr>
</tbody>
</table>

Per-allele higher mean \(\log_e\) CRP (95% CI), mg/L

Per-allele risk ratio for CHD (95% CI)
Biomarkers and Coronary Heart Disease

- Excessive number of drug-targets (e.g. CVD)

**Inflammation**
- C-reactive protein
- SAA
- Interleukins
- Soluble adhesion molecules
- sCD40 ligand

**Haemostasis/ thrombosis**
- Fibrinogen
- vWF
- tPA
- D-dimer
- PAI-1

**Oxidative stress/lipids**
- Homocysteine
- Lipoprotein associated phospholipase A2
- Myeloperoxidase

------and around 200 others....
Output of new molecular entities (NMEs) since 1950

Conclusions

1. Use of biomarkers in epidemiology provides new option in aetiology – drug-development and risk prediction

2. The sharp increase in technology and the low-cost of these techniques will provide access to an increasingly large numbers of biomarkers

3. Old known biases in epidemiology remain as the main challenges to translate findings from molecular epidemiology into risk prevention

4. Integration of different layers of biological factors, each of them exposed to a different set of biases seems to be a promising approach to accelerate the translation of biomarkers research into clinical care
**Suggested readings**

**C-reactive protein and coronary heart disease: a critical review.**
Casas JP, Shah T, Hingorani AD, Danesh J, Pepys MB.

**Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data.**
BMJ. 2011 Feb 15;342:d548

**Critical appraisal of CRP measurement for the prediction of coronary heart disease events: new data and systematic review of 31 prospective cohorts.**

**C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis.**