Abstract of Master's Dissertation

No.1

Course	Health Innovation (Master of Science)	Name	Gnamian Nouveau Kanzin Alain
Thesis Title	Next-Generation-Sequencing-based analysis of the polymorphism of malaria genes in an endogenous population of Palawan Island, Philippine		

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Background : Malaria is the leading cause of public health concern in many developing countries. More than 400 thousand people are killed every year and half of the world population is at risk of malaria in 2019. Despite a global reduction in malaria incidence rate between 2010 and 2018, the emergence of resistant parasites to insecticide and antimalarial drugs poses a serious threat. While waiting for an alternative and competitive antimalarial drug, there is an urgent need to develop a preventive vaccine. This cannot be possible without a clear understanding of the mechanisms underlying malaria immunity.

Objective : The thesis aims to characterize genetic variation of immune related or drug related *Plasmodium falciparum* (*P.f*) genes in an indigenous population of Palawan island, Philippines.

Method : Genomic sequences of the target genes were isolated from peripheral blood of 82 patients with symptomatic malaria living in Palawan island by using next generation sequencing (NGS). Each DNA extracted from individuals was amplified by using primer sets which were optimized to 8 of vaccine candidate genes of Pf, merozoite surface protein 1, 2, 3 (MSP1, MSP2 and MSP3), glutamate rich protein (GLURP), apical membrane antigen 1 (AMA1), Liver stage antigen-3 (LSA3), Ring-infected erythrocyte surface antigen (RESA) and GPI-anchor transamidase (TAM). In addition, we examined three drug resistant genes namely PfDHFR, PfDHPS and Pf10-0355. The purified and mixed PCR products were applied to the next generation sequencer, MiSeq (Illumina). We retrieved from Plasmodb the nucleotide sequence of Pf3D7, the reference strain as well as the targeted gene. GENETYX Ver.15 was used to identify the mutations. I predicted B-cell and T-cell epitope using prediction tools available in Immune Epitope DataBase (IEDB). T-cell epitope was predicted using two prediction tools consecutively. B-cell epitopes were predicted using five combined methods. This project was conducted at the Institute of Tropical Medicine (NEKKEN), Nagasaki University (Japan), in collaboration with the Research Institute for Tropical Medicine (RITM, Philippines).

＊ The abstract, containing the objective, method, result and conclusion should not exceed 300-500words and printed double sided on A4 paper)

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Result : Unique non-synonymous mutations were identified in Palawan vaccine candidate genes.

MSP2, LSA3, GLURP, AMA1 and RESA were highly mutated suggesting their high immunogenicity in the population. In contrast, the numbers of SNPs of MSP1, MSP3 and TAM were relatively lower.

Highly mutated areas in several genes were not always fitted neither to B-cell nor to T-cell epitopes predicted by the methods described. In addition, drug resistant genes carried mutations associated with resistance to Sulfadoxine-pyrimethamine and halofantrine drugs.

Conclusion : NGS-based analysis showed gene specific patterns of SNP variation which might be related to immune responsiveness determined by human host antibody or T cell receptor reactivity. Prevalence of the drug resistant malaria could be estimated at the same time.

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