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Platelet Transcriptome Profile of Acute Myocardial Infarction

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to Carla and Alice

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“The most beautiful things in the world cannot be seen or touched, they are felt with the heart.”

Antoine de Saint-Exupéry

Summary

Acute myocardial infarction (AMI) is a sudden event commonly attributed to coronary atherosclerotic plaque rupture with subsequent exposure of thrombogenic substances that promote platelet activation/aggregation as well as coagulation activation and, ultimately, intraluminal thrombus formation thus limiting coronary blood flow. Although plaque rupture or fissuring frequently occurs in atherosclerosis, only a small proportion of ruptured plaques develops thromboses. It has been suggested that individual reactivity to plaque rupture may have a causative role in provoking the clinical event of AMI.

Platelets contribute to the genesis and progression of atherosclerosis and to the precipitation of the atherothrombotic event after plaque rupture. Platelets are anucleate cells formed from the cytoplasm of megakaryocytes and circulate in the blood stream for about 7-10 days. Since platelets are without nucleus, they retain megakaryocytes–derived mRNAs that are unique in representing a nearly fixed transcriptome.

The present study was designed to test whether platelets transcriptome may predate the development of a future myocardial infarction.

The abundance of platelets transcripts levels were measured using Human Transcriptome Array 2.0 (Affymetrix). Bioinformatics analyses were conducted to identify the differentially expressed genes (p.value ≤ 0.05, fold-change ≥ ± 1.5) between patients with ST elevation myocardial infarction (STEMI, n=20), stable coronary artery disease (sCAD, n=20) and healthy donors (HD, n=20). REACTOME and DAVID analyses were performed respectively to identify pathways and biological processes associated with lists of differentially expressed genes (DEGs).

A total of 149 DEGs were observed comparing sCAD patients and HD platelet trascriptome. MNF2 and PRKCD were identified as the most up-regulated genes and CXCL8 as the most down-regulated. A number of 76 DEGs were detected in STEMI subjects compared with HD. S100A12 and CLEC4E, in STEMI patients, resulted the highest up-regulated mRNAs while CXCL8 was the most down-regulated followed by SNORD13, SNORD117 and RMRP. The comparison between STEMI and sCAD subjects allowed the identification of 138 de-regulated genes. S100A12 was again the most up-regulated gene in STEMI. From this preliminary analysis, we observed the presence of distinctive gene-expression patterns for sCAD and STEMI. These results suggested the possibility to find a more specific gene-set for STEMI condition. Thus, we focused on the characterization of a gene-signature able to discriminate patients with myocardial infarction and eventually we identified a STEMI signature consisting of 38 genes from these analyses.

In conclusion, our preliminary results show that variations in gene expression profile of megakaryocytes may occur before of an AMI and that platelet transcriptome could act as a fingerprint indicating the development of a future myocardial infarction.

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List of Acronyms

ACS: acute coronary syndrome
AMI: acute myocardial infarction
BSA: bovine serum albumin
CAD: coronary artery disease
CVDs: cardiovascular diseases
cDNA: complementary DNA
DAVID: database for annotation, visualization and integrated discovery
DCs: dendritic cells
DGEs: differentially expressed genes
DNA: deoxyribonucleic acid
FCM: flow cytometry
HbA1c: haemoglobin A1c
HD: healthy donors
HDL: high density lipoprotein
LDL: low density lipoprotein
lncRNA:long non coding RNA
LPDs: leukocyte-depleted platelets
mAb: monoclonal antibody
MI: myocardial infarction
miRNA: micro RNA
MK: megakaryocyte
MKs: megakaryocytes
mRNA: messenger RNA
oxLDL: oxidized low-density lipoprotein
PBS: phosphate buffered saline
PRP: platelet-rich plasma
qPCR: quantitative polymerase chain reaction
RNA: ribonucleic acid
RT: room temperature
sCAD: stable coronary artery disease
snRNA: small nuclear RNA
SPA: spontaneous platelet aggregation
STEMI: ST elevation myocardial infarction
TF: tissue factor
TPO: thrombopoietin
UA: unstable angina

Chapter 1

Introduction

1.1 Acute Myocardial Infarction

Acute myocardial infarction is a sudden event, fatal in approximately one third of patients with about half of deaths occurring within one hour of the event. This “out of the blue” event is commonly attributed to coronary atherosclerotic plaque rupture with subsequent exposure of thrombogenic substances that promote platelet activation/aggregation, as well as coagulation activation and ultimately intraluminal thrombus formation, thus limiting blood flow in one or more coronary arteries. Despite advances in cardiac care, myocardial infarction affects millions of people worldwide each year[1] and still remains an unpredictable event.

1.2 Pathophysiology of Acute Myocardial Infarction

Coronary atherosclerosis is the main underlying condition of acute myocardial infarction. For much of the last century, atherosclerosis was considered to be a cholesterol storage disease, characterized by the accumulation of cholesterol and thrombotic debris in the arterial wall. Nowadays, it is well known that atherosclerosis is a chronic inflammatory process of the vascular wall.[2] The evolution of atherosclerosis is a slow process, evolving over years and decades. High blood

lipoprotein levels cause LDL particles to accumulate in extracellular matrix in the artery vessel wall. These eventually become targets for oxidative processes and release phospholipids that activate endothelial cells to express leukocyte adhesion molecules and to release chemokines.[3] Leukocytes adhere to vessel wall and migrate into the intima. Monocytes differentiate into macrophages that incorporate oxLDL with the help of scavenger receptors and transform into foam cells.[4] In addition, T-cells migrate into vessel wall, recognise local antigens and secrete pro-inflammatory cytokines, contributing to local inflammation and to the growth of the atherosclerotic plaques. Most of the atherosclerotic plaques remain clinically asymptomatic, some (stable plaques) gradually become obstructive manifesting themselves with stable angina while only few become thrombosis-prone (vulnerable plaques) and may lead to an acute coronary syndrome (ACS) (FIG. 1).[5] Acute coronary syndrome is the high-risk manifestation of coronary artery disease ranging from unstable angina (UA), non ST-elevation myocardial infarction (NSTEMI), ST-elevation myocardial infarction (STEMI) to sudden death.

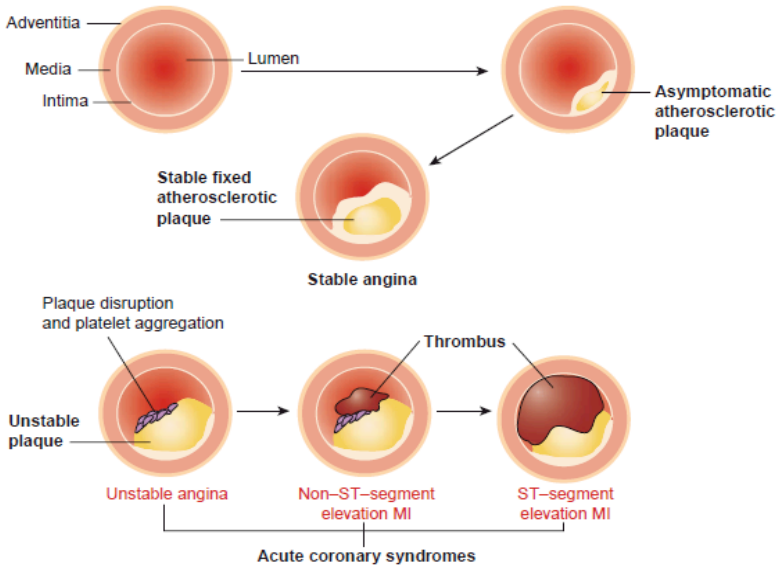

Figure 1. Atherosclerotic plaque. Stable atherosclerotic plaque in stable angina and unstable plaque with plaque disruption and platelet aggregation in acute coronary syndromes.

Coronary atherosclerotic-plaque thrombosis is a key event in the pathogenesis of MI. Recent clinical observations have confirmed that vulnerable plaque rupture is the most common cause of coronary thrombosis.[6] Vulnerable plaques are characterized by specific histopathological features that include a large lipid core, a high macrophage content and a thin fibrous cap. Although plaque rupture is a frequent event in the natural history of atherosclerosis, only a small proportion of them develop superimposed thrombosis. Necroscopy data have shown that between 8.7-16.7% of the people who die suddenly of non-cardiac causes present both thrombosed and non-thrombosed ruptured plaques.[7] Therefore, it is possible that vulnerable plaques undergo reparative processes after rupture. Given 30 days for plaque repair, it has been calculated that, in each subject, an average of 7.5 plaque ruptures per year occurs in absence of any cardiovascular event.[8] Thus, plaque rupture with local thrombin activation and subsequent healing, might simply represent a mechanism of atherosclerotic disease progression. In fact, 70% of chronic atherosclerotic plaques, determining a high grade coronary stenosis, present, at least, one rupture episode which acts as a stimulus to plaque growth.[9] However, the reason why some ruptured plaques should develop thrombosis while others do not are still unknown. Two hypotheses are currently proposed.

The first one, called *"the power of the stimulus",* considers that atherosclerotic plaques are of different types, some having such a strong thrombogenic potential that may overcome local antithrombotic mechanisms (FIG. 2). In subjects with normal blood reactivity, a very powerful thrombogenic stimulus inside the vulnerable plaque is assumed to be the primary element, which triggers and mantains the thrombotic process. Tissue factor (TF), the major initiator of the coagulation cascade,[10] is considered to have a key role in plaque thrombosis. Tissue Factor has been found in

the necrotic cores of atherosclerotic plaques and in the extracellular matrix of arterial walls.[11] Studies on coronary atherosclerotic plaques obtained during atherectomy have shown that concentrations of TF antigen and activity were higher in plaques of patients with unstable angina or myocardial infarction compared with those of patients with stable angina.[12,13] These results suggest that the TF contents in atherosclerotic plaques may determine different thrombotic responses to plaque rupture in human coronary arteries.

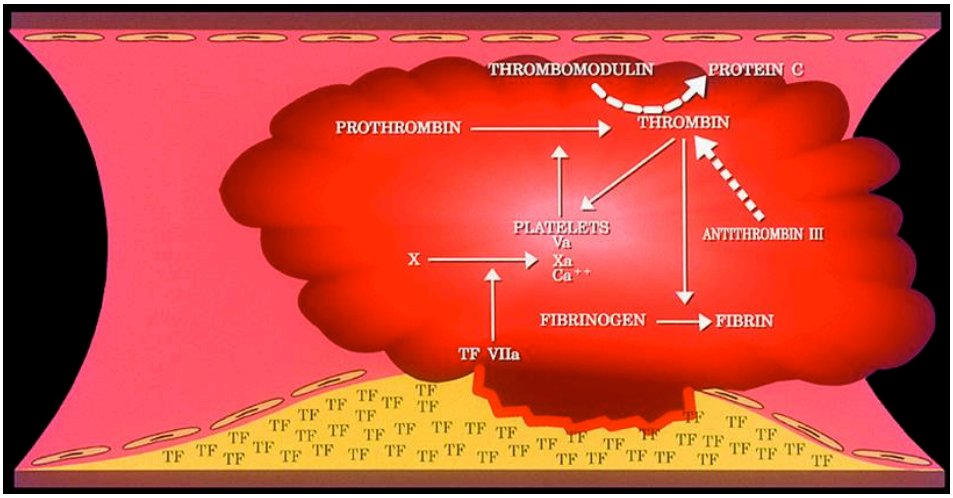

Figure 2. Pathophysiology of acute myocardial infarction. Hypothesis 1: "power of the stimulus".

The second hypothesis, called *"the power of the response"*, considers that all plaques have an equal thrombogenic potential and that a particular hypercoagulable state is needed to trigger an acute atherothrombotic event after plaque rupture (FIG. 3). High levels of factors VIIc, VIIIc and fibrinogen were associated with an increase risk of cardiovascular death in a prospective study on haemostatic function.[14] Elevated plasma concentrations of prothrombin fragment 1+2 (F1+2) and fibrinopeptide A - respectively markers of factor Xa–mediated prothrombin activation and thrombin action on fibrinogen - have been related to an early unfavorable outcome during ACS acute phase. However, after an ACS episode, a high proportion

of patients have persistently elevated levels of prothrombin F1+2, but not fibrinopeptide A.[15] It has been demonstrated that this condition of increased hemostatic system activity is associated with an increased risk of adverse events [cardiac death or myocardial (re)infarction].[16] High levels of thrombin generation may overwhelm the endogenous anticoagulant mechanisms and be prothrombotic. This may be even more critical in the setting of an unstable atherosclerotic plaque, which is associated with a loss of the antithrombotic properties of laminar flow and a normally functioning endothelium. Therefore, thrombin is a powerful agonist for platelet activation and aggregation. An icreased platelet aggregability may be another mechanism underlying the exaggerated thrombotic response to plaque disruption. Trip et al. tested spontaneous platelet aggregation (SPA) in a cohort of survivors from MI and found a positive association between platelet hyperreactivity in vitro and both mortality and cardiac events.[17] However, the pathophysiologic mechanism by which platelets contribute to the acute manifestations of coronary artery disease is not fully understood.

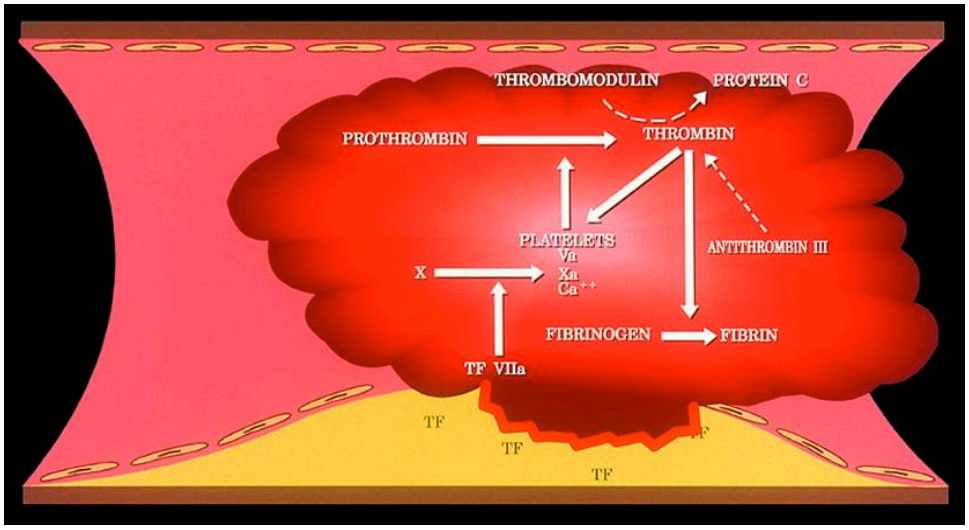

Figure 3. Pathophysiology of acute myocardial infarction. Hypothesis 2: "power of the response".

1.3 Platelets

For over 100 years platelets have been recognized as key mediators of hemostasis. At present, it has been established that platelets contribute to additional physiological processes such as angiogenesis, inflammation, innate immunity and can be responsible not only for the genesis, but also for the progression of CVDs.

