Using Mendelian randomization to explore causality

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ESC Summer School on Cardiovascular Science 2017
Drug development in the genomics era

More than half of the drugs thought promising in the lab will go on to fail.

Ernesto del Aguila, NHGRI
Drug development in the genomics era

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>25% of drugs reaching trials will be rejected as ineffective

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Drug development in the genomics era

More than half of the drugs thought promising in the lab will go on to fail.

>25% of drugs reaching trials will be rejected as ineffective.

Can we use genomic information to improve this success rate?

Ernesto del Aguila, NHGRI
The wealth of genetic data

http://www.genome.gov/gwastudies/
GWA studies of cardiovascular traits

Large-scale genome-wide association (GWA) meta-analyses, often involving trans-ethnic studies, offer large-scale information on

- Coronary heart disease (~60k cases)
- Ischaemic stroke (~12k cases)
- Lipid fractions (~200k individuals)
- Blood pressure (~350k individuals)
- Inflammatory markers, etc... (~100k individuals)
- Population biobanks (e.g. ~500k individuals in UKB)
Translational potential of genetic data

Large-scale genomic studies have the potential to be used to identify new therapeutic targets as well as to:

• Explore the causal effects of potential therapeutic pathways on disease

• Explore causality of biomarkers associated with disease risk by examining the effects of genetic variants (and genetic risk scores) that can mimic the risk factor or drug target of interest.
Randomized trials vs Mendelian randomization

**Randomized trial**

<table>
<thead>
<tr>
<th>Randomized allocation to therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
</tr>
<tr>
<td>Placebo</td>
</tr>
<tr>
<td>Biomarker lower</td>
</tr>
<tr>
<td>Biomarker higher</td>
</tr>
<tr>
<td>Event rate lower</td>
</tr>
<tr>
<td>Event rate higher</td>
</tr>
</tbody>
</table>

*Other risk factors assumed balanced on average*
Randomized trials vs Mendelian randomization

**Randomized trial**
- Randomized allocation to therapy
  - Active
    - Biomarker lower
      - Event rate lower
  - Placebo
    - Biomarker higher
      - Event rate higher
- Other risk factors assumed balanced on average

**Mendelian randomization**
- Randomization by genotype
  - allele inherited from each parent randomly determined
  - Genotype aa
    - Biomarker lower
      - Event rate lower
  - Genotype AA
    - Biomarker higher
      - Event rate higher
  - Other risk factors assumed balanced on average
Genetic variants for Mendelian Randomization

1. The genetic variant should be associated with the risk factor
2. The genetic variant should not be associated with a confounder of the risk factor – outcome association
3. There should be no alternative causal pathway from the genetic variant to the outcome, except for via the risk factor
Advantages and limitations

Hopewell et al, Stroke, 2016
MR studies of coronary heart disease

Jansen et al, EHJ, 2014
Can genetic studies anticipate causal on-target adverse effects?

