Project number: T12-705/11

RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: 1-1-2012
Project completion date: 31-12-2017

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1. Project Title:

Personalized Medicine for Cardiovascular Diseases: From Genomic Testing and Biomarkers to Human Pluripotent Stem Cell Platform

2. Names and Academic Affiliations of Project Team Members[#]

Project team member	Name / Post	Unit / Department / Institution	Average number of hours per week spent on this project in the whole project period
Project Coordinator (PC)	Prof. Hung-Fat Tse/ Chair Professor	Dept of Medicine/HKU	16
Co-Principal Investigator(s)	Prof. Hung-Fat Tse/ Chair Professor	Dept of Medicine/HKU	16
	Prof. Aimin Xu/ Professor.	Dept of Medicine/HKU	8
	Prof. Pak Sham/ Chair Professor	Dept of Psychiatry/HKU	8
	Prof. Karen SL Lam/ Chair Professor	Dept of Medicine/HKU	8
	Prof. Tai-Hing Lam/ Chair Professor	School of Public Health/HKU	8
Co-Investigator(s)	Prof. MY Cheung/ Professor	Dept of Medicine/HKU	1-2
	Prof. CB Tan/ Professor	Dept of Medicine/HKU	
	Prof. Reinhard Rennebery/ Professor	Dept of Chemistry/HKUST	
	Prof. Yu Huang/ Professor	Dept of Physiology/CUHK	
	Prof. Chung-Wah Siu/ Professor	Dept of Medicine/HKU	

	Dr. Qizhou Lian/ Assistant Professor	Dept of Medicine/HKU	
	Dr. Stacey S Cherny/ Associate Professor	Dept of Psychiatry/HKU	
	Prof. Ching-Wan Lam/ Professor	Dept of Pathology/HKU	
Collaborators	Prof. Miguel Esteban/ Professor	Key Laboratory of Regenerative Biology/GIBH, Chinese Academy of Sciences, Guangzhou Singapore Stem Cell	N.A.
	Prof. Alan Colman/ Executive Director	Consortium/Institute of Medical Biology/Singapore	
	Prof. Chao-Qiang Jiang/ Professor	Guangzhou 12th Hospital	
	Prof. Weiping Jia/ Professor	Shanghai Jiaotong University	

 # Please highlight the approved changes in the project team composition and quote the date when the RGC granted approval of such changes. For changes in the project team composition, please submit a separate request, together with the justification and the curriculum vitae of the new member(s), to the RGC three months prior to the intended effective date of the change.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
Objective 1: To validate the 95 previously identified genetic polymorphisms for blood lipids and coronary artery disease (CAD) in our Chinese population cohorts recruited in both Hong Kong and Mainland China.	100%	1. We successfully genotyped 6048 individuals using Illumina Infinium HumanExome BeadChip and performed association analyses, both on single variant and gene-based levels, to identify new genes influencing cardiovascular disease (CAD) and blood lipid traits (total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and triglyceride (TG). 2. To further increase our statistical power to detect novel association, we meta-analyzed our

Objectives*	Percentage achieved	Remarks**
		association results with PUUM-MI, a study designed to study myocardial infarction (MI) and plasma lipid levels in China (led by Dr. Cristen Willer). A total of 12,685 Chinese ancestry were included in our exome chip analysis. In addition to the validation of previously identified genetic variants related to blood lipids, we also assessed the association of 65,671 variants that are polymorphic in both Chinese cohorts and have at least 20 minor alleles. Of these variants, 58% altered protein composition and 26% were Asian-specific variants with MAF<5%. 3. In collaboration with Guangzhou Biobank Cohort Study (GBCS), we have also completed the replication studies in 17,963 subjects with completed follow-up assessment in Guangzhou to further validate the novel association signals identified in our Exome array studies for the blood lipid traits. 4. Following the identification of 3 novel lipid-associated loci (PKD1L3, PNPLA3 and TEAD2) from our exome-wide association analysis on Chinese, we further collaborated with the Global Lipids Genetic Consortium (GLGC) for the Asian meta-analysis of exomechip association (n=47,456), which identified multiple Asian-specific independent associations in known genes. 5. Moreover, we have also completed the analysis of the combined polygenic risk score of the known GLGC lipid-associated loci to predict the risk of developing CAD in our cohort.
Objective 2: To correlate these 95 genetic polymorphisms with biochemical and anthropometric parameters, and biomarkers, to provide insight into the potential mechanisms and biological pathways for dyslipidemia and atherosclerosis.	100%	1. In our meta-analysis with PUUM-MI, we confirmed several known associations between selected SNPs with blood lipids (*ABCA1* for HDL-C, *DOCK7-ANGPTL3*, *GCKR* and *MLXIPL* for TG), and identified new lead SNPs at three known genes *LIPC*, *APOB* and *DOCK6* as well as refined association signals at *LPL, APOE and APOA5.* 2. Along with the 16 known loci, two common non-synonymous variants in genes/loci not previously implicated attained exome-wide

Objectives*	Percentage achieved	Remarks**
		significance: PNPLA3 I148M (rs738409), a missense variant known to associate with non-alcoholic fatty liver disease, was shown to strongly influence triglyceride level (β=-0.072, P=4.4x10-8). The second strongest novel association mapped to a LD block encompassing the known locus of HPR at 16q22.2. Carriers of the PKD1L3 minor allele (rs7185272, encoding p.Thr429Ser) had significantly lower LDL-C (P=5.4x10-8) and TC levels (P=2.5x10-7). 3. In addition, we also identified significant association of 3 Asian-specific missense variants defined as polymorphic in Asian but monomorphic in other populations. This included a low-frequency missense *CETP* SNP independently associated with HDL-C (rs2303790, p.Asp459Gly, MAF=0.027, P=3.2x10^{-29}); two probably damaging missense changes—a rare *PCSK9* variant encoding p.Arg93Cys (rs151193009, MAF=0.014; P=7.9x10^{-32}) and a LDLR mutation encoding p.Arg257Trp (rs200990725, MAF=0.001; P=3.0x10^{-8}) for LDL-C. These two single-variant associations may, by far, represent some of the strongest effect sizes (β=-0.64 SD for *PCSK9* R93C and β=0.91 for *LDLR* R257W) for any missense variant known to associate with plasma lipid levels in Chinese. 4. More importantly, we identified a significant gene-based association with HDL-C at a novel gene, *TEAD2* (P=1.9x10^{-7}), which is mainly driven by the near exome-wide significant *TEAD2* D12N SNP (β=1.11, P=3.8x10^{-7}). Another singleton missense SNP (*TEAD2* A266V) also showed a consistent, though non-significant, HDL-C increasing effect (β=1.15, P=0.25). 5. *APOE* variants were confirmed to be associated with the response to statin therapy and the variability of lipid levels in the CAD cohort. 6. The analysis between the relationships between the genetic polymorphisms and the biomarkers, such as FGF-21, FGF-19 and RBP4 in those subjects with CAD and diabetes has been

Objectives*	Percentage achieved	Remarks**
		completed.
Objective 3: To set up, human pluripotent stem-cell (iPSC) systems (*in vitro*) from individuals with different genotypes to provide different tissue types, such as hepatocytes, vascular smooth muscle and endothelial cells and fat cells, for studying the biological pathways under the influence of those genetic polymorphisms, and thus yield novel insight into the mechanisms of dyslipidemia and atherosclerosis.	100%	1. We have successfully established the human iPSC platform using conventional retroviral vectors, episomal vectors and Sendai virus in patients with familial hypercholesterolemia as well as isogenic iPSC using Zn finger technology for LDLR mutation to model human familial hypercholesterolemia. 2. We have successfully optimized the differentiation of hepatocytes from iPSC using our modified protocol. 3. Similarly, patient-specific iPSC lines carried several targeted genetic polymorphisms, including OTUD4, *PNPLA3, and PKD1L3* have been created.
Objective 4: To use an integrated proteomics and metabonomics approach to characterize the molecular signature of iPSCs generated above, and to identify new biomarkers for dyslipidemia and atherosclerosis.	100%	1. We have completed some of the measurement of the proteomics and metabonomics iPSC derived hepatocytes-like and adipocyte-like cells generated from patients with familial hypercholesterolemia, and the detail analysis.
Objective 5: To develop a novel genotype-specific drug screening platform using an in-vitro human iPSC system, based on patient-specific iPCS generated from individual with different genotype profiles.	100%	1. Patient-derived FH iPSCs with loss-of-function in 1 *LDLR* allele and *LDLR* heterozygous knockout (KO) iPSCs were used to assess the effect of statins and PCSK9 antibodies on LDL uptake in iHeps *in vitro*. A newly generated immunodeficient mouse model homozygous KO for *ldlr* was employed to assess the effect of these medications on engrafted FH iHeps *in vivo*. PCSK9 antibodies have a stronger LDL-lowering effect than statins on FH iHeps *in vitro* and *in vivo*. Our results confirmed that FH iHeps and our humanized FH mouse model provide an excellent platform for *in vitro* and *in vivo* testing of LDL-lowering medications. 2. Several studies on the biological pathway related to metabolic syndrome and lipid metabolism, in relation to *OTUD4, PNPLA3, and PKD1L3*, as identified by exome chip studies are ongoing. 3. The functional role those three novel loci including OTUD4, PNPLA3 and PKD1L3 on

