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

Professor TSE Hung-fat
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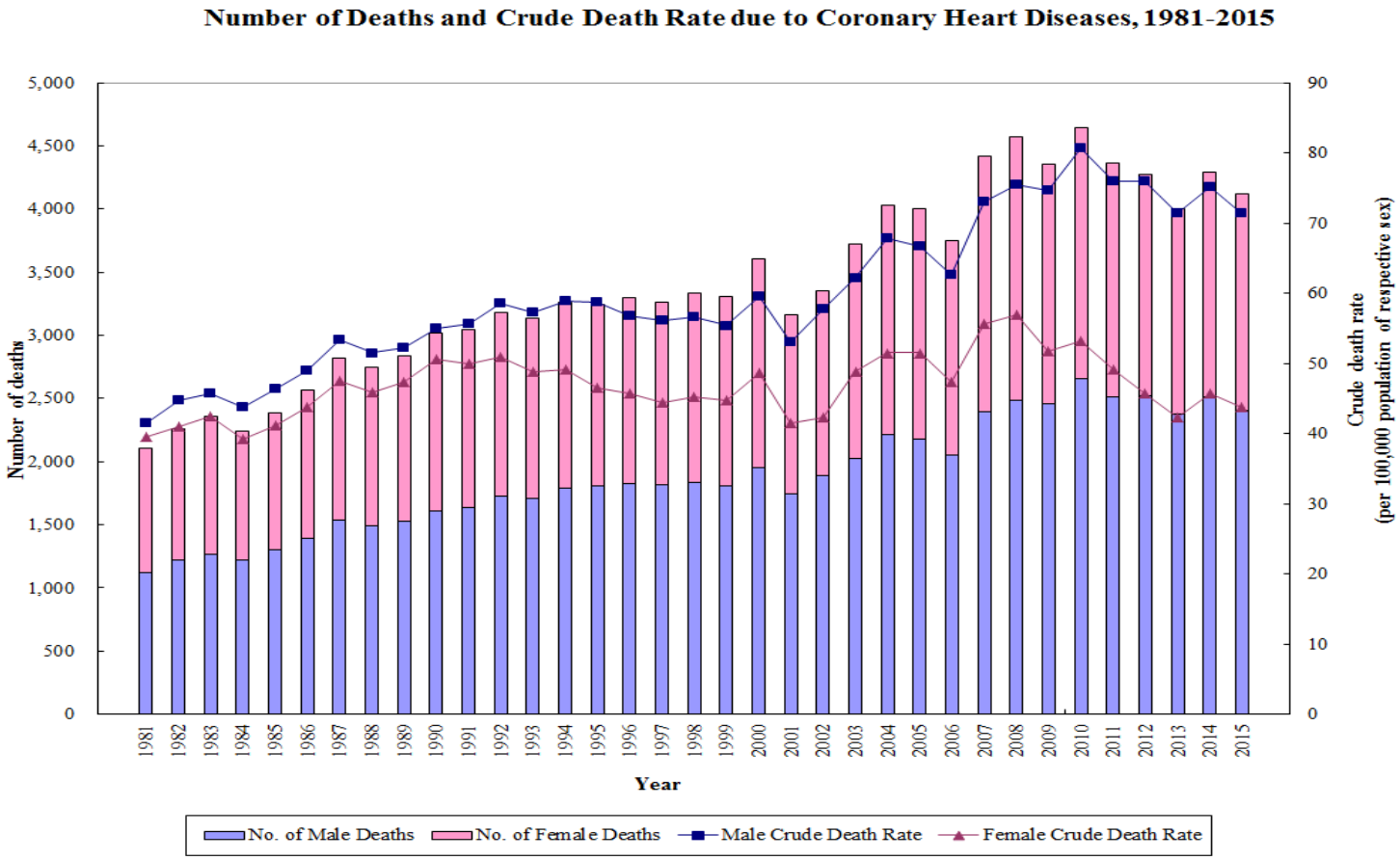
Coronary Artery Disease (CAD)

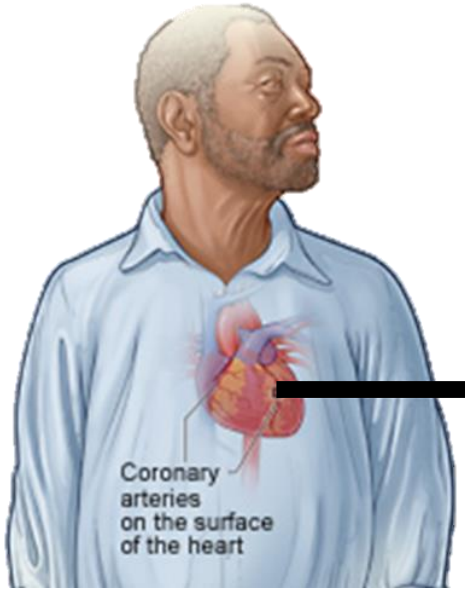


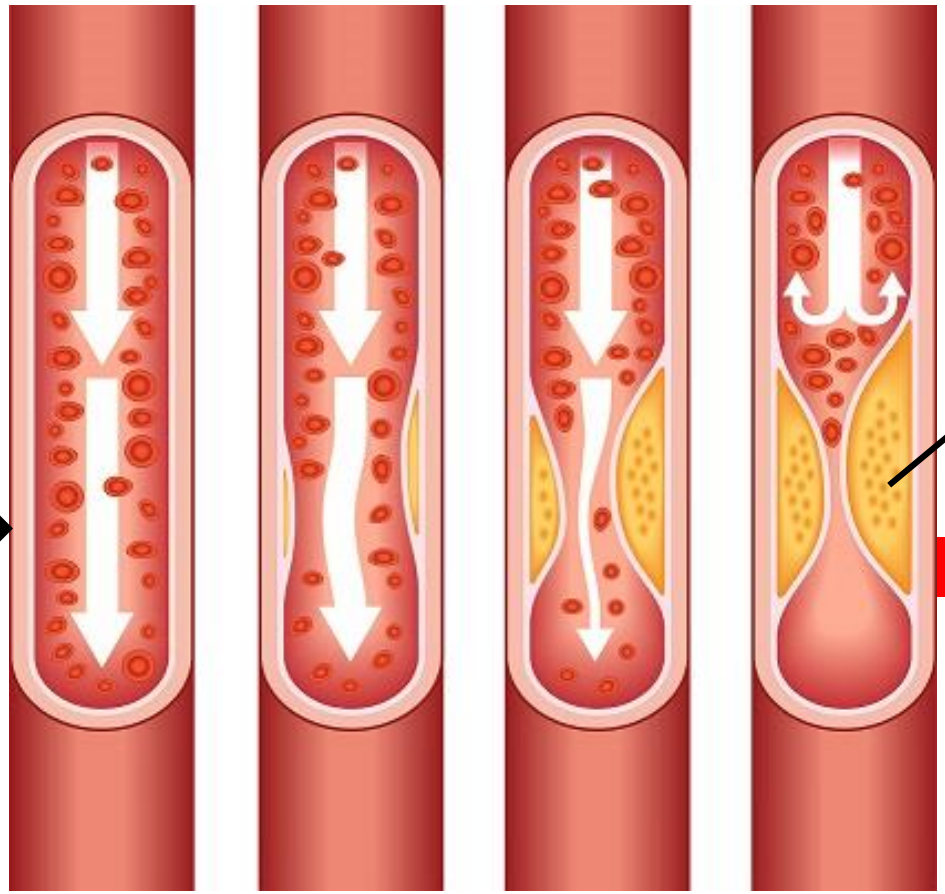
2015:

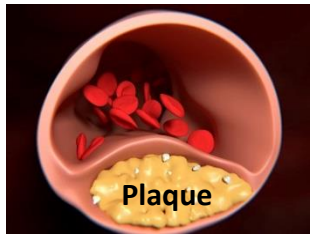
- **3rd leading cause of death in Hong Kong**
- **Account for 8.8% death in Hong Kong (total 4,123)**
- **11 persons died of CAD per day**

Department of Health (http://www.healthyhk.gov.hk/)