Statins & new-onset diabetes
Statins and diabetes – the trial evidence

- 91,140 statin trial participants, of whom 4278 developed diabetes during a mean of 4 years.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Statin Events</th>
<th>Statin Rate</th>
<th>Placebo or control Events</th>
<th>Placebo or control Rate</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCOT-LLA</td>
<td>7773</td>
<td>154</td>
<td>11.9</td>
<td>134</td>
<td>10.5</td>
<td>1.14 (0.89-1.46)</td>
</tr>
<tr>
<td>HPS</td>
<td>14573</td>
<td>335</td>
<td>9.2</td>
<td>293</td>
<td>8.0</td>
<td>1.15 (0.98-1.35)</td>
</tr>
<tr>
<td>JUPITER</td>
<td>17802</td>
<td>270</td>
<td>16.0</td>
<td>216</td>
<td>12.8</td>
<td>1.26 (1.04-1.51)</td>
</tr>
<tr>
<td>WOSCOPS</td>
<td>5974</td>
<td>75</td>
<td>5.2</td>
<td>93</td>
<td>6.5</td>
<td>0.79 (0.58-1.0)</td>
</tr>
<tr>
<td>LIPID</td>
<td>6997</td>
<td>126</td>
<td>6.0</td>
<td>138</td>
<td>6.6</td>
<td>0.91 (0.71-1.17)</td>
</tr>
<tr>
<td>CORONA</td>
<td>3534</td>
<td>100</td>
<td>20.9</td>
<td>88</td>
<td>18.5</td>
<td>1.14 (0.84-1.55)</td>
</tr>
<tr>
<td>PROSPER</td>
<td>5023</td>
<td>165</td>
<td>20.5</td>
<td>127</td>
<td>15.8</td>
<td>1.32 (1.03-1.69)</td>
</tr>
<tr>
<td>MEGA</td>
<td>6086</td>
<td>172</td>
<td>10.8</td>
<td>164</td>
<td>10.1</td>
<td>1.07 (0.86-1.35)</td>
</tr>
<tr>
<td>AFCAPS/TEXCAPS</td>
<td>6211</td>
<td>72</td>
<td>4.5</td>
<td>74</td>
<td>4.6</td>
<td>0.98 (0.70-1.38)</td>
</tr>
<tr>
<td>4S</td>
<td>4242</td>
<td>198</td>
<td>17.3</td>
<td>193</td>
<td>16.8</td>
<td>1.03 (0.84-1.28)</td>
</tr>
<tr>
<td>ALLHAT</td>
<td>6087</td>
<td>238</td>
<td>16.4</td>
<td>212</td>
<td>14.4</td>
<td>1.15 (0.95-1.41)</td>
</tr>
<tr>
<td>GISSI HF</td>
<td>3378</td>
<td>225</td>
<td>34.8</td>
<td>215</td>
<td>32.1</td>
<td>1.10 (0.89-1.35)</td>
</tr>
<tr>
<td>GISSI PREV</td>
<td>3460</td>
<td>96</td>
<td>27.5</td>
<td>105</td>
<td>30.6</td>
<td>0.89 (0.67-1.20)</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>1.09 (1.02-1.17)</strong></td>
</tr>
</tbody>
</table>

Sattar et al. Lancet, 2010
**HMGCR genetic variants mimic statin use**

- Statins exert their action by inhibiting HMGCR, leading to LDL-reduction.

- A *HMGCR* genetic variant, used as a proxy for statin use, was associated with lower LDL-C.

Swerdlow, Lancet, 2015
Is \textit{HMGCR} associated with diabetes?

- The \textit{HMGCR} genetic variant associated with lower LDL-C was also associated with higher risk of new onset diabetes.

- Impact on diabetes is an on-target effect of HMGCR inhibition.

Swerdlow, \textit{Lancet}, 2015
Implications

• Life-long inhibition of HMGCR is associated with lower LDL-C and higher risk of new onset diabetes. Effect of statins on diabetes at least partially explained by HMGCR inhibition.

• Small effect on diabetes requires large-scale studies. PheWAS may offer further insight into potential small on-target effects, albeit cannot assess off-target effects.
Can genetic studies identify a poor choice of therapeutic target? LpPLA₂ inhibition & vascular events
Lp-PLA$_2$ - observational and trial evidence

- Lp-PLA$_2$ produces pro-inflammatory mediators through hydrolysis of oxidised phospholipids carried on LDL in atherosclerotic plaques.

- Increased Lp-PLA$_2$ activity associated with higher CHD risk.
Lp-PLA$_2$ - observational and trial evidence

- Increased Lp-PLA$_2$ activity associated with CHD.
- Large-scale randomized trials of darapladib, an Lp-PLA$_2$ inhibitor, showed no effect on major vascular events.
Do genetic studies agree? Lp-PLA₂ and *PLA2G7*

- A loss-of-function variant in *PLA2G7* (V279F), encoding Lp-PLA₂, results in 50% lower Lp-PLA₂ activity per copy and is common in East Asians (5%), providing a strong genetic instrument.

- V279F was genotyped in ~90,000 individuals from the China Kadoorie Biobank and tested for association with ~7000 major vascular events as well as a range of wider diseases (to explore other on-target effects).