Objectives*	Percentage achieved	Remarks**
		lipid traits and the development of CVD will be further investigated by overexpression of these loci using adeno-associated viral (AAV) gene transfer in normal mice feed with high fat diet and atherosclerosis-prone ApoE-deficient mice.
Objective 6: To develop new genetic and biomarker-based diagnostic tools for risk stratification, prognosis and therapeutic monitoring of dyslipidemia and atherosclerosis using the knowledge obtained from the in-vitro modelling of disease mechanisms and pathways, based on the above human iPSC platform.	100%	1. We sought to investigate whether a panel of cardiometabolic biomarkers alone or combined with conventional risk factors would exhibit incremental value in the prediction of cardiovascular events. We discovered that a combination of the 3 biomarkers, lipocalin-2, A-FABP, and FGF-19, with clinical risk factors to yield the age-biomarkers-clinical risk factor model provides an optimal and validated prediction of new-onset major adverse cardiovascular events in patients with stable coronary artery disease. 2. Several biomarkers, such as fibroblast growth factor 21 (FGF21), adiponectin and adipocyte fatty acid-binding protein (AFABP), have been shown to associate with CAD risk. We therefore conducted exome-wide association analyses to identify genes influencing the circulating levels of these adipokines. We identified a missense variant rs1260326 (p.Leu446Pro) of *GCKR* significantly associated with blood FGF21 levels at genome-wide significance ($P=2.66x10^{-9}$; Beta[SE]:0.16[0.03]). This SNP also showed a significant associations with CAD (P=0.021; OR=1.10) and TG level ($P=1.21x10^{-11}$).

* *Please highlight the approved changes in objectives and quote the date when the RGC granted approval of such changes.*

****** *Please provide reasons for significantly slower rate of progress than originally planned.*

6. Research Highlights and Outputs

6.1 What are the most exciting research accomplishments of the project?

(Please list five or more of the team's best research accomplishments, such as journal and conference papers, software codes, research infrastructure, etc. For each item, please clearly justify how it has achieved international excellence (e.g. best paper award, invited presentation, citations, product licensed to industry, etc.))

Objective 1: To validate the 95 previously identified genetic polymorphisms for blood lipids and coronary artery disease (CAD) in our Chinese population cohorts recruited in both Hong Kong and Mainland China.

1. Exome-wide association analysis reveals novel coding sequence variants associated with lipid traits in Chinese (Tang CS, *et al. Nat Commun*. 2015; 6:10206).

Blood lipids are important modifiable risk factors for coronary heart disease (CHD). Our project team performed the first exome-wide association study of blood lipids on Chinese populations. Using a custom Illumina HumanExome BeadChip, we genotyped 65,671 single nucleotide polymorphisms (SNPs) of 12,685 Chinese individuals to identify novel loci influencing lipid levels. A total of 19 loci were found associated with blood lipids at exome-wide significance ($P<2.69 \times 10^{-7}$), 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 p.Thr429Ser—also influenced levels of triglycerides and low-density lipoprotein cholesterol (LDL-C), respectively. Most of these newly identified coding variants show suggestive association ($P<0.05$) with CHD. These findings demonstrated that exome-wide genotyping on samples of non-European ancestry can identify additional population-specific lipid-associated variants, shedding light on novel lipid biology and CAD. The finding was published in *Nature Communications.*

2. Exome chip meta-analysis identifies novel loci and East Asian–specific coding variants that contribute to lipid levels and coronary artery disease (Lu X, et al. Nat Genet 2017; 49(12):1722-2730).

In view of the recent success on exome-wide association study on Chinese populations, we extended the meta-analysis to include association results of 47,532 East Asian individuals in the search for novel loci associated with blood lipid levels and to clarify the mechanism of action at previously identified lipid loci. Our project team identified 255 variants at 41 loci that reached exome-wide significance, including three novel loci and 14 East Asian-specific coding variant associations. By meta-analysing with an independent cohort of >300,000 European individuals, we identified an additional of 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 regulating blood lipid levels. Our data also demonstrated that most of the low frequency or rare coding variants associated with lipids are population-specific, highlighting the importance of analysing genomic data across diverse ancestries to facilitate the identification of functional genes at associated loci. The finding was published in *Nature Genetics and* has been awarded as the *Faculty Outstanding Research Output at the LKS Faculty of Medicine 2017-2018.* .

3. An Asian-specific missense variant in PAX4 is associated with T2DM in Chinese (Cheung CY et al., Diabetologia. 2017;60:107–115)

Type 2 diabetes mellitus (T2DM) has been frequently described as a CHD equivalent. Our project team conducted an exome-chip association analysis on T2DM using the Asian-Exomechip. We assessed the associations of over 70,000 SNPs with T2DM in 5,640 Chinese subjects from Hong Kong. We subsequently selected 15 SNPs for replication analysis in an independent Chinese cohort comprising 12,362 subjects from Guangzhou. A combined analysis involving 7,189 cases and 10,813 controls was conducted. We identified an Asian-specific coding variant, p.Arg192His, in *PAX4* showing genome-wide significant association with T2DM in the combined analysis. Mutations in *PAX4* have been reported to cause the rare monogenic form of diabetes, maturity onset diabetes of the young (MODY). Our findings have provided compelling evidence that *PAX4* locus could be a possible effector gene of the 7q32 locus, previously identified in genome-wide association studies in Asian; and supported the involvement of *PAX4* in the pathogenesis of T2DM. This study was published in *Diabetologia* and was selected as one of the featured ‘up front’ articles by the Editor.

Objective 2: To correlate these 95 genetic polymorphisms with biochemical and anthropometric parameters, and biomarkers, to provide insight into the potential mechanisms and biological pathways for dyslipidemia and atherosclerosis.

1. Circulating fibroblast growth factor 21 (FGF21) as an independent predictor of incident coronary heart disease (Lee CH *et al., J Am Heart Assoc.* 2017;6(6). pii: e005344)

Fibroblast growth factor 21 (FGF21) has shown beneficial effects on lipid and carbohydrate metabolism. Conflicting results were reported on the associations of circulating FGF21 levels with CHD in previous cross-sectional studies. Our project team investigated longitudinally whether circulating FGF21 levels could be used as a risk marker for the development of CHD in patients with T2DM. Among 3,528 Chinese subjects with T2DM and no known CVD, 147 subjects developed the first CHD event over a median follow-up of 3.8 years. These subjects had significantly higher circulating FGF21 levels compared with those who did not, even after adjustment for traditional risk factors of CHD. In this study, we have provided the first demonstration that increased serum FGF21 was an independent predictor of incident CHD risk and might be usefully utilised as a biomarker for T2DM patients with increased CHD risks. This study was published in *Journal of the American Heart Association.*

2. Identification of novel genetic determinants of circulating PEDF levels (Cheung CY *et al.*, Diabetes. 2018. pii: db180500.[Epub ahead of print])

Pigment epithelium-derived factor (PEDF) is an adipocyte-secreted factor that has been previously shown to regulate lipid metabolism. Our project team conducted an exome-chip association analysis in 5385 Chinese individuals with T2DM to identify the genetic variants that influence circulating PEDF levels. In this study, we identified, for the first time, three missense variants of the *SERPINF1*, *SMYD4* and *SERPINF2* genes showing genome-wide significant associations with circulating PEDF levels in subjects with T2DM. The strongest association was detected at *SERPINF1* p.Met72Thr. *SERPINF1*, the gene that encodes the PEDF protein, appears to be the major genetic determinant of circulating PEDF levels. We postulated that *SERPINF2* and *SMYD4* may also be involved in the regulation of PEDF via the transforming growth factor beta 1 (TGFβ1) and platelet-derived growth factor (PDGF) signalling pathways, respectively. In this study, we also observed elevated circulating PEDF levels in subjects with diabetic nephropathy or diabetic retinopathy who are at higher risk of developing CVD. Our study has provided new insights into the genetic regulation of PEDF and supported its potential use as a biomarker for diabetic nephropathy and diabetic retinopathy. The findings of this study were presented in the 54th Annual Meeting of the European Association for the Study of Diabetes in Berlin, Germany; and was recently published in *Diabetes*.

