Research Progress Summary

**Genome and Epigenome Wide Association Study**

The team’s ongoing Theme-based Research Scheme (TRS) has an overarching goal to use multi-omic and functional analysis to discover genetic regulation of cardiovascular-renal complications in type 2 diabetes (T2D). A genetic analysis of over 6,000 DNA samples from the Hong Kong Diabetes Registry has now completed by the team, including subjects who developed vascular complications (coronary heart disease (CHD) and/or subjects with end stage renal disease (ESRD) in comparison with individuals without such complications. Through this effort, the team has discovered a panel of genetic variants showing highly suggestive association ($p<10^{-5}$) with cardiovascular or kidney complications in diabetes. These variants are currently undergoing validation in other independent samples and collaborating cohorts while another 7,000 samples from the Hong Kong Diabetes Biobank had been recently genotyped. Collectively, this represents one of the largest resource and datasets in the field, focused on investigation of the genetic basis of diabetic complications. The team anticipates the insights gained from these discoveries will help improving the clinical care of patients with diabetes.

**Multi-omic analysis and the Hong Kong Diabetes Biobank**

The team is undertaking a large-scale epigenome-wide association study to identify epigenetic markers associated with diabetes complications. The team has utilised the Illumina 450 methylation arrays to interrogate DNA methylation patterns in 400 patients with incident CHD, 400 patients with incident ESRD, and 600 patients without these complications despite having at least 10 years of disease. This analysis, in collaboration with Professor Kevin Yip and his team, has led to the discovery of novel methylation markers associated with diabetic complications, which are currently being validated. The group has also applied the Agilent SurePrint G3 Human miRNA high-definition arrays to identify circulating microRNAs associated with diabetic complications. Led by Professor Stephen Tsui from the School of Biomedical Sciences, the project team is currently integrating these multi-omic data, using data mining to predict their correlations with gene expressions and phenotypes.

Modelled after the Hong Kong Diabetes Registry and in collaboration with 10 diabetes centres in Hong Kong, the team has built the Hong Kong Diabetes Biobank with prospective cohorts, databases and multiple biosamples. To date, the team has already recruited over 11,000 subjects and customised a biobanking programme for cataloguing and tracking the stored samples, to be used for discovery and replication purposes.
Molecular mechanisms of cardiovascular-renal complications

In collaboration with Professor Yu Huang from the Institute of Vascular Medicine and School of Biomedical Sciences, the team is investigating the pathobiological role of the peroxisome proliferator-activated receptor α (PPARα) pathway in relation to the team's multi-omic dataset on diabetic complications. To further explore the pathobiological role and clinical significance of the tumour growth factor β (TGF-β) Smad3-dependent pathways, the team is also collaborating with Professor Huy-van Lan to tally these experimental data with the team's genomic datasets and samples in subjects with diabetic kidney disease.

Novel methodology and genotype-phenotype-treatment-outcome analysis

Through the TRS, the team has assembled a team of data and clinician scientists to develop data mining methodologies to test hypotheses based on prior knowledge and make novel discoveries. In collaboration with Professor Xiaodan Fan from the Department of Statistics, new statistical methods are being developed for detecting genotype-phenotype interaction. These methodological approaches will be highly relevant to the team's ongoing big data analysis.

In an experiment using whole transcriptome data of peripheral blood mononuclear cells from 10 patients with young onset type 2 diabetes (T2D) and 10 control subjects, we used extensive datamining to discover that the GWAS significant T2D SNP rs7636 could be functionally explained by repression of SRRT and SLC12A9, lending support to the use of mutli-level data and computational analysis to uncover regulatory pathways linked to GWAS signals.

(A) rs7636 is in perfect LD with rs11171 (SRRT), rs781190 and rs1716255 (SLC12A9) in Beijing Han Chinese in the CSRL-HapMap project (r2: 1; D’:1). Both SRRT (log2fold -0.88, adjusted p: 1.24E-4) and SLC12A9 (log2fold -0.77, adjusted p: 6.32E-3) were significantly down-regulated in T2D. Expression of ACHE was not altered (log2fold 0.19, adjusted p: 1.00). (B) The dysregulation of expression of SRRT and SLC12A9 were replicated in the independent cohort. Statistical significance in change of gene expression: * p<0.05, **p<0.01, ***p< 0.001. (C) Physical interaction of SRRT, JAZF1 and CREB5 among other proteins.