*Millwood et al, JACC, 2016*
Loss-of-function mutation in the Lp-PLA$_2$ gene

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Per F variant Odds Ratio (95% CI)</th>
<th>Uncorrected p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coronary Endpoints</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major coronary events</td>
<td>922</td>
<td>81489</td>
<td>0.96 (0.79, 1.18)</td>
<td>0.73</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>653</td>
<td>81489</td>
<td>0.91 (0.71, 1.17)</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Stroke endpoints</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemorrhagic stroke</td>
<td>1248</td>
<td>81489</td>
<td>0.88 (0.73, 1.06)</td>
<td>0.15</td>
</tr>
<tr>
<td>Ischaemic stroke</td>
<td>4843</td>
<td>81489</td>
<td>1.01 (0.92, 1.11)</td>
<td>0.81</td>
</tr>
<tr>
<td>All stroke</td>
<td>5967</td>
<td>81489</td>
<td>1.00 (0.92, 1.09)</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Composite endpoints</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major occlusive events</td>
<td>5607</td>
<td>81489</td>
<td>1.01 (0.93, 1.11)</td>
<td>0.80</td>
</tr>
<tr>
<td>Vascular death</td>
<td>1774</td>
<td>81489</td>
<td>0.94 (0.80, 1.09)</td>
<td>0.41</td>
</tr>
<tr>
<td>Major vascular events</td>
<td>7141</td>
<td>81489</td>
<td>0.98 (0.90, 1.06)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Millwood et al, JACC, 2016
PheWAS of *PLA2G7* V279F

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Per F variant Odds Ratio (95% CI)</th>
<th>Uncorrected p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infections</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious and parasitic diseases</td>
<td>2025</td>
<td>89403</td>
<td>1.05 (0.91, 1.22)</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Neoplasms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant neoplasms (excluding bronchus and lung)</td>
<td>2778</td>
<td>88650</td>
<td>0.98 (0.87, 1.10)</td>
<td>0.73</td>
</tr>
<tr>
<td>Malignant neoplasms of bronchus and lung</td>
<td>649</td>
<td>90779</td>
<td>1.22 (0.98, 1.52)</td>
<td>0.10</td>
</tr>
<tr>
<td>In situ, benign, uncertain or unknown neoplasms</td>
<td>821</td>
<td>90607</td>
<td>0.98 (0.78, 1.22)</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Diseases of the blood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseases of the blood</td>
<td>630</td>
<td>90798</td>
<td>1.16 (0.91, 1.49)</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>Endocrine, nutritional and metabolic diseases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other endocrine disorders</td>
<td>350</td>
<td>91078</td>
<td>1.23 (0.90, 1.68)</td>
<td>0.23</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3701</td>
<td>87727</td>
<td>0.91 (0.82, 1.01)</td>
<td>0.08</td>
</tr>
<tr>
<td>Malnutrition or other nutritional deficiencies</td>
<td>463</td>
<td>90965</td>
<td>1.08 (Not estimable)</td>
<td>NA</td>
</tr>
<tr>
<td>Disorders of lipoprotein metabolism</td>
<td>487</td>
<td>90941</td>
<td>0.99 (Not estimable)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mental and behavioural disorders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental and behavioural disorders</td>
<td>766</td>
<td>90662</td>
<td>0.99 (0.79, 1.24)</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>Diseases of the nervous system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseases of the nervous system</td>
<td>921</td>
<td>90507</td>
<td>0.98 (0.80, 1.21)</td>
<td>0.88</td>
</tr>
<tr>
<td>Transient cerebral ischaemic attacks</td>
<td>1606</td>
<td>89822</td>
<td>1.06 (0.91, 1.23)</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Diseases of the eye and ear</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseases of the eye and adnexa</td>
<td>2479</td>
<td>90849</td>
<td>1.02 (0.80, 1.30)</td>
<td>0.70</td>
</tr>
<tr>
<td>Diseases of the ear and mastoid process</td>
<td>926</td>
<td>90502</td>
<td>0.95 (0.77, 1.17)</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Diseases of the circulatory system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseases of the circulatory system</td>
<td>3433</td>
<td>87995</td>
<td>0.92 (0.82, 1.03)</td>
<td>0.15</td>
</tr>
<tr>
<td>Essential hypertension</td>
<td>3393</td>
<td>88035</td>
<td>0.97 (0.86, 1.09)</td>
<td>0.60</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>5517</td>
<td>85911</td>
<td>1.03 (0.94, 1.12)</td>
<td>0.55</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>7570</td>
<td>83858</td>
<td>0.98 (0.90, 1.06)</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Millwood et al, IJE, 2016
PheWAS of PLA2G7 V279F