Objective 3-5: To set up, human pluripotent stem-cell (iPSC) systems (*in vitro*) from individuals with different genotypes to provide different tissue types, such as hepatocytes, vascular smooth muscle and endothelial cells and fat cells, for studying the biological pathways under the influence of those genetic polymorphisms, and thus yield novel insight into the mechanisms of dyslipidemia and atherosclerosis; to use an integrated proteomics and metabonomics approach to characterize the molecular signature of iPSCs generated above, and to identify new biomarkers for dyslipidemia and atherosclerosis; to develop a novel genotype-specific drug screening platform using an in-vitro human iPSC system, based on patient-specific iPCS generated from individual with different genotype profiles.

1. Generation of a Human Liver Chimeric Mouse Model with Hepatocytes Derived from Familiar Hypercholesterolemia Induced Pluripotent Stem Cells. (Yang JY *et al.*, Stem Cell Report 2016 8(3):605-618.

We have established a novel human liver chimeric mouse model for testing the effect of LDL-lowering medications on iHeps *in-vivo*. In this model, we engrafted Day 17 differentiated iHeps from LDLR+/- and LDLR-/- iPSC into LDLR-/-/Rag2-/-/Il2rg-/- (LRG) mice liver by intrasplenic injection. Next, LDL-lowering medications, including statin and PCSK9 antibody were administrated to these chimeric mice and serial measurements of changes in mice LDL-C level were performed. To further determine the long-term engraftment of iHep in the LRG mice,

we further investigated the long term effect of LDL-C level in these chimeric mice engrafted with wild-type iHeps (WT-iHeps) or primary human hepatocytes (pHH). Our data showed that, under high-fat and high cholesterol (HFHC) diet, transplantation of pHH (n=3) and WT-iHeps (n=3) to LRG mice resulted in significant reduction of LDL-C level, at 4 weeks (pHH: 31.6 ± 5.9% [percentage of LDL-C change from baseline ± SEM]; WT-iHeps: 30.8 ± 13.9%) and 8 weeks (pHH: 42.0 ± 1.6%; WT-iHeps: 38.7 ± 9.0%) of engraftment. These findings suggest that engraftment of iHep is similar to pHH in our chimeric mice mode.

After successful establishment of a novel human chimeric mouse model for testing the effect of LDL-lowering medications (statins and PCSK9 antibody) on iHeps in-vivo, we further investigated the physiological outcome of these LDL-C lowering medications. Here, we assessed the vascular function of LRG mice after engraftment with iHeps by testing endothelium-dependent vasodilation in the aortae. We found that vasodilation in LRG mice engrafted with +/+ iHeps was significantly improved compared to those from mice engrafted with +/- iHeps and -/- iHeps ($P < 0.05$). Specifically, assessment on vascular function showed that 10 mg/kg/week alirocumab treatment improves vasodilation in aortae of LRG mice engrafted with +/- iHeps, whist there was no effect on vascular function in LRG mice engrafted with -/- iHeps. In contrast, 10 mg/kg/day simvastatin improved vasodilatation in aortae of both groups, indicating that simvastatin is vasoprotective independently of LDLR status and cholesterol-lowering effects. Moderate synergistic effect of simvastatin and alirocumab on vasodilation in aortae was observed in LRG mice engrafted with +/- iHeps but not -/- iHeps. Furthermore, RT-qPCR of aorta from LRG mouse engrafted with +/- iHeps showed that treatment with alirocumab and simvastatin or their combination significantly reduced the expression of pro-inflammatory cytokines, chemokine receptors and adhesion molecules that are related to vascular inflammation. This study was published in *Stem Cell Report.*

2. Familial Hypercholesterolemia Human Liver Chimeric Mouse Model Using Induced Pluripotent Stem Cell-derived Hepatocytes. (Yang J *et al*. J Vis Exp. 2018 Sep 15;(139))

Moreover, we have been investigating different approaches, including the using of urokinase to further improve the efficiency of iHep engraftment in or chimeric mice model. Urokinase, also called urinary plasminogen activator, is a serine protease. Normally, uPA is produced as a single chain (sc-uPA). In hepatocytes transduced by adenovirus vectors that express uPA, trace amounts of plasminogen are converted to plasmin, which is capable of converting the sc-uPA to the more active two-chain uPA (tc-uPA). The tc-uPA can generate more plasmin, and the protease activity of plasmin is most likely directed against a number of different cellular proteins that results in hepatocytes death. During the period of hepatocyte regeneration, it is possible to transplant hepatocytes directly in-vivo with transduction efficiencies five- to nine-fold greater to that achieved with partial hepatectomy. We employed this approach to promote the repopulation of exogenous hepatocytes in mice liver. In this study, plasma/serum human albumin level is used to monitor mature hepatocytes repopulation efficiency in chimeric mice. In our previous protocol for generation of human liver chimeric mice, we failed to detect plasma/serum human albumin in those mice even we have demonstrated 5-10% repopulation of engrafted WT, LDLR+/- and LDLR-/- iHeps using immunohistochemistry. To optimize the engraftment protocol, we employed uPA expressed by adenovirus to enhance the repopulated efficiency of primary hepatocytes and iHeps. We first used pHH to assess the effects of uPA in cell repopulation. Compared with our previous protocol using irradiation, uPA treatments elevated plasma human albumin level around 24 folds (1200ng/ml in uPA treatment vs. 50ng/ml in irradiation treatment) 9 weeks post engraftment. This method was now described in *J Vis Exp.*

3. Based on data in the exome chip studies, PCSK9, OTUD4, PKD1L3 & PNPLA3 were identified in relation to lipid metabolism. The relationships between different genetic loci on the serum biomarkers, response to statin therapy and variability of LDL-C levels have been investigated in the Hong Kong CAD cohort subjects.

A. OTUD4:

We hypothesize that mutation of the OTUD4 affect the de-ubiquitination of HMG Co-A reductase or LDLR to cause hypercholesterolemia. Specifically, we have successfully generated patient specific iPSC from blood samples of patients with hypercholesterolemia and carried a novel OTUD4 mutation (p.Val317Ala, rs146168958) as identified by the Exome chips. Interestingly, subjects carried this OTUD4 variant showed a much more responsive phenotype to statin therapy. (LDL(mmol/L): Patient 1: before drug 5.3 to after 1st dose of statin 1.6; Patient 2: before drug 5.6 to after 1st dose of statin 1.7). Furthermore, we differentiated patient-specific iPSCs into functional iHeps (OTUD4-mutant iHeps) with a previously reported 3-step protocol. After 17 days of differentiation, iHeps with equivalent morphology to human hepatocytes appeared. More importantly, these iHeps displayed markers of primary hepatocytes including albumin and α1-antitrypsin (AAT) etc, and have the ability to secret up to 8700ng/ml/24hrs human albumin as detected by ELISA.

Cholesterol metabolism is tightly regulated by a network of genes, as such, the differences in the genetic backgroud between the wildtype and mutant iHeps may significantly affected the validity of the experimental results. Adresing this issue, we pland to creat isogenic pairs of iHeps that with the endogeneous *OTUD4 gene* knocked out or replaced with the SNP sequences using the *CRISPR/Cas9*. To this end, we have obtained the CRISPR/Cas9 knockout kits (KN414054) from ORIGENE and the knockout experiments has been done.

In addition, we have created a human hepatic cell line (HepG2) that stablily expressing the shRNA against human *OTUD4 gene*. The resultant cell line are GFP-positive and showed 60% reductions in OTUD4 protein levels (See below). This OTUD4-knock down hepatic cell line may facilitate the evaluation of the potential roles of OTUD4 proteins in cholesterole metabolism. In order to evaluate the functional significance of the OTUD4 mutant in the regulation of hepatic cholesterol metabolism, we have differentiatied the patient-specific iPSCs carrying the OTUD4 mutation into hepatocytes for functional evaluation. In brief, OTUD4-mutant iHeps and WT-iHeps were cultured to day 23, and supernatant of the iHeps cultures were harvest to assess the total cholesterol and ApoB level. Our preliminary data showed OTUD4-iHeps produce more cholesterol and ApoB than WT type control.

B. PKD1L3 & PNPLA3:

We have identified several important new targets which are beyond the current scope of our investigation. In addition to confirming the association of genetic variants on LDL-Receptor (LDL-R) and proprotein convertase subtilisin/kexin type 9 (PCSK9) with increased LDL cholesterol, we have identified 3 Asian-specific missense variants, defined as polymorphic in Asians but monomorphic in other populations, that are significant associated with dyslipidemia. Most interestingly, some of the genetic variants including PKD1L3 (rs7185272) p.Th429Ser and PNPLA3(rs738409) p.lle148Met identified in our study have shown novel associations with unknown mechanism. For instance, PKD1L3 encode an ion channel of the polycystic kidney disease-like family. On the other hand, PNPLA3 has demonstrated a strong association with Non-alcoholic Fatty Liver Disorder (NAFLD) which is characterized by increase hepatic fat content and hepatic triglyceride level, and is associated with the development of liver cirrhosis and carcinoma. Indeed, we have generated patient-specific iPSCs in patients who carried those genetic variants for further studying. Unfortunately, our current in-vivo and in-vitro experimental platform are focused on LDL cholesterol metabolism in the liver. Therefore, the study of these genetic variants on dyslipidemia and there relationship to triglyceride and ion channel in kidney might provide new insight on lipid metabolism. We initially tested the expression of PKD1L3 & PNPLA3 in primary hepatocyte. However, the expression of PKD1L3 gene in both primary liver cell and iPSC derived hepatocyte is too rare and not detectable, while expression of PNPLA3 is both cell type are detectable.

