Cardiovascular diseases and coronary heart disease are the main cause of all-time mortality in highly developed countries. Familial hypercholesterolemia (FH) is one of the most common monogenic disorders responsible for coronary artery disease. FH is an autosomal, dominantly inherited disorder of lipoprotein metabolism with an estimated frequency of approximately one in 500 persons for the heterozygous form and one in a million persons for the homozygous form in most European countries. FH is mainly caused by mutations in *LDLR*, *APOB* and *PCSK9*.

LDLR gene is located on a short arm of chromosome 19. LDLR is a cell surface glycoprotein of 160 kDa that binds and internalizes LDL, and thus, regulates cholesterol homeostasis. Extracellularly, it binds the APOB (apolipoprotein-B) and APOE (apolipoprotein-E). Currently more than 1700 genetic variants, distributed across the entire length of the LDL-receptor gene, have been described, which makes FH a heterogeneous disorder at the molecular level. Mutations may be classified into five classes based on their effects on the LDLR depending on the impact of the mutation on the presence of mRNA, receptor maturation, disparity between LDL and immunoglobulin binding on the cell surface, LDL receptor degradation and trafficking. Functional assays are a direct method which permits to determine whether the activity of a mutant protein is altered by taking into account all the involved biological mechanisms. In contrast to *in silico* studies, *in vitro* experiments allow to get an precise information about a class of *LDLR* mutation.

The 42-kb *APOB* gene spanning 29 exons and 28 introns is located on a chromosome 2. It gives rise to two isoforms of the APOB protein. The APOB-100 isoform found in LDL particles is 4,563 amino acids in length is expressed by hepatocytes, while the APOB-48 isoform is expressed in cells of the small intestine. In contrast to *LDLR*, only few mutations have been reported for *APOB*, most commonly impacting exon 26. However, more recent studies revealed the presence of genetic variants located within the other exons of the *APOB*. Due to the *APOB* size, before next generation sequencing (NGS) era it was almost impossible to perform the mutational analysis of the entire gene.

More recently, *STAP1* mutations have been detected in a group of FH patients. However, the number of the tested individuals was limited and genetic alterations were found only in several patients from the Netherlands and Germany.

In a vast majority, familial hypercholesterolemia is an autosomal dominant disease. However, autosomal recessive familial hypercholesterolemia (ARH) has been also described. To date, only *LDLRAP1* mutations were detected in a group of patients with ARH. The mutational analysis of *LDLRAP1* will be performed in a current study.

Since 2000, at the Department of Biology and Genetics of the Medical University of Gdansk, mutational analysis of *LDLR*, *APOB* and *PCSK9* genes is conducted in a group of patients with familial hypercholesterolemia (FH). For this purpose, MLPA, Sanger sequencing and more recently next generation sequencing (NGS) techniques are applied. To date, approximately 1500 probands and their family members have been tested which allowed to form the National Centre of Diagnostics and Treatment of Familial Hypercholesterolemia (NCD&TFH).

The aim of the project is to perform:

a) *in vitro* studies in order to analyze the expression and activity of 20 selected *LDLR* genetic variants. In addition, the results will be compared with clinical data of FH patients harboring the particular *LDLR* mutation,
b) mutational analysis of the coding sequences of *APOB*, *STAP1* and *LDLRAP1* genes in a group of 200 patients with FH.

To summarize, the study will allow to get new insights into pathogenesis of familial hypercholesterolemia. In addition, it can allow to increase a number of individuals with molecularly confirmed FH and may allow to update an algorithm of molecular diagnosis of FH patients and members of their families.