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Gestational diabetes and developmental origins of diabetes

In collaboration with Professor Wing-hung Tam from the Department of Obstetrics and Gynaecology, the team is investigating the long-term effects of exposure to maternal hyperglycaemia and gestational diabetes. The team has recently completed a large-scale follow-up and metabolic characterisation of a cohort of offspring from mothers with or without gestational diabetes. This has facilitated the team’s ongoing multi-omics studies in relation to gestational diabetes and developmental programming, including genome-wide association studies (GWAS) for gestational diabetes, maternal hyperglycaemia, birthweight and adiposity. Supported by a Research Grant Council (RGC) General Research Fund (GRF), the team has applied novel structural equation modelling to investigate the relationship between maternal hyperglycaemia and offspring adiposity. In collaboration with Professor Chris KWong from the Hong Kong Baptist University (HKBU) and supported by the Health and Medical Research Fund (HMRF), the team is also examining the relationship between early-life exposure to environmental pollutants and the risk of cardio-metabolic diseases later in life.

Diabetes and Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorders among young women, with significant reproductive and metabolic consequences. Supported by a GRF, the team is currently conducting one of the largest longitudinal follow-up studies to characterise the development of diabetes and metabolic abnormalities among Chinese women with PCOS. Preliminary data from this project highlighted the alarming rates of developing diabetes and glucose intolerance among young women with this condition. The findings have been presented at a major International PCOS meeting held recently.

Mechanistic studies on beta cell biology

In the team’s ongoing mechanistic study led by Professor Alice Kong and in collaboration with Professors Arthur Chung and Gang Xu from HKBU, the team has made significant progress in unraveling the roles of reactive oxidative stress on beta cell function and the protective effects of Sirt3 in pancreatic beta cells. Given the importance of oxidative stress in diabetes and its complications, this model will provide a useful tool for testing various anti-diabetic compounds and their effects on expression which will complement the team’s ongoing multi-omic analysis and may lead to discovery of novel pathways and drug targets. The team has started collaboration with Professor Guy A. Rutter from Imperial College London, United Kingdom and invited Professor Rutter to deliver a talk titled “Control of pancreatic beta cell identity in health and type 2 diabetes” on 11 November 2016. Further to this talk, Professor Rutter will visit the team in May 2017 to consolidate the collaboration with support from the Research Committee, Faculty of Medicine Distinguished Scholar Scheme. Moreover, the team has also been working with Professor Andrew Miller from HKUST to use zebrafish as an efficient platform to characterise the functional significance of novel genes for diabetes focusing on beta-cell biology.
Diabetes and cancer

Diabetes, cancer and obesity are complex and closely associated chronic diseases though the nature of these associations needs clarification. In collaboration with Professors Chun-kwok Wong from the Department of Chemical Pathology and Professor Paul Chan from the Department of Microbiology, the team has developed a virus-induced cancer animal model to examine the effects of hyperglycemia and various anti-diabetic and anti-inflammatory drugs on the initiation, promotion and progression of cancer. In a series of novel experiments, the team has demonstrated the differential effects of metformin, a biguanide and an incretin analogue on cancer growth despite similar blood glucose lowering through immunomodulation involving the cytotoxic and regulatory T cells. These findings highlight the pleotropic effects of anti-diabetic drugs and given the rising trend of all-site cancer in diabetes and the proliferation of anti-diabetic drugs, these animal models will provide a useful tool for testing hypothesis with clinical implications. In addition, the team has successfully obtained funding from the Innovation and Technology Commission (ITC) to conduct a project titled “Early Detection of Liver Cancer in Type 2 Diabetes Using Serum MicroRNA” with Professor Alice Kong as Principal Investigator, Professor Juliana Chan and Ronald Ma as Co-Investigators. The results of this project may lead to identification of novel biomarkers with breakthrough in the early and non-invasive diagnosis of patients with liver cancer by testing their peripheral blood.