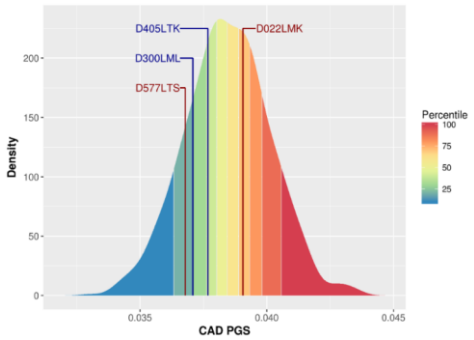
4. Familial hypercholesterolemia (FH) is characterized by highly elevated level of LDL-C that lead to atherosclerotic plaque deposition in coronary arteries at an early age, resulting in increased risk for CHD. Despite high level of LDL-C, some FH individuals are apparently "healthy". We sought to identify protective genetic factors from a FH family with all of the four siblings carrying the same protein-truncating variation in low-density lipoprotein receptor (LDLR) but only two of them developed CHD. Whole genome sequencing (WGS) was performed and variants, including single nucleotide variants, small insertion and deletions (indels) and structural variants, were called and annotated. Of the 9,034,626 variants (including 1,638,565 indels) called for 10 WGS samples, 7,523,041 passed quality controls and 5,126,450 were polymorphic (alternative allele count>1) in at least 1 of the 4 FH individuals. On average, each individual carries ~4 million variants. Most of the variants are present in dbSNP146. Among these variants, 4,769,448 variants have non-missing genotypes for all 4 FH individuals. Around half of the variants (n=2,476,556) are shared by all samples, i.e. genotypes are the same. One third of the variants (n=1,818,457) are shared in two samples with discordant phenotypes. Only 474,435 variants have genotypes completely different between cases and controls, aka "**non-shared**".

We first calculated the polygenic risk scores for these 4 WGS individuals together with 937 WGS East Asians (>80% Chinese) to estimate the polygenic contribution of multiple common to low frequency CAD-associated variants to CAD risk. The two non-CAD samples (D300LML [bottom 30%] and D405LTK [bottom 40%]) are not particularly "protected" compared to the two CAD cases (D577LTS [bottom 20%] and D022LMK [top 40%]). Other less common genetic factors might exist to explain the discordant CAD phenotype. Assuming a casual variant with additive OR=2 and CAD prevalence of 7%, the CardioGRAMplus4D + UKbiobank meta-analysis (~60K cases and 120K controls; control-to-case ratio=2) should have >80% power to detect an association if a marker of MAF>0.06% is in complete LD with the causal variant. To take into account the accurancy of imputation for rare variants, we set the MAF threshold of 0.1% in European populations, i.e. we only consider variants shared among the non-CAD controls and not shared by the CAD cases if the MAF in European populations (non-Finnish in Gnomad/ExAC) is <0.1% for heterozygotes or <3% for homozyogtes. Similarly, we required the MAF in East Asian population to be <3% for heterozygotes or <18% for homozyogtes as association of the more common variants with large effects should be detectable in the largest CAD meta-analysis on Chinese (1515 cases and 5019 controls). After the filtering by the aforementioned MAF thresholds, 40,800 biallelic variants not shared between cases and controls remained.

We further annotated these "non-shared" variants using KGGseq, revealing 5 loss-of-function (LoF; one frameshift changed from stop codon to stop codon and 1 QC- from IGV plot) rare variants and 138 other protein-alterning rare variants (exonic/nonframeshift/missense) not shared between cases and controls. Among the 5 LoF changes, the alternative rare allleles are found in controls only. One casused a frameshift in *CYP3A4*, encoding cytochrome P450 family proteins involved in drug metabolism and lipid synthesis, and another caused splicing change in *SLC30A8* (but in non-canonical isoform) where LoF mutations of *SLC30A8* protects against T2DM. Two other non-LoF variants are predicted to affect splicing based on the *in silico* prediction, the ADAscore, which include a missense change in *UTS2B* found in controls and a tandam reverse duplication in the splicing region of *CD36* found in cases. *UTS2B* encodes urotensin II-related peptide and shares the C-terminal sequence common to, the known vasoconstrictor, urotensin II. CD36 encodes the main macrophage receptors for oxidized LDL and has been implicated in atherosclerosis through mediating foam cell formation in mouse models. But there are conflicting evidence regarding the risk of cardiovascular diseases for individuals with CD36 deficiency.

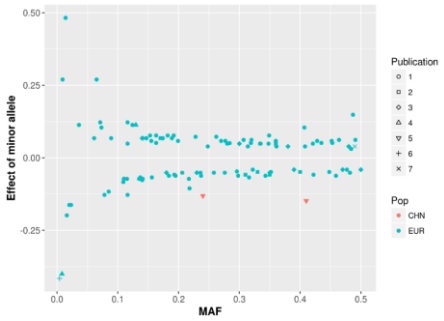
Comparing these polygenic risk scores among these four siblings and against a general population of ~1000 Chinese individuals, we did not find supporting evidence for a polygenic protection of the

non-CHD siblings, which suggested that rare protective genetic factors with large effect might exist to explain the discordant CHD phenotype. We later identified a rare missense variant in UTS2B predicted to affect splicing in the two non-CHD controls but the variation was not observed in the CHD patients. UTS2B encodes urotensin II-related peptide and shares the C-terminal sequence common to, the known vasoconstrictor, urotensin II. We hypothesized that the missense variant found in non-CHD controls might affect the function of UT2SB and hence affect the coronary vasculature. We have created iPSC derived hepatocytes-like generated from patients with FH to study their specific signatures on proteomics and metabonomics profiling as novel biomarker. Further functional analysis are still on-going.



Objective 6. To develop new genetic and biomarker-based diagnostic tools for risk stratification, prognosis and therapeutic monitoring of dyslipidemia.

1. Age-Biomarkers-Clinical Risk Factors for Prediction of Cardiovascular Events in Patients With Coronary Artery Disease. (Wong YK *et al.*, Arterioscler Thromb Vasc Biol. 2018 Oct;38(10):2519-2527.)



In a cohort of 1166 CAD patients with serial lipid level measurements and received lipid lowering agents in the Hong Kong CAD cohort included in the Exome Chips study, we have completed the measurement of 7 different biomarkers that was developed by Prof. Xu and Prof. Rennebery, included fibroblast growth factor (FGF) 19 and 21, adipocytes fatty acid-binding protein (A-FABP), lipocalin-2, retinol binding protein 4 (RBP4), adiponectin and homeobox protein pal-1 (PAI-1) that may be associated serum lipid levels and CAD to explore the potential role of these biomarkers for dyslipidemia and risk stratification of cardiovascular diseases. We prospectively follow-up those patients for the occurrence of major adverse cardiovascular events (MACE), composited of cardiovascular death, acute coronary syndrome or heart failure, while the secondary endpoints were all-cause mortality, new onset ischaemic or haemorrhagic stroke, and new onset symptomatic peripheral vascular disease requiring treatment. After a median follow-up of 35 months, 170 (incidence rate, 3.4 per 100 patient-year) patients developed new-onset MACE. Serum A-FABP, lipocalin-2, and FGF-21 levels were positively correlated with hypertension, whereas positive correlation with diabetes was only found in A-FABP (all $P<0.01$). A moderately strong correlation was observed between A-FABP and lipocalin-2 ($r=0.48$; $P<0.01$), whereas a weaker correlation was noted between A-FABP and FGF-19 ($r=0.16$; $P<0.01$). In the model with age ≥65 years and conventional risk factors, AUC for predicting MACE was 0.68. In the single biomarker approach, the best predictor value was found for the addition of high lipocalin-2 levels to the age-clinical risk factor model, with AUC increased to 0.73 (difference in AUCs, 0.05; $P<0.001$). The AUC further increased to 0.75 when a combination of high cutoff levels of lipocalin-2, A-FABP, and FGF-19 was incorporated into

