UNIVERSITATEA DE MEDICINĂ, FARMACIE, ȘTIINȚE ȘI TEHNOLOGIE „GEORGE EMIL PALADE" DIN TÂRGU MUREȘ

"GEORGE EMIL PALADE" UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE AND TECHNOLOGY OF TÎRGU MUREŞ
SCHOOL OF DOCTORAL STUDIES

ABSTRACT OF THE DOCTORAL THESIS

IDENTIFICATION OF THE GENETIC RISK PROFILE FOR CARDIO-CEREBRO-VASCULAR DISEASE THROUGH MULTIPLE GENETIC SCREENING

PhD supervisor: Prof. MD, PhD Minodora Dobreanu
Doctoral student: George-Valeriu Moldovan

Coronary heart disease is the leading cause of morbidity and mortality worldwide. One-third of patients with coronary heart disease have the onset before the age of 55 in men and 65 in women and are diagnosed with premature coronary heart disease. The most incriminated risk factor is atherosclerosis, mainly caused by hyperlipidemia. Regardless of the risk factors' presence, mutations in certain genes that influence lipid metabolism lead to coronary heart disease. In approximately 50% of cases, the clinical aspect is Familial Hypercholesterolemia (FH). This pathology is often diagnosed based on clinical criteria and laboratory tests. The definite FH diagnosis can only be made by genetic analysis, thus identifying the determining mutation. Although there are international FH registries, endorsed by several medical societies, Romania is not present with official data.

The main objective of the present paper was to obtain a definite FH diagnosis in patients with clinically manifest premature ischemic heart disease. Another research goal was achieving a genetic profile specific for the patient with a predisposition to cardiovascular pathology. The intent was to emphasize the importance of a definite molecular diagnosis in patients with familial hypercholesterolemia.

This prospective study included patients clinically diagnosed with premature coronary heart disease (uni-, bi- or trivascular), confirmed by cardiac catheterization. Subjects presented elevated serum LDL-cholesterol levels and serum triglyceride levels within the physiological limits and were hospitalized in the Adult Cardiology Clinics of the Emergency Institute for Cardiovascular Diseases and Transplantation from Tîrgu Mureş between January 2016-August 2018. Exclusion criteria were the presence of associated pathologies that can lead to the occurrence of coronary heart disease, or changes in circulating serum lipids. Clinically healthy adults were included in the control group.

In order to identify the most frequent mutations involved in the occurrence of familial hypercholesterolemia in the Romanian area, this paper was structured in three distinct studies:

1. Genetic investigation of familial hypercholesterolemia using validated methods for clinical diagnosis;
2. Use of Sanger sequencing in familial hypercholesterolemia diagnosis;
3. Identification of new biomarkers in atherosclerotic risk assessment.

The first study aimed to identify point mutations associated with FH, analyze the involvement of copy number variations in FH etiology, and the applicability of clinically validated tests on the Romanian population. To achieve these objectives, a number of 158 individuals from the study group and 92 subjects from the control group were included. Serum lipids were investigated using validated diagnosis methods. Identification of point mutations was performed using the Familial Hypercholesterolemia Array I and II (Randox Laboratories, Crumlin, UK), and copy number variation analysis with the MLPA technique, using the SALPA MLPA Probemix P062 LDLR kit (MRC Holland, Amsterdam, The Netherlands).

FH causing point mutations were identified in four patients. Copy number variation analysis within the *LDLR* gene was not involved in FH occurrence. Although the present study identified a low genetic diagnosis rate, the use of clinically validated tests for the FH molecular diagnosis may be an accessible option for the investigation of a target population.

With the use of Sanger sequencing, the second study aimed to identify *LDLR* gene variants in a family whose members presented clinical and biochemical features suggestive for FH. Further intent was to identify a causal relationship between the molecular findings and the severity of the manifest disease and to investigate whether the identified variants were involved in the FH etiology. Sanger sequencing allowed the identification of seven mononucleotide variants in the nucleic acid sample from the index case. Some of the exon variants identified in the index case were also present in its first-degree relatives.

The identified genetic changes, even though individually not considered pathogenic, in combination with other predisposing factors, could increase the risk for coronary heart disease. The results highlight the complexity of the genetic etiology of this pathology, which involves in addition to the main *LDLR* gene, various other genes whose individual changes can lead to the presence of FH, as well as the possibility of polygenic determinism.

The third study aimed to assess the atherosclerotic risk by evaluating the contribution of *APOE* allelic variants in the etiology of coronary heart disease, estimating the atherosclerosis risk by identifying new potential biomarkers, and evaluating the accuracy of LDL-C calculation formulas. A number of 104 patients were included in the study group and 87 healthy people in the control group. The subjects' inclusion criteria were those presented in the general methodology to which the presence of long-term lipid-lowering therapy was added in the case of patients previously diagnosed with coronary heart disease. Biochemical investigation of the main serum lipids, as well as plasma proteins, was performed using dedicated analytical systems and diagnosis-validated reagents. LDL-C was directly measured, but also calculated, using the Friedewald equation and Equation_2 (Sampson et al.). All subjects were tested for the presence of *APOE* variants rs429358 and rs7412 using two different PCR-based techniques. SPSS 23.0 software was used for statistical analysis and graphical representations of the data.

The coronary heart disease risk was not influenced by *APOE* genotypes. In patients treated with lipid-lowering medication, the ApoB/ApoA1 ratio was not relevant as a biomarker, but there was a positive association between non-HDL-C and the ApoB/ApoA1 ratio, being further possibly useful in the assessment of coronary heart disease recurrence risk. The results obtained proved the accuracy of Sampson's Equation2, particularly for serum levels considered targets in the lipid-lowering treatment.

The degree of originality of this thesis is conferred by the investigation of Romanian patients clinically diagnosed with premature coronary heart disease with state-of-the-art molecular genetic techniques. Considering the addressability of the clinic from which the patients were enrolled, an accurate FH mapping of the central region of Romania was performed. Therewith, an etiological genetic diagnosis for these patients was proposed for the first time, knowing that obtaining a definite molecular diagnosis in familial hypercholesterolemia is essential in approaching the patient with premature ischemic heart disease.