Paris, June 16, 2021

Final Report

(The 2nd Wael Al-Mahmeed & IAS Research Training Grants and Fellowships for the MENA Region & Africa project)

To Whom it may concern

Dear Sir or Madam,

I am grateful to inform you that Dr. Youmna GHALEB, a Post-Doctoral fellow in my team at INSERM U1148, Paris, France and a researcher in the laboratory of Biochemistry and Molecular Therapeutics (LBTM) in Saint-Joseph University of Beirut where she also has a Faculty position in the school of Pharmacy, has successfully finished her six-month fellowship at my team, from November 1, 2020 to April 30, 2021.

A close collaboration has been established years ago between my team at INSERM U1148, Paris, France and the team of Pr. Marianne ABI FADEL at Saint-Joseph University of Beirut, Lebanon. This collaboration has enabled us to obtain major results in the cholesterol field and led us to identify *PCSK9*, the third gene implicated in familial hypercholesterolemia. This project is part of this collaboration. Youmna GHALEB did the experiments at INSERM U1148 and analyzed the results during her stay in Paris as well as during her presence in Lebanon.

Project Title

Identification of new gene in Autosomal Dominant Hypercholesterolemia with Next Generation Sequencing

Project Summary

Atherosclerosis and its cardiovascular complications are one of the leading causes of morbidity and mortality in the industrialized countries. Hypercholesterolemia, which is characterized by high blood cholesterol levels (≥ 240 mg/dl), is one of the major cardiovascular risk factors and affects 1 in 20 individuals in the general population. Autosomal Dominant Hypercholesterolemia (ADH) is a genetic disorder characterized by markedly elevated plasma levels of LDL-C and the presence of characteristic clinical signs (xanthomas, xanthelasmas, arcus cornealis). When left untreated, ADH can lead to premature atherosclerosis, cardiovascular disease and often death at a very young age. ADH is caused by mutations in the following genes: *LDLR*, *APOB*, *PCSK9* and *APOE*. Note that rare cases of

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autosomal recessive hypercholesterolemia (ARH) have been reported and are caused by mutations in the *LDLRAP1* gene.

Genetic studies play an important role in the diagnosis and especially in the discovery of new therapeutic targets and approaches for treating dyslipidemia and its cardiovascular complications. Mutations identified in the *LDLR*, *APOB*, *PCSK9* and *APOE* genes account for 80% of ADH cases. The remaining 20% are mutation negative patients (ADH/M-). Several reasons can explain these ADH/M- cases including a possible existence of other genes which are yet to be discovered and might become new targets for lipid-lowering therapy. The objective of this project is to discover new genes, major genetic factors and modifiers involved in ADH. The first step will be the development of a Next Generation Sequencing (NGS) "gene panel". Then functional studies will be performed on selected candidate genes to evaluate their pathogenicity in ADH using cellular models built with the CRISPR/Cas9 technology. Once the ADH-causing effect confirmed, we will study the functional candidate gene(s) in molecularly homogeneous ADH populations to search for frequent variants with an effect on the variability of LDL levels.

Scientific Strategy

1. NGS Gene Panel

a. Selection of the genes:

The genes of the panel were selected based on different criteria:

- Genes identified in other ADH studies (obtained from bibliography)
- Genes identified in GWAS studies
- Genes presented in different congress and meetings (IAS, EAS, ESHG, ELC, …)
- Genes identified in whole exome sequencing (WES) and whole genome sequencing (WGS) performed in ADH families with no mutations in *LDLR*, *APOB*, *PCSK9* and *APOE*.
- LDL-C rising SNP defining the polygenic score

b. Methodology and Technique

- NGS panel is developed with the "KAPA HyperPlus" kits by Roche®. These kits are the most advanced library preparation option and provide a streamlined workflow that includes fully automatable fragmentation and library preparation in a single tube. The kits are compatible with the Illumina sequencing platform.
- The sequencing is performed using Illumina sequencing platform

c. Data analysis and pipeline development

2. Functional analysis of selected ADH candidate genes

Functional studies of the genes where rare variants are identified in several ADH/M- probands will be performed in order to confirm the implication of the genes in ADH.

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3. Search for modifier genes

Once its implication in ADH confirmed, the candidate gene(s) will be studied in other different populations already available and well characterized:

- in a large collection resulting from an international collaboration involving France, Lebanon, Canada and Sweden to identify new ADH-variants in these genes
- in Lebanese FH patients carrying the same mutation (p.Cys681ter or "Lebanese mutation" in the *LDLR* gene). These patients form a homogeneous group to test the hypothesis that the new identified gene(s) could carry frequent polymorphisms with an effect on the variability of LDL levels and thus might constitute a modifier gene in FH

We will also sequence the new identified genes in:

- hypocholesterolemic proband especially from France and Lebanon to identify LDL-lowering variants
- populations with high cardiovascular risk to identify new mutations responsible for these phenotypes.

Problems encountered and Preliminary Results

This six-month fellowship and therefore the project were supposed to start in September 2020. However, due to the COVID-19 pandemic and travel restrictions, Youmna GHALEB's stay in Paris was delayed and begun in November 2020. During this period (September to November 2020), Youmna GHALEB worked on the selection of the genes for the panel. A total of 285 genes were selected:

- genes from the analysis of the results of the WES and WGS
- genes involved in intracellular trafficking
- genes involved in the spliceosome
- genes interacting with the LDL receptor
- genes interacting with or regulating PCSK9
- genes from studies presented in congresses and scientific meetings
- genes identified in other studies on ADH
- genes identified in GWAS studies

Once the list of genes established, we encountered another problem which is the time taken by Roche® to validate and then produce the panel. A period of three weeks was proposed to validate the panel but finally we had to wait two months. Then the delay to receive all the reagents has been extended by two months and the experiments could not start until mid-April 2021.

We validated our protocol in the first experiment where samples with known variants were sequenced. The results obtained with our NGS panel allowed us to find the same variants previously identified by either WES or WGS. The results of this first run are currently being analyzed in order to identify a new variant in an ADH gene or to identify a new candidate gene. In parallel, Youmna GHALEB is working on the preparation and the sequencing of the samples. Five NGS run have been done till now (24 samples for each run for a total of 120 samples) and the results are under analysis.

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Dr. Youmna GHALEB possesses excellent professional qualities as a researcher. She is hard-working, regular and autonomous in her work. During her PhD in my team, she developed several new approaches which have enabled her to obtain significant results recognized by the scientific community. She also gained expertise in cell culture, molecular biology and flow cytometry. Her great skills confirmed my choice to keep her in my team as a post-doctoral researcher. In addition, she works on collaborative projects between our team at INSERM U1148 and the team of Pr. Marianne ABI FADEL at Saint-Joseph University of Beirut, Lebanon.

Dr. Youmna GHALEB has already published several peer-reviewed articles as a first author or as a co-author and she is now working on an article concerning the implication of a new gene in ADH. I am persuaded that the data we will obtain after the analysis of the gene panel sequencing of the whole ADH/M- cohort we have collected should allow publishing article in a good impact factor journal.

Best regards

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