the model with age ≥65 years, diabetes and hypertension, incremental benefit was found in comparison to single biomarker model (difference in AUCs, 0.02; *P*=0.038). Kaplan-Meier event-free survival was significantly lower in elderly patients with both diabetes and hypertension compared with young patients without diabetes or hypertension (log-rank test, *P*<0.001). Moreover, CAD patients with lipocalin-2, A-FABP, and FGF-19 levels above the cutoff values had significantly worse adjusted event-free survival than those with levels below the cutoff values (logrank test, *P*<0.001). The adjusted hazard ratio on MACEs for lipocalin-2, A-FABP, and FGF-19 levels above optimal cutoffs were 2.23 (95% CI, 1.62–3.08), 1.99 (95% CI, 1.43–2.76), and 1.65 (95% CI, 1.15–2.35), respectively. We further validated the predictive performance of this age-biomarker-clinical factor risk model in another cohort of 1262 CAD patients with diabetes. Optimal cutoff level of biomarkers derived from the discovery cohort was used. The model with age ≥65 years and hypertension yielded an AUC of 0.58 (*P*<0.001). Incorporating a combination of lipocalin-2 and A-FABP at high levels into the model with age ≥65 years and hypertension increased the AUC to 0.62 (difference in AUCs versus age and risk factors model, 0.04; *P*<0.001). The AUC further increased to 0.63 when high FGF-19 levels were added into the age-biomarker-clinical factor risk model with a combination of A-FABP and lipocalin-2, providing significant incremental value in comparison to single biomarker model (difference in AUCs, 0.03; *P*=0.005). The approach using multiple biomarkers was confirmed to provide good discrimination and calibration over the conventional risk factor alone for prediction of MACE (Hosmer-Lemeshow goodness-of-fit, *P*>0.5). *This study was published in Arteriosclerosis,Thrombosis, and Vascular Biology.*

2. A functional missense variant of *GCKR* regulates circulating FGF21 levels (Cheung CY *et al., Diabetes.* 2017;66(6):1723-1728)

Our project team also investigated the genetic determinants of circulating FGF21 levels in an exome-chip association analysis by genotyping 5,169 Chinese individuals from a community-based cohort and two clinical based cohorts. We examined over 70,000 single nucleotide polymorphisms in the single variant association test and identified a novel association with circulating FGF21 levels at the *GCKR* locus. The common missense variant, p.Pro446Leu, of *GCKR* showed genome-wide significant association with circulating FGF21 levels in the combined analysis. This missense variant may influence FGF21 expression through its ability to enhance glucokinase (GCK) activity. This can lead to increased FGF21 expression via elevated fatty acid synthesis, consequent to the inhibition of carnitine/palmitoyl-transferase by malonyl-CoA, and via increased glucose-6-phosphate-mediated activation of the carbohydrate response element binding protein, which is known to modulate the gene expression of FGF21. *GCKR* is a highly pleiotropic gene. The common variants of *GCKR*, including p.Pro446Leu and its proxy SNPs, have been shown to be associated with various metabolic traits. We demonstrated that *GCKR* p.Pro446Leu may play a role in the regulation of FGF21 levels that is independent from the effect of body mass index, fasting plasma glucose and triglyceride. We also showed that *GCKR* p.Pro446Leu was strongly associated with triglyceride but only modestly correlated with fasting plasma glucose. These findings suggest that FGF21 may play a more prominent role in lipid metabolism and adiposity rather than blood glucose. Our study has further highlighted the pleiotropic role of *GCKR* and provided new insights into the genetic regulation of FGF21 levels and its relationship with metabolic diseases. The interesting findings of this study have been published in *Diabetes*.

6.2 What was the added value of the TRS funding, rather than standard project grant funding?
(For example, could this work have been achieved with other funding scheme, such as the General Research Fund or Collaborative Research Fund? If not, why?)

This TRS project provide a unique platform which allow us to perform the first exome sequencing study and meta-analysis in collaboration with centers in China and overseas related to

dyslipidemia and cardiovascular disease in Chinese. These findings were published in Nature Communication and Nature Genetic which are the top ranking international journals. Moreover, the TRS project allow us to perform validation study on our genomic analysis with our collaborators in China.

6.3 If the project has not met its original objectives, why?

The project met all the original goal.

6.4 (a) Peer-reviewed journal publication(s) arising directly from this project:

(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference. Please mark the symbol "#" next to the publications involving inter-institutional collaborations)

Publication List:

(**Bold**-TRS team members, **#**-inter-institutional collaborations**)**

A. Original publications arising directly related to TRS projects

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2018	2018			Wong YK, **Cheung CYY**, **Tang CS**, **Au KW,** Hai JSH, Lee CH, Lau KK, **Cheung BMY**, **Sham PC**, **Xu A***, **Lam KSL***, **Tse HF***	Age-Biomarkers-Clinical Risk Factors for Prediction of Cardiovascular Events in Patients With Coronary Artery Disease. *Arterioscler Thromb Vasc Biol.* 2018;38:2519-2527. (*JIF =6.086, co-corresponding author**)	2019 Completion Report	Yes	Yes	Yes
2018	2018			**Cheung CYY**, Lee CH, **Tang CS, Xu A**, **Au KW**, Fong CHY, Ng KKK, Kwok KHM, Chow WS, Woo YC, Yuen M, Hai J, **Tan KCB, Lam TH**, **Tse HF***, **Sham PC*, Lam KSL***.	Genetic Regulation of Pigment Epithelium-Derived Factor (PEDF): An Exome-Chip Association Analysis in Chinese Subjects with Type 2 Diabetes. *Diabetes.* 2018 Oct 10. pii: db180500. doi: 10.2337/db18-0500. [Epub ahead of print] (*JIF=7.273, co-corresponding author**)	2019 Completion Report	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2018	2018			**#** Yang J, Wong LY, Tian XY, Wei R, Lai WH, **Au KW**, Luo Z, Ward C, Ho WI, Ibañez DP, Liu H, Bao X, Qin B, **Huang Y**, **Esteban MA***, **Tse HF***	A Familial Hypercholesterolemia Human Liver Chimeric Mouse Model Using Induced Pluripotent Stem Cell-derived Hepatocytes. *J Vis Exp.* 2018 Sep 15;(139). doi: 10.3791/57556. (*JIF=1.232, corresponding author*)*	2019 Completion Report	Yes (Abstract) This is a Video Journal	Yes	Yes
2017	2017			Lee CH, Woo YC, Chow WS, **Cheung CY**, Fong CH, Yuen MM, **Xu A**, **Tse HF***, **Lam KS***	Can circulating fibroblast growth factor 21 play a role in primary prevention of coronary heart disease among Chinese patients with type 2 diabetes? *J Am Heart Assoc.* 2017;6. pii: e005344. doi: (*JIF =4.45, *co-corresponding author*)	2019 Completion Report	Yes	Yes	Yes
2017	2017			**Cheung CY**, **Tang CS**, **Xu A**, Lee CH, **Au KW,** Xu L, Fong CH, Kwok KH, Chow WS, Woo YC, Yuen M, **Cherny SS**, Hai J, **Cheung BM**, **Tan K**, **Lam TH**, **Tse HF***, **Sham PC***, **Lam KS***	An exome-chip association analysis in Chinese reveals a functional missense variant of *Gckr* that regulates FGF21 levels. *Diabetes.* 2017:6:1723-1728 (*JIF =7.273,*co-corresponding author*)	2019 Completion Report	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2017	2017			Mak TSH, Porsch RM, Choi SW, Zhou X, **Sham PC***	Polygenic scores via penalized regression on summary statistics. *Genet Epidemiol.* 2017;41:469-480. (*JIF =2.544, *corresponding author*)	2019 Completion Report	Abstract	Yes	No
2017	2017			**#** Yang J, Wang Y, Zhou T, Wong LY, Tian XY, Hong X, Lai WH, **Au KW**, Wei R, Liu Y, Cheng LH, Liang G, Huang Z, Fan W, Zhao P, Wang X, Ibañez DP, Luo Z, Li Y, Zhong X, Chen S, Wang D, Li L, Lai L, Qin B, Bao X, Hutchins AP, **Siu CW**, **Huang Y**, **Esteban MA***, **Tse HF***	Generation of human liver chimeric mice with hepatocytes from familial hypercholesterolemia induced pluripotent stem cells. *Stem Cell Reports.* 2017;8:603-618. (*JIF =6.537, *co-corresponding author*)	2019 Completion Report	Abstract	Yes	No

