Fine-mapping and functional evaluation of DNA methylation markers predictive of Type-2 Diabetes in Asians

Background
Type-2 diabetes (T2D) is a leading global cause of morbidity and mortality, and a major risk factor for myocardial infarction, stroke and renal failure. Our recent work has identified that DNA methylation, a key epigenomic regulatory mechanism influencing gene expression and cellular differentiation, may account for the underlying differences in T2D between ethnic populations in United Kingdom, as well as act as a novel independent predictor of future T2D in high-risk normoglycaemic individuals. In addition, we have showed that fine-mapping of methylation at T2D associated genetic loci via targeted resequencing is likely to reveal patterns of methylation that are even more strongly associated with T2D than the markers revealed by methylation arrays that only cover <3% of the epigenome, thus providing opportunities for improved risk stratification.

More recently, we have extended our investigation of the association of DNA methylation with incident T2D to the Singapore population on ~1,500 individuals across the three major ethnic groups (Chinese, Malay and Indians) as part of the TOAST-T2D (Translating ‘omics’ into stratified approach for prevention of type 2 diabetes) study. Initial analyses have identified ~150 methylation markers that are significantly associated with incident T2D, including both known and novel associations.
To extend on our study, Assistant Prof Marie Loh will now build on our current findings by fine-mapping of selected top identified methylation loci, in combination with transcriptomics analysis. This will further enhance risk stratification for T2D, as well as our understanding of the underlying biological mechanisms.

**Aims**
The primary aim of the current proposed PhD project will be to build on the state-of-the-art initial results emerging from the TOAST-T2D study to enhance risk stratification and our understanding of the underlying biological mechanisms for T2D.

Specifically, potential candidates will need to:
1. Perform fine-mapping of selected top methylation signals identified from TOAST-T2D
2. Evaluate and fine-map the relationship of above-mentioned methylation sites with gene expression
3. Perform Mendelian randomization experiments to investigate causal relationships for above-mentioned methylation sites
4. Undertake laboratory experiments e.g. CRISPR to explore and evaluate potential molecular mechanisms of the candidate methylation sites identified

**Approach**
The Illumina Infinium MethylationEPIC array assays ~4% of the estimated ~30 million CpG sites in the human genome. Fine-mapping of top loci will enable better understanding of the methylation markers that influence risk of T2D, and will enable optimisation of the molecular risk score for T2D. In addition, as a secondary benefit, results will provide potential insights into underlying genomic mechanisms, enabling the design of functional experiments into causal pathways.

We will perform targeted resequencing on a 10kb region (5kb either side) around the top 3-5 identified CpG sites in genomic DNA from 100 Singapore Chinese, 100 Malay and 100 Indians. A distance of 5kb will be more than sufficient as previous publications has suggested that correlation between methylation markers rarely extends beyond 1kb. Bioinformatics analysis to determine percentage methylation will be performed according to established protocols. Any CpG sites that cannot be analysed by MiSeq due to design constraints will be analysed by pyrosequencing. RNAseq/qPCR will also be performed in parallel in the same samples to assess the relationship of these epigenetic changes on gene expression.

We will use the concept of Mendelian randomisation to investigate possible causal relationships between methylation and transcription levels at the above selected sites. Methylation quantitative trait loci (methQTL) and expression quantitative trait loci (eQTL) analyses will be performed in order to obtain estimated effects for the
The relationship between genetic variants with methylation levels respectively. Genotyping data are already available on these selected samples.

Upon identification of potential causal mechanisms, we will embark on laboratory experiments including but not restricted to CRISPR knock-outs to further explore and evaluate potential molecular mechanisms of the candidate methylation sites identified.

**Brief summary of main Methodologies and/or Techniques to be learned during the proposed PhD (experimental or analytical):**

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<td>1.</td>
<td>Knowledge on DNA methylation quantification, both array-based and next generation sequencing (NGS)-based</td>
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<td>2.</td>
<td>Knowledge on transcriptomic analysis, specifically RNAseq</td>
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<td>3.</td>
<td>Bioinformatics and statistical analytical skills related to points 1 and 2 above, including integrative omics analysis</td>
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<td>4.</td>
<td>Laboratory techniques e.g. CRISPRT and pyrosequencing</td>
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**Keywords:** metabolic disease, metabolic disturbance, type-2-diabetes (T2D), epigenome, transcriptome
### Supervisor(s)

#### Primary Supervisor
Name of Supervisor: Marie Loh  
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#### Co-Supervisor *(need not be determined at this time)*
Name of Supervisor: John Chambers  
Designation: Professor  
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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  
   
   If “Yes”, is the IRB’s approval in place?  
   - [ ] Yes  
   - [x] No  

2. Does the project involve contact with patients?  
   - [ ] Yes  
   - [x] No  

3. Does the project involve contact with animals  
   - [ ] Yes  
   - [x] No  
   
   If “Yes”, is the NTU-Institutional Animal Care and Use Committee approval in place?  
   - [ ] Yes  
   - [ ] No  

4. Is there a potential for overseas academic exchange as part of this research project?  
   - [ ] Yes  
   - [ ] No  
   
   If “Yes”, please specify:  
   
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