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

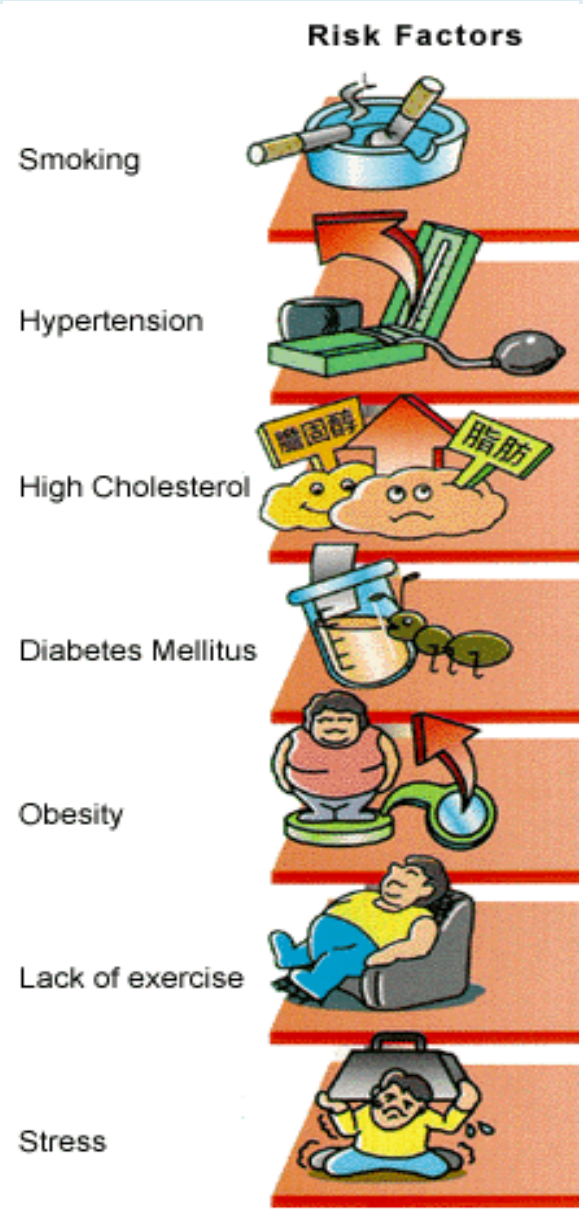
Modifiable risk factors of CAD

Abnormal blood lipid levels is the major risk factor of CAD

1mmol/L

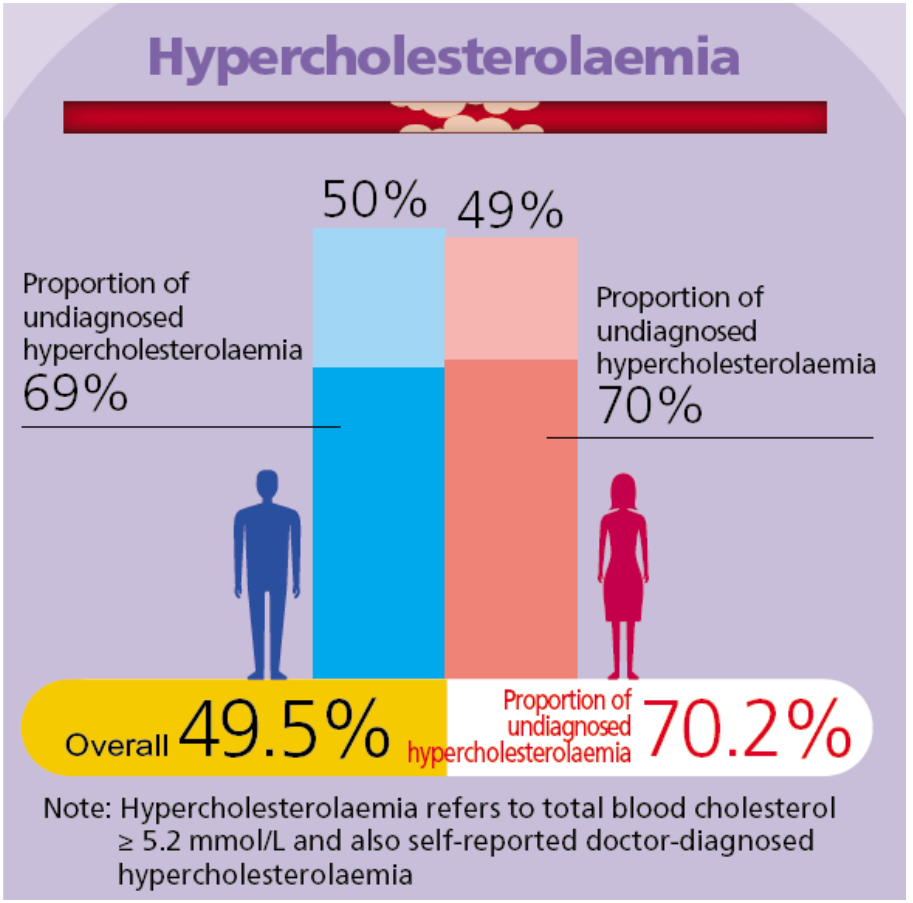
risk of CAD death 35%

- **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)**



Abnormal blood level lipids are prevalent among general population

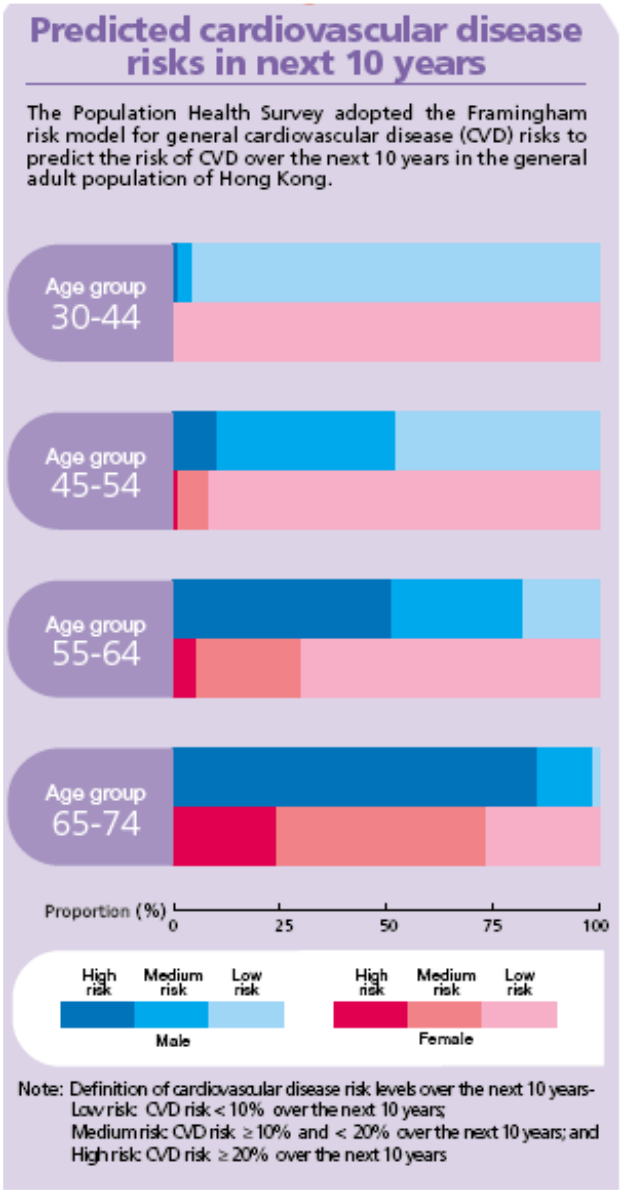
- **The second Population Health Survey released by the Department of Health reported that abnormal blood lipid levels (hypercholesterolaemia) are common in Hong Kong**
  - 49.5% of Hong Kong people aged 15 to 84 have hyper-cholesterolaemia
  - Compared to 2003/04, hypercholesterolaemia becomes more prevalent with age-standardised rate increases from 35.3% to 42.1%
  - 70.2% were only picked up by health examination



Department of Health

Cardiovascular (CVD) risk

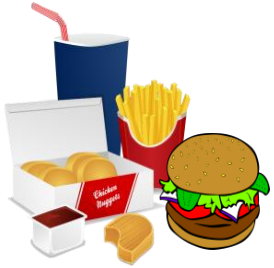
- By adopting the Framingham risk model, the Survey predicted that, among people aged 30 to 74, the mean cardiovascular (CVD) risk over the next 10 years is 10.6%
- 8/10 of males and 1/4 of females aged 65-74 were predicted as having high CVD risk
- Around half of the major non-communicable diseases were only picked up by health examination



Department of Health

Factors affecting blood lipids levels

- 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



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

Background

ARTICLES

nature genetics

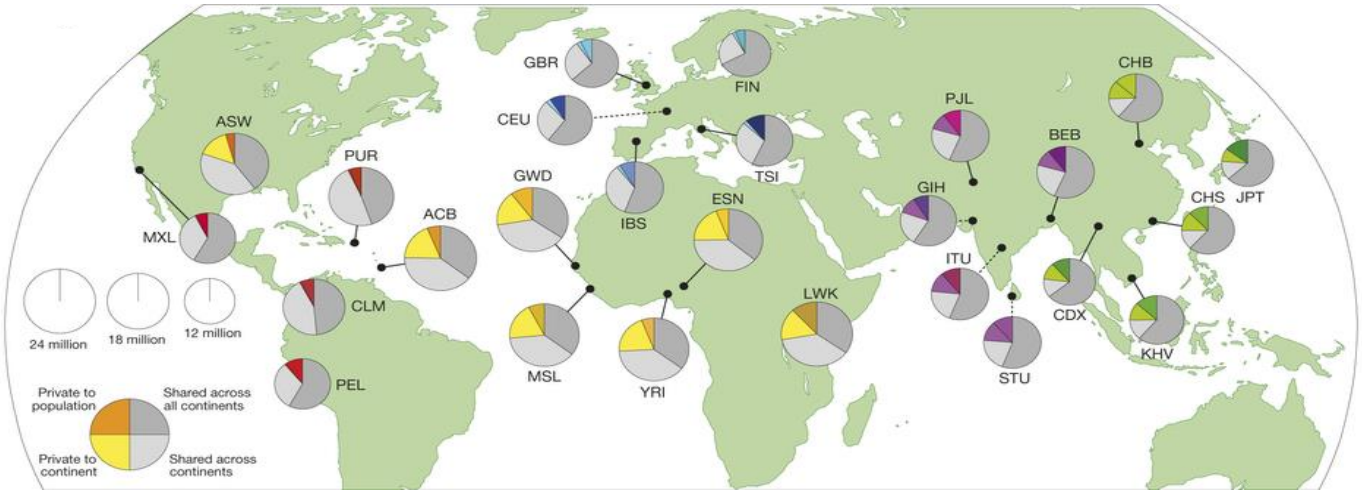
Exome chip meta-analysis identifies novel loci and East Asian–specific coding variants that contribute to lipid levels and coronary artery disease

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

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

Adapted from 1000 Genomes Project Consortium. *Nature* **526**, 68–74. doi:10.1038/nature15393

The first Chinese exome-wide genetic study on blood lipids and CAD

nature COMMUNICATIONS

ARTICLE

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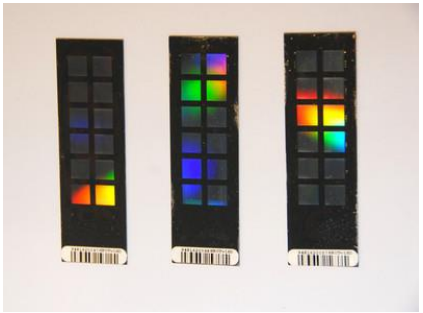
DOI: 10.1038/ncomms10206 OPEN

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

Clara S. Tang[1,*], He Zhang[2,*], Chloe Y.Y. Cheung[3,*], Ming Xu[4,*], Jenny C.Y. Ho[3], Wei Zhou[2,5], Stacey S. Cherny[1,6,7], Yan Zhang[8], Oddgeir Holmen[9,10], Ka-Wing Au[3], Haiyi Yu[4], Lin Xu[11], Jia Jia[8], Robert M. Porsch[1], Lijie Sun[4], Weixian Xu[4], Huiping Zheng[4], Lai-Yung Wong[3], Yiming Mu[12], Jingtao Dou[12], Carol H.Y. Fong[3], Shuyu Wang[13], Xueyu Hong[3], Liguang Dong[14], Yanhua Liao[14], Jiansong Wang[14], Levina S.M. Lam[6], Xi Su[15], Hua Yan[15], Min-Lee Yang[2], Jin Chen[2], Chung-Wah Siu[3,16], Gaoqiang Xie[17], Yu-Cho Woo[3], Yangfeng Wu[18], Kathryn C.B. Tan[3,16], Kristian Hveem[9], Bernard M.Y. Cheung[3,16,19], Sebastian Zöllner[20], Aimin Xu[3,16,19,21], Y. Eugene Chen[2], Chao Qiang Jiang[22], Youyi Zhang[23], Tai-Hing Lam[11], Santhi K. Ganesh[2,24], Yong Huo[8], Pak C. Sham[1,6,7,**], Karen S.L. Lam[3,16,19,**], Cristen J. Willer[2,5,24,**], Hung-Fat Tse[3,16,25,**] & Wei Gao[26,**]

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 ($P<2.69\times10^{-7}$), 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* p.Thr429Ser—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.