1.3.1 Platelets biology

Platelets are small anucleate cell fragments with a discoid shape and a range in diameter from 1 to 3 μm. Platelets are formed from cytoplasm of megakarycytes (MKs) which reside in the bone marrow. In a cytoskeleton-driven maturation process, MKs become polyploid by endomitosis, their cytoplasm is packaged into multiple long processes called proplatelets and the nucleus is extruded. A mature MK may extend 10-20 proplatelets, each of which starts as a blunt protusion that over time elongates, thins, and branches repeatedly. Proplatelets migrate through bone marrow endotelial cells of the sinusoidal blood vessel and release preplatelets (formed selectively at the tips of proplatelets) directly into the marrow vascular sinusoidal space [Fig 4]. The terminal platelet formation continues in the blood stream where preplatelets undergo subsequent fission events to generate discoid platelets.[18] A conversion from pre- to proplatelet is also possible and it is driven by microtubule-based forces. Some authors have hypothesized that, as proplatelets extending into the lumen, they could have a monitoring function of circulating levels of proteins, such as TPO, or even platelets numbers. This would allow the MK to receive information and instruct the MK in processes such as protein translation, granule packaging and platelets production. Interestingly, as platelets develop, they receive their granule and organelle content as streams of individual particles transported from the MK cell

body.[19] Platelets retain also a specific set of megakaryocyte-derived RNAs and are capable of *de novo* protein synthesis. The time required for MKs to complete polyploidization, mature, and release platelets is ~ 5 days. Once released into the bloodstream, platelets circulate throughout the body for 7–10 days until clearance by liver and spleen.[20]

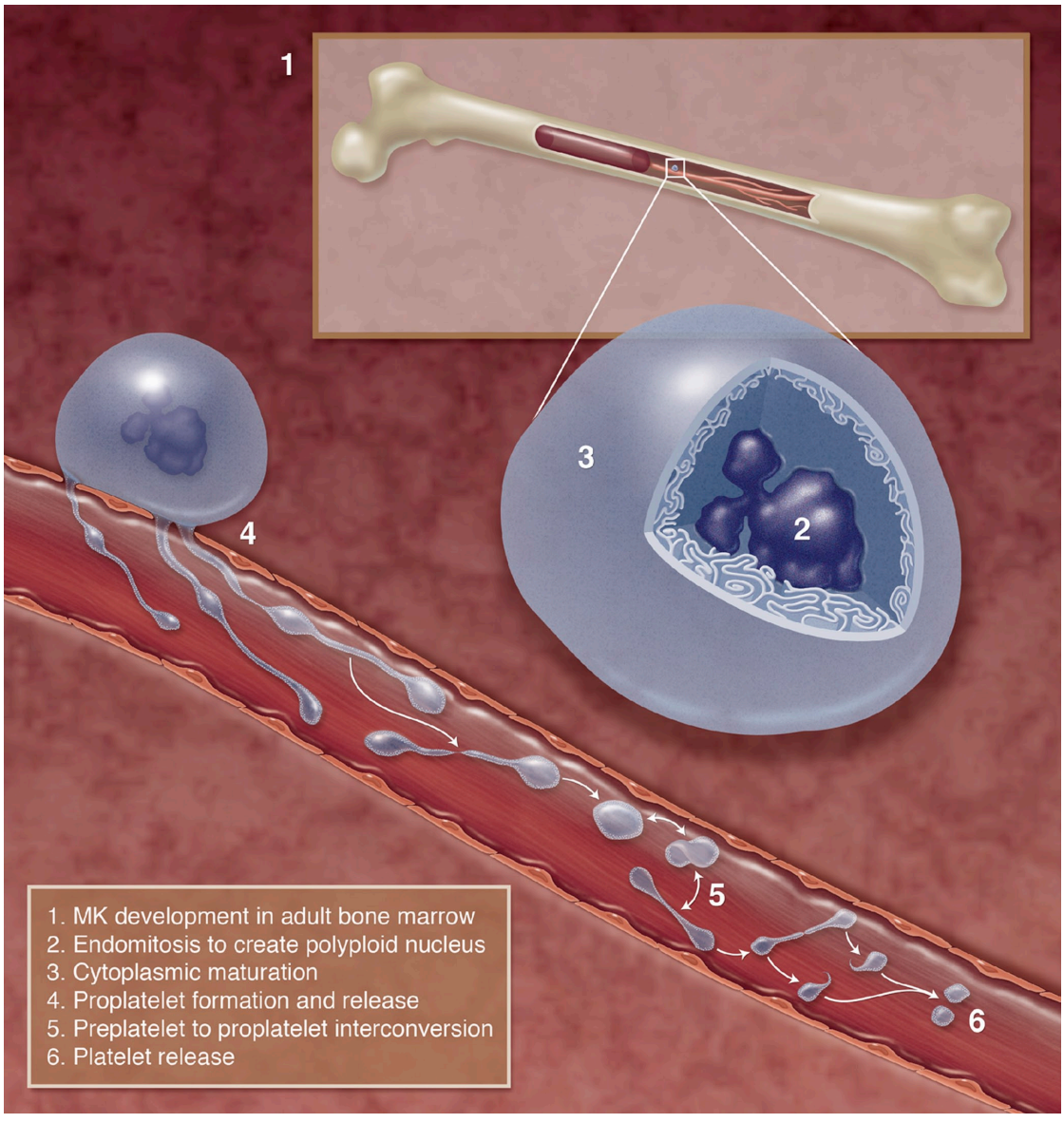

Figure 4. Schematic of platelet generation and development.From Machuls KR et al. J Cell Biol. 2013.

1.3.2 Platelets and atherosclerosis

Platelets are known to contribute to early steps of atherosclerosis (endothelial dysfunction), but also to final events (plaque rupture). Platelets participate in atherogenesis by chemokine release, surface association of oxLDL, direct cell–cell interaction (leukocytes, DCs), release of microparticles, and provision of potent inflammatory mediators that regulate homotypic and heterotypic intercellular aggregation, chemotaxis, angiogenesis, matrix degradation, and signalling events in target cells.[21] Platelets conceived as immune cells and mediators of vascular/tissue remodeling have a strong impact on atheroprogression. The net effect of platelet-mediated inflammation may be an atheropromoting one, although these anucleate cells may mediate distinct atheroprotective mechanisms as well.[22] Platelets participate in events that immediately precede acute myocardial infarction (AMI) and platelet-dependent thrombus formation is a key event in the pathogenesis of AMI.

1.3.3 Platelet trascriptome

Platelets lack nuclear DNA but retain megakaryocyte-derived RNAs and so the platelet transcriptome could provide a novel window on gene expression preceding acute coronary events.[23] Despite the absence of a nucleus, platelets contain protein synthesis machinery and RNAs, including mRNA, lncRNA, miRNA, accounting for more than 9000 transcripts in healthy human donors.[24,25] Platelet RNAs primarily derive from MKs, indeed genome-wide expression studies of platelets from MKs derived by culture from CD34+ hematopoietic progenitor cells, have shown that approximately half of the ~ 10000 transcripts present in MKs can also be detected in platelets.[26,27,28] Moreover, some studies have suggested a biological and pathophysiological role of platelet RNA finding a correlation between specific RNA

profiles with a number of disease and platelet function states. [29,30,31,32,33] Gene expression analyses, using micro-array technology, are growing exponentially due to their clinical application with diagnostic and prognostic implications in many pathological conditions, including cardiovascular diseases.[34] Specifically, concerning ACS, few studies have identified differentially expressed genes with a potential pathological role in the development and progression of this disease.[35,36] Nevertheless, some of these studies have been performed in nucleated cells, which are able to modify their transcripts in few hours and so their mRNA expression may be strongly modified by the acute event itself lacking a predictive value. Conversely, platelet RNA is unique in representing a nearly fixed transcriptome. Indeed, they are without transcriptional activity and, although there may be some flux or degradation, the changes may be minimal and a significant turnover in transcript levels is reflected in days compared with hours for nucleated cells. Therefore, since platelets realesed from MKs circulate in the blood stream for about 7-10 days, profiling platelet transcriptome at the time of AMI could identify the up or down-regulation of platelet-specific transcripts that may predict a future coronary acute event. Currently a very limited number of studies examining RNA from platelets have demonstrated the presence of a specific platelet phenotype that is direclty associated with atherothrombotic diseases.[23,37,38]

1.4 Aim of the research

The present study was designed to test whether platelets transcriptome may predate the development of a future myocardial infarction.

Chapter 2

Materials and Methods

2.1 Study population

The subjects eligible for enrolment were:

1. *Hospitalised patients with ST elevation myocardial infarction (STEMI)*. STEMI was defined as chest pain with electrocardiogram documentation of new ST elevation in two contiguous leads [ST elevation at the J point with the cut-points: ≥0.1 mV in all leads other than leads V2–V3 where the cut points apply were: ≥0.2 mV in men ≥40 years; ≥0.25 mV in men <40 years, or ≥0.15 mV in women] or evidence of new left bundle branch block. Blood samples were collected as soon as possible within 12 hours of the onset of chest pain and before any invasive procedures were performed.
2. *Hospitalised patients with stable coronary artery disease (sCAD)*.

   sCAD was defined as presence of stable angina and/or evidence of inducible ischemia upon usual cardiac stress testing.
3. *Healthy donors (HD)*.

   HD were defined as subjects with absence of cardiovascular disorders, chronic inflammatory and hematological disease.

The three groups were matched for age (+/- 3 years) and gender.

A coronary angiography was performed in both STEMI and sCAD patients enrolled,

and only STEMI with confirmed presence of intraluminal thrombus in one or more of the coronary arteries and sCAD subjects with evidence of critical epicardial atherosclerotic lesion, with an indication to revascolarization, were selected for the further protocol analysis.

The inclusion criteria were:

- age > 18 years
- ability to provide informed consent

The exclusion criteria were:

- inability to provide informed consent;
- ongoing treatment with fibrinolytic agents, IIb/IIIa glycoprotein inhibitors, unfractionated/fractionated heparin, oral anticoagulants or anti-platelet drugs [except aspirin];
- known hematological disease;
- severe anemia (hemoglobin levels < 8 g/dL).
- any condition not assuring optimal participation in the study or which was deemed dangerous for the patient after medical evaluation;

2.2 Sample source

Citrate anti-coagulated blood samples (50 mL) were taken from each patient and collected in vacutainers (BD Vacutainer, Becton Dickinson, San Diego, CA) at a final sodium citrate concentration of 3.8%. Blood samples were collected within 12 hours of the onset of chest pain in STEMI patients, before administration of heparin, IIb/IIIa glycoprotein inhibitors, P2Y12 receptor inhibitors and before any invasive procedures were performed. Platetes were isolated within 3-6 hours after blood sample collection.

2.3 Platelet purification and cryopreservation

The 50 mL samples were centrifuged at 160 g for 20 minutes at room temperature (RT) in order to obtain platelet-rich plasma (PRP). Platelets were then purified by negative separation using magnetic beads coated with anti-CD45 monoclonal antibody (mAb) (DynabeadsH, Invitrogen, Carlsbad, CA), to deplete nucleated cells. Briefly, PRP was stained with the magnetic beads-coated mAb anti-CD45 for 20 minutes at RT on a rotator. PRP was placed in a magnetic field and the leukocyte-depleted platelets (LDPs) were collected as the negative fraction. The purified platelets were washed 3 times in PBS/BSA solution, counted, tested for purity by anti-CD41 staining and flow cytometry (FCM) analysis (only samples containing >98% CD41+ were used). Finally aliquots of 1 mL from each of the samples of highly purified platelets were treated with an appropriate amount of TRIzolTM (Invitrogen) for cell lysis and RNA cryopreservation.[39,40]

2.4 Microarray gene expression profiling

RNA was extracted by TRIzolTM (Invitrogen) in accordance with the manufacturer's protocol. RNA was quantified on a Nanodrop ND-100 spectrophotometer, followed by RNA quality assessment by analysis on an Agilent 2200 TapeStation (Agilent Tehnologies, Palo Alto, CA). Fragmented biotin labeled cDNA (from 10 ng of RNA) was synthesized using the GeneChip WT Pico kit (Affymetrix, Santa Clara, CA). Affymetrix gene chips, Human Transcriptome Array 2.0 (Affymetrix, Santa Clara, CA), were hybridized with 5 µg fragmented and biotin-labeled cDNA in 200 µl of hybridization cocktail. Target denaturation was performed at 99°C for 5 min and then 45°C for 5 min, followed by hybridization with rotation 60 rpm for 16 hour at 45°C. Arrays were then washed and stained using Gene chip Fluidic Station 450, using

Affymetrix GeneChip hybridization wash & stain kit. Chips were scanned on an Affymetrix Gene Chip Scanner 3000, using Command Console Software.

2.5 Bioinformatics Analysis

The signals from all samples were converted to expression values by the SST-RMA algorithm implemented in Expression Console (Affymetrix). Quality controls of the gene-expression data were perfomed using Expression Console. Samples with low-quality controls were excluded from the analysis. The remaining samples were normalized using SST-RMA. Differentially Expression Analysis were perfomed using one-way subset between anova (unpaired) impletemeted in Transcriptome Console Analysis. In the comparison between STEMI, sCAD and HD we considered the genes differentially expressed (DEGs) with a p.value ≤ 0.05 and a fold change between ≤ -1.5 and ≥ + 1.5.

2.5.1 Identification of the gene-signature

In order to define a genes-signature able to distinguish STEMI from sCAD patients, we filtered the genes considering different thresholds of fold-change and p.values. We eventually applied a combination of bootstrap and knn to estimate the performances of prediction to classify and stratify the STEMI and sCAD patients. We first, applied bootstrap on our dataset (n=10.000). Secondly, we applied k-nearest neighbor (k=3 with euclidean distance), a common classification algorithm,[41,42,43,44,45] to study patients using the gene lists generated in the identification of DEGs. All analysis were performed using R-3.31 and CMA[46] R-package.

2.5.2 Identification of biological processes and enriched pathways.

DAVID[47] and REACTOME[48] were used respectively to identify biological processes and pathways associated with lists of differentially expressed genes.

2.5.3 Identification of known genes involved in our phenotypes

To assess whether a gene of the DEGs was already known related with CAD we followed two approaches. First, CADgene database was used to query each gene. Secondly, a manual research of the gene and phenotypes on pubmed was perfomed.

2.5.4 Heatmaps

Heatmaps were generated using GENE-E (https://software.broadinstitute.org/GENE-E/). In detail, hierarchical clustering analysis were perfomed using 1-minus pearson correlation and average linkage. To underline the gene-expression levels, “subtract row mean, divide by row standard deviation” option was applied. The plot of the common genes between all comparisons of STEMI vs sCAD was generated using UpSetR package.

2.5.6 Statistical methods

Continuous variables are presented as mean ± standard deviation, categorical variables are reported as frequencies and percentages. The comparisons between variables continuous were conducted using the Mann-Whitney-U test. The binary categorical variables were compared using Chi-square test. All hypothesis were perfomed considering a type I error rate of 0.05.

Chapter 3

Results

3.1 Baseline demographic and clinical characteristics of study population

Clinical characteristics of the 20 Healthy Donors, 20 STEMI and 20 sCAD patients, whose samples were used for platelets transcriptome microarray analysis, are described in Table 1. The three groups were matched for age (50-82 years) and gender (15 males vs 5 females). There were significant differences in clinical risk factors between sCAD patients and STEMI patients that included positive history of arterial hypertension (80 vs 50%), current smoking status (10 vs 40%), current drug therapy with aspirin (85 vs 5 %) and current drug therapy with beta blocker (85 vs 20%). In addition, STEMI patients had higher levels of white blood cells than sCAD patients (10.3 ± 2.6 vs 6.9 ± 1.7). In constrast sCAD patients had high level of platelets compared to STEMI (268 ± 90 vs 209 ± 34).

However, other risk factors such as diabetes, cholesterol levels, family history of CAD did not differ between the two coronary disease groups. These findings indicate that the sCAD and STEMI population were quite homogenous. Healthy donors had a lower Body Mass Index than sCAD subjects but family history and smoking status were similar to that of sCAD population.

