LKCMedicine PhD Research Project Submission Form

Research Theme (Please indicate as appropriate)

☐ Dermatology & Skin Biology  ☐ Family Medicine & Primary Care
☒ Health Systems & Population Health  ☐ Infection & Immunity
☒ Metabolic Disorders  ☐ Neuroscience & Mental Health
☐ Medical Education  ☐ Others (Please specify):

Research Project Title:

Developing stratified approaches for preventing type-2-diabetes

Project Description:

Background
Type-2 diabetes (T2D) is a major public health problem, driven by a global epidemic of overweight and obesity. Prof Chambers’ recent studies identify DNA methylation, a key epigenomic regulatory mechanism influencing gene expression and cellular differentiation, may explain the underlying differences in T2D between populations and can be used as a novel independent predictor of future T2D in high-risk normoglycaemic individuals. DNA methylation is influenced by both genetic and environmental factors, e.g., diet, physical activity and weight. This epigenomic regulatory mechanism represents a potential biomarker of an individual's genetic and environmental exposures AND a potential molecular pathway determining phenotypic pattern and underlying disease pathogenesis.

To extend on his study, Prof Chambers will combine DNA methylation with other serum metabolites to be associated with T2D. This will further enhance the discrimination for T2D.

Aims
Thus, this project aims to investigate if molecular information (DNA methylation and other biomarkers) can be used to develop stratified approaches for health promotion and therapeutic intervention to prevent type-2-diabetes (T2D). Specifically, potential candidates will need to:

1. Replicate and extend the association of DNA methylation and serum metabolites with incident T2D in Singapore Chinese.
2. Fine-map DNA methylation at the identified genetic loci to identify the methylation markers that best predict T2D and optimise assays for accurate and reproducible measurement of DNA methylation at the identified CpG sites.
3. Develop, calibrate and validate molecular risk scores for prediction of T2D amongst samples from completed and ongoing multi-ethnic population cohorts across South and South East Asia (N=6000, 3000 with incident T2D).
Approach

DNA methylation in samples from Singapore Chinese will be quantified to confirm the relationship between DNA methylation and risk of future T2D. Samples will be selected from study participants who are normoglycaemic at baseline (HbA1c <6% and fasting glucose <6mmol/L). Cases of incident T2D (N=500) will be selected for analysis based on: i. physician diagnosis, ii. HbA1c >=6.5%, or fasting glucose >7mmol/L at follow-up. Controls (N=500) will be documented to be free from T2D at follow-up, but otherwise selected at random from the cohort. DNA methylation and serum metabolites from these samples will be quantified via the IlluminaMethyEPIC array and AbsoluteIDQTM platform, respectively.

To better understand methylation markers that influence risk of T2D and enable optimisation of the molecular risk scores for T2D, fine-mapping of DNA methylation at identified genetic loci will be carried out. Fine-mapping of DNA methylation will be carried out in two stages. For the first stage, targeted resequencing on a 10kb region (5kb either side) around each of the identified CpG sites in genomic DNA will be carried out in 100 Chinese, 100 European and 100 Indian Asian people, to identify the CpG sites that show intermediate methylation. In the second stage, CpG sites identified to show differential methylation at the respective genomic loci will be analysed in up to 1,000 Chinese, 1,000 European and 1,000 Indian Asian samples (50% with incident T2D in each population). Methylation will be quantified by pyrosequencing, bisulfite sequencing or by microarray; depending on which approach provides the most accurate and cost-effective strategy for methylation quantification.

Genomic data derived from methylation and metabolites analyses will then be used to derive, calibrate and validate molecular risk scores for prediction of future T2D. To derive the model for T2D prediction, molecular risk factors that appear independently associated with incident T2D will be identified, using the newly generated data for Chinese and existing data for Europeans and Indian Asians (LOLIPOP and iHEALTH, both of which are large-scale prospective population studies lead by Prof Chambers). Independent relationships of DNA methylation and serum metabolites associated with T2D from EPIC methylation assays, BIOCRATES assays and fine mapping of DNA methylation at identified genetic loci will be quantified. Interaction terms will be used to test for population specific effects. A parsimonious set of molecular markers will be identified, with evidence for independent association with T2D, for potential inclusion in molecular risk scores for prediction of T2D.

Validation of the molecular risk score will be carried out in up to 3,000 further independent samples (1,500 cases of incident T2D and 1,500 controls). Validation will include assessment of calibration and discrimination in each of the separate population studies contributing to the validation set, and in key subgroups (gender, age, ethnicity).

The major output of this work will be the release of the first, calibrated and validated molecular risk score for prediction of risk of T2D amongst Chinese, European and Indian Asian peoples. The risk score will enable classification of individuals into low, intermediate and high risk of future T2D, along with the respective sensitivity, specificity and predictive values of the risk thresholds.
### Brief summary of main Methodologies and/or Techniques to be learned during the proposed PhD (experimental or analytical):

1. Knowledge on DNA methylation quantification assays.
2. Bioinformatics-based analytical skills for large-scale population based study.
3. Development of predictive tools based on data obtained from Pt. 1 and Pt. 2.

**Keywords:** metabolic disease, metabolic disturbance, type-2-diabetes (T2D), epigenome
### Supervisor(s)

**Primary Supervisor**

<table>
<thead>
<tr>
<th>Name of Supervisor:</th>
<th>Prof John Chambers</th>
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<td>Designation:</td>
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**Co-Supervisor (need not be determined at this time)**

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### Main Location of Research Work (Please indicate as appropriate)

- [ ] LKCMedicine Experimental Medicine Building @ Yunnan Campus
- [x] LKCMedicine Clinical Sciences Building @ Novena Campus
- Others (Please specify):  

### Other Information

1. Does the proposal need IRB’s approval?  
   - [x] Yes  
   - [ ] No
2. Does the project involve the use of animals?  
   - [ ] Yes  
   - [x] No
3. Does the project involve contact with patients?  
   - [ ] Yes  
   - [x] No
4. Is there a potential for overseas academic exchange as part of this research project?  
   - [ ] Yes  
   - [x] No

   **If “Yes”, please specify:**  

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