Theme-based Research Scheme (TRS) on Personalised Medicine for Cardiovascular Disease: From Genomic Testing and Biomarkers to Human Pluripotent Stem Cell Platform

HKU and international collaborative research teams discover novel genetic markers for blood lipids and coronary artery disease

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Coronary Artery Disease (CAD)

2015:
- 3rd leading cause of death in Hong Kong
- Account for 8.8% death in Hong Kong (total 4,123)
- 11 persons died of CAD per day
CAD pathogenesis

Normal inner vessel wall

CAD inner vessel wall

References: National Heart, Lung and Blood Institute (NHLBI), National Institute of Health (NIH); Centers for Disease Control and Prevention
Modifiable risk factors of CAD

Abnormal blood lipid levels is the major risk factor of CAD

1mmol/L
risk of CAD death 35%

- Total cholesterol (TC, <5.2)
- Low-Density Lipoprotein cholesterol (LDL, <3.4; Optimal : <2.6)
- High-Density Lipoprotein cholesterol (HDL >1.0; Optimal : >1.6)
- Triglycerides (TG, <1.7)
The second Population Health Survey released by the Department of Health reported that abnormal blood lipid levels (hypercholesterolaemia) are common in Hong Kong:

- 49.5% of Hong Kong people aged 15 to 84 have hypercholesterolaemia.
- Compared to 2003/04, hypercholesterolaemia becomes more prevalent with age-standardised rate increases from 35.3% to 42.1%.
- 70.2% were only picked up by health examination.

Note: Hypercholesterolaemia refers to total blood cholesterol ≥ 5.2 mmol/L and also self-reported doctor-diagnosed hypercholesterolaemia.
Cardiovascular (CVD) risk

- By adopting the Framingham risk model, the Survey predicted that, among people aged 30 to 74, the mean cardiovascular (CVD) risk over the next 10 years is 10.6%.
- 8/10 of males and 1/4 of females aged 65-74 were predicted as having high CVD risk.
- Around half of the major non-communicable diseases were only picked up by health examination.
A large proportion of blood lipids in the human body is manufactured by the liver. A small proportion is directly derived from diet (10-15%). Variation of blood lipid levels depends on:

- Genetics (50-70%)
  - Genetic variations inherited from parents
- Environment (<50%)
  - Diet (saturated fat, trans fat, and cholesterol)
  - Exercise
  - Lifestyle
Exome-chip meta-analysis on blood lipids and CAD on East Asians

Exome chip meta-analysis identifies novel loci and East Asian-specific coding variants that contribute to lipid levels and coronary artery disease

Most genome-wide association studies have been of European individuals, even though most genetic variation in humans is seen only in non-European samples. To search for novel loci associated with blood lipid levels and clarify the mechanism of action at previously identified lipid loci, we used an exome array to examine protein-coding genetic variants in 47,532 East Asian individuals. We identified 255 variants at 41 loci that reached chip-wide significance, including 3 novel loci and 14 East Asian-specific coding variant associations. After a meta-analysis including >300,000 European samples, we identified an additional nine novel loci. Sixteen genes were identified by protein-altering variants in both East Asians and Europeans, and thus are likely to be functional genes. Our data demonstrate that most of the low-frequency or rare coding variants associated with lipids are population specific, and that examining genomic data across diverse ancestries may facilitate the identification of functional genes at associated loci.
Background of this study

- Frequencies of genetic variations vary across populations

- Contribution of genetic factors affecting blood lipid levels also varies across populations

- Most genetic studies on blood lipids focus on Europeans

- Genetic factors influencing blood lipid levels on East Asians (including Chinese) remain largely unknown

Exome-wide association analysis reveals novel coding sequence variants associated with lipid traits in Chinese

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Blood lipids are important risk factors for coronary artery disease (CAD). Here we perform an exome-wide association study by genotyping 12,685 Chinese, using a custom Illumina HumanExome BeadChip, to identify additional loci influencing lipid levels. Single-variant association analysis on 65,671 single nucleotide polymorphisms reveals 19 loci associated with lipids at exome-wide significance (P<2.69 x 10^{-7}), including three Asian-specific coding variants in known genes (CETP p.Asp459Gly, PCSK9 p.Arg93Cys and LDLR p.Arg257Trp). Furthermore, missense variants at two novel loci—PNPLA3 p.Ile148Met and PKD1L3 pThr429Ser—also influence levels of triglycerides and low-density lipoprotein cholesterol, respectively. Another novel gene, TEAD2, is found to be associated with high-density lipoprotein cholesterol through gene-based association analysis. Most of these newly identified coding variants show suggestive association (P<0.05) with CAD. These findings demonstrate that exome-wide genotyping on samples of non-European ancestry can identify additional population-specific possible causal variants, shedding light on novel lipid biology and CAD.
Results of Chinese exome-wide genetic study

