ERNDIM TRAINING GRANT REPORT

Foremost, my sincere appreciation to ERNDIM for organizing this Training Support grant for the purpose of providing financial assistance to participants especially from countries outside of Europe to obtain training and learn relevant techniques and information pertinent to the diagnosis of inborn errors of metabolism.

Specifically, my deepest gratitude to Dr. George Ruijter who accommodated my request to undertake a brief training pertinent to the use of MS/MS for the quantification of GAGs (MPS) at Erasmus University Medical Center. Further, he was the one who brought up the idea that I apply for an ERNDIM Training Support grant in order for me to proceed with this proposed visit. Also, he provided me a recommendation to contact Dr. Laura Steinbusch, my generous host at Maastricht University Medical Center, for the training on purines and pyrimidine analysis (PUPY).

This short-term training in the Netherlands is pivotal to the attainment of the 5-year strategic plan of the Biochemical Genetics Laboratory, Institute of Human Genetics (IHG), National Institutes of Health, University of the Philippines-Manila. Currently, the laboratory is continuously contributing to the diagnosis of rare diseases such as inborn errors of metabolism (IEM), through its demonstrated capability of detecting Maple Syrup Urine Disease (MSUD), with 354 cases being managed but also in the diagnosis of Phenylketonuria (n = 66), Carnitine Uptake Deficiency (n = 15), and Methylmalonic Acidemia (n = 24). However, in terms of other ultra-rare disorders such as Holocarboxylase Deficiency, Krabbe Disease, Lesch-Nyhan Disease, and Tay-Sachs Disease, the Institute has only seen a single case of each while disorders under the mucopolysaccharidoses (MPS) group, with MPS II being the most common with 67 cases. These statistics underscore the need to strengthen the capability of the Biochemical Genetics Laboratory in the Philippines to diagnose a wide spectrum of rare diseases.

For instance, the Lesch-Nyhan disease impacts purine metabolism. Through this exposure visit at Maastricht University Medical Center, facilitated by Dr. Steinbusch, I had an enriching discussion with Mr. Huub Waterval and Ms. Sandra van den Linden-Coeren, who are both knowledgeable in the conduct of analysis of PUPY via LC-MS/MS including method validation. This was supplemented by lectures provided by both Dr. Jorgen Bierau of Erasmus University Medical Center and Dr. Laura Steinbusch. The theoretical discussions of PUPY metabolic pathway provided to me has deepened by understanding about the PUPY metabolism. Both Dr. Bierau and Dr. Steinbusch have emphasized the importance of setting up the PUPY analysis in the laboratory in the Philippines, in order to increase the diagnosis rate of not only the Lesch-Nyhan Disease but also other deficiencies arising from the disruptions in the PUPY metabolic pathway.

Further, having been equipped with best practices from both world-class metabolic laboratories of Erasmus MC and Maastricht UMC, I am confident that the Biochemical Genetics Laboratory of the IHG will also be able to participate in research studies which shows clinical value in the early, rapid and noninvasive diagnosis of Tuberculosis (TB). Purine and pyrimidine metabolites such as hypoxanthine and xanthine have been shown to discriminate between those groups with latent tuberculosis infection (LTBI) and healthy controls. Tuberculosis is the 6th

leading cause of mortality in the Philippines and thus, is a major public health problem in the Philippines.

Dr. Ruijter's team, meanwhile, have demonstrated the conduct of MPS screening using the dimethylene blue (DMB) test using a Hamilton robot as well as the Quantitative analysis of methanolyzed Chondroitin, Dermatan, and Heparan sulfate in urine using Tandem mass spectrometry for diagnostic purposes. Further, Ms. Elly Bogaerts-Taal, Quality Manager, Clinical Genetics, Hereditary Metabolic Diseases provide a detailed discussion on method validation as well as means of implementing quality control in the metabolic laboratory. Tucked with these learnings, I am more confident to proceed with the planned method validation for the LC-MS/MS analysis of chondroitin, dermatan and heparin sulfate in urine for the differentiation of the different MPS particularly MPS II and IV which are quite common in the Philippines.

Dr. Ruijter is also generous to allow me to observe the conduct of the following biochemical assays that are all useful and relevant to the capacity-building initiatives of our laboratory:

1. Targeted metabolomics in plasma and urine samples for therapy monitoring using the high resolution mass spectrometer (HRAM-MS);
2. Semi-quantitative method for the determination of markers specific for the identification of oligosaccharides
3. Organic acid analysis in urine using solid-phase extraction (SPE) followed by quantitation using GC-MS. This was complimented by a demonstration of post run analysis using the Shimadzu GC-MS software.

Dr. Marne C Hagemeijer also provided a very good discussion about the analysis of urinary oligosaccharide excretion patterns by UHPLC/HRAM mass spectrometry for screening of lysosomal storage disorders (LSD).

Meanwhile, at Maastricht University Medical Center (MUMC), I have gained considerable amount of information about PUPY protocol, highlighting some critical issues related to optimization of the mass spectrometric (MS) method. Mr. Reza Rezaie, demonstrated the process by which the MS raw data is integrated to provide the final results for the PUPY. Dr. Steinbusch also presented some of those patients who have been diagnosed with Lesch-Nyhan Disease and discussed the manner by which they interpret the results.

I was also able to get a good grasp about the dihydropyrimidine dehydrogenase (DPD) protocol through Mr. Martijn Lindhout, an enzyme assay technician, which they perform to assess the patient's DPD enzyme activity for pharmacogenetics application. In addition, Dr. Irene M.L.W. Körver-Keularts, a Clinical Chemist at MUMC, who provided me with relevant publications from their group, is so keen to give a webinar on targeted urine metabolomics for rapid diagnosis of inborn errors of metabolism.

Overall, this training in the Netherlands, has equipped me, as Head of the Biochemical Genetics Laboratory, the only confirmatory laboratory for the diagnosis and management of IEM in the Philippines, with a clear perspective leading to the design of a concrete plan towards expansion of biochemical services that we can provide to the Philippine community, improvement on the analytical methods which are already in place in the laboratory, and new approaches and strategies for consideration in the diagnosis of inborn errors of metabolism. Importantly, this training enabled me to connect with some of the metabolic experts'/laboratory scientists in the

Netherlands who, could provide technical guidance and could be potential research collaborators.

Meanwhile, the visit in the Netherlands is truly a memorable experience for me. Indeed, the beauty of the Netherlands is mesmerizing. I am amaze with the people proactively participating in the effort to minimize environmental pollution, thus protecting the environment. I have witnessed the use of solar panels, 100% electric cars, recycling of materials, etc. The ambience in the Netherlands is so relaxing. I really enjoyed my time at Rijksmuseum, it was really a culturally enriching activity for me.

Again, my heartfelt gratitude to everyone who made this short term training as fruitful as possible as well as to ERNDIM which provides an avenue for networking and collaboration between and among scientists, analysts, laboratory technicians, chemists, quality managers and other clinical personnel involved in the diagnosis and management of inborn errors of metabolism.

Sincerely,

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