Form 3

Report for Joint/Usage Research Program for Endocrine/Metabolism (Fiscal Year 2023)

Date：2024/4/4

To Director of Institute for Molecular and Cellular Regulation, Gunma University

Principal Applicant	
Institution	Faculty of Medicine Siriraj Hospital, Mahidol University
Position	Instructor-Head of Cellular and Molecular Diabetes Research Group
Name	Prapaporn Thamtarana

We report on the results of joint research in fiscal year 2023 as below.

（Program No.　　）

1．Research Title	Characterization of Dnajc3, a candidate gene for autosomal dominant diabetes
2．Purpose and Significance of the research project	Functional characterization of novel autosomal dominant diabetes associated gene Dnajc3 in human pancreatic alpha cells and beta cells.
3．Period of The Program	April 1, 2023 ～ March 31, 2024

4．Project Members

Name	Age	Sex	Affiliation	Position	Role
(Principal Applicant) Prapaporn Thamtarana	42	F	Mahidol University/ Faculty of Medicine Siriraj Hospital/Research Department	Position：Instructor Degree：PhD Acquisition date：2011.6.17	Project director
(Research Collaborators) Pa-thai Yenchitsoma-nus	69	M	Mahidol University/ Faculty of Medicine Siriraj Hospital/Research Department	Professor	Consultant
Siriporn Riyajan	31	F	Mahidol University/ Faculty of Medicine Siriraj Hospital/Research Department	Research Assistant	Cell analysis
Chutima Chanprasert	41	F	Mahidol University/ Faculty of Medicine Siriraj Hospital/Research Department	Research Assistant	Genetic and Genomic analyses

※If additional space is required, please attach a separate sheet.

5．Collaborating Researcher of IMCR	Name of Laboratory	Diabetes and Metabolic Disorders	Name	Jun Shirakawa

6． Research Plans

Monogenic diabetes is a heterogeneous group of distinct subtypes of diabetes characterized by different modes of inheritance and age of onset. Familial diabetes with autosomal dominant inheritance is more common and has more variable age of onset. Maturity-onset diabetes of the young (MODY) is a form of autosomal dominant diabetes that generally occurs during childhood and adolescence. Mutations in at least fourteen different genes (MODY1-MODY14) have been described to date as the cause of MODY in different families. However, in Asian populations, such as Chinese, Japanese, Korean, and Thai, up to 85% of MODY cases have unknown genetic causes.

We previously identified a missense mutation (c.T1424C:p.L475P) in ZYG11A identified by exome sequencing as segregating with hyperglycemia in a Thai family with autosomal dominant diabetes. ZYG11A functions as a target recruitment subunit of an E3 ubiquitin ligase complex that plays an important role in the regulation of cell cycle. We recently reported that ZYG11A as a cell cycle regulator required for beta-cell growth (Mol Cell Endocrinol. 2021) by this Joint/Usage Research Program for Endocrine/Metabolism in 2021.

Majority of Thai patients with diabetes are caused by unknown genetic etiology. Through whole exome sequencing, a heterozygous mutation (NM_006260; c.C712A; p.H238N) in a gene encoding DnaJ heat shock protein family (Hsp40) member C3 (DNAJC3) was identified in two families with early onset T2D. The mutation segregated with diabetes in the families and, thereby, was proposed as diabetic cause. DNAJC3 is a Bip co-chaperone. Loss of DNAJC3 increases pancreatic β-cell apoptosis in mice. Homozygous *DNAJC3* mutations have been reported as the causes of a rare monogenic diabetes with multisystemic neurodegeneration. However, an effect of heterozygous mutation in more common and milder forms of diabetes has never been investigated.

This work aims to *i*) investigate the association of *DNAJC3*-p.H238N with hyperglycemia in Thais, *ii*) screens for other mutation(s) in *DNAJC3*, and *iii*) generate the patient-derived induced pluripotent stem cells (iPSCs) from carriers for modeling of diabetes associated with *DNAJC3*-p.H238N.

The segregation of a mutation (p.H238N) in *DNAJC3*, a gene encoding co-chaperone, with early onset type 2 diabetes (T2D) in two families has been recently reported. However, a role of the mutation in diabetes development and the mechanism underlying its pathogenic effect have never been investigated. Through genetic-case control association study, our unfinished work shows a trend toward statistically significant (p = 0.069) association between the *DNAJC3*-p.H238N mutation and hyperglycemia in Thai population. We also identify another variation (p.G473G) that possibly activates cryptic splice site and leads to alternative splicing in a T2D patient. Moreover, we are establishing the induced pluripotent stem cells (iPSCs) from peripheral blood mononuclear cells (PBMCs) of the carriers for modeling of diabetes pathogenesis associated with the *DNAJC3*-p.H238N mutation. We found that all generated iPSC lines express the pluripotent markers studied and show normal karyotypes. The authentication of all lines is affirmed. Their differentiation capacity is being undertaken.

7． Research results:
Please describe the details of the contribution of the joint research with IMCR in obtaining the results.

To assess the expression of Dnajc3 in endocrine pancreas, western blotting, qPCR, or immunohistochemical analysis for Dnajc3 were conducted in mouse islets and human islets. The changes in the expression of Dnajc3 under ER stress and oxidative stress were confirmed.

The presence of DNAJC3-p.H238N in DNA of 2,064 and 1,514 subjects with hyperglycemia and normoglycemia, respectively, was investigated using the rhAmp SNP Genotyping System. Statistical differences of allele frequency between case and control were analyzed using Chi-square test. The results suggested the causal relationship between the DNAJC3 mutation and diabetes.

Six iPSC lines from peripheral blood mononuclear cells (PBMCs) of two affected family members carrying DNAJC3-p.H238N were generated using Sendai viral reprogramming under feeder free condition. The

pluripotency was affirmed by immunofluorescence staining of pluripotent markers. The differentiation capacity was demonstrated by embryoid bodies (EBs) formation, in which the marker of each germ layer was determined by immunofluorescence staining. An authentication of the generated iPSCs was verified by short tandem repeat (STR) analysis and Sanger sequencing. Chromosomal integrity was investigated by standard Giemsa staining.

8. Present status of academic conference presentations and research papers associated with the results of the joint research, and exchange of information on the joint research with the collaborating researcher at IMCR.
(As much as possible, please state papers that include the names of the collaborating researcher at IMCR or papers stating that the research was supported by the Joint Research Program with IMCR.
Regarding papers, please send a PDF file together with the report to the email address of the general affairs section of the Institute.) Office of General Affairs: kk-msomu4@ml.gunma-u.ac.jp

① Please list the publications that include the name of the collaborating researcher from IMCR and send a reprint of each publication to IMCR.

We are now submitting the collaborative paper.

② Please list the publications that include a description that the research was supported by the Joint Research Program with IMCR and send a reprint of each publication to IMCR.

Same as above

③ List up to 3 conferences (name of conference, date of conference, and title of the presentation).

Asian Islet Study Group (AISG), 16th November 2022

④ Exchange of information exchange with collaborating researcher from IMCR (please list main points of communication).

We usually have web meetings monthly to share the experimental results.