Table 1. Baseline demographic and clinical characteristics of study population; p-values <0.05 were considered statistically significant and are indicated by an asterisk (*)

Demographics	**Control (n=20)**	**sCAD (n=20)**	**STEMI (n=20)**	**sCAD vs Control**	**STEMI vs Control**	**STEMI vs Scad**
Age (years)	63.3 ± 8.9	64.1 ± 8.1	64 ± 9.4	0.72	0.84	0.96
Gender						
Male	15	15	15			
Female	5	5	5			
Body Mass Index (kg/m^2)	23.9 ± 1.9	27.9 ± 4.3	26.7 ± 3.6	0.001*	0.009*	0.34
Risk factors, n (%)						
Family History of coronary artery disease	8 (40)	10 (50)	7 (35)	0.52	0.52	0.33
Hypertension	2 (1)	16 (80)	10 (50)	0.000009*	0.005*	0.046*
Diabetes mellitus	0	9 (45)	4 (20)	0.0006*	0.035*	0.09
Hypercholesterolemia	3 (15)	14 (70)	10 (50)	0.0004*	0.018*	0.20
Previous CAD	0	2 (10)	3 (15)	0.15	0.07	0.63
Currently smoking	3 (15)	2 (10)	8 (40)	0.63	0.07	0.028*
History of smoking	7 (35)	12 (60)	9 (45)	0.11	0.52	0.34
Never smoking	10 (50)	6 (30)	2 (10)	0.20	0.006*	0.113
Medications, n (%)						
Aspirin	-	17 (85)	1 (5)	-	-	≤ 0.0001*
ACE inhibitor	2 (1)	11 (55)	3 (15)	0.0024*	0.63	0.008*
Statin	1	10 (50)	6 (30)	0.0014*	0.0374*	0.20
Beta blocker	2 (1)	17 (85)	4 (20)	0.000002*	0.37	0.000039*
Vital signs						
Systolic blood pression (mmHg)	124 ± 11	131 ± 14.8	133 ± 24	0.12	0.26	0.88
Diastolic blood pression (mmHg)	76 ± 8.4	78.0 ± 9.0	75.7 ± 12	0.68	0.80	0.44
Heart Rate (bpm)	66.2 ± 7.4	67.1 ± 9.9	74.3 ± 15.0	0.88	0.08	0.12
Labs						
White blood cells count x 10^3 mL	-	6.9 ± 1.7	10. 3 ± 2.6	-	-	0.0002*
Red blood cells count x 10^{6}mL	-	4.7 ± 0.5	4.6 ± 0.6	-	-	0.54
Hemoglobin (g/dL)	-	13.8 ± 2	13.7 ± 2.4	-	-	0.74
Platelet count x 10^3 mL	-	268 ± 90	209 ± 34	-	-	0.028*
Total Cholesterol (mg/dL)	-	189 ± 6.7	177 ± 3.6	-	-	0.88
HDL (mg/dL)	-	48.0 ± 13.1	41.7 ± 15	-	-	0.35
LDL (mg/dL)	-	117 ± 56	111± 34.1	-	-	0.65
Triglycerides (mg/dL)	-	129 ± 66	114 ± 71	-	-	0.98
Glucose (mg/dL)	-	122 ± 70	151 ± 47	-	-	0.0035*
HbA1c (mmol/mol)	-	55 ± 7.3	41.1 ± 8.4	-	-	0.0423*

3.2 Exploratory analysis

An exploratory analysis allowed to assess that the platelets transcriptomes of STEMI patients, sCAD patients and Healthy Donors (HD) were highly correlated (average spearman correlation of 0.89, range= 0.79 - 0.89). High level of correlation were also observed considering each group. These results are consistent with other studies which demonstrated that the platelets gene-expression profiles, in different ACS conditions, were highly correlated allowing the identificantion of few differentially expressed genes (DEGs).[38] Although we identified similar characteristics in our samples, we perfomed extensive bioinformatics analysis to discover DEGs and specific genes associated with our CAD phenotypes (FIG.5).

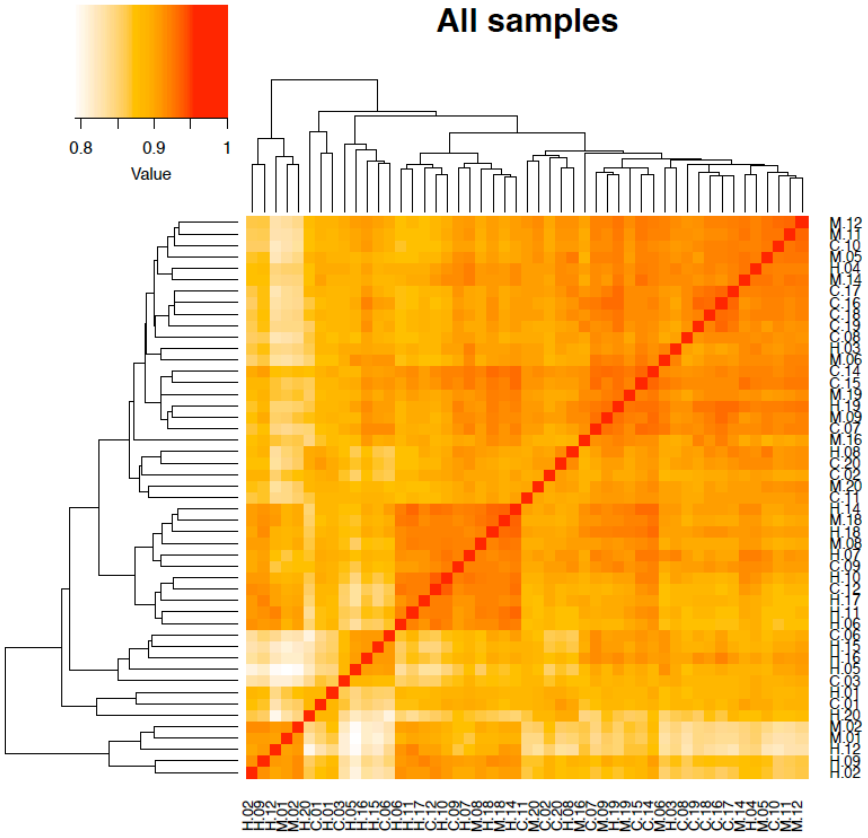

Figure 5. Spearman correlation analysis considering all samples.

3.3 Differentially expressed genes between patients with stable coronary artery disease (sCAD) and healthy donors (HD).

Gene expression data have been firstly analyzed to identify modulated genes between patients with stable coronary artery disease (sCAD) and healthy donors (HD). Applying a one-way between subset anova, considering a p.value of 0.05 and a fold-change of 1.5, a total of 149 differentially expressed genes (DEGs) were observed. A number of 17 and 132 genes were identified respectively up-regulated and down-regulated in sCAD patients (Supplemental Table 1-2). The hierarchical clustering of the DEGs showed that the gene expression patterns between sCAD and healthy subjects were distinct (Fig 6). Using a REACTOME analysis, the 17 up-regulated genes in sCAD were found to be enriched for different pathways as innate immune system (p.value ≤ 0.05), hemostasis (p.value ≤ 0.01), neutrophil degranulation, platelet activation signaling aggregation (p.value ≤ 0.01) and platelet degranulation (Table 2). TGFB1 (Transforming growth factor beta 1), PPIF (Peptidylprolyl Isomerase F), ATP2A3 (ATPase Sarcoplasmatic/Endoplasmatic Reticulum Ca2+ Transporting 3), MFN2 (Mitofusin 2), PRKCD (Protein Kinase C Delta) were identified important for the hemostasis and platelets activation. TGFB1, NF-kappaB and several inflammatory cytokines up-regulate TG2 (Transglutaminase 2), a gene of the transglutaminases family involved in several inflammatory diseases and atherosclerosis.[49,50,51] Cyclophilin (Ppif) is a mitochondrial matrix peptyl-prolyl isomerase known to modulate opening of the mitochondrial permeability transition pore (MPTP).[52,] Mitochondria have been noted to be involved in ATP-controlled thrombotic signalling and platelets apoptosis.[53]

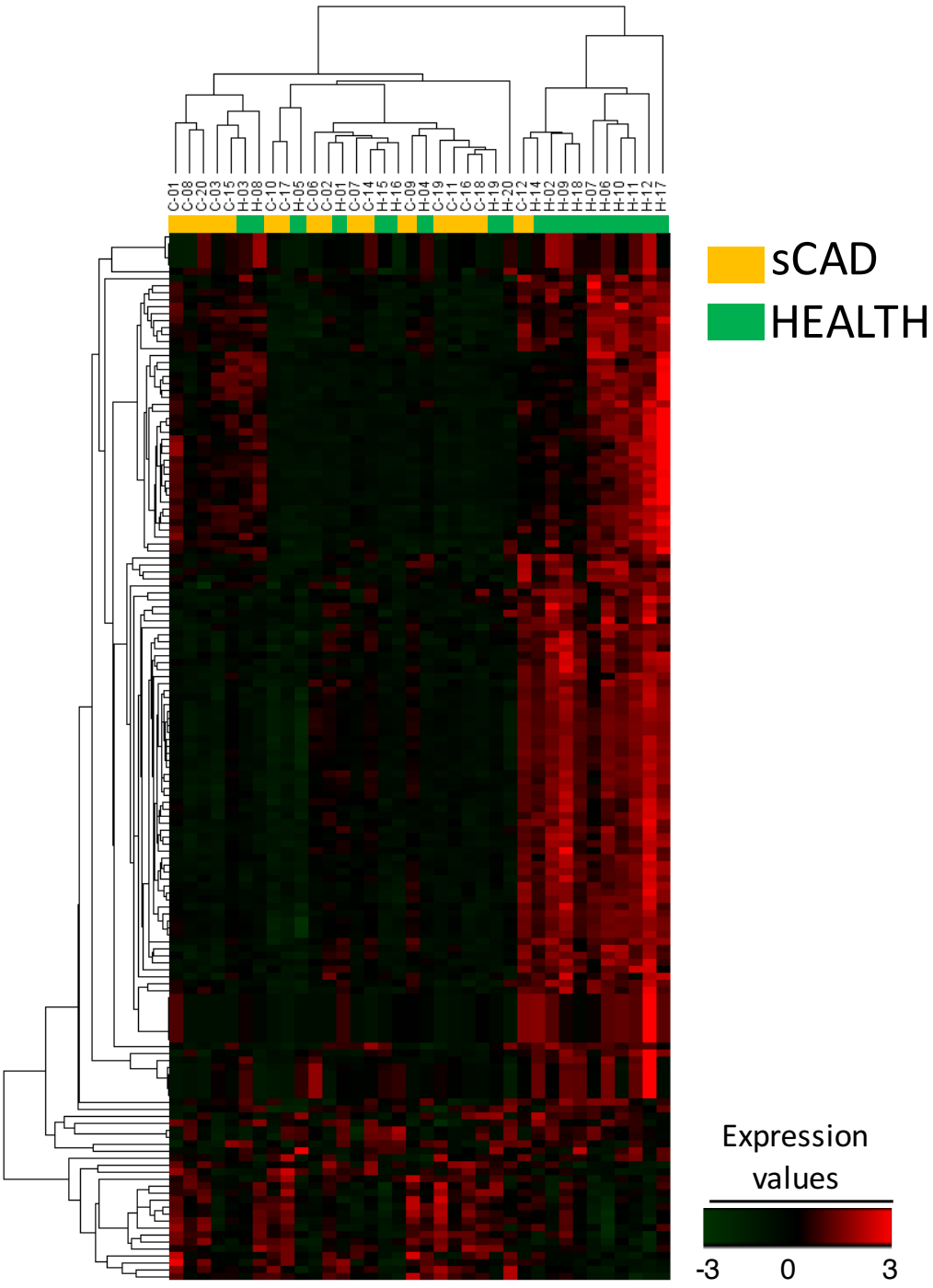

Figure 6. Heatmap of the DEGs between sCAD and healthy phenotype

Table 2. REACTOME analysis results: up-regulated genes in the comparison between patients with sCAD and HD

Pathway name	Submitted entities found	p. value
Innate Immune System	MAP2K3;PKM;PRKCD;ITGA2B;COTL1;PPIF;CD68	0.023145
Hemostasis	TGFB1;PRKCD;ITGA2B;PPIF;ATP2A3;MFN2	0.001201
Neutrophil degranulation	PKM;PRKCD;COTL1;PPIF;CD68	0.002773
Platelet activation, signaling and aggregation	TGFB1;PRKCD;ITGA2B;PPIF	0.002514
Platelet degranulation	TGFB1;ITGA2B;PPIF	0.002367
Response to elevated platelet cytosolic Ca2+	TGFB1;ITGA2B;PPIF	0.002637

We also performed the identification of the involved biological processes using gene ontology analysis (DAVID). Biological processes related with cellular calcium ion homeostasis (ATP2A3, TGFB1, VDR p.value ≤ 0.01), positive regulation of superoxide anion generation (PRKCD, TGFB1, p.value ≤ 0.01) were identified (Supplemental Table 3). Four of the up-regulated genes (MFN2, PRKCD, VDR, TGFB1) were already known related with CAD according to CADgene database[54] and literature.

MFN2 was the most up-regulated gene (FIG. 7). MFN2 is a gene that codifies for a protein that promotes the mitochondrial fusion. Recent evidence suggest that the fusion/fission factors in adult cardiomyocytes play essential noncanonical roles in cardiac development, Ca2+ signaling, mitochondrial functions and cell death. The impairment of this circuit causes cardiomyocyte dysfunction and death leading to heart injury and hearth failure.[55]

PRKCD was the second gene highly expressed in sCAD patients (FIG. 7). Previous studies on protein kinases family have reported their involvement in the regulation of myocardial contraction, hyperthropy and pathological cardiac remodelling.[56] Protein kinase D (PKD) was demonstrated to be highly expressed in platelets by receptors coupled to heterotrimeric G-proteins. [57]

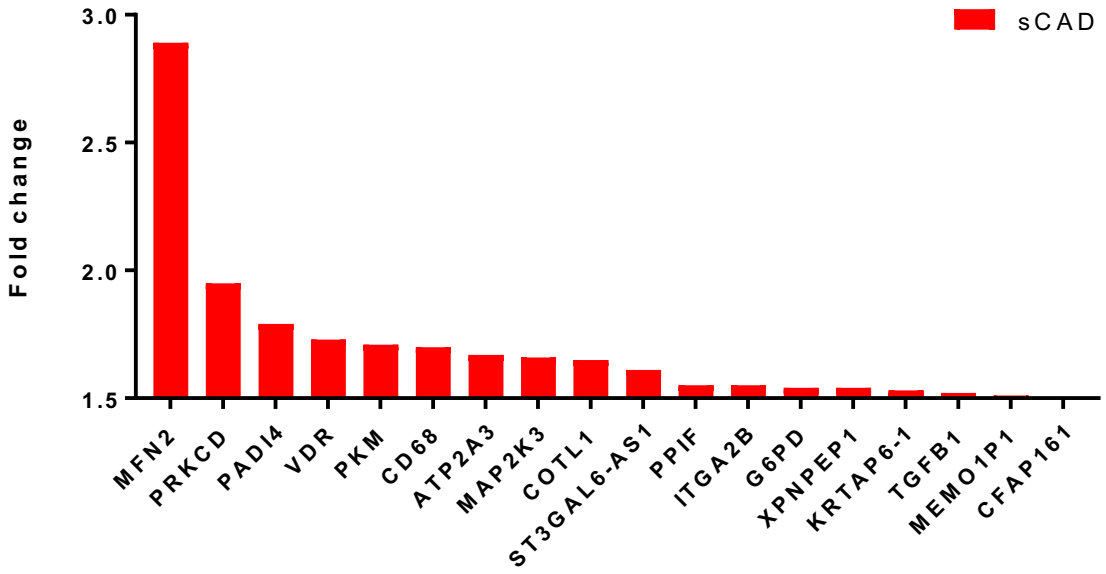

Figure 7. Up-regulated genes in sCAD patients vs Healthy Donors

Analysis of the 132 down-regulated genes with REACTOME allowed to identify enriched pathways related with translocation of zap-70 to immunological synapse (p.value ≤ 0.01), phosphorylation of CD3 and TCR zeta chains (p.value ≤ 0.01) and PD-1 signalling (p.value ≤ 0.01). Three of the down-regulated genes (PLAUR, NAMPT, PTGS2) were already known related with CAD according to CADgene database[54] and literature. DAVID analysis of the down-regulated genes showed biological processes related with immune response, inflammatory response and cellular response to cytokine stimulus (see Supplemental Table 4 for details).