- **TG**: Triglycerides (TG)
  - Genes: APOA5, DOCK7, GCKR, MLXIPL, LPL, TM6SF2, PNPLA3
  - Lipid-associated genes not reported before

- **HDL**: High-density lipoprotein cholesterol (HDL-C)
  - Genes: CETP, LPL, ABCA1, LIPC, APOA5, APOE-APOC4

- **LDL**: Low-density lipoprotein cholesterol (LDL-C)
  - Genes: PCSK9, CELSR2, APOB, LDLR, PKD1L3

- **TC**: Total cholesterol (TC)
  - Genes: PCSK9, CELSR2, APOB, APOE, DOCK6, PKD1L3

- **LHD genes**:
  - PNPLA3
  - PKD1L3
  - TEAD2

- **Lipid levels**:
  - TG: Increased
  - HDL: Increased
  - LDL: Increased
  - TC: Increased
## Known lipid-associated genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Lipid type</th>
<th>Frequency (%)</th>
<th>Effect</th>
<th>(P)</th>
<th>CAD risk</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCSK9 (R93C)</td>
<td>LDL</td>
<td>1.3</td>
<td>-24%</td>
<td>8x10^{-32}</td>
<td>(\downarrow) 52%</td>
<td>4x10^{-7}</td>
</tr>
<tr>
<td>CETP (D459G)</td>
<td>HDL</td>
<td>2.7</td>
<td>+17%</td>
<td>3x10^{-29}</td>
<td>(\downarrow) 3%</td>
<td>0.73</td>
</tr>
<tr>
<td>LDLR (R257W)</td>
<td>LDL</td>
<td>0.1</td>
<td>+32%</td>
<td>3x10^{-8}</td>
<td>(\uparrow) 366%</td>
<td>1x10^{-4}</td>
</tr>
</tbody>
</table>

## Novel lipid-associated genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Lipid type</th>
<th>Frequency (%)</th>
<th>Effect</th>
<th>(P)</th>
<th>CAD risk</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNPLA3 (I148M)</td>
<td>TG</td>
<td>36.7</td>
<td>-3%</td>
<td>4x10^{-8}</td>
<td>(\downarrow) 7%</td>
<td>0.011</td>
</tr>
<tr>
<td>PKD1L3 (T429S)</td>
<td>LDL</td>
<td>74.0</td>
<td>+3%</td>
<td>5x10^{-8}</td>
<td>(\uparrow) 5%</td>
<td>0.11</td>
</tr>
<tr>
<td>TEAD2</td>
<td>HDL</td>
<td>0.09</td>
<td>+37%</td>
<td>2x10^{-7}</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Results of Chinese exome-wide genetic study**
Replication of association between *PNPLA3* variants and TG on Europeans

**Exome-wide association study of plasma lipids in >300,000 individuals**

We screened variants on an exome-focused genotyping array in >300,000 participants (replication in >280,000 participants) and identified 444 independent variants in 250 loci significantly associated with total cholesterol (TC), high-density-lipoprotein cholesterol (HDL-C), low-density-lipoprotein cholesterol (LDL-C), and/or triglycerides (TG). At two loci (*JAK2* and *ATCF*), experimental analysis in mice showed lipid changes consistent with the human data. We also found that: (i) beta-thalassemia trait carriers displayed lower TC and were protected from coronary artery disease (CAD); (ii) excluding the *CETP* locus, there was not a predictable relationship between plasma HDL-C and risk for age-related macular degeneration; (iii) only some mechanisms of lowering LDL-C appeared to increase risk for type 2 diabetes (T2D); and (iv) TG-lowering alleles involved in hepatic production of TG-rich lipoproteins (*TM6SF2* and *PNPLA3*) tracked with higher liver fat, higher risk for T2D, and lower risk for CAD, whereas TG-lowering alleles involved in peripheral lipolysis (*LPL* and *ANGPTL4*) had no effect on liver fat but decreased risks for both T2D and CAD.
Leveraging power by meta-analysis