Asian Exome-chip

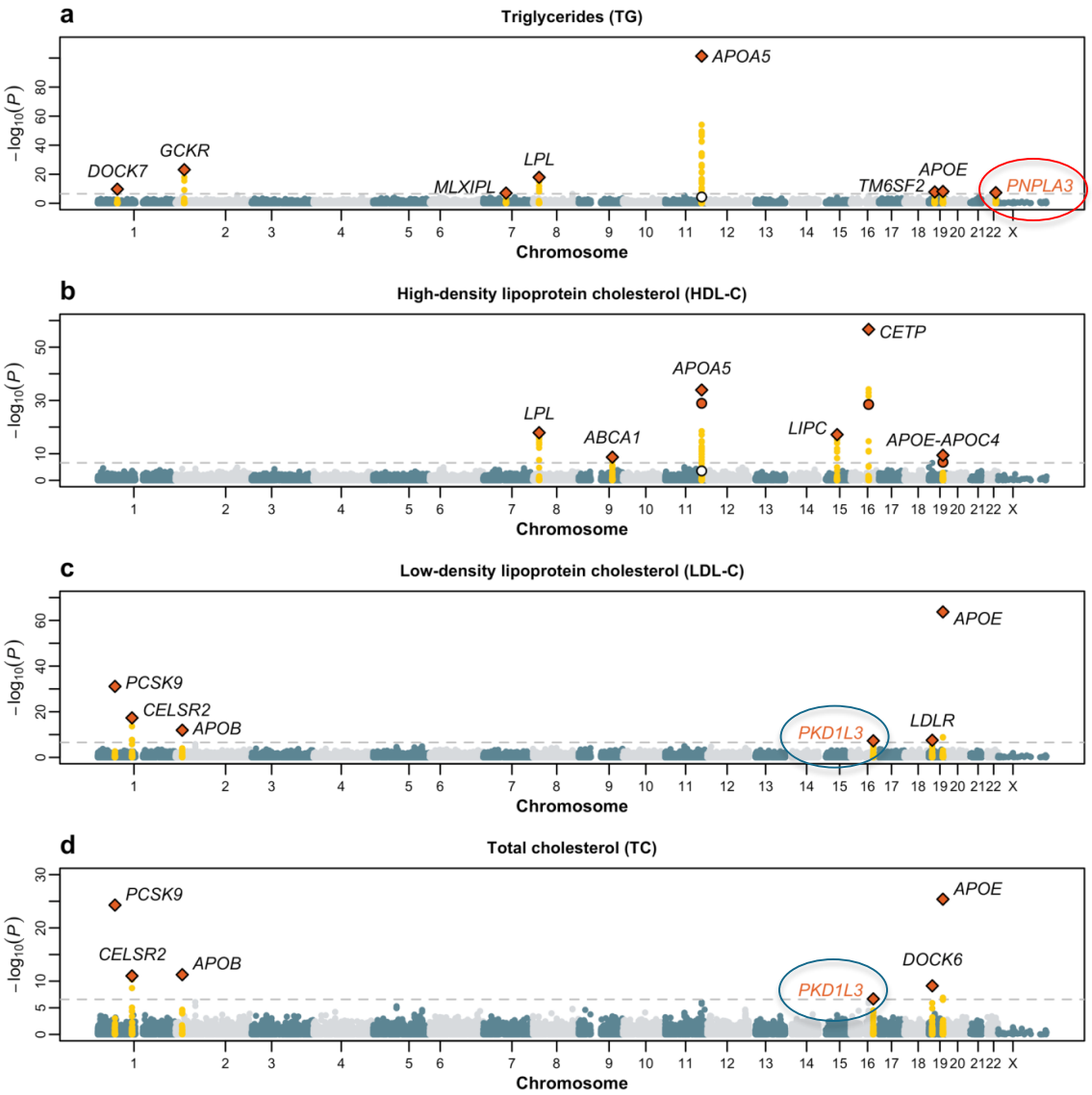
Results of Chinese exome-wide genetic study

TG

HDL

LDL

TC



Lipid-associated genes not reported before

PNPLA3 → TG

PKD1L3 → LDL, TC

TEAD2 → HDL

Results of Chinese exome-wide genetic study

Known lipid-associated genes			Blood lipid level		CAD	
Gene	Lipid type	Frequency (%)	Effect	*P*	risk	*P*
PCSK9 (R93C)	LDL	1.3	-24%	$8x10^{-32}$	↓ 52%	$4x10^{-7}$
CETP (D459G)	HDL	2.7	+17%	$3x10^{-29}$	↓ 3%	0.73
LDLR (R257W)	LDL	0.1	+32%	$3x10^{-8}$	↑ 366%	$1x10^{-4}$

Novel lipid-associated genes			Blood lipid level		CAD	
Gene	Lipid type	Frequency (%)	Effect	*P*	risk	*P*
PNPLA3 (I148M)	TG	36.7	-3%	$4x10^{-8}$	↓ 7%	0.011
PKD1L3 (T429S)	LDL	74.0	+3%	$5x10^{-8}$	↑ 5%	0.11
TEAD2	HDL	0.09	+37%	$2x10^{-7}$	-	-

Replication of association between *PNPLA3* variants and TG on Europeans

LETTERS

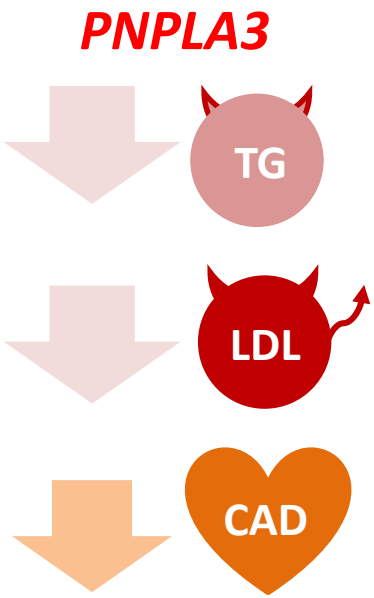
nature genetics

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 *A1CF*), 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.

Global Lipid Genetics Consortium (GLGC)

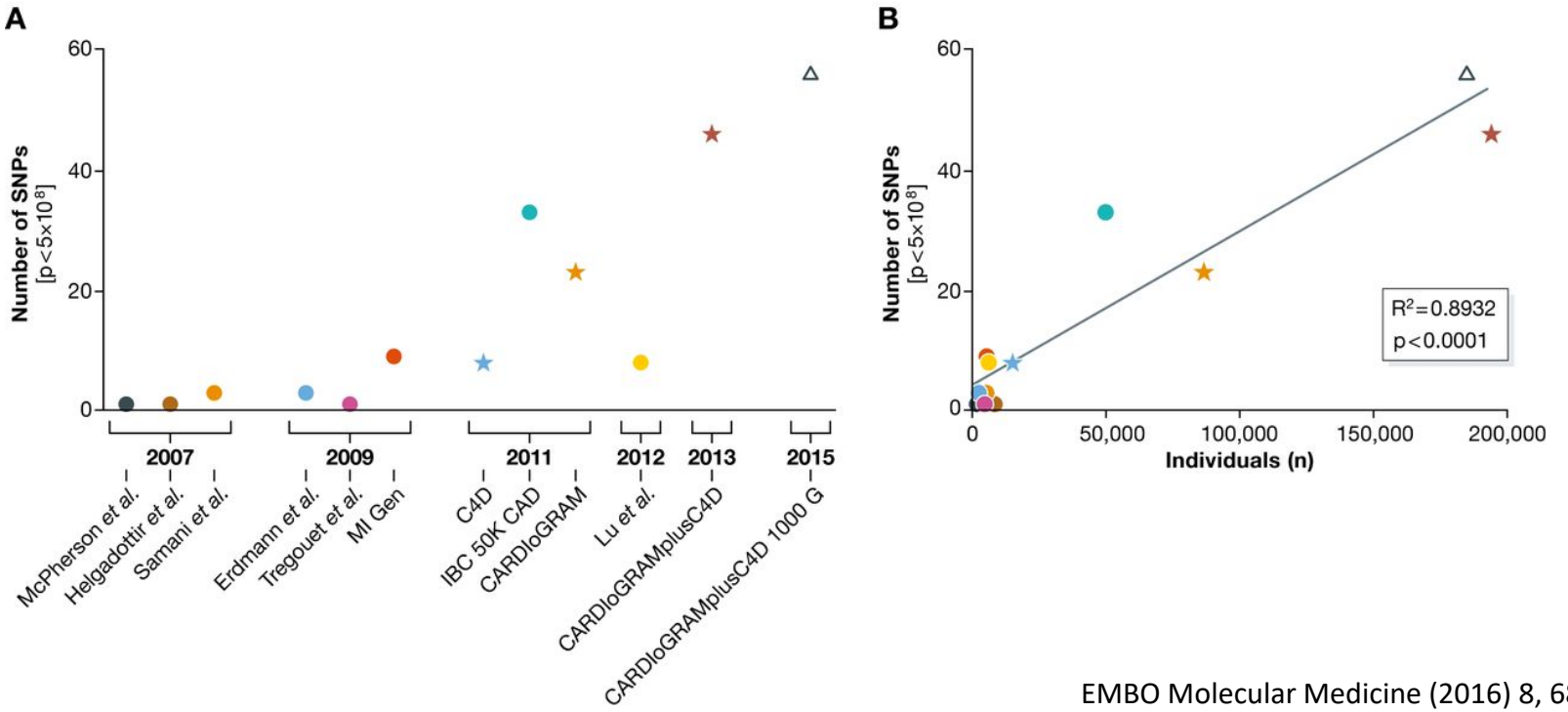
>300,000 samples (84% Europeans)



Liu *et al.*, Nature Genetics, 2017

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



EMBO Molecular Medicine (2016) 8, 688-701

Aims of this study

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

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

Materials and Methods

ARTICLES

nature genetics

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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