Table 3. REACTOME analysis results: down-regulated genes in the comparison between patients with sCAD and HD

Pathway name	Submitted entities found	p. value
Translocation of ZAP-70 to Immunological synapse	HLA-DRB4;HLA-DQA1	0.006739
Phosphorylation of CD3 and TCR zeta chains	HLA-DRB4;HLA-DQA1	0.008129
PD-1 signaling	HLA-DRB4;HLA-DQA1	0.008129
Generation of second messenger molecules	HLA-DRB4;HLA-DQA1	0.016002

CXCL8 (Interleukin-8) was the most down-regulated gene (Fig. 8). The plasmatic levels of IL8 have been shown to be predictive for future cardiac events, however its role is still unclear. In fact, a recent review describes that serum levels of CXCL8 in CAD patients can be lower than in health patients.[58]

Several miRNAs were also identified as highly down-regulated in sCAD patients: mir548AI, mir548I4, mir548AJ2. These results suggest that differences in gene-expression patterns exist between sCAD and Healthy patients. In general, we observed that patients with sCAD had a consistent down-regulation of the gene-expression levels. Only a few groups of genes were identified as up-regulated in patients with sCAD. Of these, MFN2, TGF1B and other genes were identified relevant in pathways analysis and according to literature.

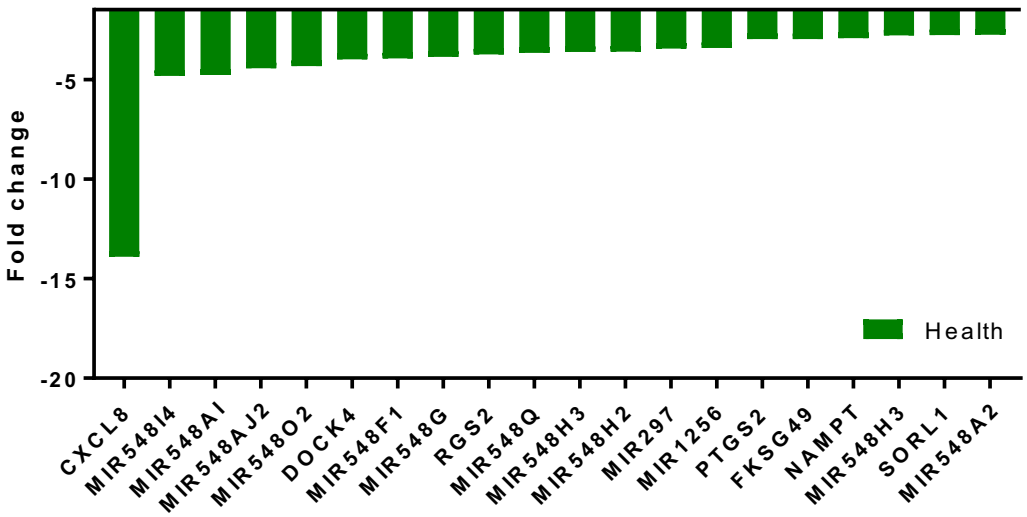

Figure 8. Down-regulated genes in sCAD vs Healthy Donors

3.4 Differentially expressed genes between patients with ST-elevation myocardial infarction (STEMI) and healthy donors (HD).

The analysis of DEGs was perfomed to assess the transcriptomic differences between patients with STEMI and HD. A number of 76 gene, 41 up-regulated and 35 down-regulated were identified in STEMI group (Supplemental Table 5-6). The hierarchical clustering of the DEGs showed that the gene expression patterns between STEMI subjects and HD were distinct (Fig. 10).

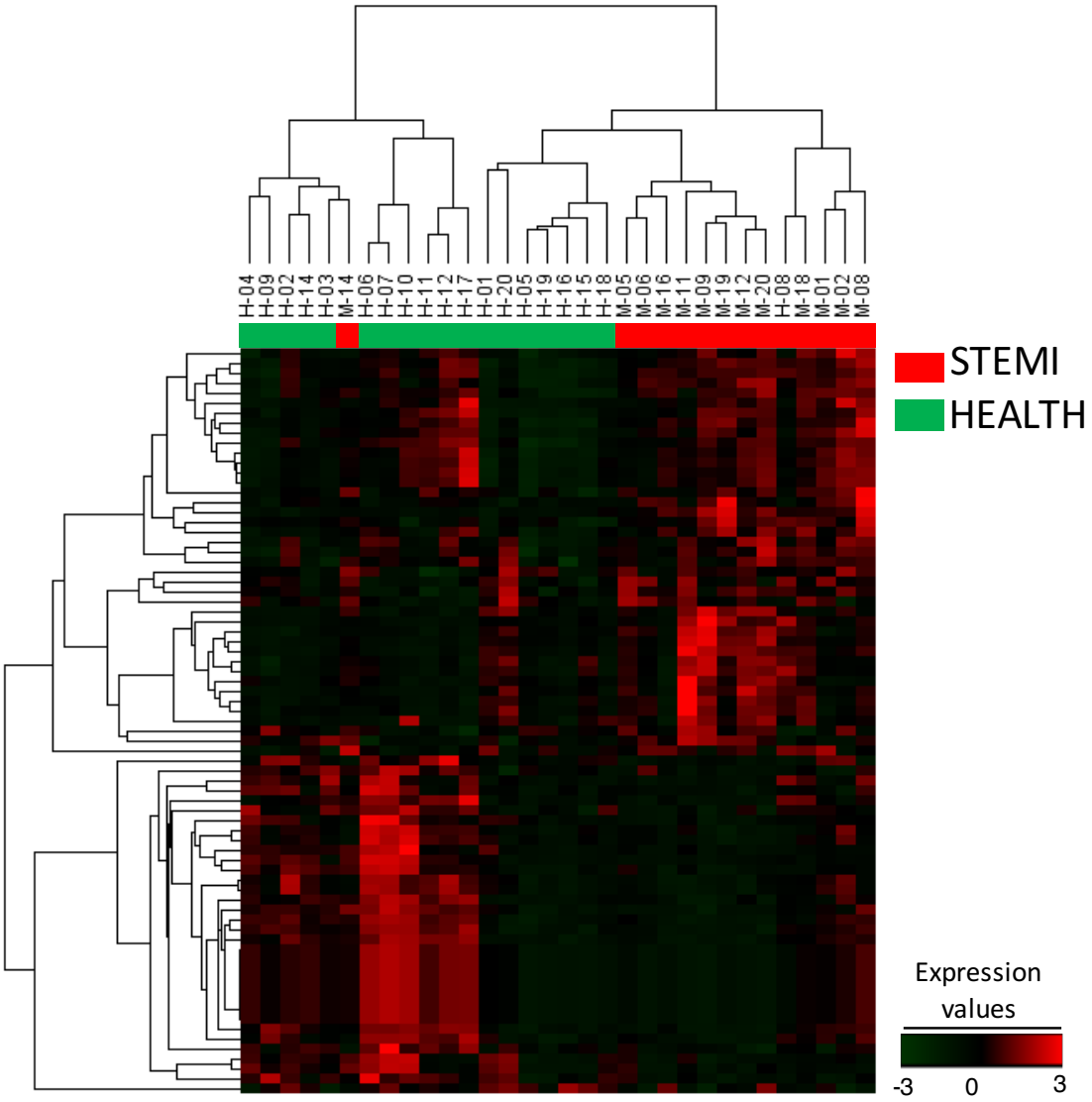

Figure 9. Heatmap of the DEGs between STEMI and healthy phenotype

REACTOME analysis of the up-regulated genes allowed us to detect pathways related to the neutrophil degranulation (p.value ≤ 0.01), innate immune system (p.value ≤ 0.01) and Toll-Like Receptor cascades (p.value ≤ 0.01) (Table 4). The genes in our list associated with neutrophil degranulation were TNFAIP6, ARG1, MMP1, RAB18, S100A12, MNDA, S100P, MCEMP1, S100A9, GLA, S100A8 and the genes identified associated with innate immune-system were TNFAIP6, ARG1, MMP1, IRAK3, IRS2, MCEMP1, TXN, RAB18, S100A12, S100P, MNDA, CLEC4E, S100A9, GLA, S100A8. Considering the list of the up-regulated genes, 14 of these were already known to be correlated with myocardial infarction (CLEC4E, S100A9, S100P, MMP1, S100A8, H19, FKBP5, ARG1, EPAS1, IRS2, NAT8B, MIR675, IRAK3, RAB18).

Table 4. REACTOME analysis results: up-regulated genes in the comparison between patients with STEMI and HD

Pathway name	Submitted entities found	p. value
Neutrophil degranulation	TNFAIP6;ARG1;MMP1;RAB18;S100A12; MNDA;S100P;MCEMP1;S100A9;GLA;S100A8	6.77E-07
Innate Immune System	TNFAIP6;ARG1;MMP1;IRAK3;IRS2;MCEMP1; TXN;RAB18;S100A12;S100P;MNDA;CLEC4E;S100A9;GLA;S100A8	4.01E-05
Toll-Like Receptors Cascades	S100A12;IRAK3;S100P;S100A9;S100A8	4.43E-04
Immune System	TNFAIP6;ARG1;MMP1;IRAK3;IRS2;MCEMP1; TXN;IFI27;RAB18;S100A12;S100P;MNDA;CLEC4E;S100A9;GLA;S100A8	0.003086
MyD88:Mal cascade initiated on plasma membrane	S100A12;IRAK3;S100P;S100A9	0.006479
Toll Like Receptor TLR6:TLR2 Cascade	S100A12;IRAK3;S100P;S100A9	0.006479
Toll Like Receptor TLR1:TLR2 Cascade	S100A12;IRAK3;S100P;S100A9	0.006988
Toll Like Receptor 2 (TLR2) Cascade	S100A12;IRAK3;S100P;S100A9	0.006988
Activated TLR4 signalling	S100A12;IRAK3;S100P;S100A9	0.010537
Toll Like Receptor 4 (TLR4) Cascade	S100A12;IRAK3;S100P;S100A9	0.013903

Interestingly, S100A12 and CLEC4E were the most up-regulated genes in STEMI patients (Fig. 10).

S100A12 is a calgranulin family protein and member of S100 genes which are known to be involved in chronic inflammatory diseases. S100A8 and S100A9 were previously described as the strongest predictors of ST elevation myocardial infarction.[59] Recently, S100A12 is emerging as biomarker for human atherosclerosis.[60] **SAMSN1, SPARCL1** and **MNDA** were other genes expressed at high level but their roles in cardiovascular disease is not clear. The gene ontology analysis of the up-regulated genes showed biological processes associated with positive regulation of inflammatory response (p.value ≤ 0.01) and cytokine production (p.value ≤ 0.01), (Supplemental Table 7). The analysis of our data supports that also S100A12 could be a marker of STEMI in platelets. **CLEC4E**, the second most up-regulated gene, is another putative marker of acute MI.

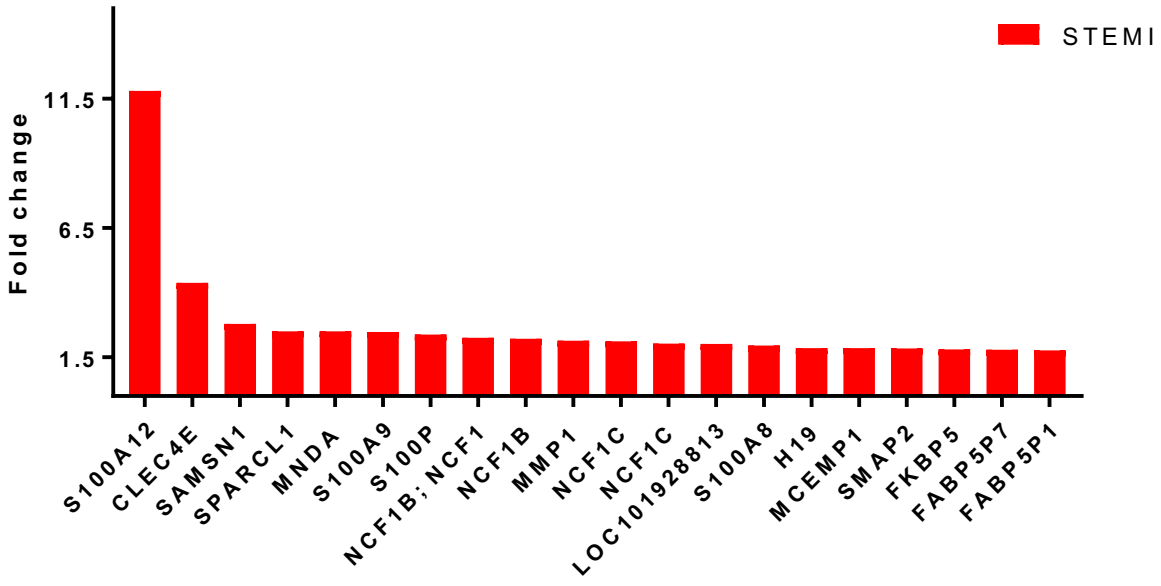

Figure 10. Up-regulated genes in STEMI patients vs Healthy Donors

Of the 35 down-regulated genes detected from the comparison between STEMI and HD, 5 genes (TGFBR3, EGR1, HLA-DRB4, ZNF667-AS1, CXCL8) were already known to be involved in the biology of cardiovascular disease. REACTOME analysis identified that the down-regulated genes were enriched for MCH class II antigen presentation (p.value ≤ 0.01) and are associated with negative regulation of killer cell chemotaxis (p.value ≤ 0.01), signal transduction, adaptive immune response (p.value ≤ 0.01) and biological processes terms (Table 5). For DAVID analysis see Supplemental Table 8.

Table 5. REACTOME analysis results: down-regulated genes in the comparison between patients with STEMI and HD

Pathway name	Submitted entities found	p. value
MHC class II antigen presentation	CD74;HLA-DRB4	0.041571

CXCL8 was the most down-regulated genes followed by SNORD13, SNORD117 and RMRP (Fig.11).

In conclusion, our results suggest that days before a MI there is in platelets an activation of genes related to inflammation. Moreover, we identified several genes already known to be involved in CVD and MI and 3 highly expressed genes (SAMSN1, SPARCL1, MNDA) for which the function in MI is unknown.

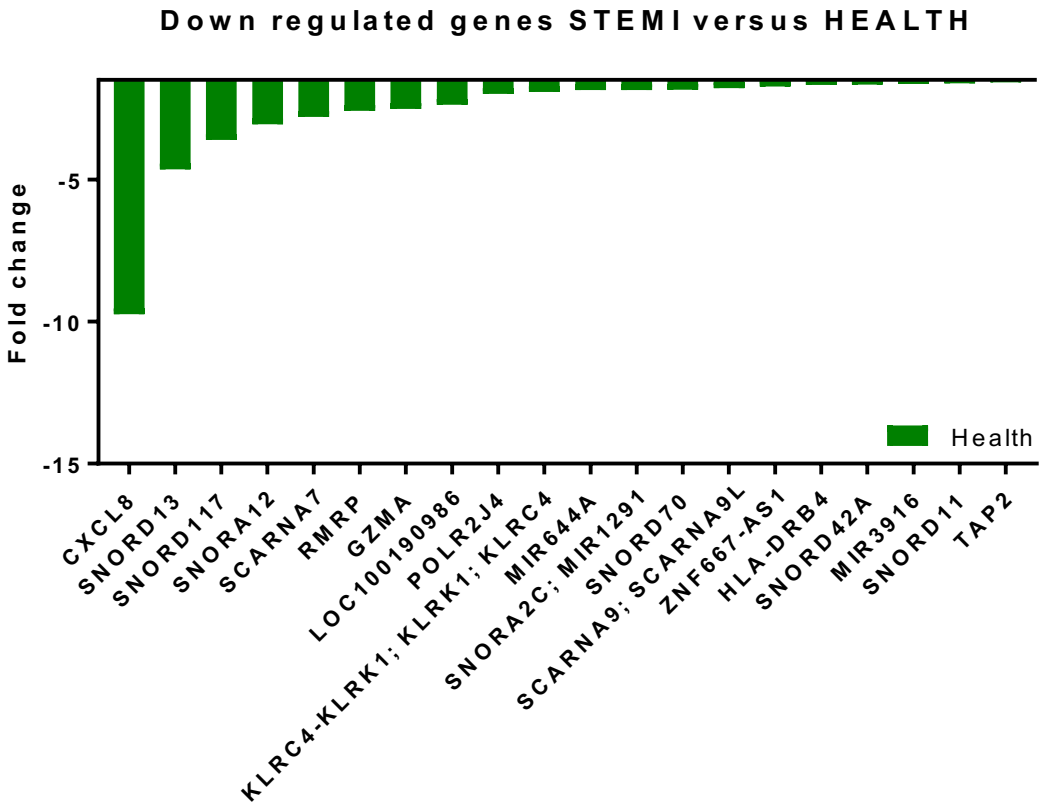

Figure 11. Down-regulated genes in STEMI patients vs Healthy Donors

3.5 Differentially expressed genes between patients with ST elevation myocardial infarction (STEMI) and stable coronary disease (sCAD): exploratory analysis.