Academic exchanges and capacity building

The group is currently training a dozen or more postgraduate students, who are working in inter-related projects, supported by the TRS, RGC, HMRF private funds and CUHK postgraduate studentships.

1. Collaborations with The Surrogate Markers for Micro- and Macro-vascular Endpoints for Innovative Diabetes Tools (SUMMIT) Consortium (Leif Groop, Lund University, Sweden and Mark McCarthy, Oxford University, UK) and The Fenofibrate in Event Lowering in Diabetes (FIELD) Study Cohort (Tony Keech, University of Sydney, Australia) for cross validation of loci associated with diabetic complications.

2. Joint analysis with the Genetic Investigation of ANthropometric Traits (GIANT) Consortium in one of the largest genetic projects to date, a multi-ethnic meta-analysis of GWAS of obesity traits with around 1 million individuals.

3. Participation in other international consortia on multi-ethnic genetic studies for diabetes, gestational diabetes and related metabolic diseases including the T2D Portal funded by the Foundation of the National Institute of Health as well as collaboration with Bendix Carstensen and Dorte Vistisen at the Steno Diabetes Center, Denmark for a East-West comparative study on diabetic nephropathy.

4. Collaboration with the Diabetes Research on Patient Stratification (DIRECT) Study (Ewan Pearson, University of Dundee, UK) which is a pan-European Consortium funded by the Innovative Medicines Initiative to discover biomarkers and clinical subtypes associated with various treatment responses for precision medicine.

5. Collaboration with Cecilia Lindgren and Alex Drong of the Wellcome Trust Centre for Human Genetics for analysis of epigenome wide association study.

6. Collaboration with Professor Assam El-Osta from the Baker IDI Heart and Diabetes Institute and Monash University, Melbourne, Australia and Professor Yu Huang of the Institute of Vascular Medicine, CUHK to identify epigenomic markers in diabetes patients with coronary heart disease.


8. Collaboration with Professor Johan Auwerx from Switzerland to use pancreatic specific Sirt3 floxed mice in future experiments.

9. Collaboration with Professor Philip Clarke, University of Melbourne, Australia to develop diabetes outcome models for cost-effectiveness analysis which will pave the way for economic analysis of precision medicine using clinico-genomic-treatment data.
Recognitions

Awards and Fellowships

<table>
<thead>
<tr>
<th>Member’s Full Name</th>
<th>Details</th>
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<tr>
<td>Claudia Tam</td>
<td>Travel grant to attend the Novo Nordisk Foundation Symposium on Early Growth Genetics and Metabolic Diseases, Copenhagen, Denmark, April 2016.</td>
</tr>
<tr>
<td>Guozhi Jiang</td>
<td>Travel grant to attend the 11th International Diabetes Federation-Western Pacific Region Congress 2016 and 8th Asian Association for the Study of Diabetes Scientific Meeting, Taipei, Taiwan, October 2016.</td>
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<tr>
<td>Greg Tutino</td>
<td>Conference grant from the Hong Kong Society of Endocrinology, Metabolism and Reproduction (HKSEMIR) to attend and present at the 76th Scientific Sessions of the American Diabetes Association, New Orleans, USA, June 2016.</td>
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<tr>
<td>Noel Ng</td>
<td>Conference grant from the Hong Kong Society of Endocrinology, Metabolism and Reproduction (HKSEMIR) to attend and present at the 14th Annual Meeting of the Androgen Excess -Polycystic Ovary Syndrome Society, Lorne, Victoria, Australia, November 2016.</td>
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<td>Ming Waie Yeung</td>
<td>Travel grant to attend the 52nd International Diabetes Federation (EASD) Annual Meeting, Munich, Germany, September 2016.</td>
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<tr>
<td>Chenzhao Ding</td>
<td>Travel grant to attend the 11th International Diabetes Federation-Western Pacific Region Congress 2016 and 8th Asian Association for the Study of Diabetes Scientific Meeting, Taipei, Taiwan, October 2016.</td>
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<tr>
<td>Chenzhao Ding</td>
<td>Travel grant to attend the 12th International Congress of Endocrinology (ICE) and 15th Annual Conference of Chinese Society of Endocrinology (CSE), Beijing, China, August 2016.</td>
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<tr>
<td>Baoqi Fan</td>
<td>Travel grant to attend the 11th International Diabetes Federation-Western Pacific Region Congress 2016 and 8th Asian Association for the Study of Diabetes Scientific Meeting, Taipei, Taiwan, October 2016.</td>
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Grants and Consultancy