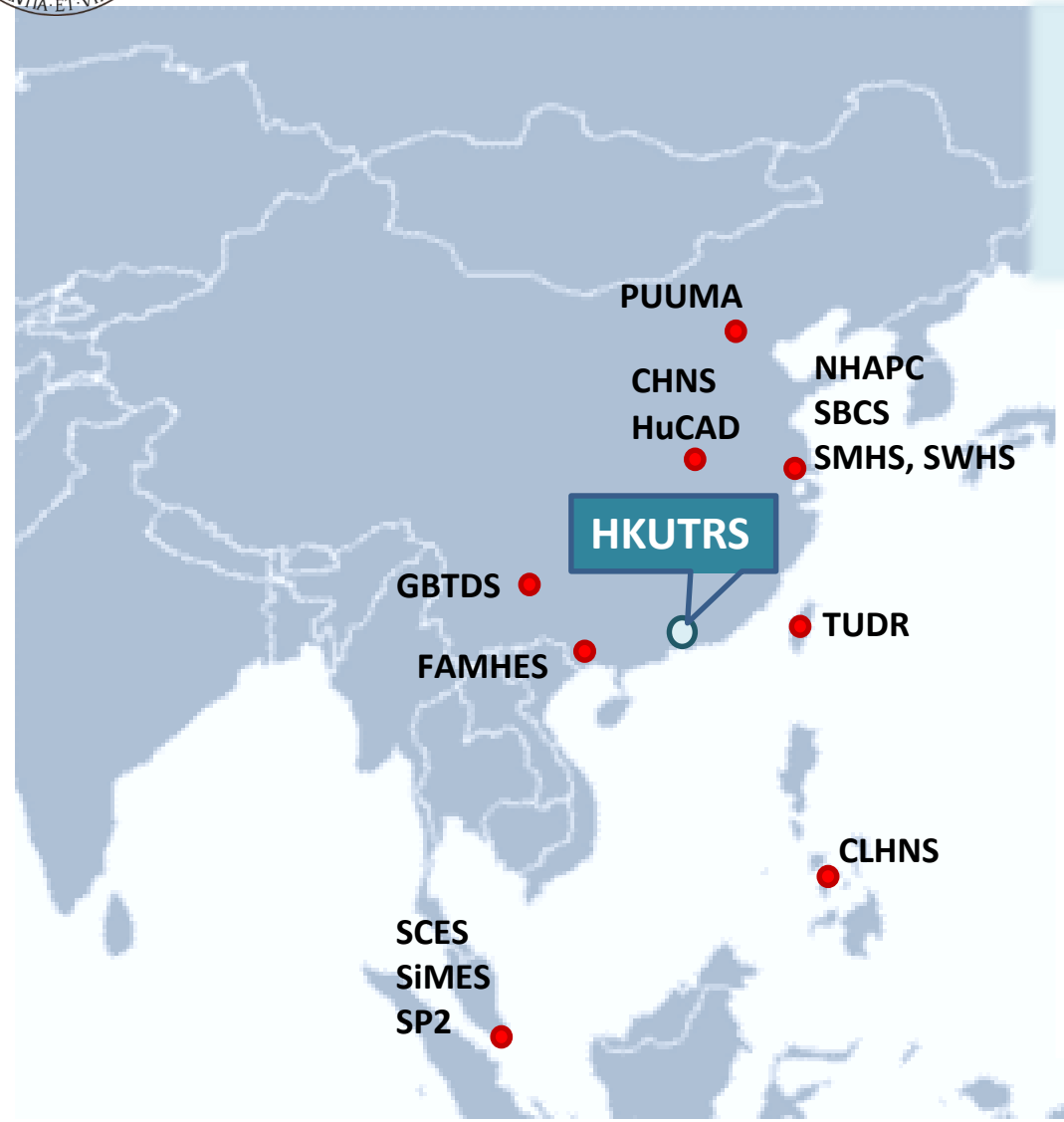
Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS)

- 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

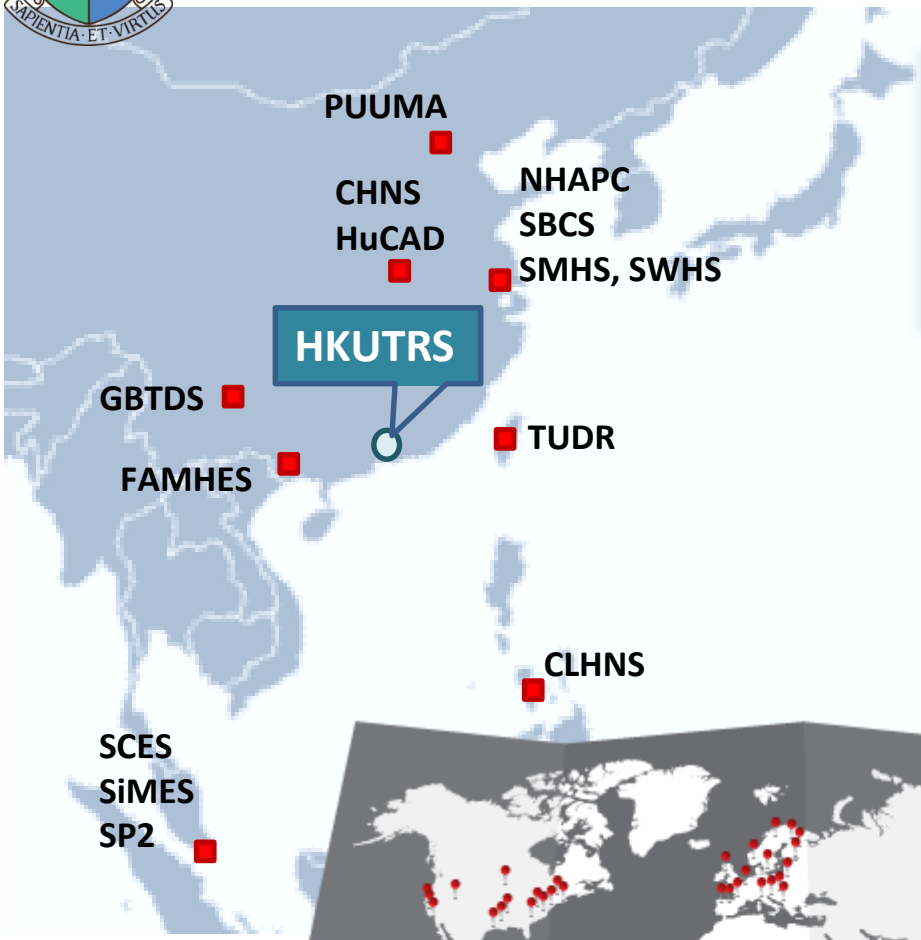
Study subjects



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

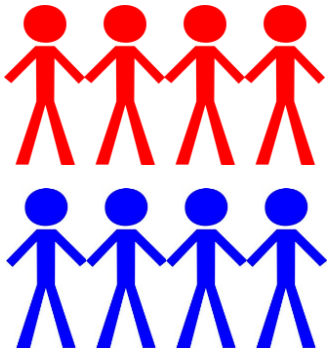
Study subjects



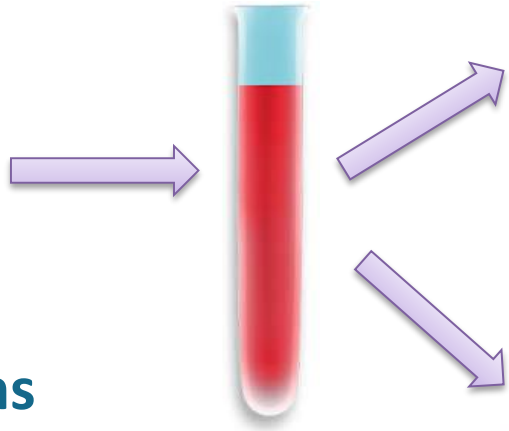
Meta-analysis of East Asian + GLGC exome chip association analyses