The characterization of the transcriptome between patients with STEMI and sCAD is important to define the molecular differences at the base of these two conditions. To this aim, the identification of the DEGs was perfomed as described in “Material and Methods”. A number of 138 genes were found demodulated comparing STEMI and sCAD platelet transcriptome. In detail 114 and 24 genes were identified respectively up and down-regulated in STEMI subjects (Supplemental Table 9-10).

The hierarchical clustering allowed us to underline the presence of two gene expression patterns specific for STEMI and sCAD patients (Fig. 12).

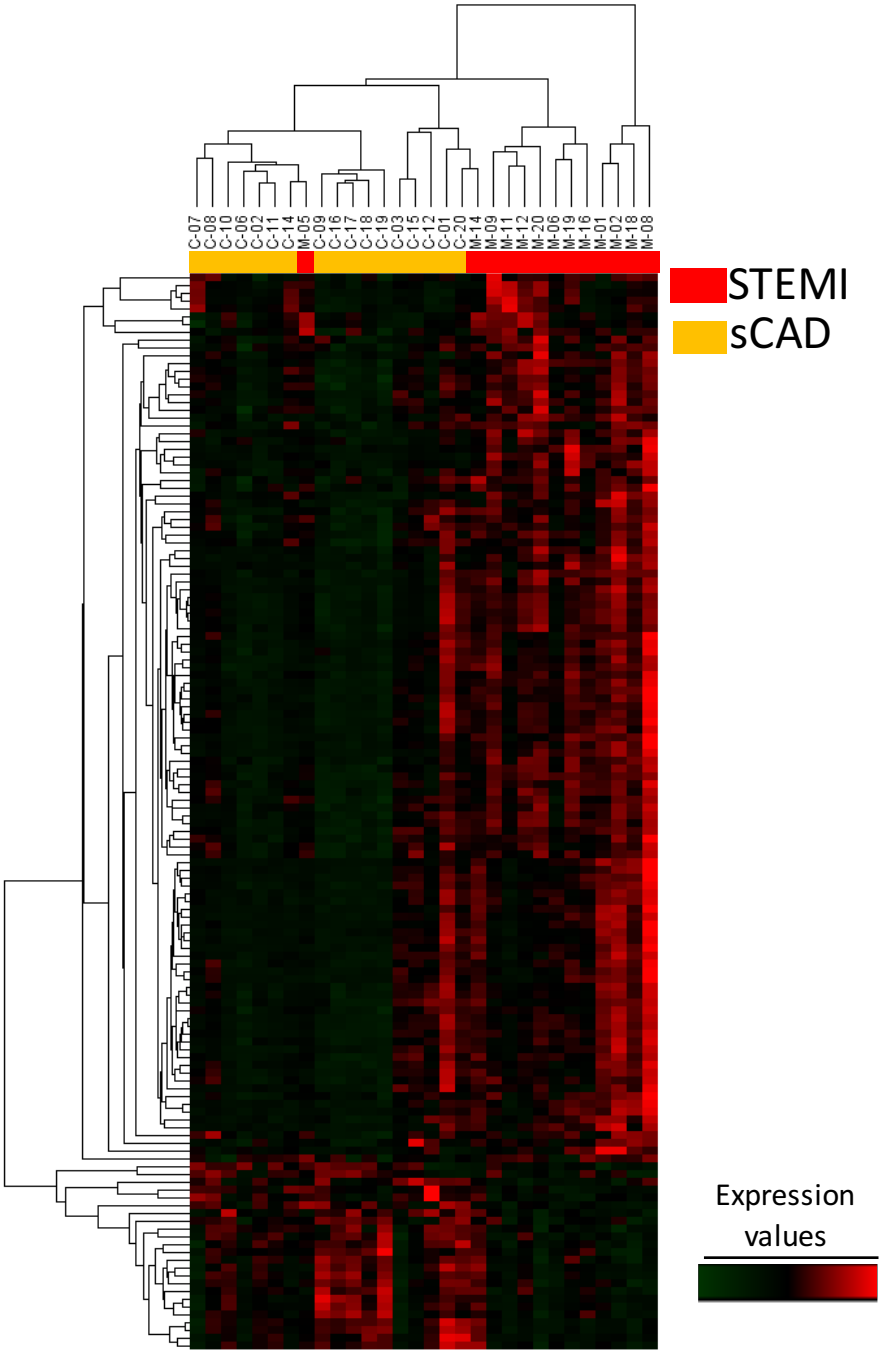

Figure 12. Heatmap of the DEGs between STEMI patients and sCAD patients.

REACTOME analysis of the up-regulated genes allowed the identification of a prevalence of pathways associated with immune system and inflammation. The complete lists of pathways and associated genes are reported in Table 6.

Table 6. REACTOME analysis results: up-regulated genes in the comparison between patients with STEMI and sCAD

Pathway name	Submitted entities found	Entities pValue
Immune System	CDA;SERPINA1;NCF1;TNFAIP6;NCF2;NCF4; UBE2D1;IRS2;TREM1;FCAR;FCGR3A; FCGR3B;IL18RAP;ADGRE3;AMICA1;PGLYRP1;CR1; MME;ARG1;IL1R2;RNASE6;IRAK3;MMP9;CLEC4D; PELI1;PADI2;TXNIP;CLEC4E;S100A9;TLR4;S100A8; C5AR1;FPR1;LY96;TXN;IQGAP1;FPR2;MEFV;PAK1; CXCR1;ALOX5;CXCR2;S100A12;S100A11;MGAM; GCA;EIF2AK2;CPPED1;NFKBIA;SELL;AGO4;QPCT; RAB18;S100P;MNDA	2.96E-14
Innate Immune System	CDA;SERPINA1;TNFAIP6;C5AR1;FPR1;UBE2D1;LY96; IRS2;TXN;IQGAP1;FPR2;MEFV;TREM1;FCAR;FCGR3A; PAK1;FCGR3B;CXCR1;ALOX5;ADGRE3;CXCR2;S100A12; PGLYRP1;S100A11;MGAM;CR1;MME;GCA;ARG1; RNASE6;IRAK3;MMP9;CPPED1;NFKBIA;CLEC4D; SELL;AGO4;QPCT;RAB18;PELI1;PADI2;TXNIP; S100P;MNDA;CLEC4E;S100A9;TLR4;S100A8	1.11E-16
Neutrophil degranulation	CDA;SERPINA1;TNFAIP6;C5AR1;FPR1;IQGAP1;FPR2; FCAR;FCGR3A;FCGR3B;CXCR1;ALOX5;ADGRE3; CXCR2;S100A12;PGLYRP1;S100A11;MGAM;CR1; MME;GCA;ARG1;MMP9;CPPED1;CLEC4D;SELL;QPCT; RAB18;PADI2;S100P;MNDA;S100A9;S100A8	1.11E-16
Toll-Like Receptors Cascades	NFKBIA;PELI1;UBE2D1;S100A12;LY96;IRAK3;S100P;S100A9;TLR4;S100A8	1.24E-06
Activated TLR4 signalling	NFKBIA;PELI1;UBE2D1;S100A12;LY96;IRAK3;S100P;TLR4;S100A9	3.76E-06
Toll Like Receptor 4 (TLR4) Cascade	NFKBIA;PELI1;UBE2D1;S100A12;LY96;IRAK3;S100P;TLR4;S100A9	8.59E-06
MyD88:Mal cascade initiated on plasma membrane	NFKBIA;PELI1;S100A12;LY96;IRAK3;S100P;TLR4;S100A9	8.55E-06
Toll Like Receptor TLR6:TLR2 Cascade	NFKBIA;PELI1;S100A12;LY96;IRAK3;S100P;TLR4;S100A9	8.55E-06
Toll Like Receptor TLR1:TLR2 Cascade	NFKBIA;PELI1;S100A12;LY96;IRAK3;S100P;TLR4;S100A9	1.04E-05
Toll Like Receptor 2 (TLR2) Cascade	NFKBIA;PELI1;S100A12;LY96;IRAK3;S100P;TLR4;S100A9	1.04E-05
Peptide ligand-binding receptors	CXCR1;C5AR1;CXCR2;FPR1;FPR2	0.002226
MyD88-independent TLR3/TLR4 cascade	NFKBIA;UBE2D1;S100A12;LY96;S100P;TLR4;S100A9	5.72E-04
Toll Like Receptor 3 (TLR3) Cascade	NFKBIA;UBE2D1;S100A12;LY96;S100P;TLR4;S100A9	5.72E-04
TRIF-mediated TLR3/TLR4 signaling	NFKBIA;UBE2D1;S100A12;LY96;S100P;TLR4;S100A9	5.72E-04
MyD88 cascade initiated on plasma membrane	NFKBIA;PELI1;S100A12;S100P;S100A9	0.001738
Toll Like Receptor 10 (TLR10) Cascade	NFKBIA;PELI1;S100A12;S100P;S100A9	0.001738
Toll Like Receptor 5 (TLR5) Cascade	NFKBIA;PELI1;S100A12;S100P;S100A9	0.001738
TRAF6 mediated induction of NFkB and MAP kinases upon TLR7/8 or 9 activation	NFKBIA;PELI1;S100A12;S100P;S100A9	0.00191
MyD88 dependent cascade initiated on endosome	NFKBIA;PELI1;S100A12;S100P;S100A9	0.002093
Toll Like Receptor 7/8 (TLR7/8) Cascade	NFKBIA;PELI1;S100A12;S100P;S100A9	0.002093
Toll Like Receptor 9 (TLR9) Cascade	NFKBIA;PELI1;S100A12;S100P;S100A9	0.002392
Regulation of TLR by endogenous ligand	LY96;TLR4;S100A9;S100A8	2.24E-04
Interleukin-1 signaling	IL1R2;PELI1;IRAK3	0.001138
RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways	NFKBIA;UBE2D1;S100A12;S100P;S100A9	0.009307
Cross-presentation of particulate exogenous antigens (phagosomes)	NCF1;NCF2;NCF4	2.67E-04
The NLRP3 inflammasome	TXNIP;TXN;MEFV	8.00E-04
RHO GTPases Activate NADPH Oxidases	NCF1;NCF2;NCF4	0.00175
IKK complex recruitment mediated by RIP1	UBE2D1;LY96;TLR4	0.001956
RIP-mediated NFkB activation via ZBP1	NFKBIA;S100A12;S100P;S100A9	0.00241
Inflammasomes	TXNIP;TXN;MEFV	0.002659
TRAF6 mediated NF-kB activation	NFKBIA;S100A12;S100P;S100A9	0.002924
ZBP1(DAI) mediated induction of type I IFNs	NFKBIA;S100A12;S100P;S100A9	0.003814
Diseases of Immune System	NFKBIA;LY96;TLR4	0.00449
Diseases associated with the TLR signaling cascade	NFKBIA;LY96;TLR4	0.00449

Using DAVID analysis, S100A8, S100A9, S100A12 were identified associated with "inflammatory response pathway" (see Supplemental Table 11 for the complete list of genes). Moreover, analysis of the up-regulated genes revealed a "positive regulation of NF-kappaB transcription factor activity" (NFKB1A; S100A12; S100A8; S100A9; EIF2AK2; IRAK3; TLRK4). NF-kB is important to cardiac responses to ischemia and reperfusion. It is activated by pro-inflammatory cytokines and endogenous ligands for TLRs that are generated in response to ischemia. It is known that after myocardial ischaemia there is an activation of NF-kB in several cell types of myocardium, which induces pro-inflammatory proteins.[61] In the same way, it is possible that platelets can express high level of NF-kB before a STEMI. This is consistent with previous results in which it was demonstrated that patients with unstable angina, who are at risk of plaque rupture, have high levels of activated NF-kB in their white blood cells. Considering the list of the up-regulated genes, 51 of these were already known involved in myocardial infarction.

S100A12 was again the most up-regulated gene (Fig.13). This evidence suggests that high expression of S100A12 is specific of patients with STEMI in comparison with sCAD and healthy subjects.

RGS2 was another highly up-regulated gene. This is well known to play an important role in cardiovascular diseases. RGS2 is a potent and selective regulator of Gq/11 signaling.[62] Gq/11-mediated signaling transduction is well recognized for its role in regulating both vascular tone and cardiac remodeling.[63] The deregulation of RGS2 was demonstrated to promote chronic constriction of peripheral vasculature leading to hypertension. The ablation of RGS2 renders the heart more vulnerable and results in premature death.[64] The expression of RGS2 in our patients could suggest a protective mechanism in STEMI phenotype.

FCAR mRNA levels were also high in STEMI. FCAR is a member of the immunoglobulin gene superfamily and encodes a receptor for the Fc region of IgA. This receptor is a transmembrane glycoprotein present on the surface of myeloid lineage cells such as neutrophils, monocytes, macrophages, and eosinophils, where it mediates immunologic responses to pathogens. However, it was demonstrated that human platelets also express the IgA receptor which is capable of mediating platelet activation.[65] Therefore, it was established an association between FCAR gene polymorphism and myocardial infarction.[66]

Other 4 genes (MNDA, NCF1B, KCNJ5, SAMSN1) were highly expressed in the platelets of STEMI patients but their role in infarction is not well described.

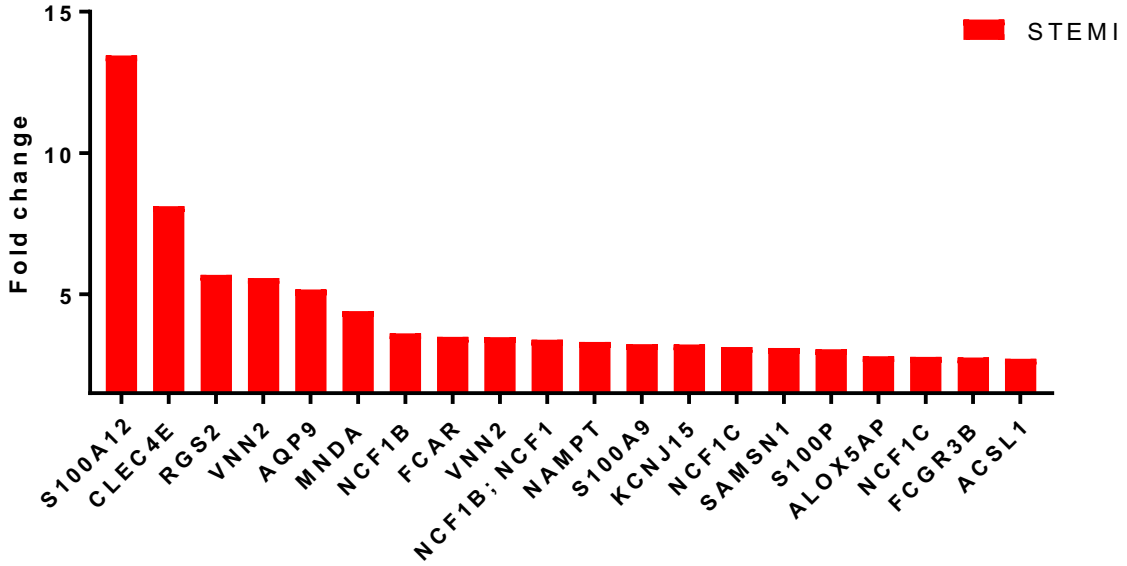

Figure 13. Up-regulated genes in STEMI patients vs sCAD patients

The REACTOME analysis of the down-regulated genes showed their involvement in hemostatis (p.value ≤ 0.01), metabolism of carbohydrates (p.value ≤ 0.05) and

extracellular matrix organization (p.value ≤ 0.05) (Table 7). See Supplemental Table 12 per DAVID analysis.

