BASIC SCIENCE

PP-107

Is Alzheimer's Disease Risk Factor, Apolipoprotein E Polymorphism, A Risk Factor of Type-2 Diabetes Mellitus?

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INTRODUCTION
Individuals with Type-2 Diabetes Mellitus (T2DM) are known to have higher risk of developing Alzheimer's Disease (AD). One of the most prominent risk factors of AD is Apolipoprotein E (ApoE) polymorphisms. While ε2 allele is known to have suppression effect, ε4-carriers have 5-30-fold increased risk of developing AD compared to ε3-carriers. In this study, we have developed an efficient blood-based APOE genotyping method to investigate if APOE polymorphism plays roles in T2DM.

METHODOLOGY
Whole blood were collected from 409 participants (88 pre-diabetes, 202 diabetes, 119 healthy) under approved study protocol NMRR-15-980-26563. Four allele-specific primers were designed with intentional mismatches at each single nucleotide polymorphism (SNP) sites at rs429358 and rs7412, and two common primers flanking the non-allele-specific region. Single plex polymerase chain reaction (PCR) was carried out using a panel of 5 primer sets, whole blood 2 μL/reaction and KAPA Biosystem's Blood PCR Mix. Analysis of APOE genotypes against T2DM status were compared using ANOVA tests and chi-square tests (α=0.05).

RESULTS
Definitive APOE genotype were obtained for 407 participants (99.5% of total subjects). Genotype of two participants were further confirmed using follow-up samples. As expected, ε3 allele (84.23%) is the most common, followed by ε2 (9.05%) and ε4 (6.72%). Interestingly, ε4 is significantly linked to Malays (10.69%, p-value=0.0035). However, no statistical difference is found in the allele distribution across pre-diabetic, diabetic, and healthy participants (p-value=0.763), suggesting there is no direct association of ApoE genotype and T2DM in our cohort. Further analysis also found no particular links of fasting blood glucose, HbA1c and BMI with ε2-, ε3- and ε4-carriers.

CONCLUSION
ApoE polymorphism is not directly indicative of T2DM in our cohort. However, given that ε4-carriers have increased risk of developing AD, it is imperative to follow up with T2DM ε4-carriers, especially the Malay ethnic, who has a significant prevalence as ε4-carrier.

PP-108

Identification of Chromosome Conformation Signatures involved in Progression of Type-2 Diabetes Mellitus Using EpiSwitch™

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INTRODUCTION
Prevalence of Type-2 Diabetes Mellitus (T2DM) has increased more than 50% in 2006-2015 (National Health and Morbidity Survey, 2015). Oxford Biodynamics has demonstrated using Chromosome Conformation signatures (CCS) (Salter M. et al, 2018), as the most informative molecular entity in epigenetics for stratification of phenotypes. The proprietary EpiSwitch™ biomarker discovery platform is employed to find markers informative of T2DM progression.

METHODOLOGY
We have recruited a total of 409 subjects and categorised them into 4 groups, i.e. healthy, pre-diabetes, diabetes treatment naïve and treated diabetes; n=122, 90, 77, 120, respectively, based on glycated haemoglobin (HbA1C), fasting blood glucose (FBG) and oral glucose tolerant test. HbA1C, FBG and relevant clinical data of these subjects were followed up for 2 years at a 6-month interval. Five pre-diabetes, 6 diabetes treatment naïve and 6 treated diabetes samples were selected based on baseline data and compared against a pool of 16 healthy controls using proprietary microarray.

RESULTS
Follow-up blood tests showed 2 pre-diabetes samples progressed to diabetes. After comparing against samples regressing either from pre-diabetes to healthy, diabetes to pre-diabetes, diabetes to healthy and samples that are constantly diabetes, 59 unique progression markers have been identified. Functional analysis of the 59 markers

using STRING database showed that the markers are significantly involved in T2DM and insulin resistance pathway; false discovery rate 1.87e-09 and 5.39e-09 respectively.

CONCLUSION

Four markers overlapped and interconnected between insulin resistance and T2DM pathways demonstrate the significance and robust outcome of the methodology. CCS of these markers can be used to stratify risk among healthy and pre-diabetes people. The developed blood-based testing not only provides unprecedented early solutions for management, diagnosis and treatment of T2DM but also promising clues on the mechanism of T2DM progression. A further validation with cohort collected is to follow.

PP-109

Islet Cell Autoantibody Profile in a Malaysian Type-2 Diabetes Mellitus Population

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INTRODUCTION

Latent autoimmune diabetes in adults (LADA) accounts for 2-12% of all adult diabetes, where progressive islet cell failure leads to insulin deficiency. Insulin sensitivity is a major factor in the development of Type-2 diabetes mellitus (T2DM). This study aims to investigate islet cell autoantibody profile in a multi-ethnic T2DM cohort.

METHODOLOGY

ELISA assays of insulin antibodies, glutamic acid decarboxylase 65 (GADA65) and tyrosine phosphatase-related islet antigen 2 (IA2) (EUROIMMUN AG, Germany) were performed for 88 subjects (50 diabetes, 31 pre-diabetes and 7 healthy). Assay was performed using subjects' sera in duplicates and absorbance was read at wavelength 405 nm using a microplate reader. Titer positivity for anti-GAD65 and anti-IA2 were defined at ≥10 IU/ml. Subsequently, subjects were categorised into good control (HbA1C <5.6%, n=8) and bad control (≥5.6%, n=80). Statistical analysis of demographic factors was performed using chi-square test and ANOVA.

RESULTS

Eleven anti-GAD65 positives subjects were detected, and 1 subject presented positivity for both anti-GAD65 and anti-IA2. Among them, none are healthy subjects, 2 are pre-diabetic and 9 are diabetic, reflecting a gradual increment of β-cells failure from pre-diabetic to diabetic. A higher anti-GAD65-positives was also observed in Chinese (n=6/11, 54.5%) and in females (n=8/11, 72.7%). Comparing to anti-GAD65-negatives, anti-GAD65-positive subjects tend to be older (mean=57.6 years,±10.2), with higher HbA1c (mean=7.7%,±2.1), and higher BMI (5 overweight, 45.5% and 3 obese, 27.3%).

CONCLUSION

Anti-GAD65-positives detected is higher (18%) compared to studies in Singapore (7%) and China (5.9%). No statistical significance was observed when comparing autoantibody profiles with demography factors probably due to the small sample size. However, the higher prevalence of β-cell failure in T2DM diagnosed individuals in Malaysia indicates the necessity to study with a larger cohort.