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

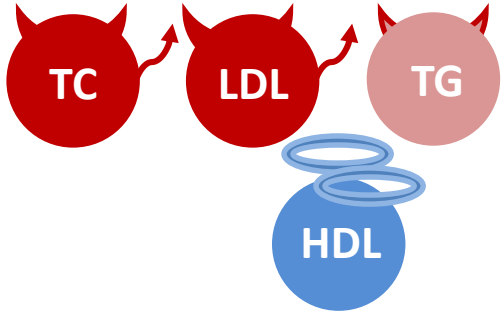
Sample preparation



47,532 East Asians



Blood lipid levels



DNA

Meta-analysis of
East Asian exome chip
association analyses

Exome-chip meta-analysis on East Asians

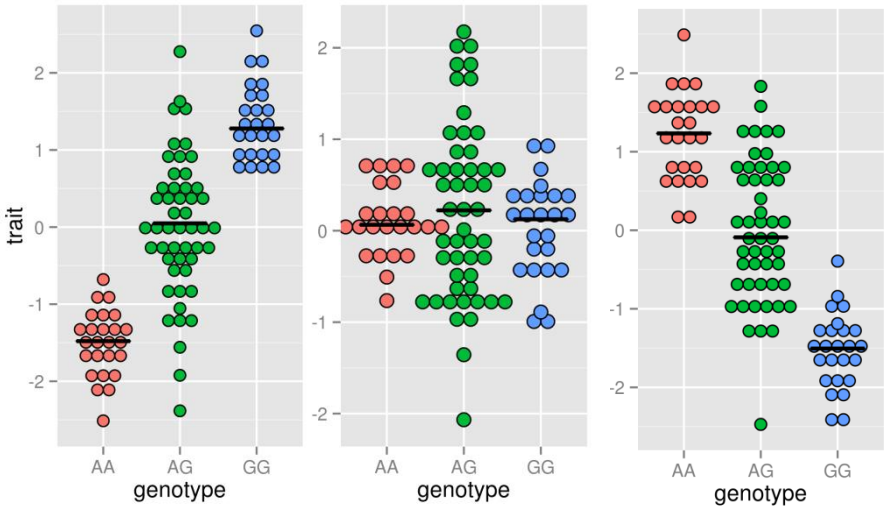
- **Blood lipids**

- **CAD**

47,532 East Asian subjects passing quality controls

9,661 CAD cases

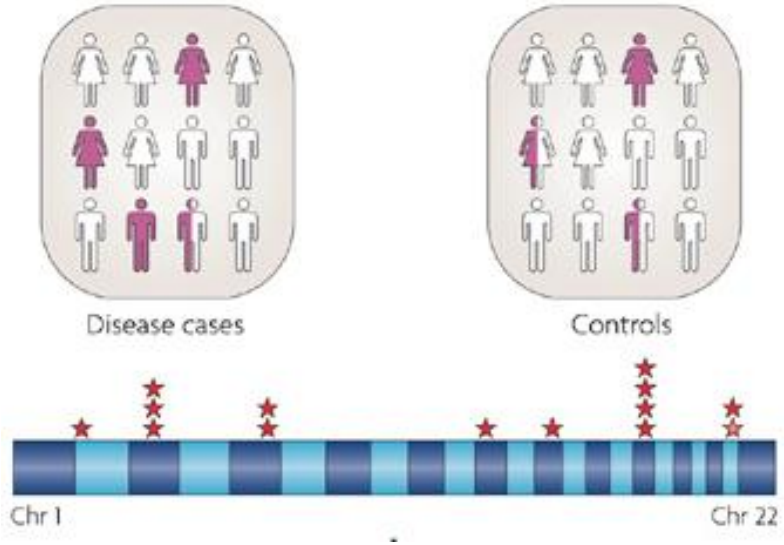
18,558 Non-CAD controls



Positive association

No association

Negative association



Adapted from Mathew. (2008) *Nat Rev Genet* **9(1)**, 9–14

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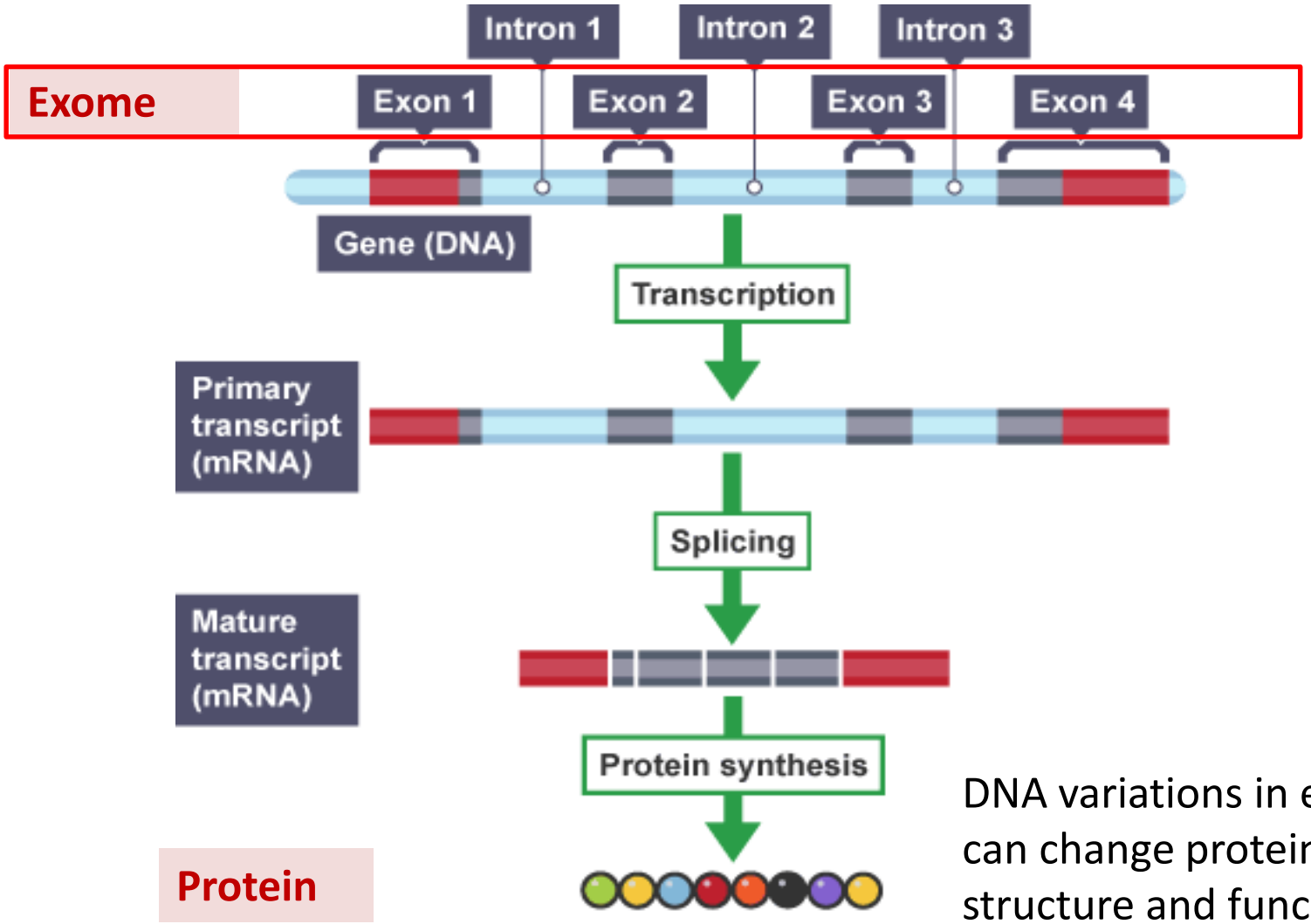
Asian Exome-chip

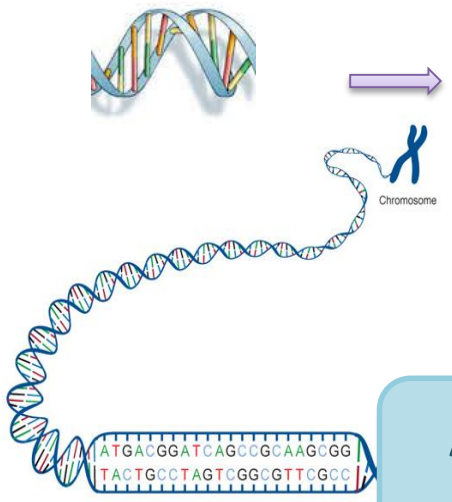
Exome



DNA variations in exome can change protein structure and function

Asian Exome-chip

CENTRE FOR GENOMIC SCIENCES
LI KA SHING FACULTY OF MEDICINE
THE UNIVERSITY OF HONG KONG
香港大學李嘉誠醫學院基因研究中心



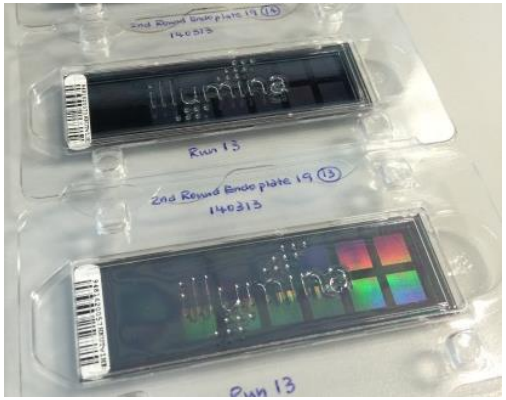
Asian Exome-chip
303,719 variants

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

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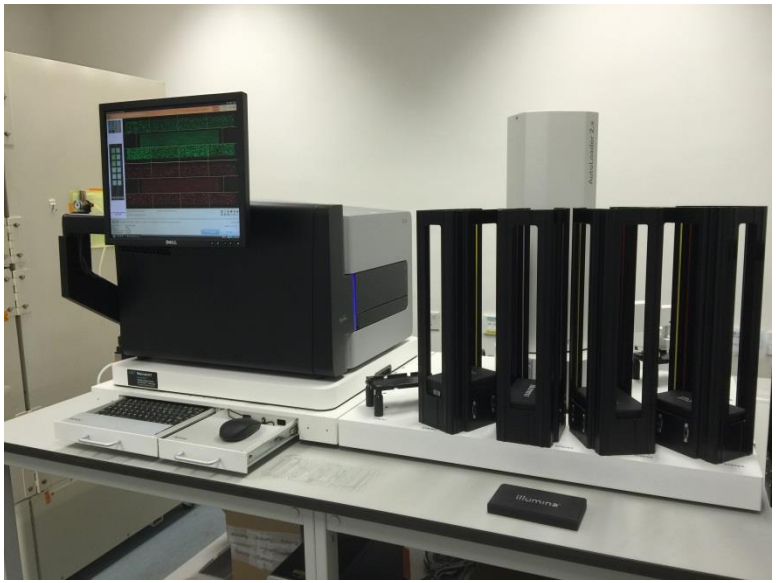
香港大學李嘉誠醫學院基因研究中心



Theme-based Research Scheme (TRS)

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Results

ARTICLES

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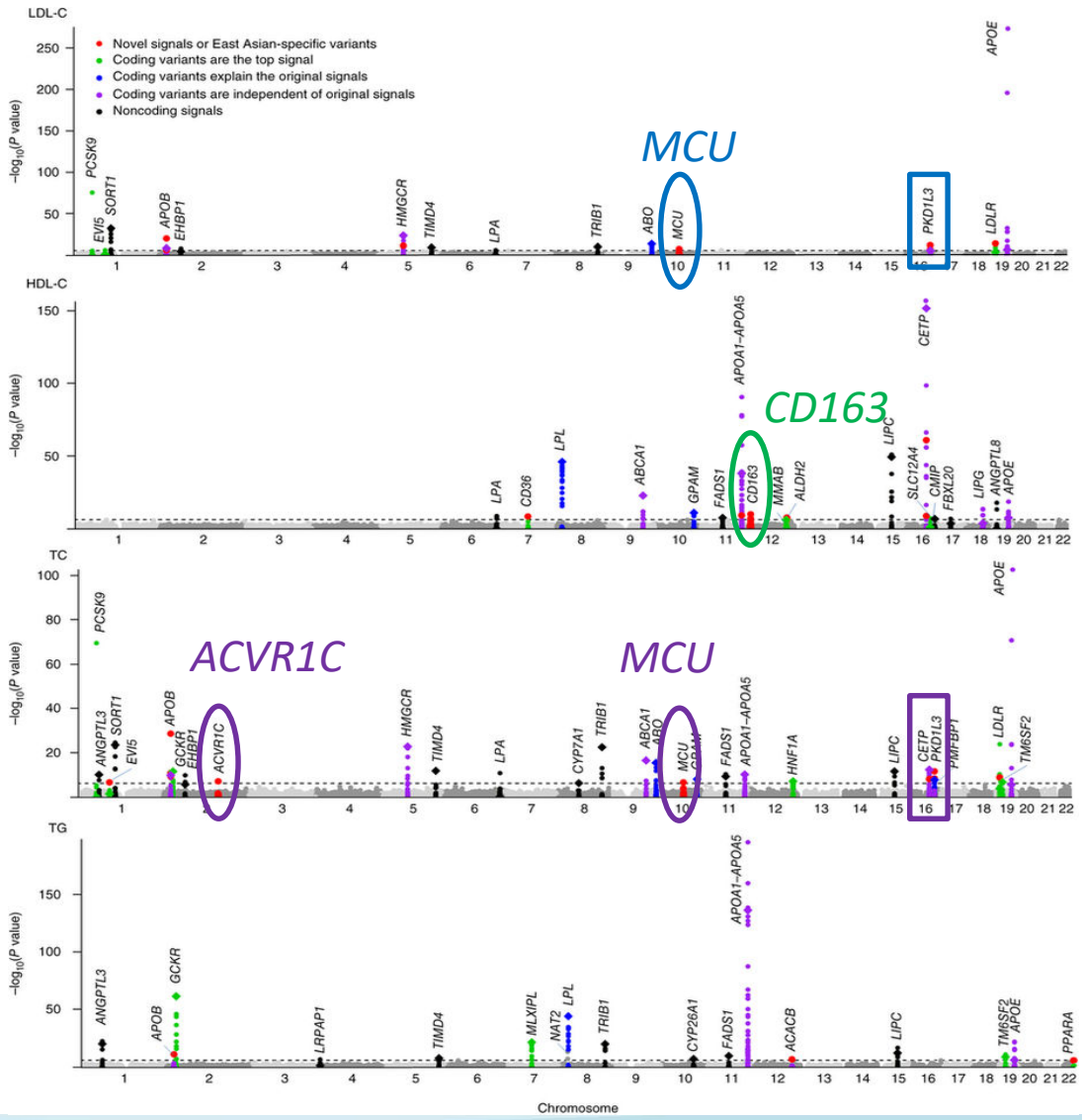
Lu *et al.*, Nature Genetics, 2017