Table 7. REACTOME analysis results: down-regulated genes in the comparison between patients with STEMI and sCAD

Pathway name	Submitted entities found	Entities pValue
Signaling by NOTCH	ATP2A3;FURIN;ARRB1	0.002884
Hemostasis	CD74;TGFB1;BSG;ATP2A3;ARRB1;PRKACB	0.003724
Metabolism of carbohydrates	PKM;TAL1;PRKACB	0.006751
Deubiquitination	TGFB1;FKBP8;ARRB1	0.03471
Extracellular matrix organization	TGFB1;BSG;FURIN	0.039238
Developmental Biology	TGFB1;MYH9;KRTAP6-1;FURIN;ARRB1;PRKACB	0.04978

RNU6ATAC was the most down-regulated gene (Fig.14). It is a small nuclear RNA and is not clear as its de-regulation is involved in cardiovascular diseases.

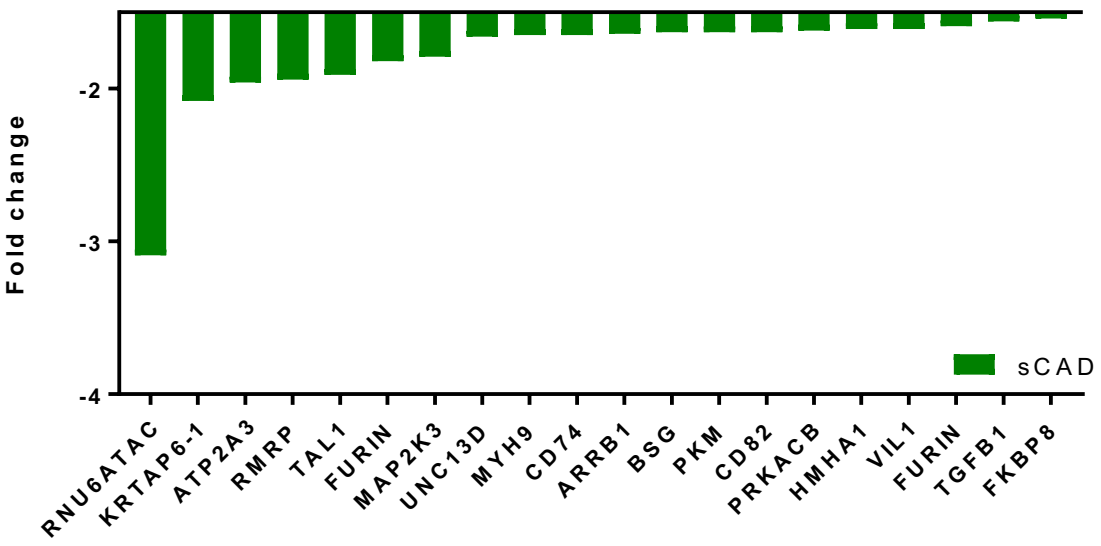

Figure 14. Down-regulated genes in STEMI patients vs sCAD patients

These results suggest that platetes have distinctive gene-expression patterns in STEMI and sCAD patients. In platetes of STEMI subjects we identified highly

expressed inflammatory genes and, interestingly, we also detected a positive regulation of NFKB1A. According to previous results, it is possible that NFKB1A plays a pathogenitic role in the stages before an acute myocardial infarction.

3.6 The identification of gene-signature of STEMI patients

The exploratory analysis between STEMI and sCAD showed two distinct gene-expression patterns specific for the two phenotypes of patients. However, the identification of specific genes able to distinguish STEMI and sCAD patients is relevant to further characterize STEMI condition. For this aim, we applied a custom pipeline for the identification of a gene-signature of AMI (Fig. 15). As described in section “Material and Methods”, different thresholds of fold-change and p.values were considered for the identification of DEGs between patients with sCAD and STEMI.

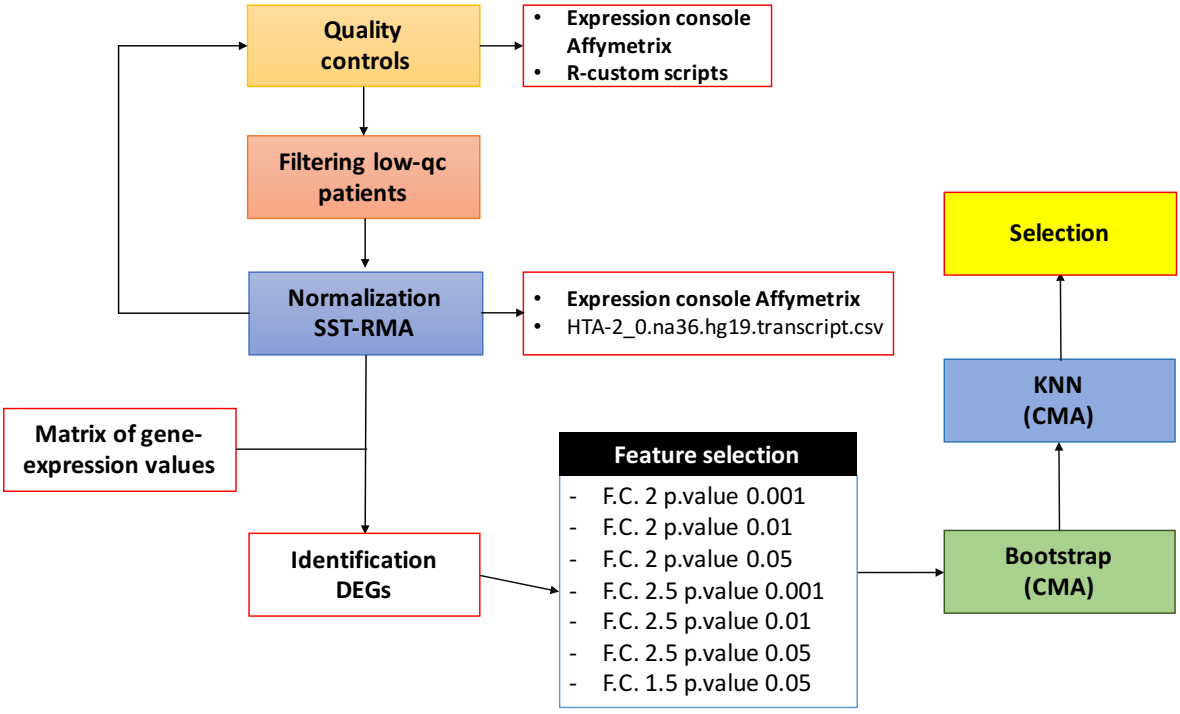

Figure 15. Workflow for the identification of a STEMI-signature

Fifteen genes were identified in common in all these comparison (Table 8). For each gene-list boostraping and k-nearest neighbor models (kNN) were applied to estimate the performance of classification of each gene-list. Supplementale Table 13 contains the performance of prediction in terms of specificity and sensitivity. The list that provided the best results in terms of specificity (0.865) and sensitivity (0.884) was then obtained considering a fold-change of 2 and a p.value of 0.001 but with 26 DEGs (Fig. 16). However, this threshold was more conservative and excluded important genes related to CVDs.

Table 8. List of the common genes of all comparisons between patients with STEMI and sCAD

Gene ID	Gene Name/Gene-symbol	Panther Protein Class
CLEC4E	C-type lectin domain family 4 member E;CLEC4E;ortholog	cell adhesion molecule(PC00069);immunoglobulin receptor superfamily(PC00090)
S100A9	Protein S100-A9;S100A9;ortholog	calmodulin(PC00060);signaling molecule(PC00131)
AQP9	Aquaporin-7;AQP7;ortholog	transporter(PC00227)
S100A12	Protein S100-A12;S100A12;ortholog	calmodulin(PC00060);signaling molecule(PC00131)
FCAR	Immunoglobulin alpha Fc receptor;FCAR;ortholog	immunoglobulin receptor superfamily(PC00090);membrane-bound signaling molecule(PC00124)
MNDA	Myeloid cell nuclear differentiation antigen;MNDA;ortholog	transcription factor(PC00218)
FCGR3B	Low affinity immunoglobulin gamma Fc region receptor III-B;FCGR3B;ortholog	cell adhesion molecule(PC00069)
S100P	Protein S100-P;S100P;ortholog	calmodulin(PC00060);signaling molecule(PC00131)
SAMSN1	SAM domain-containing protein SAMSN-1;SAMSN1;ortholog	
NCF1B	Putative neutrophil cytosol factor 1B;NCF1B;ortholog	
NCF1C	Putative neutrophil cytosol factor 1C;NCF1C;ortholog	
NCF1	Neutrophil cytosol factor 1;NCF1;ortholog	
NAMPT	Nicotinamide phosphoribosyltransferase;NAMPT;ortholog	cytokine(PC00207)
ACSL1	Long-chain-fatty-acid--CoA ligase 1;ACSL1;ortholog	ligase(PC00142)
RGS2	Regulator of G-protein signaling 2;RGS2;ortholog	G-protein modulator(PC00095)

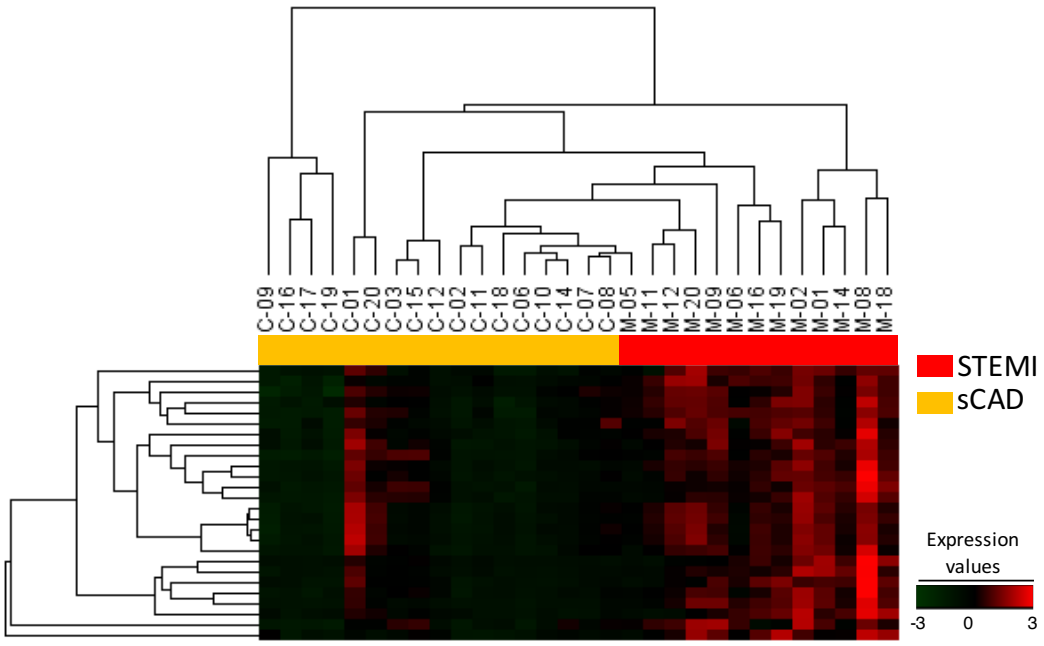

Figure 16. Heatmap of the DEGs between patients with STEMI and sCAD(fold change of 2, p. value 0.001)

Therefore, we selected the gene-list that provided the second best results in terms of specificity and sensitivity. This list of 38 genes was obtained considering a fold-change of 2 and a p.value of 0.01 (Fig. 17; Fig. 18). The specificity and sensitivity of this gene list in classification of our classes were respectively of 0.88 and 0.78. (Supplemental Table 14). Therefore, the performance of classification was conserved (Fig.19). In order to capture the ability of gene-signature (fold change of 2, p.value ≤0.01) to discriminate STEMI from sCAD patients, principal component analysis (PCA) was performed on this gene-list. PCA showed a good discrimination of our phenotypes. In fact, separation along the first principal component (PC1) divided STEMI (red) from sCAD (orange) samples. The heatmap in Figure 19 was generated ordering the samples by the PC1.

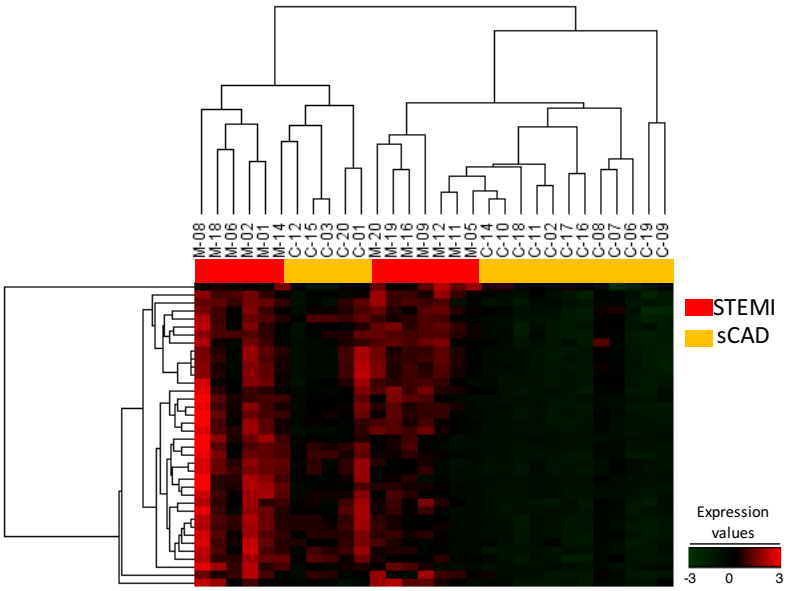

Figure 17. Heatmap of the DEGs between patients with STEMI and sCAD (fold-change of 2, p.value ≤ 0.01)

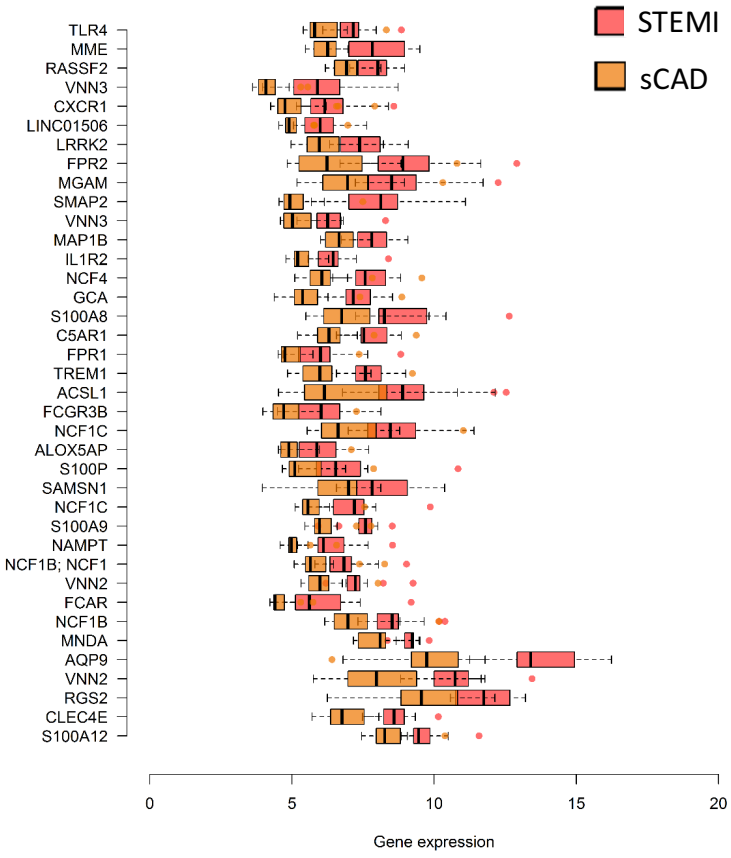

Figure 18. DEGs between patients with STEMI and sCAD (fold-change of 2, p.value ≤ 0.01)

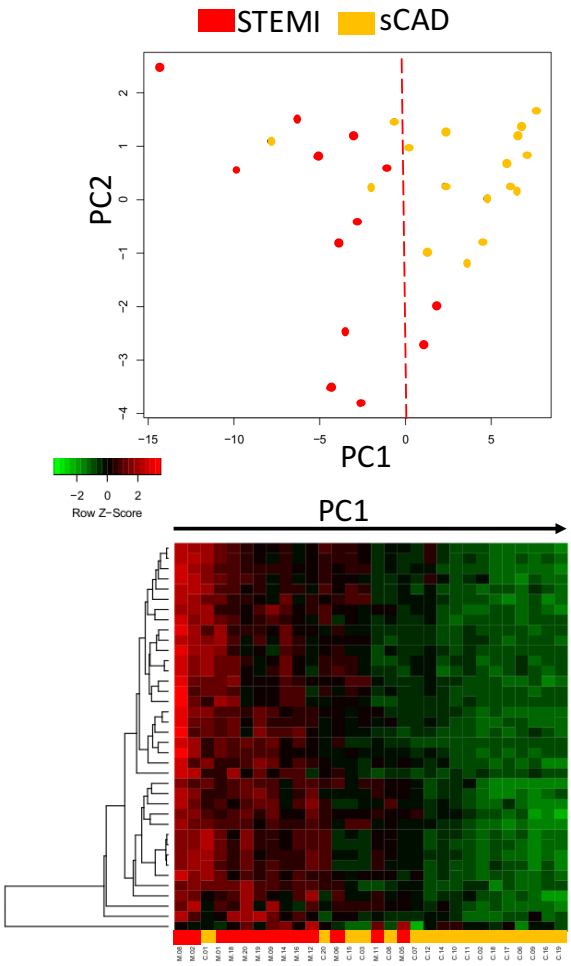

Figure 19. PCA of the DEGs between patients with STEMI and sCAD (fold change of 2, p.value ≤ 0.01).

