

Heterozygous familial hypercholesterolaemia (HeFH) is the most frequent autosomal dominant genetic disease with prevalence 1 in 250 individuals, is characterized by elevation of LDL cholesterol leading to premature atherosclerosis. The risk of cardiovascular disease (CVD) in patients with FH increases at least 10-fold. Therefore, effective lipid-lowering therapy is extremely important. Treatment with proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) is recommended in very-high-risk HeFH patients if the treatment goal is not achieved on maximal tolerated the dose of lipid-lowering therapy- statin plus ezetimibe. Studies have shown that the use of PCSK9i resulted in a reduced all-cause mortality, which may indicate that these drugs exhibit pleiotropic properties, not just a reduction in LDL-C levels. The latest literature data prove that, epigenetic mechanisms (that is, those responsible for activating/silencing gene expression, but these changes do not result directly from DNA sequence) might be involved in the regulation of interindividual lipid level variability and thus may contribute to the cardiovascular risk profile. Moreover, these processes are also influenced by oxidative stress, which is very detrimental to the health of every human being. We hypothesize that several processes regulating lipid level are controlled by epigenetic mechanisms.

The implementation of this project aims to identify and verify the pleiotropic properties of PCSK9i related to their influence on the human genome and epigenome. **The main aim of the project** is to assess whether PCSK9i restore epigenome balance and reduce DNA damage in patients with HeFH. The study group will consist of 40 patients with genetic or clinical diagnosis of HeFH and 40 patients with low cardiovascular risk and concentration of LDL-C (<100 mg/dL). The control and research group studies will include the isolation of peripheral blood mononuclear cells (PBMCs), plasma and serum from peripheral whole blood from patients with HeFH (before and after 6-month PCSK9i therapy). We plan to carry out following tests at the DNA, RNA and protein levels including the assessment of PCSK9i for these processes in patients with HeFH: (1) global DNA methylation level, (2) methylation level and (3) determination of expression of genes related to the process: methylation (*DNMT1*, *DNMT3A*), demethylation (*TET2*, *TET3*) and modification of histones (*EHMT1*, *EHMT2*, *HDAC3*, *HDAC5*) and genes related to oxidative stress and with LDL-C levels (*LOX-1*, *NOS3*, *GSTT1* and *PRDM16*), (4) determination of expression of the selected elements of epigenome at the protein level particularly related to histone acetylation and methylation (H3ac, H3K4/K9me3, H3K27me3).

Although patients with HeFH are exposed to elevated levels of LDL-C from birth and ox-LDL may will be induce other oxidation pathways leading to atherosclerosis and cardiovascular disorders, the level of DNA damage in FH patients has not yet been established. Our preliminary studies shown that DNA damage occurs at the level of about 20% in HeFH patients, and the 6-month lipid-lowering therapy with PCSK9i reduces the damage to 12%. The implementation of this project will allow the continuation of research on a more accurate determination of the level of DNA damage, including oxidative damage to purines and pyrimidines, and will also allow the assessment of the impact of PCSK9i on the reduction of DNA damage. Specific objectives of the project include the assessment of following parameters and processes in patients HeFH and the influence of PCSK9i on these processes: (5) determination of single/double DNA strand-breaks will be assessed by means gel electrophoresis in alkaline and neutral conditions (comet assay), (6) oxidative damage to purines and pyrimidines using comet assay with restriction enzymes, (7) detection of 8-OHdG, (8) analysis the total antioxidant capacity of the plasma and (9) lipid peroxidation markers such as: antiox-LDL, ox-LDL and *PONI*.

The results of our research will allow to identify new potential pleiotropic properties of PCSK9i. Taking into account the sublime disease entity selected for research, modern lipid-lowering therapy, analyzed DNA damage and epigenetic mechanisms as well as the innovative character of the research, its undoubted that the implantation of this project will allow the acquisition of new knowledge in the field of pharmacology, medicine, epigenetics and molecular biology.