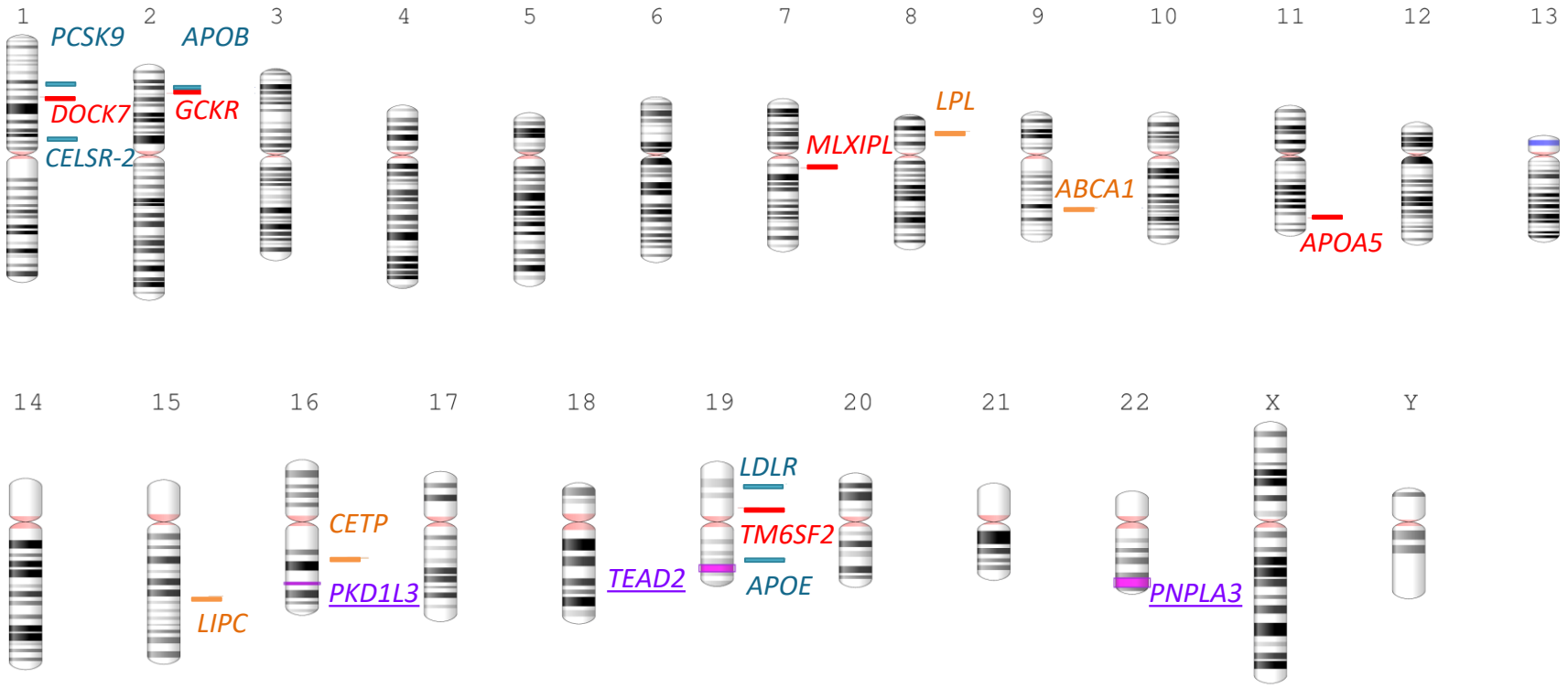
Results of meta-analysis of exome chip association analysis on East Asians



- We identified 255 chip-wide significant variants at 41 loci
- **3 novel associated loci**

- *MCU* rs7901016
  ↓ LDL ↓ TC
  EA 27% GLGC 9%
- *CD163* (I342V)
  ↑ HDL
  EA 31% GLGC 6%
- *ACVR1C* rs4377290
  ↓ TC

Results of exome-wide meta-analysis analysis on East Asians

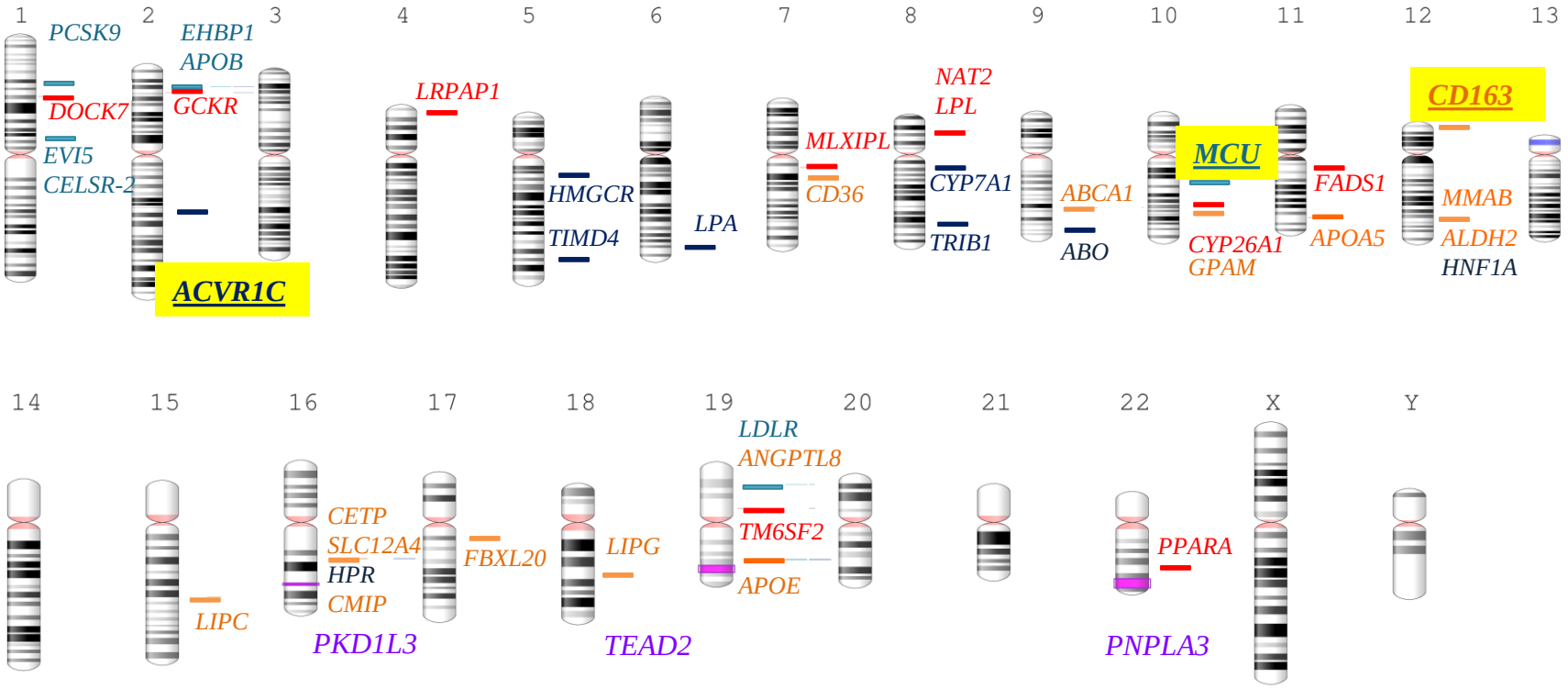


TG **HDL**

TC **LDL**

Tang CS *et al.*, Nature Communication, 2015

Novel associations identified from exome-wide meta-analysis analysis on East Asians