Following this approach we recovered 12 genes not present in the first gene-list. Of these, 6 (CXCR1[67], TREM1[68], VNN3[69], ALOX5AP[70], C5AR1[71], FPR1[72]) were already known related to myocardial infarction and CVDs. This second gene-signature contains about 15 genes already known related with MI. The remaining 23 genes are not described to be involved in CVDs. Between the top 10 DEGs without a function related with CVDs there were: MNDA and NCF1B. **MNDA** is a gene that codifies for the myeloid cell nuclear differentation antigen, a member of interferon-inducible of

p200. MNDA expression is correlated with granulocyte and monocyte differentation, however its role in platelets and cardiovascular diseases is not well described. **NCF1** is a component of the nicotinamide adenine dinucleotide phospate (NADPH) oxidase complex. The role of NCF1 in platelets and infarction is not clear. However, recent evidences suggests that NCF1 and its pseudogenes (NCF1B, NCF1C) were linked with the Williams Syndrome. Therefore, our data suggests that these pseudogenes could be expressed by megakaryocytes and shared with the platelets before of an acute myocardial infarction. Finally, we used this list of genes to perform a clustering analysis considering also the healthy patients. Also in this case, we observed that our gene signature was able to distinguish STEMI subjects from sCAD and HD (Figure 20).

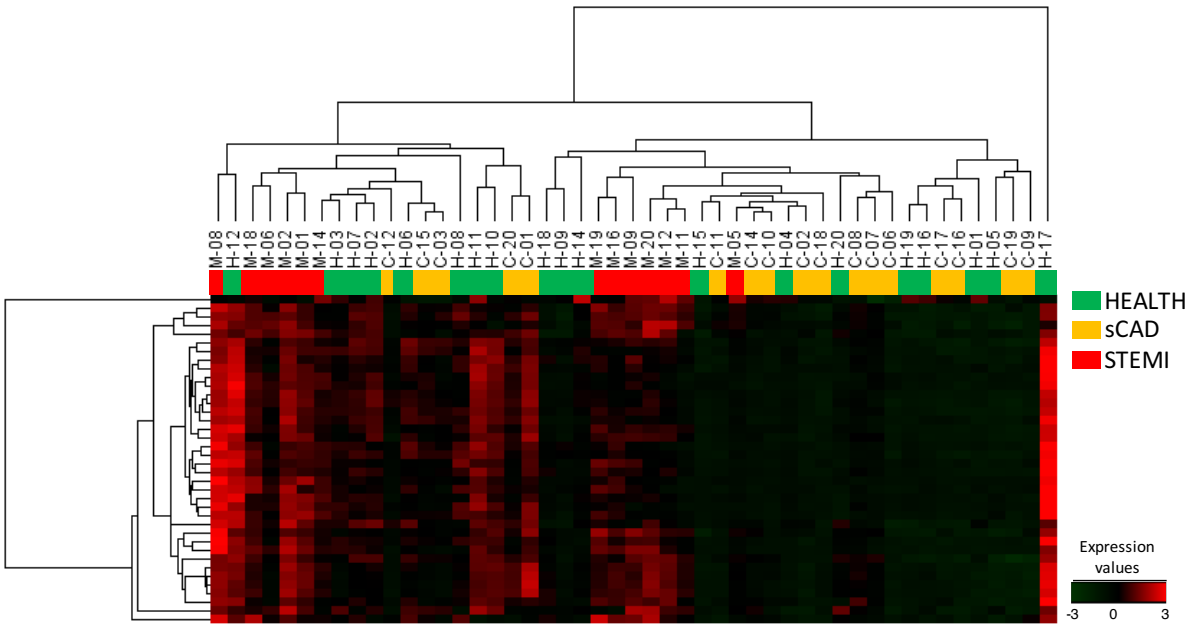

Figure 20. Heatmap of the DEGs between STEMI and sCAD patients and HD, considering a fold change of 2 and p.value ≤ 0.01

Chapter 4

Discussion

Genomic technologies have provided new opportunities to identify gene expression profiles related to cardiovascular disease [73,74], including acute coronary syndromes (ACS) and acute myocardial infarction (AMI).[75] However, many microarray gene-expression studies in ACS and AMI were performed on white blood cells transcriptome and those performed on platelets showed high leukocytes contamination, thus their findings could reflect either triggering events or downstream consequences of the acute event since nucleated cells are able to modify their transcripts in few hours. Platelets, both anuclear and without transcriptional activity, show the unique feature that the acute event itself can not lead to new gene transcription. Therefore, transcriptional profile of platelets is virtually stable and, being the general lifespan of platelets about 7-10 days, the identification of changes in platelets mRNA transcripts of subjects with AMI may provide information on gene expression that precedes the acute event. Platelet transcriptome profiling could consequently offer an earlier diagnosis, many hours before myocardial injury. Currently, a very limited number of studies examining RNA from platelets have demonstrated that there is a specific platelet phenotype that is direclty associated with atherothrombotic diseases.[23,37,38] Using a similar strategy based on platelet transcriptome analysis, we have found that strong differences in platelets gene

expression exist between patients affected by ST elevation MI (STEMI) and subjects with stable coronary artery disease (sCAD) or healthy donors (HD). Many of the differentially expressed genes (DEGs) are associated with disease pathways that strictly correlate with ACS and AMI. In fact, platelets of STEMI patients show an increased expression in genes involved in immune response and inflammation. mRNA levels of S100A12 (the highest up-regulated gene, with a fold change > 13) show increased expression in STEMI patients both in the comparison with sCAD and HD. It is known that S100/calgranulin family members (S100A8, S100A9 and S100A12) bind to RAGE (receptor for advance-glycation end products) and trigger vascular inflammation and prothrobotic response. Serum S100A12 has recently been shown to predict future cardiovascular events in a longitudinal population study and it is emerging as a biomarker for human atherosclerosis.[60,76] Therefore, importantly, S100A12 and the RAGE axis can be modified pharmacologically and they might soon become a therapeutic target for atherosclerosis. In our study, we have also observed that STEMI patients show a significant augmentation in the expression of CLEC4E (C-type lectin receptor 4e). This gene encodes a member of the C-type lectin/C-type lectin-like domain (CTL/CTLD) superfamily. Members of this family share a common protein fold and have diverse functions, such as cell adhesion, cell-cell signalling, glycoprotein turnover, and roles in inflammation and immune response. The activation of CLEC4E signaling is causally involved in atherogenesis. Recently, CLEC4E was demonstrated to coordinate biological pathways involved in the progression of advance atherosclerotic plaques.[77] Futhermore, among the down-regulated genes, CXCL8 (C-X-C motif chemokine ligand 8) has been detected as the less expressed mRNA in platelets of STEMI and sCAD patients in the comparison with HD (fold change rispectivelly of ~10 and 14). It is well known that chemokines

are inflammatory cytokines characterized by their ability to cause directed migration of leukocytes into inflamed tissue, and raised levels are found in atherosclerosis, both systemically and within the atherosclerotic plaques. Chemochine CXCL8 (IL-8) plays a key role in neutrophils activation and transmigration. Through activation of the transcriptional factor, nuclear factor kB, hypoxia and oxidative stress are potent inducers of CXCL8. In various animal models, IL-8 promotes cardiomyocyte apoptosis and impaired cardiomyocyte contractility.[78] Therefore, serum levels of interleukin-8/CXCL8 are increased during MI and in some studies it was shown to be predictive for future cardiac events although its role as clinical biomarkers is still unclear.[58] At any rate the reason why the signal for this specific mRNA on microarrays analysis is in the negative range needs to be investigated.

In short, the preliminary analysis on the DEGs supports evidence linking inflammation in the pathogenesis of AMI and suggests that days before an AMI there is a strong demodulation of inflammation related genes in platelets. Futhermore, our data show the presence of distinctive gene-expression patterns for STEMI and sCAD condition. Therefore, looking for a more specific gene-set for STEMI patients, we have characterized a gene-signature able to discriminate the STEMI phenotype. The signature consists of 38 genes: S100A12, S100A9, S100A8, CLEC4E, RGS2, VNN2, AQP9, MNDA, NCF1B, FCAR, VNN2, NCF1B; NCF1, NAMPT, NCF1C, SAMSN1, S100P, ALOX5AP, FCGR3B, ACSL1, TREM1, FPR1, C5AR1, GCA, NCF4, IL1R2, MAP1B, VNN3, SMAP2, MGAM, FPR2, LRRK2, LINC01506, CXCR1, VNN3, RASSF2, MME, TLR4. This gene-signature was obtained considering a fold change of 2 and a p.value of 0.01 and it is able to distinguish STEMI subjects from sCAD and HD with a good specifity (0.78) and sensibility (0.88). However, this biosignature has to be confirmed and validated in a large population. Moreover, the most differentially

expressed genes must be validated with qPCR analysis while protein expression has to be tested by Western Blot.

In conclusion, our microarrays preliminary data suggest that platelet transcriptome could be predictive of an AMI. The identification of a “genes signature” specific for acute MI could have a putative clinical implication as a tool to predate the onset of an acute event in patients with cardiovascular risk. Thus, transcriptional profiling may provide a novel window on platelets contribution to MI onset, development and prognosis. Therefore, combining genomic information and clinical data may lead to:

1) *detection of predictive biosignatures* with significant predictive power that may be useful to identify MI predisposed subjects

2) *classification/redefinition of cardiovascular diseases on a molecular basis* in order to improve diagnostic and prognostic precision

3) *detection of new molecular targets* useful to optimize treatment options in AMI patients.

This research has been giving us the possibility to identify and characterise genes and related pathways, that could enhance the prediction of AMI risk and improve prevention, treatment, and quality of care in individual patients. However, longitudinal studies assessing the incremental value of genetic factors over traditional risk factors in predicting AMI and its recurrence are necessary to allow genetic information to be incorporated into clinical practice. Cardiovascular medicine may soon have new means of predicting and treating cardiovascular diseases more effectively, but the challenge is still to translate these results into practical knowledge that will help clinicians to deliver this benefit to patients.

Chapter 5

Conclusion

Great efforts in the fields of molecular biology and omics sciences have been done to investigate the precise molecular events immediately preceding an acute MI. However, these mechanisms remain yet uncertain.

The present study suggests that variations in gene expression profile of megakaryocytes may occur before of an acute myocardial infarction and the platelets transcriptome could act as powerful fingerprint indicating the development of a future myocardial infarction. The clinical implication of these important findings are obvious, but their validation in external populations must still be accomplished before reaching a definitive conclusion.

This study is the result of a strong collaboration between the Cardiology Division of University Hospital of Parma and the Human Anatomy Unit of Department of Biomedical, Biotechnological and Translational Sciences (S.Bi.Bi.T.) of University of Parma.

Chapter 6

Supplementary Materials

Supplemental Tables

Supplemental Table 1. Differentially expressed genes. Genes up-regulated in patients with sCAD in the comparison between sCAD and Healthy Donors

Transcript Cluster ID	Gene Symbol	Description	Fold Change (linear) (C vs. H)	ANOVA p-value (C vs. H)
Transcript Cluster	MFN2	mitofusin 2	2.89	0.003759
Transcript Cluster	PRKCD	protein kinase C, delta	1.95	0.034974
Transcript Cluster	PADI4	peptidyl arginine deiminase, type IV	1.79	0.024266
Transcript Cluster	VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor	1.73	0.037694
Transcript Cluster	PKM	pyruvate kinase, muscle	1.71	0.011072
Transcript Cluster	CD68	CD68 molecule	1.7	0.006386
Transcript Cluster	ATP2A3	ATPase, Ca++ transporting, ubiquitous	1.67	0.042952
Transcript Cluster	MAP2K3	mitogen-activated protein kinase kinase 3	1.66	0.031603
Transcript Cluster	COTL1	coactosin-like F-actin binding protein 1	1.65	0.011656
Transcript Cluster	ST3GAL6-AS1	ST3GAL6 antisense RNA 1	1.61	0.008627
Transcript Cluster	PPIF	peptidylprolyl isomerase F	1.55	0.028174
Transcript Cluster	ITGA2B	integrin alpha 2b	1.55	0.047477
Transcript Cluster	G6PD	glucose-6-phosphate dehydrogenase	1.54	0.012275
Transcript Cluster	XPNPEP1	X-prolyl aminopeptidase (aminopeptidase P) 1,	1.54	0.032637
Transcript Cluster	KRTAP6-1	keratin associated protein 6-1	1.53	0.011181
Transcript Cluster	TGFB1	transforming growth factor beta 1	1.52	0.027832
Transcript Cluster	MEMO1P1	mediator of cell motility 1 pseudogene 1	1.51	0.035633
Transcript Cluster	CFAP161	cilia and flagella associated protein 161	1.5	0.019745

Supplemental Table 2. Differentially expressed genes. Genes down-regulated in patients with sCAD in the comparison between sCAD and Healthy Donors.