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<tr>
<th>Full Name of PI</th>
<th>Project Title</th>
<th>Funding Source</th>
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<tr>
<td>Ronald CW Ma</td>
<td>Maternal Exposure to Perfluorooctane Sulfoate (PFOS) and the Risk of Childhood Obesity and Metabolic Abnormalities in the Offspring - Analysis of a Longitudinal Birth Cohort</td>
<td>Food and Health Bureau - Health and Medical Research Fund</td>
<td>01/04/2016</td>
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<td>Ronald CW Ma</td>
<td>Accelerating Medicines Partnership Type 2 Diabetes Project - Genetic Variants for Type 2 Diabetes and Diabetic Complications in East Asians: The Hong Kong Diabetes Registry</td>
<td>Foundation for the National Institutes of Health</td>
<td>01/06/2016</td>
<td>31/05/2019</td>
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<td>Juliana CN Chan</td>
<td>Identifying the Epigenomic Fingerprint of Coronary Heart Disease in Chinese Adults with Type 2 Diabetes</td>
<td>National Health and Medical Research Council - National Natural Science Foundation of China Joint Research Scheme</td>
<td>01/06/2016</td>
<td>31/12/2020</td>
<td>RMB 3,500,000</td>
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<td>Alice PS Kong</td>
<td>Early Detection of Liver Cancer in Type 2 Diabetes Using Serum MicroRNA</td>
<td>The Hong Kong Anti-Cancer Society</td>
<td>01/04/2016</td>
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<td>Alice PS Kong</td>
<td>Early Detection of Liver Cancer in Type 2 Diabetes Using Serum MicroRNA</td>
<td>Innovation and Technology Fund</td>
<td>01/01/2017</td>
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<td>Lorena TF Cheung (PhD student)</td>
<td>Lifestyle Factors and Their Relationships with the Weight and Glycemic Control in Non-obese and Obese Hong Kong Chinese Type 2 Diabetic Patients</td>
<td>Hong Kong Association for the Study of Obesity Research Grant 2016</td>
<td>01/01/2017</td>
<td>31/12/2018</td>
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Publications

A. Journal Papers


5. Kong AP, Luk AO, Chan JC. Detecting people at high risk of type 2 diabetes: How do we find them and should they be treated? Best Practice & Research Clinical Endocrinology & Metabolism. 2016; 30(3):345-55. (Review/Editorial)


B. Conference Papers

1. Hu M, Tam HT, So WY, Chan JC, Tomlinson BT, Ma RC. Genetic variants and lipid traits in the Hong Kong Chinese patients with type 2 diabetes. In: The 84th European Atherosclerosis Society Congress; Innsbruck, Austria; 2016 May 29-Jun 1.


8. Ma RC, Tam CH, Jiang G, Luk AO, Lee HM, Lim CK, Tsui SK, Yu W, Tsui SK, So WY, Chan JC, Ma RC. Genome-wide association study in Chinese identifies novel loci associated with End Stage Renal Disease among Chinese patients with Type 2 Diabetes. In: The 52nd Annual Meeting of the European Association for the Study of Diabetes; Munich, Germany; 2016 Sep 12-16.


10. Kong AP, Choi KC, Ding C, Zhang J, Luk AO, Ma RC, So WY, Cheung K, Wong YK, Chan JC. A randomized controlled trial to investigate the impact of sleep education program on glycemic control in Hong Kong Chinese type 2 diabetic patients with short sleep duration – An interim analysis at 6 months of intervention, In: The 17th International Congress of Endocrinology (ICE) and 15th Annual Conference of Chinese Society of Endocrinology (CSE); Beijing, China; 2016 Aug 31-Sep 4.