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Analysis and characterization of genetic and epigenetic variants associated with cardiovascular disease

Cardiovascular disease (CVD) is one of the main causes of death and disability in the world: more people die annually from this type of disease than from any other causes. CVD refers to various types of conditions that can affect heart function and blood vessels. The leading cause of death in industrialized countries is specifically myocardial infarction (MI). Although the pathophysiological spectrum extending from ischemia to mechanical complications of the infarct is well known, the atherosclerotic pathogenesis is multifactorial and is constantly being studied.

In addition to tobacco usage, air pollution, unhealthy diet, alcohol abuse and physical inactivity, it is increasingly suggestive to believe that genetic factors influencing the excitation-contraction mechanisms could play an important role in modifying the individual's risk for MI. Different publications illustrate the enormous progress that has been made in understanding calcium signaling in the heart and the excitation-contraction coupling. The events that occur in E–C coupling depend not only by changes in intracellular calcium concentration ($[Ca^{2+}]_i$) or by the combination of the properties of Ca^{2+} channels and transporters but also by the proteins that regulate the Ca^{2+} channels. Among all the proteins involved in this complex mechanism, it is assumed that calmodulin could be an important regulator of excitation-contraction coupling because of its ability to bind calcium modulating Ca^{2+} channels, even if its precise role has not yet been defined.

Since several works show how some polymorphic variants can be considered predisposing factors to complex pathologies, we hypothesize that the identification of some polymorphic variants of proteins involved in the CalM pathway, could be important to understand if MI and other CVD have genetics bases.

Among the proteins involved in this pathway, a recent study has investigated the role of CAMKK1, calcium calmodulin kinase kinase 1, in myocardial infarction. Marc Penn et al have hypothesized that CAMKK1 behaves as a modifier of the functional effects of mesenchymal stem cells, improving cardiac function after myocardial infarction.

Because of its possible role in improving post-infarction conditions and being an upstream regulator in the kinase cascade pathway in major cells, CAMKK1 could play a role in regulating calcium in the heart. Furthermore, CAMKK1 presents polymorphic variants in the population of clinical interest. For example, the polymorphism rs7214723, which determines an amino acid change from glutamic acid (E) to glycine (G), has been associated with an increased risk of developing lung cancer [18, 19]. This polymorphism is very represented in the population (MAF index: C = 0.3954 / 1980 (1000 Genomes)).

Since the regulation of calcium is the protagonist of the cardiac excitation-contraction mechanism and since CAMKK1 is probably involved in this process, it could be interesting to study whether this known CAMKK1 polymorphism in lung cancer can also affect myocardial infarction, because it is known that some polymorphic variants can be considered predisposing factors to complex pathologies.
The identification of the association of some polymorphisms of the calcium pathway to atherosclerotic pathology could be relevant both from the clinical, for the severity of the coronary pathology, and epidemiological, for the spread of the pathology in the general population, point of view.

Thus during this first year of my PhD, I studied the association between some polymorphisms of the calcium pathway and the onset of infarction, specifically I focused my attention on CAMKK1 (Calcium-calmodulin kinase kinase 1) and NOS3 (Nitric oxide synthase 3) genes. The goal of this study is to identify new biomarkers that could contribute to classify high-risk patients from low-risk patients.

The study has provided the drafting and subsequent approval of an ethics committee since it was necessary the recruitment of 300 subjects, identified by the S.C. Cardiac surgery of ASST Settelaghi of Varese, divided into two groups. The first population consists of patients suffering from severe and critical coronary artery disease (stenosis> 70%) demonstrated angiographically (coronarography), the second population consists of patients who are certainly not affected by atherosclerotic coronary artery disease. The patients were subjected to a blood sample, stored on a FTA Card Whatman at room temperature. The cards were then transported to the University of Parma where I worked on, studying the genetic profile and deepening the correlation with the subject's pathological history. The purification of nucleic acids from blood on FTA was done following a specific protocol. To amplify the specific region of the gene containing the SNP (single nucleotide polymorphism) of interest, we proceeded with PCR reaction and subsequently the amplicon was identified by electrophoretic run on 2% agarose gel. The samples , in which the amplification worked correctly, were then subjected to RFLP analysis with a restriction enzyme capable of cutting the sequence based on the present SNP. This allowed to discriminate, with a subsequent electrophoretic run, if the sample under examination belongs to a homozygous or heterozygous individual. The data obtained were statistically analyzed to assess if there is a relationship between polymorphism of interest and the onset of atherosclerotic coronary artery disease.

I finished to analyze 300 samples and logistic regression analysis was applied between CAMKK1 rs7214723 polymorphism and risk of myocardial infarction.

The study of the rs1799983 polymorphism of NOS3 gene is currently underway: so far 250 samples have been analyzed.