- Increasing sample size by meta-analysis increases power to detect disease-associated variants
- Meta-analysis between and within populations allows identification of variants with common and population-specific effects
By meta-analysing the exome chip association studies including >47,000 East Asians and >300,000 GLGC samples, we aim to identify genetic factors

- specifically affecting blood lipid levels in East Asian populations and
- contributing to the risk of CAD in East Asian populations and/or
- affecting blood lipid levels across populations
Theme-based Research Scheme (TRS) on Personalized Medicine for Cardiovascular Disease: From Genomic Testing and Biomarkers to Human Pluripotent Stem Cell Platform

Exome-chip meta-analysis on blood lipids and CAD on East Asians

Materials and Methods

Lu et al., Nature Genetics, 2017
Study subjects

HKUTRS

6,048 Southern Chinese (HK)
• 2,372 CAD patients
• 3,388 non-CAD controls

Hong Kong Chinese CAD Cohort
• Started in 2004-2005 in Queen Mary Hospital
• On-going prospective cohort study on the risk factors and clinical outcomes in Chinese patients with established CAD

Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS)
• On-going population-based prospective study of cardiovascular risk factors in Hong Kong, which was started in 1995

Hong Kong West Diabetes Registry (HKWDR)
• commenced in 2008 at medical specialist clinics of the Hong Kong West Cluster
• Prospective study on control of diabetes and related cardiovascular risk factors, and development of diabetic complications in type 2 diabetes patients
Study subjects

Meta-analysis of East Asian exome chip association analyses

- 47,532 East Asians
  - 12,685 Chinese samples from previous study
  - ~35,000 additional samples from
    - Mainland China
    - Taiwan
    - Singapore
    - The Philippines
Study subjects

Meta-analysis of East Asian + GLGC exome chip association analyses

- 47,532 East Asians
- >300,000 samples from GLGC
  - 84% Europeans
  - 16% of South Asians, Africans, Hispanics and others
Sample preparation

47,532 East Asians

Meta-analysis of East Asian exome chip association analyses

Blood lipid levels
- TC
- LDL
- TG
- HDL

DNA
Exome-chip meta-analysis on East Asians

- **Blood lipids**
  - 47,532 East Asian subjects passing quality controls

- **CAD**
  - 9,661 CAD cases
  - 18,558 Non-CAD controls

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DNA variations in exome can change protein structure and function.
Asian Exome-chip

303,719 variants

HKU custom panel
1,501 variants

Asian custom panel
59,317 variants

HumanExome BeadChip
242,901 variants
Assaying genetic variations

Asian Exome-chip
303,719 variants

iScan microarray scanner
Exome-chip meta-analysis on blood lipids and CAD on East Asians

Results

Exome chip meta-analysis identifies novel loci and East Asian–specific coding variants that contribute to lipid levels and coronary artery disease

Most genome-wide association studies have been of European individuals, even though most genetic variation in humans is seen only in non-European samples. To search for novel loci associated with blood lipid levels and clarify the mechanism of action at previously identified lipid loci, we used an exome array to examine protein-coding genetic variants in 47,532 East Asian individuals. We identified 255 variants at 41 loci that reached chip-wide significance, including 3 novel loci and 14 East Asian–specific coding variant associations. After a meta-analysis including >300,000 European samples, we identified an additional nine novel loci. Sixteen genes were identified by protein-altering variants in both East Asians and Europeans, and thus are likely to be functional genes. Our data demonstrate that most of the low-frequency or rare coding variants associated with lipids are population specific, and that examining genomic data across diverse ancestries may facilitate the identification of functional genes at associated loci.

Lu et al., Nature Genetics, 2017
Results of meta-analysis of exome chip association analysis on East Asians

- We identified 255 chip-wide significant variants at 41 loci
- 3 novel associated loci
  - **MCU** rs7901016
    - LDL ↓
    - TC ↓
    - EA 27%
    - GLGC 9%
  - **CD163** (I342V)
    - LDL ↓
    - HDL ↑
    - EA 31%
    - GLGC 6%
  - **ACVR1C** rs4377290
    - TC ↓
Results of exome-wide meta-analysis analysis on East Asians

Tang CS et al., Nature Communication, 2015
Novel associations identified from exome-wide meta-analysis analysis on East Asians