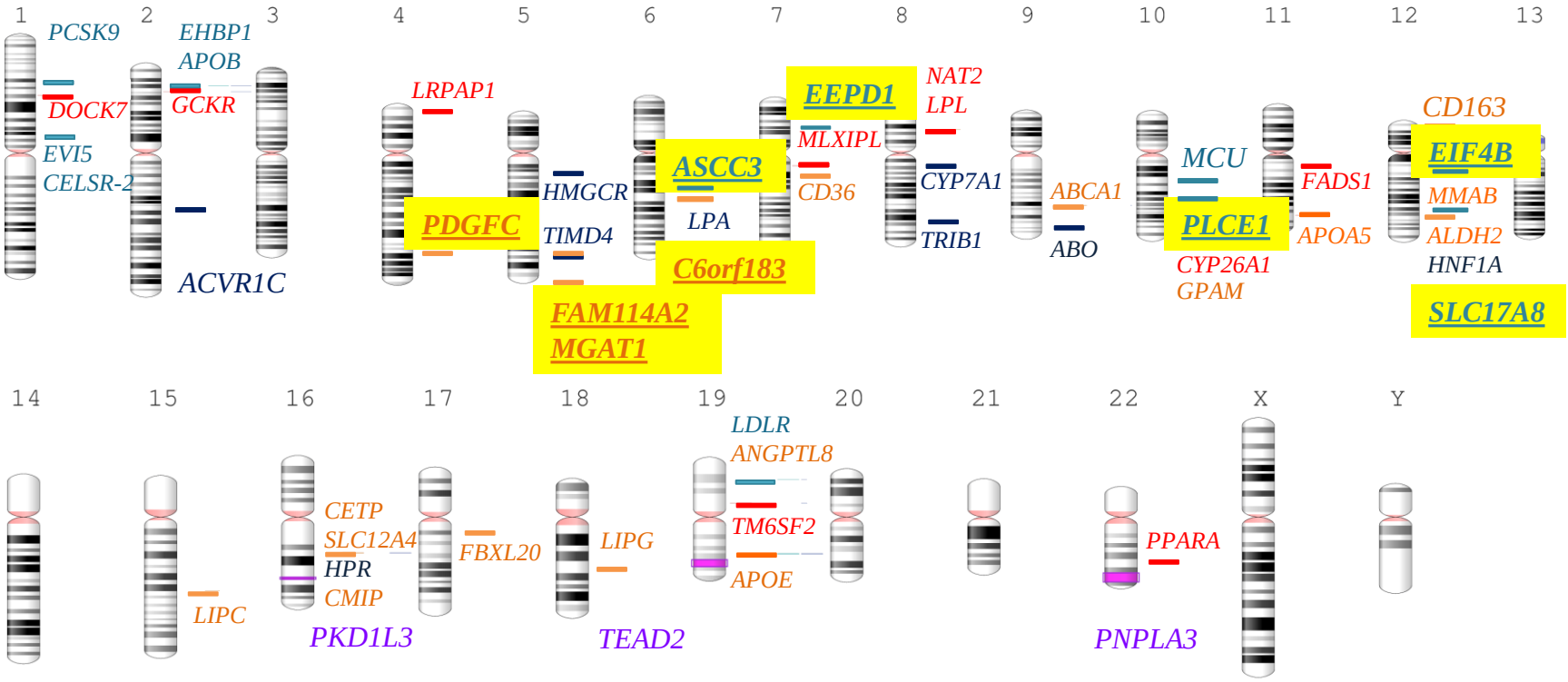
TG HDL

TC LDL

- **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



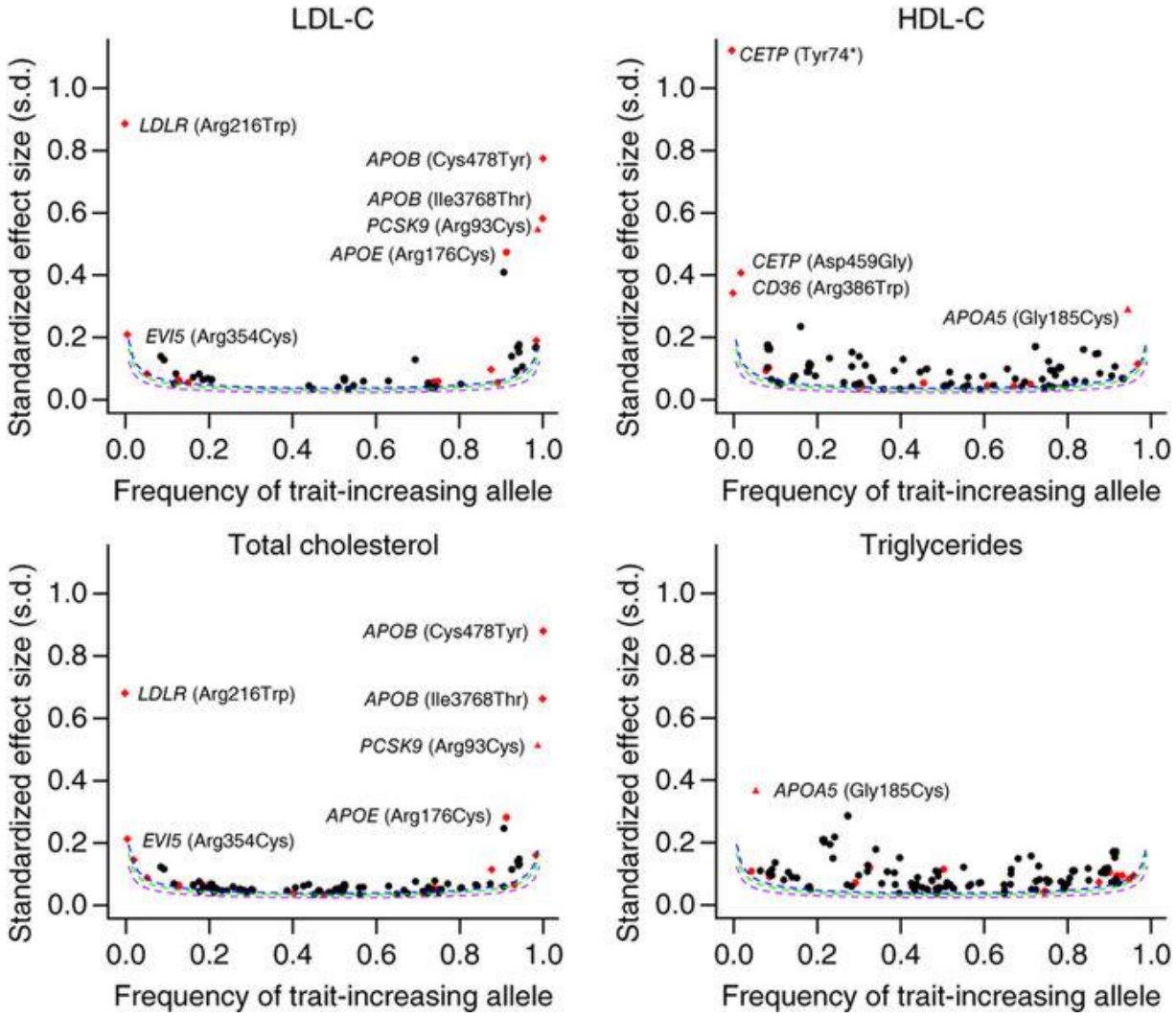
TG **HDL**

TC **LDL**

- **9 novel associated loci from East Asian + GLGC meta-analysis (n>350,000)**

Lu X *et al.*, Nature Genetics, 2017

Results of exome-wide meta-analysis analysis on East Asians

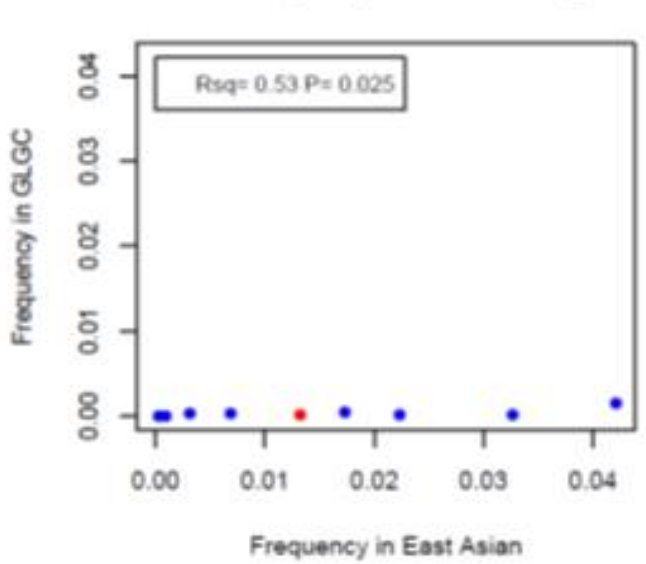


- Frequencies of variants are inversely proportional to effect size
- Rare and low frequency coding variants generally have larger effects

Lu X *et al.*, Nature Genetics, 2017

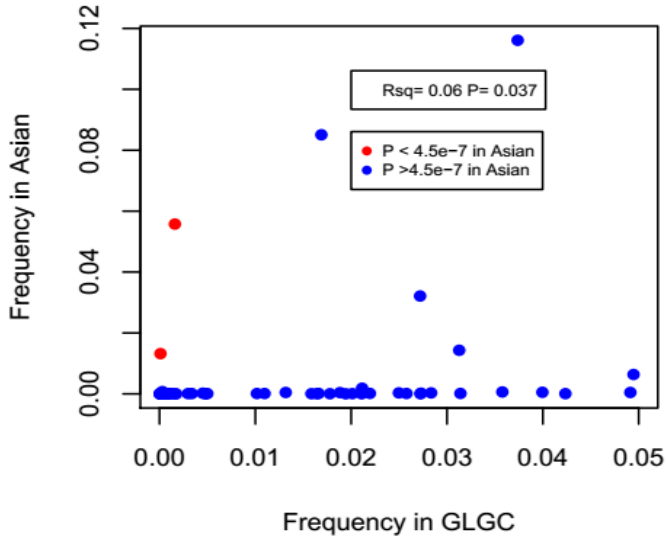
Population-specific association from East Asian and GLGC samples

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

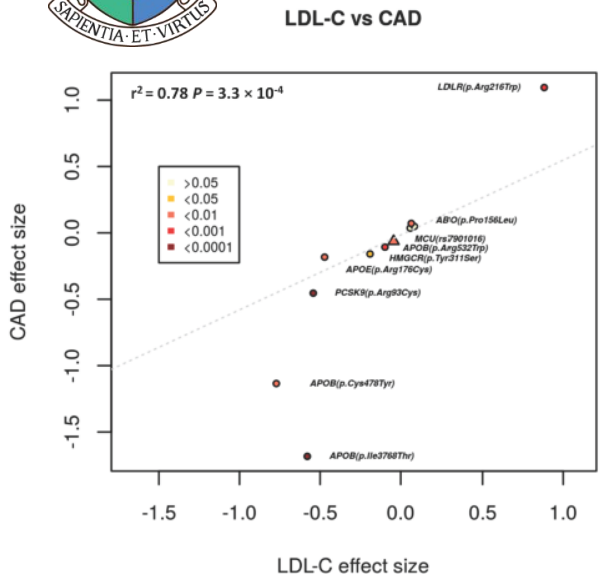
Lu X *et al.*, Nature Genetics, 2017

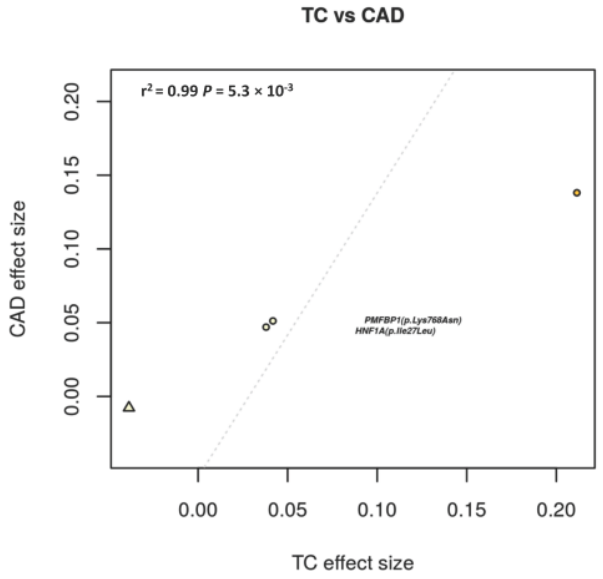
14 novel East Asian-specific association with blood lipids