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2017	2017			**# Cheung CYY**, **Tang CS**, **Xu A**, Lee CH, **Au KW**, Xu L, Fong CHY, Kwok KHM, Chow WS, Woo YC, Yuen MM, Hai JSH, Jin YL, **Cheung BM**, **Tan KC**, **Cherny SS**, Zhu F, Zhu T, Thomas GN, Cheng KK, Jiang CQ, **Lam TH***, **Tse HF***, **Sham PC***, **Lam KS***	Exome-chip association analysis reveals an Asian-specific missense variant in PAX4 associated with type 2 diabetes in Chinese. *Diabetologia* 2017;60:107-115. (*JIF =6.023, *co-corresponding author*)	2019 Completion Report	Yes	Yes	Yes
2016	2016			Kwan JS, Li MX, Deng JE, **Sham PC***.	FAPI: Fast and accurate P-value Imputation for genome-wide association study. Eur J Hum Genet. 2016;24:761-6. (*JIF =3.636, *corresponding author*)	2019 Completion Report	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2015	2015			**# Tang CS**, Zhang H, Cheung CY, Xu M, Ho JC, Zhou W, **Cherny SS**, Zhang Y, Holmen O, Au KW, Yu H, Xu L, Jia J, Porsch RM, Sun L, Xu W, Zheng H, Wong LY, Mu Y, Dou J, Fong CH, Wang S, Hong X, Dong L, Liao Y, Wang J, Lam LS, Su X, Yan H, Yang ML, Chen J, **Siu CW**, Xie G, Woo YC, Wu Y, **Tan KC**, Hveem K, **Cheung BM**, Zolner S, **Xu A**, Chen Y, Jiang CQ, Zhang Y, **Lam TH**, Ganesh S, Huo Y, **Sham PC***, **Lam KS***, Willer CJ*, **Tse HF***, Gao W*	Exome-wide association analysis reveals novel coding sequence variants associated with lipid traits in Chinese. *Nat Comm* 2015;6:10206. (*JIF =12.353, *co-corresponding author*)	2019 Completion Report / 2016 Extension Report	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2015	2015			Deng JE, **Sham PC**, Li MX	SNPTracker: A Swift Tool for Comprehensive Tracking and Unifying dbSNP rs IDs and Genomic Coordinates of Massive Sequence Variants. *G3* (Bethesda). 2015;6:205-7. (*JIF =NA)*	2019 Completion Report / 2016 Extension Report	Yes	Yes	Yes
2015	2015			Li MJ, Deng J, Wang P, Yang W, Ho SL, **Sham PC**, Wang J, Li M	wKGGSeq: A Comprehensive Strategy-Based and Disease-Targeted Online Framework to Facilitate Exome Sequencing Studies of Inherited Disorders. *Hum Mutat.* 2015;36:496-503. (*JIF =5.359*)	2019 Completion Report / 2016 Extension Report	Yes	Yes	Yes
2015	2015			Van der Sluis S, Dolan CV, Li J, Song Y, **Sham P**, Posthuma D, Li MX	MGAS: a powerful tool for multivariate gene-based genome-wide association analysis. *Bioinformatics.* 2015;31:1007-15. (*JIF =7.481*)	2019 Completion Report / 2016 Extension Report	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2015	2015			**#** Lin Z, Pan X, Wu F, Ye D, Zhang Y, Wang Y, Jin L, Lian Q, **Huang Y**, Ding H, Triggle C, Wang K, Li X, **Xu A***	Fibroblast growth factor 21 prevents atherosclerosis by suppression of hepatic sterol regulatory element-binding protein-2 and induction of adiponectin in mice. *Circulation.* 2015;131:1861-71. (*JIF =18.88,*corresponding author*)	2019 Completion Report / 2016 Extension Report	Yes	Yes	Yes
2014	2014			Hui E, **Xu A**, Chow WS, Lee PC, Fong CH, Cheung SC, **Tse HF**, Chau MT, **Cheung BM**, **Lam KS***	Hypoadiponectinemia as an independent predictor for the progression of carotid atherosclerosis: a 5-year prospective study. *Metab Syndr Relat Disord.* 2014;12:517-22. (*JIF =1.744,*corresponding author*)	2019 *Completion Report / 2015 Progress Report*	Yes	Yes	Yes
2014	2014			Cheung CY, Hui EY, **Cheung BM**, Woo YC, **Xu A**, Fong CH, Ong KL, Yeung CY, Janus ED, **Tse HF**, **Sham PC, Lam KS***	Adiponectin gene variants and the risk of coronary heart disease: a 16-year longitudinal study. *Eur J Endocrinol.* 2014;171:107-15. (*JIF =4.333,*corresponding author*).	2019 *Completion Report / 2015 Progress Report*	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2013	2013			Chow WS, **Xu A**, Woo YC, Tso AW, Cheung SC, Fong CH, **Tse HF**, Chau MT, **Cheung BM, Lam KS***	Serum fibroblast growth factor-21 levels were associated with carotid atherosclerosis independent of established cardiovascular risk factors. *Arterioscler Thromb Vasc Biol.* 2013;33:2454-9 (*JIF=6.086, *corresponding author)*	2019 *Completion Report / 2014 Progress Report*	Yes	Yes	Yes
2013	2013			Chow WS, Tso AW, **Xu A**, Yuen MM, Fong CH, **Lam TH**, Lo SV, **Tse HF**, **Cheung BM, Lam KS***	Elevated circulating adipocyte-fatty acid binding protein levels predict incident cardiovascular events in a community-based cohort: A 12-year prospective study. *J Am Heart Assoc.* 2013;2:e004176 (*JIF= 4.45, *corresponding author*)	2019 *Completion Report / 2014 Progress Report*	Yes	Yes	Yes
2013	2013			Lin Z, Tian H, Lam KS, Lin S, Hoo RC, Konishi M, Itoh N, Wang Y, Bornstein SR, **Xu A,** Li X	Adiponectin mediated the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. Cell Metab 2013;17:779-789 (*JIF= 20.56, *corresponding author*)	2019 *Completion Report / 2014 Progress Report*	Yes	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2012	2012			Li MX, Kwan JS, **Sham PC***	HYST: a hybrid set-based test for genome-wide association studies, with application to protein-protein interaction-based association analysis. *Am J Hum Genet*. 2012;91:478-88 (*JIF= 8.855, *corresponding author*)	2019 *Completion Report / 2014 Progress Report*	Yes	Yes	Yes
2012	2012			**Cheng KK**, **Lam KS**, Wu D, Wang Y, Sweeney G, Hoo RL, Zhang J, **Xu A***	APPL1 potentiates insulin secretion in pancreatic β cells by enhancing protein kinase Akt-dependent expression of SNARE proteins in mice. *Proc Natl Acad Sci U S A.* 2012;109:8919-24. (*JIF= 9.504, *corresponding author*)	2019 *Completion Report / 2014 Progress Report*	Yes	Yes	Yes