- 3 novel associated loci from East Asian meta-analysis

Lu X et al., Nature Genetics, 2017
Novel associations identified from exome-wide meta-analysis analysis of East Asian and GLGC samples

- 9 novel associated loci from East Asian + GLGC meta-analysis (n>350,000)
Results of exome-wide meta-analysis analysis on East Asians

- Frequencies of variants are inversely proportional to effect size
- Rare and low frequency coding variants generally have larger effects

Lu X et al., Nature Genetics, 2017
Population-specific association from East Asian and GLGC samples

Rare and low frequency **coding** variants found from **East Asian** meta-analysis

Higher frequencies in East Asians compared to Europeans

Rare and low frequency **coding** variants found from **GLGC** study (n>300,000)

Higher frequencies in Europeans than East Asians

Lu X et al., Nature Genetics, 2017
14 novel East Asian-specific association with blood lipids

<table>
<thead>
<tr>
<th>Gene</th>
<th>Lipid type</th>
<th>East Asian</th>
<th>GLGC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Freq (%)</td>
<td>Effect</td>
</tr>
<tr>
<td>EVI5 (R354C)</td>
<td>TC</td>
<td>0.69</td>
<td>0.21</td>
</tr>
<tr>
<td>APOB (I3768T)</td>
<td>TC</td>
<td>0.15</td>
<td>-0.66</td>
</tr>
<tr>
<td>(C478Y)</td>
<td></td>
<td>0.09</td>
<td>-0.88</td>
</tr>
<tr>
<td>(R532W)</td>
<td></td>
<td>12.4</td>
<td>-0.11</td>
</tr>
<tr>
<td>HMGCR (Y311S)</td>
<td>LDL</td>
<td>1.7</td>
<td>-0.19</td>
</tr>
<tr>
<td>CD36 (R386W)</td>
<td>HDL</td>
<td>0.31</td>
<td>0.34</td>
</tr>
<tr>
<td>APOA1 (A61T)</td>
<td>HDL</td>
<td>3.3</td>
<td>-0.12</td>
</tr>
<tr>
<td>ACACB (V2141I)</td>
<td>TG</td>
<td>74.3</td>
<td>0.04</td>
</tr>
<tr>
<td>ALDH2 (Q457K)</td>
<td>HDL</td>
<td>20.4</td>
<td>-0.05</td>
</tr>
<tr>
<td>CETP (Y74*)</td>
<td>HDL</td>
<td>0.03</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>(N459G)</td>
<td>2.23</td>
<td>0.41</td>
</tr>
<tr>
<td>PKD1L3 (R1572H)</td>
<td>LDL</td>
<td>5.4</td>
<td>0.09</td>
</tr>
<tr>
<td>LDLR (R257W)</td>
<td>TC</td>
<td>0.09</td>
<td>0.68</td>
</tr>
<tr>
<td>PPARA (V227A)</td>
<td>TG</td>
<td>4.2</td>
<td>-0.09</td>
</tr>
</tbody>
</table>

Lu X et al., Nature Genetics, 2017
• All non-HDL-related variants showed a consistent direction of effects between lipid traits and CAD

• Nearly all LDL-associated coding variants demonstrated association with CAD ($r^2=0.78; P=3.3\times 10^{-4}$)

Lu X et al., Nature Genetics, 2017
Summary

- Meta-analysis of exome chip association analysis of 47,532 East Asians and >300,000 GLGC samples on blood lipid levels identified
  - Association of DNA variations in 12 genes not previously reported to be associated with lipids
    - 3 genes (MCU, CD163 and ACVR1C) from East Asian meta-analysis
    - 9 genes from trans-ethnic meta-analysis with GLGC
    - 14 Asian-specific association involving coding variants
- Most of the novel non-HDL-associated variants also influence risk of CAD
Individuals carrying more lipid-increasing allele tends to have higher blood lipid levels.
The Promise of Precision Medicine

**Conventional Approach**

Clinical risk factor assessment

- High risk
- Low risk

**Precision Medicine Approach**

Clinical + Genetic risk factors assessment

- High risk
- Low risk

Gene-chip
The Promise of Precision Medicine

- Genetic risk score improves risk prediction on top of family history
- Individuals with high genetic risk may have the larger clinical benefit with statin treatment
- Early intervention of high-risk individuals may have larger relative risk reduction for incident or recurrent CAD
Acknowledgements

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