A loss-of-function variant in \textit{CETP} and risk of CVD in Chinese adults

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for the CKB collaborative group

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HDL-C and CHD: observational evidence

**LDL-C**

Baseline HDL-C (mmol/L): ≤1.25

Hazard ratio (95% CI)

Usual non-HDL cholesterol (mmol/L)

3.0  4.0  5.0  6.0

1.0  2.0  3.0  4.0

**HDL-C**

Baseline non-HDL-C (mmol/L): 5+

Hazard ratio (95% CI)

Usual HDL cholesterol (mmol/L)

1.0  1.5

0.5  1.0

**ERFC:** ~25% lower CHD risk per 1 SD (15 mg/dL) higher HDL-C

PSC, Lancet 2007; ERFC, JAMA 2009
HDL cholesterol and CVD risk

- HDL-C is strongly inversely associated with CVD risk, especially CHD, but causal effects are unclear
- Drugs that raise HDL-C (e.g. CETP inhibitors) have the potential for further reducing CVD risk
- REVEAL study should confirm or refute inconclusive results from previous trials of CETP inhibition

REVEAL
Randomized EValuation of the Effects of Anacetrapib through Lipid-modification
CETP and HDL metabolism

CETP: exchanges cholesterol esters for triglycerides between HDL and VLDL particles.
Genetic studies of \textit{CETP} and CVD risk

- \textit{CETP} variants are associated with higher HDL-C and also with lower LDL-C and triglycerides.
- Common \textit{CETP} variants associated with reduced CHD risk, but previous findings are inconclusive.
- East Asians functional variant rs2303790 (c.1376A>G, p.D459G) results in lower CETP mass and activity.
- \textit{CETP} rs2303790 greatly increases HDL-C, with effect size >2 times greater than lead SNP in Europeans.

MR studies can help to assess the effects of lifelong lower CETP activity on CVD (and non-CVD) risks.
China Kadoorie Biobank (CKB)
(Mean age 51, 41% men, 4% obese, 99.98% sample collection)

- 512,891 recruited from 10 localities in 2004-08
- Participants interviewed, measured, and gave plasma and DNA for long-term storage
- All followed up indefinitely via electronic record linkage to deaths and ALL hospital episodes
- Periodic resurvey of 5% surviving participants (allow for enhancements and sources of variation)

Consent for unspecified research use of stored samples
CKB: Location of the 10 survey sites in China
(with different risk exposure and disease patterns)

CETP MR study: design & methods

- 5 genetic variants in CETP gene:
  - 1 East Asian functional variant (D459G gene)
  - 4 other SNPs associated with HDL-C

- 91,500 CKB participants (meta-analysed):
  - Population-based: 75,000
  - CVD case-control: 16,500
  - No prior CVD and statin use

- Lipids and NMR-metabolomics in a subset
  - Mean LDL-C: 92 mg/dL; HDL-C: 48 mg/dL

- 3300 MCE, 8800 IS and 12,000 MCVE

Linear and logistic regressions yielded adjusted per allele effects for traits and incident CVD events
**CKB: Lipoprotein subtypes and CVD risk**
(A nested case-control study of NMR-metabolomics in 5K)

### Myocardial infarction

- Extremely Large VLDL
- Very Large VLDL
- Large VLDL
- Medium VLDL
- Small VLDL
- Very Small VLDL
- IDL
- Large LDL
- Medium LDL
- Small LDL

### Ischaemic stroke

- Extremely Large VLDL
- Very Large VLDL
- Large VLDL
- Medium VLDL
- Small VLDL
- Very Small VLDL
- IDL
- Large LDL
- Medium LDL
- Small LDL

* P<0.05, ** P<0.01, *** P<0.0001, with Bonferroni correction
CETP SNPs and major lipid concentrations
(lipids measured using conventional methods in 20K)

<table>
<thead>
<tr>
<th>SNP/Trait</th>
<th>MAF</th>
<th>NObs</th>
<th>HDL-C Estimate (95% CI)</th>
<th>LDL-C Estimate (95% CI)</th>
<th>Triglycerides Estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3764261 (A)</td>
<td>0.16</td>
<td>17834</td>
<td>3.62 (3.30-3.94)</td>
<td>0.94 (0.16-1.71)</td>
<td>-4.75 (-8.74-0.76)</td>
</tr>
<tr>
<td>rs1800775 (A)</td>
<td>0.53</td>
<td>17840</td>
<td>1.38 (1.14-1.62)</td>
<td>0.62 (0.04-1.19)</td>
<td>-3.22 (-6.17-0.27)</td>
</tr>
<tr>
<td>rs708272 (A)</td>
<td>0.41</td>
<td>17828</td>
<td>1.94 (1.70-2.19)</td>
<td>0.86 (0.28-1.44)</td>
<td>-4.34 (-7.32-1.36)</td>
</tr>
<tr>
<td>rs9939224 (G)</td>
<td>0.88</td>
<td>17840</td>
<td>2.32 (1.95-2.69)</td>
<td>1.24 (0.35-2.13)</td>
<td>-3.07 (-7.64-1.50)</td>
</tr>
<tr>
<td>rs2303790 (G)</td>
<td>0.02</td>
<td>17837</td>
<td>6.07 (5.24-6.89)</td>
<td>-0.43 (-2.42-1.55)</td>
<td>-9.07 (-19.25-1.12)</td>
</tr>
<tr>
<td>Gene Score (W)</td>
<td></td>
<td>17763</td>
<td>11.48 (10.62-12.34)</td>
<td>2.82 (0.73-4.91)</td>
<td>-16.22 (-26.94-5.50)</td>
</tr>
</tbody>
</table>