B. **Original publications partially support by TRS project**

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2018	2018			Mak TS, Lee YK, **Tang CS**, Hai JJ, **Sham PC***, **Tse HF***	Coverage and diagnostic yield of Whole Exome Sequencing for the Evaluation of Cases with Dilated and Hypertrophic Cardiomyopathy. *Sci Rep. 2018;8:10846.* (*JIF=4.609, co-corresponding author**)	2019 Completion Report	No	Yes	Yes
2018	2018			**#** Zhuang Q, Li W, Benda C, Huang Z, Ahmed T, Liu P, Guo X, Ibañez DP, Luo Z, Zhang M, Abdul MM, Yang Z, Yang J, Huang Y, Zhang H, Huang D, Zhou J, Zhong X, Zhu X, Fu X, Fan W, Liu Y, Xu Y, Ward C, Khan MJ, Kanwal S, Mirza B, Tortorella MD, **Tse HF**, Chen J, Qin B, Bao X, Gao S, Hutchins AP, **Esteban MA***	NCoR/SMRT co-repressors cooperate with c-MYC to create an epigenetic barrier to somatic cell reprogramming. *Nat Cell Biol.* 2018;20:400-412. (*JIF=19.064, corresponding author**)	2019 Completion Report	No	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2017	2017			Lu, J., Y. K. Lee, **X. Ran**, W. H. Lai, R. A. Li, W. Keung, K. Tse, **H. F. Tse***, X. Yao	"An abnormal TRPV4-related cytosolic Ca2+ rise in response to uniaxial stretch in induced pluripotent stem cells-derived cardiomyocytes from dilated cardiomyopathy patients." Biochim Biophys Acta (*JIF=5.34, corresponding author*)*	2019 Completion Report	No	Yes	Yes
2016	2016			**#** Ng KM, Mok PY, Butler AW, Ho JC, Choi SW, Lee YK, Lai WH, **Au KW**, Lau YM, Wong LY, **Esteban MA**, **Siu CW**, **Sham PC**, Colman A, **Tse HF***	Amelioration of X-linked related autophagy failure in Danon disease with DNA methylation inhibitor. *Circulation.* 2016;134:1373-1389. (*JIF =18.88, corresponding author**)	2019 Completion Report	No	Yes	Yes
2016	2016			**#** Xu J, Lee YK, Ran X, Liao SY, **Yang J, Au KW**, Lai WH, **Esteban MA**, **Tse HF***	Generation of induced cardiospheres via reprogramming of skin fibroblasts for myocardial regeneration. *Stem Cells* 2016:34:2693-2706. (*JIF =5.587, corresponding author**)	2019 Completion Report / 2016 Extension Report	No	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2016	2016			Law CY, **Siu CW**, Fan K, L**ai WH, Au KW, Lau YM**, **Wong LY**, **Ho JCY**, **Lee YK**, **Tse HF***, **Ng KM**	Lysosomal membrane permeabilization is involved in oxidative stress-induced apoptotic cell death in LAMP2-deficient iPSCs-derived cerebral cortical neurons. Biochem Biophys Rep. 2016 Jan 14;5:335-345 (*JIF =0.93, *corresponding author*)	2019 Completion Report	No	Yes	Yes
2015	2015			Liu J, Wang L, Tian XY, Liu L, Wong WT, Zhang Y, Han QB, Ho HM, Wang N, Wong SL, Chen ZY, Yu J, Ng CF, Yao X, **Huang Y***	Unconjugated bilirubin mediates heme oxygenase-1-induced vascular benefits in diabetic mice. *Diabetes.* 2015;64:1564-75. (*JIF =7.273,*corresponding author*)	2016 Extension Report	No	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2014	2014			Liu L, Xu Y, He M, Zhang M, Cui F, Lu L, Yao M, Tian W, Benda C, Zhuang Q, Huang Z, Li W, Li X, Zhao P, Fan W, Luo Z, Li Y, Wu Y, Hutchins AP, Wang D, **Tse HF***, Schambach A, Frampton J, Qin B, Bao X, Yao H, Zhang B, Sun H, Pei D, Wang H, Wang J, **Esteban MA**	Transcriptional pause release is a rate-limiting step for somatic cell reprogramming. Cell Stem Cell. 2014 Nov 6;15(5):574-88. (*JIF = 23.29,*corresponding author*)	2015 Progress Report	No	Yes	Yes
2014	2014			Li X, Zhang Y, Yeung SC, Liang Y, Liang X, Ding Y, Ip MS, **Tse HF***, Mak JC, **Lian Q**	Mitochondrial transfer of induced pluripotent stem cell-derived mesenchymal stem cells toairway epithelial cells attenuates cigarette smoke-induced damage. Am J Respir Cell Mol Biol. 2014 Sep;51(3):455-65. (*JIF = 4.10,*corresponding author*)	2015 Progress Report	No	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2014	2014			Wong CM, Zhang Y, **Huang Y**	Bone morphogenic protein-4-induced oxidant signaling via protein carbonylation for endothelial dysfunction. Free Radic Biol Med. 2014 Oct;75:178-90. (*JIF = 6.02,*corresponding author*)	2015 Progress Report	No	Yes	Yes
2014	2014			Zhang J, **Ho JC**, Chan YC, **Lian Q**, **Siu CW, Tse HF***	Overexpression of myocardin induces partial transdifferentiation of human-induced pluripotent stem cell-derived mesenchymal stem cells into cardiomyocytes. Physiol Rep. 2014 Feb 25;2(2) (*JIF= 0.667, *corresponding author*)	2015 Progress Report	No	Yes	Yes
2014	2014			**Yiu KH, Tse HF***	Specific role of impaired glucose metabolism and diabetes mellitus in endothelialprogenitor cell characteristics and function. Arterioscler Thromb Vasc Biol. 2014 Jun;34(6):1136-43. (*JIF= 6.607, *corresponding author*)	2015 Progress Report	No	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2014	2014			**Xu JY, Lee YK, Wang Y, Tse HF***	Therapeutic application of endothelial progenitor cells for treatment of cardiovascular diseases. Curr Stem Cell Res Ther. 2014;9(5):401-14. (*JIF= 2.194, *corresponding author*)	2015 Progress Report	No	Yes	Yes
2013	2013			**# Tse HF***, Ho JC, Buter AW, Lee YK, Choi SW, Ng KM, **Siu CW**, Simpson MA, Lai WH, Chan YC, Zhang J, Lay KW, **Esteban MA**, Colman A, **Sham PC**	Patient-specific induced pluripotent stem cells derived cardiomyocytes recapitulates the pathogenic phenotypes of dilated cardiomyopathy due to a novel desmin gene mutation identified by whole exome sequencing. *Hum Mol Genet* 2013;22:1395-1403 (*JIF= 4.902, *corresponding author*)	2014 Progress Report	No	Yes	Yes
2013	2013			Dong J, Wong SL, Lau CW, Liu J, Wang YX, Dan He Z, Fai Ng C, Yu Chen Z, Yao X, **Xu A**, Ni X, Wang H, **Huang Y***	Calcitriol restores renovascular function in estrogen-deficient rats through downregulationof cyclooxygenase-2 and the thromboxane-prostanoid receptor. Kidney Int. 2013 Jul;84(1):54-63 (*JIF= 3.36, *corresponding author*).	2014 Progress Report	No	Yes	Yes

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2013	2013			Ting S, Lecina M, Chan YC, **Tse HF***, Reuveny S, Oh SK	Nutrient supplemented serum-free medium increases cardiomyogenesis efficiency of human pluripotent stem cells. World J Stem Cells. 2013 Jul 26;5(3):86-97. (*JIF= 4.376, *corresponding author*)	2014 Progress Report	No	Yes	Yes
2013	2013			**Lai WH**, **Ho JC**, Chan YC, Ng JH, **Au KW**, **Wong LY**, **Siu CW, Tse HF***	Attenuation of hind-limb ischemia in mice with endothelial-like cells derived from different sources of human stem cells. PLoS One. 2013;8(3):e57876. (*JIF= 2.766, *corresponding author*)	2014 Progress Report	No	Yes	Yes
2013	2013			Chan YC, Ting S, L**ee YK, Ng KM**, Zhang J, Chen Z, **Siu CW**, Oh SK, **Tse HF***	Electrical stimulation promotes maturation of cardiomyocytes derived from humanembryonic stem cells. J Cardiovasc Transl Res. 2013 Dec;6(6):989-99. (*JIF= 2.319, *corresponding author*)	2014 Progress Report	No	Yes	No

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and journal/book *(with the volume, pages and other necessary publishing details specified)*	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged the support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2013	2013			**Liao SY, Tse HF***	Multipotent (adult) and pluripotent stem cells for heart regeneration: what are the pros andcons? Stem Cell Res Ther. 2013 Dec 24;4(6):151. (*JIF= 4.967, *corresponding author*)	2014 Progress Report	No	Yes	Yes
2012	2012			**Siu CW**, Lee YK, **Ho JC**, Lai WH, Chan YC, Ng KM, Wong LY, **Au KW**, Lau YM, Zhang J, Lay KW, **Colman A, Tse HF***	Modeling of lamin A/C mutation premature cardiac aging using patient-specific inducedpluripotent stem cells. Aging (Albany NY). 2012 Nov;4(11):803-822 (*JIF= 4.867, *corresponding author*)	2014 Progress Report	No	Yes	Yes

(b) Recognised international conference(s) in which paper(s) related to this project was/were delivered: *(Please attach a copy of each conference abstract)*

Month/Year/ Place	Title	Conference name	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of the RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
05/2014/HK	Strategic Research on Stem Cell and Regenerative Medicine	Joint TRS Symposium	2019 Completion Report	Yes	Yes	No
12/2015/HK	Patient specific induced pluripotent stem cells as a platform for disease diagnosis and drug screening.	Hong Kong & Guangzhou International Conference on Stem Cell & Regenerative Medicine: Stem Cell Applications and Regulatory Principles	2019 Completion Report/ 2016 Extension Report	Yes	Yes	No
03/2016/HK	Generation of a Familial Hypercholesterolemia Chimeric Mouse Model with Human LDLR Knockout Induced Pluripotent Stem Cells-Derived Hepatocytes	11th International Symposium on Healthy Aging "Science and Aging: An Era of Discovery"	2019 Completion Report	Yes	Yes	No
04/2016/Japan	Patient-Specific Induced Pluripotent Stem Cell as Model for Hypertrophic Cardiomyopathy Study	The International Society for Stem Cell Research (ISSCR)	2019 Completion Report	Yes	Yes	No
07/2016/Germany	Investigation in the Role of Lamin A (LMNA) Deficiency in Heart Block and Dilated Cardiomyopathy (DCM) Using Human Induced Pluripotent Stem Cell Derived Cardiomyocytes	10th International Meeting on Substrate-Integrated Mircoelectrode Arrays	2019 Completion Report	Yes	Yes	No
10/2018/Germany	Genetic regulation of pigment epithelium-derived factor (PEDF), a multifunctional anti-tumour factor: an Exome-chip association analysis in subjects with type 2 diabetes.	54th European Association for the Study of Diabetes (EASD) Annual Meeting	2019 Completion Report	Yes	Yes	No

(c) RGC funding should have been acknowledged in all publication(s)/conference papers listed in (a) and (b) above. If no acknowledgement has been made in any of the publications/ papers, please indicate and provide explanations.