Millwood et al, IJE, 2016
LpPLA₂ – Trials vs Genetic studies

**Randomized trial**
- Randomized allocation to therapy
  - Darapladib
  - Placebo
  - Lp-PLA₂ inhibited
  - Lp-PLA₂ normal
  - No difference in cardiovascular event rate

**Genetic study**
- Randomization by PLA2G7 variant
  - V279F (carrier)
  - V279F (noncarrier)
  - Lp-PLA₂ inhibited
  - Lp-PLA₂ normal
  - No difference in cardiovascular event rate or other major diseases
Implications

• Concordance between the effects of genetic markers and clinical trials; Lp-PLA$_2$ inhibition does not lower rate of MVEs. Thus, the biological mechanism isn’t causal for disease.

• PheWAS indicates no large effects on other disease outcomes, suggesting no major safety concerns or other potential promising indications.
Do trials and Mendelian randomization always agree?

PCSK9 inhibitors, CHD and ischaemic stroke
**PCSK9 genetic mutations to drug discovery**

- **PCSK9 gain-of-function mutations**
  - High LDL-C
  - Premature heart disease

- **PCSK9 loss-of-function mutations**
  - Low LDL-C
  - Reduction in CHD incidence
  - Not associated with other detectable abnormalities

→ Development of PCSK9 inhibitors

Abifadel et al, Nat Gen, 2003
Cohen et al, Nat Gen, 2005; Cohen et al, NEJM, 2006
What does PCSK9 do?

PCSK9 regulates surface expression of LDLRs by targeting for lysosomal degradation.

Qian et al, J Lipid Res, 2007
Horton et al, J Lipid Res, 2009
How might PCSK9-inhibition impact different vascular outcomes?

• Statin trials suggest that LDL-lowering lowers the risk of both CHD and of ischaemic stroke comparably.

• However, observational evidence suggests a weaker effect of LDL-C on ischaemic stroke than on CHD.

• Prior to very recent Phase 3 studies of PCSK9 inhibition, there was no reliable direct evidence for the impact of PCSK9 on vascular outcomes.
**Impact of PCSK9 inhibition on vascular outcomes**

**FOURIER – a Phase 3 clinical trial**

Evolocumab resulted in a 27% risk reduction in myocardial infarction and a 25% risk reduction in ischaemic stroke.

---

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Evolocumab (N = 13,784)</th>
<th>Placebo (N = 13,780)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary end point: cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization</td>
<td>1344 (9.8)</td>
<td>1563 (11.3)</td>
<td>0.85 (0.79–0.92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Key secondary end point: cardiovascular death, myocardial infarction, or stroke</td>
<td>816 (5.9)</td>
<td>1013 (7.4)</td>
<td>0.80 (0.73–0.88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>468 (3.4)</td>
<td>639 (4.6)</td>
<td>0.73 (0.65–0.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hospitalization for unstable angina</td>
<td>236 (1.7)</td>
<td>239 (1.7)</td>
<td>0.99 (0.82–1.18)</td>
<td>0.89</td>
</tr>
<tr>
<td>Stroke</td>
<td>207 (1.5)</td>
<td>262 (1.9)</td>
<td>0.79 (0.66–0.95)</td>
<td>0.01</td>
</tr>
<tr>
<td>Ischemic</td>
<td>171 (1.2)</td>
<td>226 (1.6)</td>
<td>0.75 (0.62–0.92)</td>
<td></td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>29 (0.21)</td>
<td>25 (0.18)</td>
<td>1.16 (0.68–1.98)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>13 (0.09)</td>
<td>14 (0.10)</td>
<td>0.93 (0.44–1.97)</td>
<td></td>
</tr>
</tbody>
</table>

Sabatine et al, NEJM 2017
What do the genetics suggest?