rs2303790 (MAF 2.2%) per allele effect: 6 mg/dL (0.16 mmol/L) HDL-C
(P value = 8.1E-47)
## CETP SNPs and apolipoproteins

### Apo A1

<table>
<thead>
<tr>
<th>SNP/Trait</th>
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<td>2.37 (1.87-2.86)</td>
</tr>
<tr>
<td>rs9939224</td>
<td>0.88</td>
<td>17840</td>
<td>3.07 (2.32-3.83)</td>
</tr>
<tr>
<td>rs2303790</td>
<td>0.02</td>
<td>17837</td>
<td>8.53 (6.85-10.21)</td>
</tr>
</tbody>
</table>

### Apo B

<table>
<thead>
<tr>
<th>SNP/Trait</th>
<th>NObs</th>
<th>Estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3764261</td>
<td>17834</td>
<td>-0.02 (-0.63-0.58)</td>
</tr>
<tr>
<td>rs1800775</td>
<td>17840</td>
<td>0.11 (-0.33-0.56)</td>
</tr>
<tr>
<td>rs708272</td>
<td>17828</td>
<td>0.21 (-0.24-0.66)</td>
</tr>
<tr>
<td>rs9939224</td>
<td>17840</td>
<td>0.30 (-0.39-0.99)</td>
</tr>
<tr>
<td>rs2303790</td>
<td>17837</td>
<td>-1.44 (-2.98-0.10)</td>
</tr>
</tbody>
</table>

### Gene Score (W)

- **Apo A1**: 15.13 (13.37-16.89)
- **Apo B**: -0.27 (-1.89-1.35)
Associations of \textit{CETP} rs2303790 (per allele) with lipid composition in $^1$H-NMR metabolomics

Changes in composition and particle size (not shown) are consistent with inhibition of CETP, even though it has no major effect on LDL-C concentration.
### Associations of CETP rs2303790 with CVD risk

#### Meta-analysis of 3 East Asia studies:
- 8379 CHD cases, OR=0.95 (0.85-1.07), p=0.41

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Per G allele Odds Ratio (95% CI)</th>
<th>Uncorrected p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary endpoints</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major coronary events</td>
<td>3297</td>
<td>73232</td>
<td>1.17 (0.98, 1.39)</td>
<td>0.09</td>
</tr>
<tr>
<td>Ischaemic stroke</td>
<td>8852</td>
<td>73232</td>
<td>1.02 (0.90, 1.15)</td>
<td>0.78</td>
</tr>
<tr>
<td>Major occlusive events</td>
<td>11612</td>
<td>73232</td>
<td>1.04 (0.94, 1.16)</td>
<td>0.44</td>
</tr>
<tr>
<td><strong>Secondary endpoints</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>1742</td>
<td>73232</td>
<td>1.24 (0.98, 1.58)</td>
<td>0.07</td>
</tr>
<tr>
<td>Haemorrhagic stroke</td>
<td>5494</td>
<td>73232</td>
<td>1.08 (0.92, 1.26)</td>
<td>0.36</td>
</tr>
<tr>
<td>Total stroke</td>
<td>13588</td>
<td>73232</td>
<td>1.02 (0.92, 1.13)</td>
<td>0.76</td>
</tr>
<tr>
<td>Fatal occlusive vascular events</td>
<td>2106</td>
<td>73232</td>
<td>1.27 (1.02, 1.57)</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Associations of CETP rs2303790 with non-CVD risk

Significant excess risk of eye diseases, as in one Asia study
Summary and implications

- A LOF variant in *CETP* strongly affects HDL metabolism, mimicking the pharmacological inhibition of CETP.
- The LOF variant had no significant effects on CVD risk.
- In East Asians, increasing HDL-C by CETP inhibition is unlikely to confer appreciable protection against CVD.
- Prospective biobanks with cohort-wide genetic and multiple outcome data can inform drug development.

All 0.5 million CKB samples will be genotyped using custom designed 800K SNPs array (>80K missense/LOF variants)
Acknowledgements

Key members of CKB genetic working group
I Millwood, M Holmes, D Bennett, R Boxall, R Clarke, R Walters, Z Chen

Study website: www.ckbiobank.org