Form 3

Report for Joint/Usage Research Program for Endocrine/Metabolism (Fiscal Year 2024)

Date：2025/3/21

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

Principal Applicant	
Institution	The Hong Kong University of Science and Technology
Position	Associate Professor
Name	Yukinori Hirano

We report on the results of joint research in fiscal year 2024 as below.

（Program No.　22003　）

1．Research Title	Study of the link between metabolome and age-related sleep dysfunction in Drosophila
2．Purpose and Significance of the research project	Aging process can be variable between individuals, although underlying mechanisms are unknown. One of the prominent phenotypes showing aging individuality found in our lab is sleep fragmentation in flies, which can be seen also in human, known as insomnia（不眠症）. In the previous year, we found the metabolome change specific to the sleep fragmented aged flies. In the coming year, we will investigate the causes of the metabolome change linked to sleep fragmentation. These will provide a reasonable and novel concept of biological diversity and aging process.
3．Period of The Program	April 1, 2024 ～ March 31, 2025

4．Project Members

Name	Age	Sex	Affiliation	Position	Role
（Principal Applicant） Yukinori Hirano	44	M	The Hong Kong University of Science and Technology, Division of Life Science	Position：Assistant professor Degree：PhD Acquisition date：2008.3.31	Project director
（Research Collaborators） Priyanshu Bhargava	34	M	The Hong Kong University of Science and Technology, Division of Life Science	Position：Postdoc Degree：PhD Acquisition date：2018.9.30	Researcher

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

5．Collaborating Researcher of IMCR	Name of Laboratory	Metabolic Regulation and Genetics	Name	Takashi Nishimura

6． Research Plans

We aim to uncover the mechanisms of aging individuality, by conducting phenotypic analyses followed by metabolomic analyses that separate the aged population according to phenotype, namely sleep fragmentation (SF) and epileptic seizure. A widely targeted metabolome analysis will be conducted by Dr. Nishimura lab at IMCR, Gunma University. Preparation of the sample flies will be performed in our laboratory.

1) Causal relationship of leaky gut to sleep fragmentation

To test the significance of gut homeostasis in sleep phenotype, we will induce leaky gut pharmacologically or genetically. Hyperproliferation of gut stem cells could induce leaky gut, so that we will utilize this method. After generating flies with leaky gut, we will examine sleep and metabolome.

2) Causal relationship of microbiota to sleep fragmentation

Antibiotics feeding suggested that a specific bacteria could be related to sleep fragmentation: tetracycline suppressed sleep fragmentation, but other antibiotics did not. To understand the specific bacteria for sleep fragmentation, we are characterizing the microbiome via bacterial genomic sequencing using flies with sleep fragmentation or normal sleep, and also after feeding of tetracycline. The candidate bacteria will be cultured and fed to flies, to examine sleep and metabolome. The target metabolome will include metabolite from the bacteria.

3) Lipid-related metabolome related to epileptic seizure.

The epileptic seizure was negatively correlated to the expression of Lsd2. Lsd2 limits catalysis of triacylglycerol (TAG) in fat body. Aging is accompanied by the accumulation of fat. We hypothesize that over-usage of accumulating fat in aged flies is linked to epileptic seizure, therefore low expression of Lsd2 is involved in epileptic seizure. We will first test this hypothesis by overexpressing and knocking down Lsd2. We will then test how metabolome is changed by Lsd2 expression.

7． Research results:

1) Causal relationship of leaky gut to sleep fragmentation

We conducted the metabolomic analysis by separating the aged male flies with leaky gut or without leaky gut displaying SF and found that the abundance of monoamines, such as dopamine and octopamine, are increased in the aged flies without leaky gut but displaying SF. Mutations in the dopamine receptors, Dop1R2 and Dop2R alleviated age-dependent SF. To further obtain insights into the mechanisms underlying the monoamine change correlated to SF, we performed RNA-seq analyses to investigate the transcriptome change correlated to SF. Further analysis will be conducted in the following year. Inducing hyperproliferation of gut stem cells was not working, so that analyses were not performed.

2) Causal relationship of microbiota to sleep fragmentation

We identified SF-causing bacteria. With the help of other collaborators, we determined a metabolite which is secreted from the specific bacteria. Feeding the metabolite induced SF. Therefore, we determined the microbiome-brain axis that is mediated by the metabolite from a specific bacteria.

3) Lipid-related metabolome related to epileptic seizure.

We have tested the genetic manipulation of Lsd2, but it didn't affect the age-dependent epileptic seizure. We are now conducting forward genetic screening of the mechanisms.

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.

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.

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

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