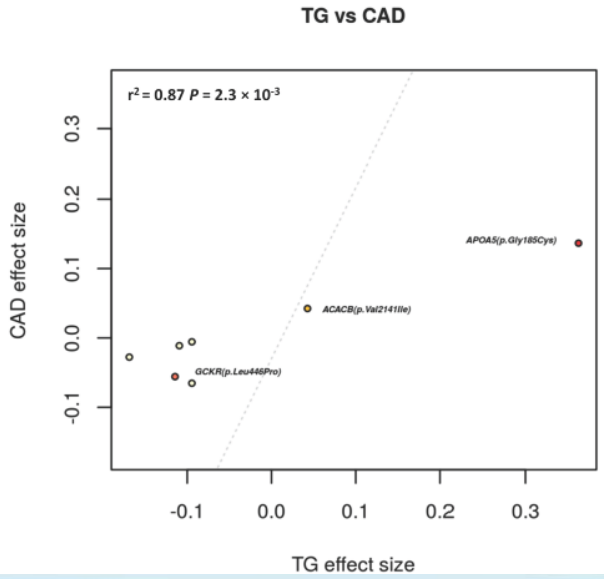
		East Asian			GLGC		
Gene	Lipid type	Freq (%)	Effect	*P*	Freq (%)	Effect	*P*
EVI5 (R354C)	TC	0.69	0.21	1.4×10^{-7}	0.03	0.10	0.25
APOB (I3768T)	TC	0.15	-0.66	8.4×10^{-12}			
(C478Y)		0.09	-0.88	2.1×10^{-10}			
(R532W)		12.4	-0.11	1.5×10^{-19}	0.19	-0.08	6.7×10^{-3}
HMGCR (Y311S)	LDL	1.7	-0.19	2.2×10^{-13}	0.04	-0.12	0.08
CD36 (R386W)	HDL	0.31	0.34	3.2×10^{-9}	0.02	0.22	0.01
APOA1 (A61T)	HDL	3.3	-0.12	5.5×10^{-10}	0.02	0.08	0.45
ACACB (V2141I)	TG	74.3	0.04	4.0×10^{-8}	80.2	0.01	5.3×10^{-4}
ALDH2 (Q457K)	HDL	20.4	-0.05	1.2×10^{-8}	0.08	-0.01	0.93
CETP (Y74*)	HDL	0.03	1.12	9.0×10^{-10}	0.001	0.72	0.04
(N459G)		2.23	0.41	7.5×10^{-62}	0.02	0.38	3.2×10^{-5}
PKD1L3 (R1572H)	LDL	5.4	0.09	2.1×10^{-8}	24.4	-0.01	8.5×10^{-5}
LDLR (R257W)	TC	0.09	0.68	5.6×10^{-10}	0.001	1.90	1.6×10^{-4}
PPARA (V227A)	TG	4.2	-0.09	3.2×10^{-7}	0.15	-0.06	0.12

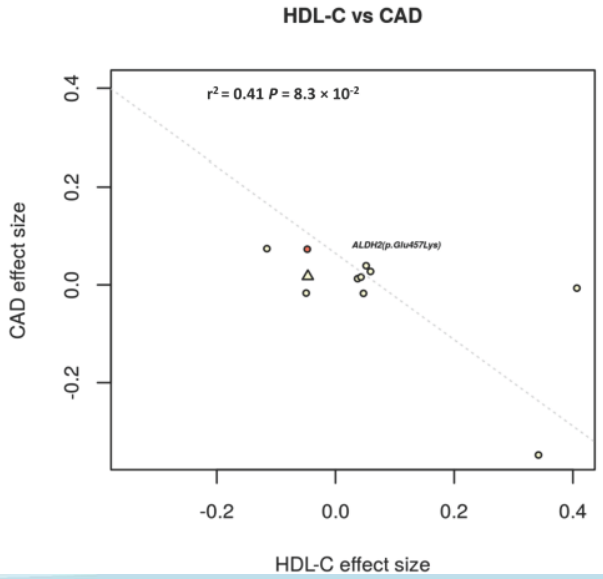
Lu X *et al.*, Nature Genetics, 2017

Association of novel lipid-associated variants with CAD

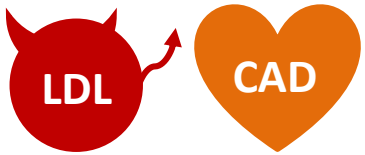








- All non-HDL-related variants showed a consistent direction of effects between lipid traits and CAD
- Nearly all LDL-associated coding variants demonstrated association with CAD (r^2=0.78;P=3.3× 10^{-4})



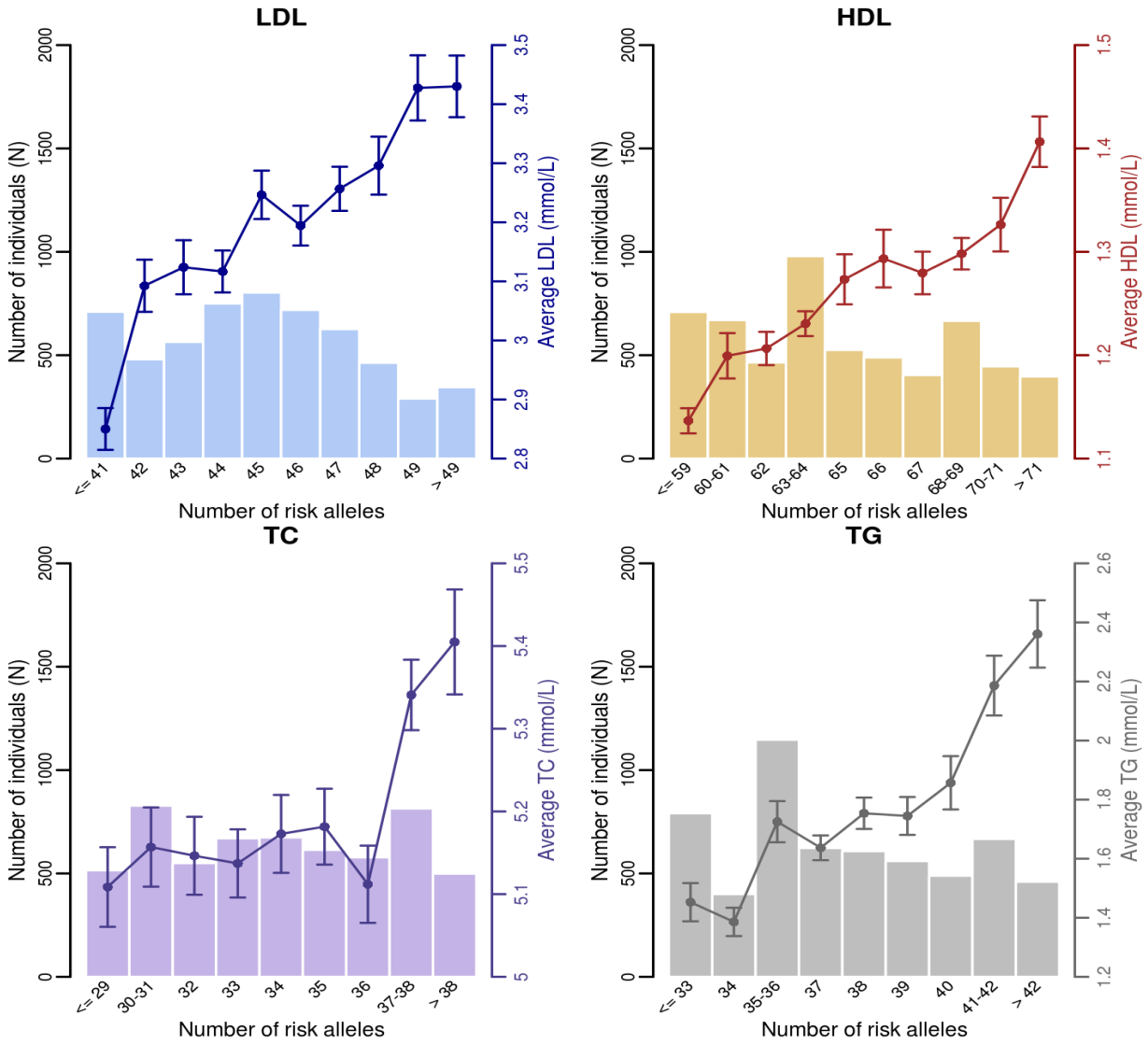
Lu X *et al.*, Nature Genetics, 2017

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

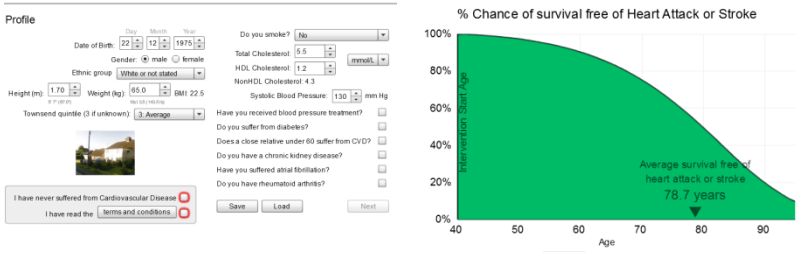
- **Most of the novel non-HDL-associated variants also influence risk of CAD**

Individuals carrying more lipid-increasing allele tends to have higher blood lipid levels



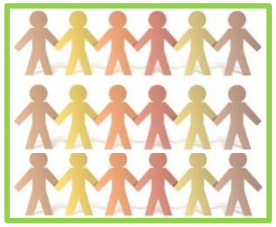
The Promise of Precision Medicine

Conventional Approach



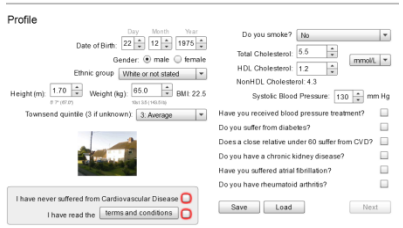
Clinical risk factor assessment

High risk



Low risk

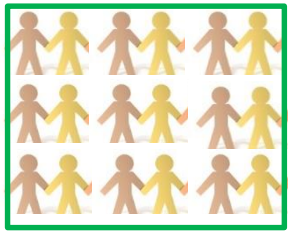
Precision Medicine Approach



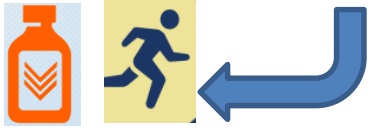
Gene-chip

Clinical + Genetic risk factors assessment

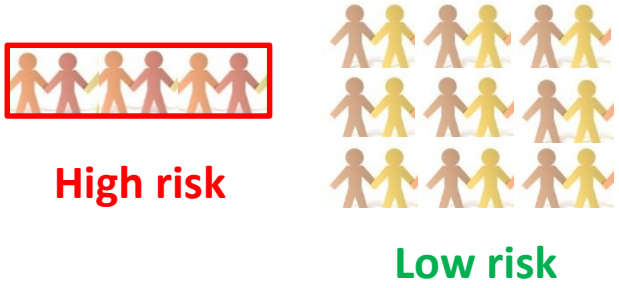
High risk



Low risk



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

Theme-based Research Scheme (TRS)

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From Genomic Testing and Biomarkers to
Human Pluripotent Stem Cell Platform**

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