All the publications listed have acknowledged the support from the TRS funding

6.5 To what extent this project has strengthened inter-institutional collaborations and other partnerships?

As shown in the publication lists, the project has significantly strengthened our collaboration between different institutions in Hong Kong and overseas on genomic, stem cell and clinical researches related to dyslipidemia and cardiovascular diseases in Hong Kong.

6.6 Research students trained (registration/awards):

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Jiayin Yang	PhD	7/2014	7/2018
Xinru Ran	PhD	2/2015	On-going
Jianyong Xu	PhD	7/2013	6/2016
Kelvin Kwok	PhD	9/2013	8/2017
Robert Porsch	PhD	9/2013	8/2017
Debbie Wong	PhD	1/2017	On-going

6.7 Specific products (e.g. software or netware, instruments or equipment developed):

NIL

6.8 Other education activities and/or training programmes developed:

This project also allow us to provide training to several post-doc fellows and RAP (Cheng KK, Tang CS, Cheung CY, and Mak TS) on genomic, stem cell and clinical researches on cardiovascular diseases.

6.9 Please highlight any deliverables indicated in the project implementation timetable endorsed by the RGC which have not been covered or achieved as per sections 6.1 to 6.8 above, and explain/ elaborate.

Our teams have achieved all the milestones as endorsed by RGC.

Project Management

6.10 Please elaborate how the PC has played his/her role in coordinating and managing the project.

The PC is responsible for coordination, management, and financial monitoring of the whole project. The PC has been in regular contact with the PIs and co-Is of this project, and ensure the progress of the project met the Milestones.

As shown in the Appendix minutes, our research teams have held regular meeting in monthly basis to discuss the results and progress of the studies. Moreover, PC held management team meeting to overlook the whole project overview, management and budget as well as the upcoming research plan for this TBRS projects. This meeting was attended by all the PIs and Co-Is of this projects.

Furthermore, regular research meeting and seminar have been organized between different research teams for exchange and discussion of research ideas and data (See Appendix list).

7. Awards and Recognition

7.1 Have any research grants been awarded that are directly attributable to the results obtained from this project?

1. 2012: Health and Medical Research Fund-Full Grant (10111531): Mendelian randomization to causally infer the impact of life-long vitamin D deficiency on Cardiovascular disease and death: implications for community-based cardiovascular prevention **(Amount HK$999,000)**
2. 2016: Croucher-CAS Joint Lab: Discovery of New Approaches for Treating Hyperlipidemia Using an Induced Pluripotent Stem Cell (iPSC)-Based Platform (**Amount HK$ 1,000,000**)
3. 2017: Innovation and Technology Support Programme (Tier 3) (GHP/046/17GD ITSP TCFS project "Generation of a familial hypercholesterolemia human liver chimeric rabbit model" (**Amount HK$ 1,200,000**)
4. 2018: NSFC Grant 建立家族性高胆固醇血症人肝嵌合小鼠模型 (**Amount CNY 920,000**)

7.2 Have any project team members participated as invited speakers in or organisers of international conferences as a result of this project?

Invited talks

Mak TSH, PC Sham. The choice of the tuning parameter in lassosum (**Best paper of the year award presentation, 2017**) **(14th – 16th Oct 2018**) 27th *International Genetic Epidemiology Society Annual Meeting, San Diego, USA.*

7.3 Have any project team members taken leadership positions in editorial boards, scientific and professional organisations?

Nil

7.4 Any documentary proof of the application of technologies arising directly from this project?

Nil

7.5 Other awards and recognitions as a result of this project (please specify):

1. **Best paper of the year award, 2017** for the paper **"**Polygenic scores via penalized regression on summary statistics" published in *Genet Epidemiol.*
2. **Faculty Outstanding Research Output** for the paper "Exome chip meta-analysis identifies novel loci and East Asian-specific coding variants contributing to lipid levels and coronary artery disease." published in *Nat Genet.*

8. Impacts

8.1 What are the current and expected impacts of the project on the long-term development of Hong Kong (social or economic development, e.g. patent, technology transfer, collaboration with external organisations, etc.)?

To our knowledge, our study is the first that used the exome chip to study impact of genetic traits

on lipid and CAD among Southern Chinese. Overall, our findings demonstrated that exome-wide genotyping on samples of Southern Chinese: non-European ancestry can identify additional population-specific, possibly causal variants, shedding light on novel lipid biology and CAD. It also implicated the important contribution of population-specific rare variants to CAD risk, whose effect could only be detected by family-based study or population-based association analysis of large sample size. We have now further extended our investigations to other risk factors of CAD, such as obesity and T2DM in patient cohort.

Moreover, our novel in-vito cell based platform and in-vivo chimeric mice model established in this project can be used for future R&D for drug screening and discovery for dyslipdemia, which are major modifiable risk factors for CAD. In this project, we have been collaborated with Sanofi on the testing of the human PSCK9 antibody for treatment of dyslipidemia and other collaborations different drug companies are being discussed.

In addition, the gene risk scores for LDL-C and biomarkers panel developed in this study can improve the early diagnosis and prediction of cardiovascular events in our Chinese population. Indeed, we have been discussed with different local and overseas biotech companies to develop a gene chip for dyslipidemia as well as testing of other biomarkers in our patient cohort.

8.2 Others (please specify):

NIL

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?

We have identified novel SNPs, and biomarkers related to dyslipidemia and cardiovascular diseases in Chinese that can be used to develop new therapies and screening platform for treatment and prevention of cardiovascular diseases.

9.2 Whether there are new projects evolved directly from this project?

We have several ongoing research and grants on further development of chimeric mice and rabbits with human livers for drug screening for dyslipidemia and cardiovascular diseases

9.3 Whether there are new collaborations developed directly from this project?

We have established new collaborations with industries (Sanofi and CIB) on the development of chimeric mice and rabbits with human livers for drug screening for dyslipidemia and cardiovascular diseases

9.4 Please give details on how much money and from which sources has been obtained/requested for the specific purpose of continuing the work started under this project.

As shown above, we have obtained over 4 million HKD from external grants for further development of this project.

10. Statistics on Research Outputs

(Please ensure the statistics in this section are consistent with the information presented in other

sections of this report.)

	Peer-reviewed journal publications	Conference papers	Scholarly, books, monographs and chapters	Patents awarded	Other research outputs (please specify)	
					Type	No.
No. of outputs arising directly from this research project	41	6	0	0		

12. The Layman's Summary

(describe in layman's language the abstracts and research impact of the project.)

Cardiovascular diseases (CVD) are leading global cause of morbidity and mortality. Despite recent advances in the management of cardiovascular risk factors such as hypertension, diabetes, dyslipidemia and obesity, the prevalence of CVD continues to increase worldwide. Although the prevalence of some cardiovascular risk factors and events in high-income countries are decreasing, they are rising in Asia and other low-income countries. It is well established that the level of circulating blood lipids is one of the cardiovascular risk factors strongly associated with risk of coronary artery disease. Indeed, recent studies have shown that a general increase in serum total cholesterol level in Asian countries, including China, Japan and Thailand, has contributed to the increasing prevalence of CVD. In addition to life-style and environmental factors, blood lipid levels are under tighter genetic control than the related CVD. This highlights the need for new approaches beyond monitoring of conventional serum biochemical parameters to prevent, identify and treat individuals who are at risk of developing CVD. In this study, we first performed whole exome sequencing in 12,685 Chinese individuals and identify 19 novel loci associated with blood lipids, including three Asian-specific coding variants in known genes (CETP, PCSK9 and LDLR). Furthermore, missense variants at two novel loci: PNPLA3 and PKD1L3 also influenced levels of triglycerides and low-density lipoprotein cholesterol (LDL-C), respectively. Next, we extended these findings and performed meta-analysis to include association results of 47,532 East Asian individuals in the search for novel loci associated with blood lipid levels and to clarify the mechanism of action at previously identified lipid loci. Our project team identified 255 variants at 41 loci that reached exome-wide significance, including three novel loci and 14 East Asian-specific coding variant associations. By meta-analysing with an independent cohort of >300,000 European individuals, we identified an additional of 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 regulating blood lipid levels. This change in the serum LDL-C level related to these genetic variants conferred a significantly increased risk of CAD. Moreover, we have established a novel human in-vitro platform as well as liver chimeric mouse model for testing the effect of lowering medications. Furthermore, the use of multiple biomarkers with or without associated with different genomic loci can also enhance the prediction of adverse cardiovascular events. Our study emphasizes that genetic studies can offer novel insight into the complex pathophysiology of human diseases that might translate into new approaches to personalized medicine for prevention, diagnosis and treatment CVD.