

Familial hypercholesterolemia (FH) is a common genetic disorder that affects more than 25 million people worldwide. It is characterized by an elevation of low-density lipoprotein (LDL) cholesterol concentration in the bloodstream and a markedly increased risk of premature coronary artery disease. The leading cause of FH is pathogenic variants of *LDLR*, *APOB*, and *PCSK9* genes, which encode proteins involved in cholesterol metabolism. Proprotein convertase subtilisin/kexin type 9 (PCSK9) protein is an important regulator of cellular LDLR receptor level. The PCSK9 binds to the LDLR on the cell surface and initiates its degradation. Due to this process, the number of LDLRs available for LDL-C is reduced, resulting in decreased cholesterol levels in the cell.

Genetic studies have revealed that the gain-of-function *PCSK9* variants may excessively reduce the number of LDLRs available to LDL particles, consequently leading to FH. On the other hand, the loss-of-function *PCSK9* alterations may attenuate the interaction between PCSK9 and LDLR and increase cellular LDLR protein levels. In humans carrying these variants, plasma LDL concentrations are significantly reduced and protect against premature cardiovascular disease.

In order to restore correct cholesterol metabolism, patients with FH receive specific treatment: statins (reducing the synthesis of endogenous cholesterol) or PCSK9 inhibitors (abolishing the interaction between LDLR and PCSK9). The attenuated LDLR-PCSK9 interaction increases the number of LDLRs available for LDL molecules on the cell surface and improves the capacity to clear the LDL from blood. However, administration of PCSK9 inhibitors requires a patient's genetic test, proper variant classification, and confirmation of FH.

The project aims to conduct a functional analysis of *PCSK9* gene variants of unknown clinical significance detected in patients with FH and to perform their correct genetic classification. The project aims to understand the nature of the PCSK9 protein defect and its interaction with the LDLR receptor based on *in vitro* cultures. The obtained results will allow us to determine the individual impact of changes in the PCSK9 gene on the receptor degradation process, which may reflect the level of LDL fraction in the blood serum of patients with FH. Moreover, understanding the interaction mechanism between the PCSK9 variant and the LDLR receptor may help diagnose FH patients and their families, influence the selection of lipid-lowering treatment, and contribute to developing a more effective treatment program for FH.