- International collaboration of studies examining the genetics of ischaemic stroke (IS), involving ~10,000 IS cases from 12 studies (about 50% with TOAST subtyping).

- Effects of PCSK9 variants on IS and IS subtypes in the METASTROKE genome-wide meta-analysis were estimated.

- Estimates of the effects of PCSK9 variants on LDL-C and CHD risk were obtained from the Global Lipids Consortium and CARDIoGRAMPlusC4D Consortium respectively.

Hopewell et al, EHJ, In Press
PCSK9 R46L and risk of vascular disease

The R46L loss-of-function variant (~1.5% frequency in Europeans), is associated with lower PCSK9 levels, and ~0.5 mmol/L lower LDL-C levels (as well as small effects on other biomarkers).

Hopewell et al, EHJ, In Press
**PCSK9 R46L and risk of vascular disease**

The R46L loss-of-function variant (~1.5% frequency in Europeans), is associated with lower PCSK9 levels, and ~0.5 mmol/L lower LDL-C levels (as well as small effects on other biomarkers).

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**Table:**

<table>
<thead>
<tr>
<th>Vascular disease</th>
<th>Events</th>
<th>Odds ratio (95% CI) per LDL-C lowering allele</th>
<th>P(Het)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary heart disease</td>
<td>37748</td>
<td>0.77 (0.69-0.87)</td>
<td>0.02</td>
</tr>
<tr>
<td>Ischaemic stroke</td>
<td>10307</td>
<td>1.04 (0.84-1.28)</td>
<td></td>
</tr>
</tbody>
</table>

Hopewell et al, EHJ, In Press
Impact of *PCSK9* variants on LDL-C lowering and vascular risk

Hopewell et al, EHJ, in press
PCSK9 – Trials vs Genetic studies

Randomized trial

Randomized allocation to therapy

Evolocumab

PCSK9 inhibited

CHD & IS event rate lower

Placebo

PCSK9 normal

CHD & IS event rate higher

Genetic study

Randomization by PCSK9 variant

R46L (carrier)

PCSK9 inhibited

CHD event rate lower

R46L (noncarrier)

PCSK9 normal

CHD event rate higher

No difference in IS event rate
Implications

• *PCSK9* variants that produce life-long lower LDL-C levels, and that are associated with lower risk of CHD, appear to have a significantly weaker (if any) effect on the risk of IS.

• By comparison, PCSK9-inhibitor therapy (similarly to statin and ezetimibe) reduces the risk of CHD and IS comparably.

• Findings illustrate potential limitations with the use of Mendelian randomization to predict the effects of novel therapeutic interventions on different health outcomes.
Mendelian randomization
Take home messages

• Mendelian randomization is a powerful tool that can help to elucidate the causal relevance of therapeutic mechanisms and biomarkers.

• Mendelian randomization studies can be complex and interpretation challenging, thus findings should be considered alongside other sources of evidence.

• Statistical approaches are available that may facilitate better understanding of Mendelian randomization studies and the potential impact of pleiotropy.
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Maysson Ibrahim
Fielder Camm
Hanning Zhu
Sarah Parish
Rory Collins

METASTROKE Collaboration
Rainer Malik
Brad Worrall
Sudha Seshadri

Funding from the BHF
Back-up slides
MR of specific LDL-C lowering variants also suggests weaker effect on IS than on CHD risk.

Observe heterogeneity between effects on CHD and on IS.

Preliminary analyses - not for citation or publication.
Statins & PCSK9

Statins increase PCSK9 (limiting their impact)

\[ \downarrow \]

PCSK9 inhibitors decrease PCSK9 expression

\[ \downarrow \]

Statin in the presence of PCSK9 inhibition should be more effective!

Statins and fibrates induce increased PCSK9 expression, thus PCSK9 inhibition could induce robust LDL reduction as add-on therapy.