Transcript Cluster ID	Gene Symbol	Description	Fold Change (linear) (C vs. H)	ANOVA p-value (C vs. H)
TC04002067.hg.1	CXCL8	chemokine (C-X-C motif) ligand 8	-13.91	0.001478
TC07001990.hg.1	MIR548I4	microRNA 548i-4	-4.82	0.018506
TC14000999.hg.1	MIR548AI	microRNA 548ai	-4.77	0.040134
TC04001081.hg.1	MIR548AJ2	microRNA 548aj-2	-4.43	0.045434
TC08001104.hg.1	MIR548O2	microRNA 548o-2	-4.33	0.017361
TC07003113.hg.1	DOCK4	dedicator of cytokinesis 4	-3.99	0.002371
TC01003634.hg.1	MIR548F1	microRNA 548f-1	-3.95	0.036114
TC03001607.hg.1	MIR548G	microRNA 548g	-3.87	0.04061
TC01001624.hg.1	RGS2	regulator of G-protein signaling 2	-3.75	0.027401
TC09001442.hg.1	MIR548Q	microRNA 548q	-3.67	0.048397
TC09001222.hg.1	MIR548H3	microRNA 548h-3	-3.62	0.039796
TC03000824.hg.1	MIR548H2	microRNA 548h-2	-3.6	0.036762
TC04001473.hg.1	MIR297	microRNA 297	-3.45	0.001136
TC10001393.hg.1	MIR1256	microRNA 1256	-3.42	0.008049
TC01003638.hg.1	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	-2.97	0.015317
TC20000823.hg.1	FKSG49	FKSG49	-2.97	0.037314

TC07001738.hg.1	NAMPT	nicotinamide phosphoribosyltransferase	-2.92	0.015823
TC06001958.hg.1	MIR548H3	microRNA 548h-3	-2.78	0.030603
TC11002916.hg.1	SORL1	sortilin-related receptor, L(DLR class) A repeats containing	-2.77	0.037463
TC03000403.hg.1	MIR548A2	microRNA 548a-2	-2.76	0.02871
TC05000257.hg.1	FKSG52		-2.76	0.043443
TC07000964.hg.1	MIR548F3	microRNA 548f-3	-2.6	0.038505
TC0X000613.hg.1	LOC101928495	uncharacterized LOC101928495	-2.6	0.027222
TC12002007.hg.1	MIR4472-2	microRNA 4472-2	-2.57	0.017964
TC06001717.hg.1	TREM1	triggering receptor expressed on myeloid cells 1	-2.51	0.022709
TC01006308.hg.1	FCGR3B	Fc fragment of IgG, low affinity IIIb, receptor (CD16b)	-2.45	0.032461
TC20000383.hg.1	FKSG49	FKSG49	-2.44	0.04881
TC06000267.hg.1	FKSG49	FKSG49	-2.37	0.017105
TC19000676.hg.1	C5AR1	complement component 5a receptor 1	-2.32	0.019767
TC11002730.hg.1	MALAT1	metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)	-2.31	0.049652
TC09001166.hg.1	MIR1299	microRNA 1299	-2.24	0.02029
TC05001142.hg.1	MIR4454	microRNA 4454	-2.21	0.029769
TC12001453.hg.1	SNORA2C; MIR1291	small nucleolar RNA, H/ACA box 2C; microRNA 1291	-2.21	0.015334
TC11000580.hg.1	RNU6-45P	RNA, U6 small nuclear 45, pseudogene	-2.14	0.029608
TC06001523.hg.1	HCG27	HLA complex group 27 (non-protein coding)	-2.1	0.031983
TC19001241.hg.1	ADGRE3	adhesion G protein-coupled receptor E3	-2.08	0.035566
TC02004133.hg.1	BRE-AS1	BRE antisense RNA 1	-2.06	0.008833
TC17001816.hg.1	MIR548AA2; MIR548D2	microRNA 548aa-2; microRNA 548d-2	-2.05	0.007886
TC04000408.hg.1	CXCL8	chemokine (C-X-C motif) ligand 8	-2.03	0.006148
TC0X001246.hg.1	MIR1256	microRNA 1256	-2.03	0.016472
TC21000008.hg.1	LOC644450		-1.99	0.002804
TC10000148.hg.1	RNU6-15P	RNA, U6 small nuclear 15, pseudogene	-1.92	0.034177
TC19001593.hg.1	PLAUR	plasminogen activator, urokinase receptor	-1.89	0.040199
TC0X000518.hg.1	MIR548AN	microRNA 548an	-1.88	0.034094
TC12002520.hg.1	PLXNC1	plexin C1	-1.88	0.02177
TC19002254.hg.1	ZNF667-AS1	ZNF667 antisense RNA 1 (head to head)	-1.88	0.015025
TC02002378.hg.1	CXCR4	chemokine (C-X-C motif) receptor 4	-1.87	0.049627
TC11002177.hg.1	RNU6-16P	RNA, U6 small nuclear 16, pseudogene	-1.86	0.030795
TC01002292.hg.1	MIR1290	microRNA 1290	-1.85	0.009923
TC02002145.hg.1	RNF149; SNORD89	ring finger protein 149; small nucleolar RNA, C/D box 89	-1.85	0.033307
TC08001341.hg.1	CASC9	cancer susceptibility candidate 9 (non-protein coding)	-1.85	0.010669
TC12001178.hg.1	CLEC4E	C-type lectin domain family 4, member E	-1.85	0.021048
TC22001185.hg.1	RPL23AP82	ribosomal protein L23a pseudogene 82	-1.85	0.03749
TC20000241.hg.1	MIR644A	microRNA 644a	-1.84	0.049003
TC04001810.hg.1	SLED1	proteoglycan 3 pseudogene	-1.83	0.013868
TC11000256.hg.1	HTATIP2	HIV-1 Tat interactive protein 2	-1.83	0.032995
TC19002237.hg.1	FCAR	Fc fragment of IgA receptor	-1.8	0.013077
TC02000623.hg.1	IL18RAP	interleukin 18 receptor accessory protein	-1.77	0.014738
TC05002066.hg.1	DUSP1	dual specificity phosphatase 1	-1.77	0.036255
TC07001356.hg.1	RNU7-76P	RNA, U7 small nuclear 76 pseudogene	-1.74	0.031048
TC11003160.hg.1	OR7E5P	olfactory receptor, family 7, subfamily E, member 5 pseudogene	-1.74	0.012324
TC0X000953.hg.1	MIR548AJ2	microRNA 548aj-2	-1.73	0.015494
TC10000099.hg.1	RNU6-1; RNU6-2	RNA, U6 small nuclear 1; RNA, U6 small nuclear 2	-1.73	0.047964
TC16000868.hg.1	MIR548H2	microRNA 548h-2	-1.73	0.017595
TC02003396.hg.1	IGKC	immunoglobulin kappa constant	-1.72	0.012839
TC07000899.hg.1	MGAM	maltase-glucoamylase	-1.72	0.031213
TC08001077.hg.1	MIR548H4	microRNA 548h-4	-1.72	0.016988
TC11000910.hg.1	SCARNA9; SCARNA9L	small Cajal body-specific RNA 9; small Cajal body-specific RNA 9-like	-1.72	0.030177
TC12001788.hg.1	MIR548AL	microRNA 548al	-1.72	0.022696
TC19000924.hg.1	ZNF667-AS1	ZNF667 antisense RNA 1 (head to head)	-1.72	0.01814
TC01004998.hg.1	G0S2	G0/G1 switch 2	-1.71	0.016149
TC12001215.hg.1	CLEC7A	C-type lectin domain family 7, member A	-1.71	0.038464
TC14000471.hg.1	FOS	FBJ murine osteosarcoma viral	-1.71	0.01646

		oncogene homolog		
TC01006307.hg.1	FCGR3A	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)	-1.7	0.034029
TC09000252.hg.1	RP11-327I22.4		-1.69	0.018115
TC17000361.hg.1	LOC400590	uncharacterized LOC400590	-1.68	0.013534
TC05000487.hg.1	POM121L9P	POM121 transmembrane nucleoporin-like 9, pseudogene	-1.67	0.006349
TC15000084.hg.1	SNORD115-11; SNORD115-36; SNORD115-43; SNORD115-29	small nucleolar RNA, C/D box 115-11; small nucleolar RNA, C/D box 115-36; small nucleolar RNA, C/D box 115-43; small nucleolar RNA, C/D box 115-29	-1.67	0.027045
TC15000099.hg.1	SNORD115-43; SNORD115-29; SNORD115-11	small nucleolar RNA, C/D box 115-43; small nucleolar RNA, C/D box 115-29; small nucleolar RNA, C/D box 115-11	-1.67	0.027045
TC15000105.hg.1	SNORD115-43; SNORD115-36; SNORD115-11; SNORD115-29	small nucleolar RNA, C/D box 115-43; small nucleolar RNA, C/D box 115-36; small nucleolar RNA, C/D box 115-11; small nucleolar RNA, C/D box 115-29	-1.67	0.027045
TC15000112.hg.1	SNORD115-29; SNORD115-43; SNORD115-11	small nucleolar RNA, C/D box 115-29; small nucleolar RNA, C/D box 115-43; small nucleolar RNA, C/D box 115-11	-1.67	0.027045
TC03001939.hg.1	CCNL1	cyclin L1	-1.66	0.034228
TC12000730.hg.1	PLXNC1	plexin C1	-1.65	0.0331
TC05002451.hg.1	HEXB	hexosaminidase B (beta polypeptide)	-1.64	0.016663
TC09001372.hg.1	HSD17B3	hydroxysteroid (17-beta) dehydrogenase 3	-1.64	0.022903
TC07002775.hg.1	RNF216	ring finger protein 216	-1.63	0.041498
TC09001296.hg.1	LOC440173	uncharacterized LOC440173	-1.63	0.019197
TC11001418.hg.1	SNORD97	small nucleolar RNA, C/D box 97	-1.62	0.028443
TC12000309.hg.1	LRRK2	leucine-rich repeat kinase 2	-1.61	0.03125
TC06001121.hg.1	MIR548U	microRNA 548u	-1.59	0.025573
TC08002406.hg.1	REXO1L2P	REX1, RNA exonuclease 1 homolog-like 2, pseudogene	-1.59	0.010887
TC09001565.hg.1	SNORD90	small nucleolar RNA, C/D box 90	-1.59	0.047882
TC12000573.hg.1	MIR548C; MIR548Z	microRNA 548c; microRNA 548z	-1.59	0.005221
TC15001719.hg.1	BCL2A1	BCL2-related protein A1	-1.58	0.029697
TC04002881.hg.1	SLED1	proteoglycan 3 pseudogene	-1.57	0.01769
TC05000309.hg.1	POM121L9P	POM121 transmembrane nucleoporin-like 9, pseudogene	-1.57	0.009648
TC05000321.hg.1	POM121L9P	POM121 transmembrane nucleoporin-like 9, pseudogene	-1.57	0.009648
TC06000218.hg.1	POM121L9P	POM121 transmembrane nucleoporin-like 9, pseudogene	-1.57	0.009648
TC06000691.hg.1	POM121L9P	POM121 transmembrane nucleoporin-like 9, pseudogene	-1.57	0.009648
TC06002162.hg.1	GVQW2	GVQW motif containing 2	-1.57	0.008933
TC06002355.hg.1	OR2J2	olfactory receptor, family 2, subfamily J, member 2	-1.57	0.039287
TC0X000818.hg.1	LOC101928201	uncharacterized LOC101928201	-1.57	0.024142
TC13000163.hg.1	SMIM2-AS1	SMIM2 antisense RNA 1	-1.57	0.024371
TC15000094.hg.1	SNORD115-22	small nucleolar RNA, C/D box 115-22	-1.57	0.049048
TC10002856.hg.1	CACUL1	CDK2-associated, cullin domain 1	-1.56	0.045483
TC18000455.hg.1	INO80C	INO80 complex subunit C	-1.56	0.016053
TC6_mann_hap4000161.hg.1	HLA-DRB4; HLA-DRB1	major histocompatibility complex, class II, DR beta 4; major histocompatibility complex, class II, DR beta 1	-1.55	0.018221
TC01002853.hg.1	RP11-302M6.2		-1.54	0.038456
TC07001599.hg.1	MIR1285-1	microRNA 1285-1	-1.54	0.025538
TC10002134.hg.1	LOC101929165	uncharacterized LOC101929165	-1.54	0.014295
TC16000322.hg.1	NPIPB3	nuclear pore complex interacting protein family, member B3	-1.54	0.002469
TC16001238.hg.1	RNU6-23P	RNA, U6 small nuclear 23, pseudogene	-1.54	0.025801
TC01003065.hg.1	LOC100996920; RP11-423O2.5	uncharacterized LOC100996920	-1.53	0.007629
TC03000105.hg.1	RNU7-10P	RNA, U7 small nuclear 10 pseudogene	-1.53	0.00734
TC04000007.hg.1	MIR571	microRNA 571	-1.53	0.00153
TC02004978.hg.1	IGKV2D-28	immunoglobulin kappa variable 2D-28	-1.52	0.014153
TC20000678.hg.1	LINC00837	long intergenic non-protein coding RNA 837	-1.52	0.030538
TC01004068.hg.1	MIR3916	microRNA 3916	-1.51	0.029188
TC08000487.hg.1	LY96	lymphocyte antigen 96	-1.51	0.025657
TC13000776.hg.1	RP11-75N6.2		-1.51	0.012795
TC02000547.hg.1	ANKRD36BP2	ankyrin repeat domain 36B pseudogene 2	-1.5	0.033393
TC06000359.hg.1	POU5F1	POU class 5 homeobox 1	-1.5	0.005592
TC06002502.hg.1	HLA-DQA1	major histocompatibility complex,	-1.5	0.002078

		class II, DQ alpha 1		
TC12001914.hg.1	KCCAT198	renal clear cell carcinoma-associated transcript 198	-1.5	0.003992
TC15000092.hg.1	SNORD115-15; SNORD115-20; SNORD115-21; SNORD115-34	small nucleolar RNA, C/D box 115-15; small nucleolar RNA, C/D box 115-20; small nucleolar RNA, C/D box 115-21; small nucleolar RNA, C/D box 115-34	-1.5	0.029038
TC17001150.hg.1	MIR548H3	microRNA 548h-3	-1.5	0.018221
TC6_cox_hap2000056.hg.1	POU5F1	POU class 5 homeobox 1	-1.5	0.005592
TC6_dbb_hap3000049.hg.1	POU5F1	POU class 5 homeobox 1	-1.5	0.005592
TC6_mann_hap4000050.hg.1	POU5F1	POU class 5 homeobox 1	-1.5	0.005592
TC6_mcf_hap5000043.hg.1	POU5F1	POU class 5 homeobox 1	-1.5	0.005592
TC6_qbl_hap6000049.hg.1	POU5F1	POU class 5 homeobox 1	-1.5	0.005592
TC6_ssto_hap7000043.hg.1	POU5F1	POU class 5 homeobox 1	-1.5	0.005592

Supplemental Table 3. DAVID analysis: identification of the biological processes related to the up-regulated genes in patients with sCAD in the comparison between sCAD and Healthy Donors

Term	Genes in the category	P-Value	Benjamini
cellular calcium ion homeostasis	ATP2A3; TGF1B; VDR	2,4E-3	9,7E-1
positive regulation of superoxide anion generation	PRKCD; TGFB1	6,9E-3	9,6E-1
positive regulation of protein import into nucleus	PRKCD; TGFB1	1,2E-2	9,1E-1
positive regulation of protein import into nucleus	PRKCD; TGFB1	1,6E-2	8,9E-1
ATP biosynthetic process	PKM; TFGB1	2,2E-2	9,0E-1
cellular response to hydrogen peroxide	PPIF; PRKCD	4,8E-2	8,7E-1
organ regeneration	PKM; TFGB1	5,0E-2	9,2E-1

Supplemental Table 4. DAVID analysis: identification of the biological processes related to down-regulated genes in patients with sCAD in the comparison between sCAD and Healthy Donors

Term	Genes in the category	P-Value	Benjamini
immune response	CXCL8; FCAR; FCGR3A ; FCGR3B; C5AR1; IGKC; IL18RAP; HLA-DQA1; HLA-DRB1; HLA-DRB4; SLED1	2,9E-8	1,3E-5
inflammatory response	CXCL8; CXCR4; CLEC7A; FOS; C5AR1; IL18RAP; LY96; PTGS2	2,5E-5	5,7E-3
antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	HLA-DQA1; HLA-DRB1; HLA-DRB4	1,1E-3	1,5E-1
chemotaxis	CXCL8; CXCR4; C5AR1; PLAUR	2,9E-3	2,8E-1
negative regulation of MAP kinase activity	DUSP1; RGS2; SORL1	3,5E-3	2,7E-1
calcium-mediated signaling	CXCL8; CXCR4; LRRK2	6,5E-3	3,9E-1
response to lipopolysaccharide	FOS; C5AR1; LY96; PTGS2	9,0E-3	4,5E-1

Supplemental Table 5. Differentially expressed genes. Genes up-regulated in patients with STEMI in the comparison between STEMI and Healthy Donors

Transcript Cluster ID	Gene Symbol	Description	Fold Change (linear) (M vs. H)	ANOVA p-value (M vs. H)
TC01003260.hg.1	S100A12	S100 calcium binding protein A12	11.81	0.000006
TC12001178.hg.1	CLEC4E	C-type lectin domain family 4, member E	4.38	0.001147
TC21001069.hg.1	SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1	2.8	0.001317
TC04002623.hg.1	SPARCL1	SPARC like 1	2.51	0.002273
TC01001346.hg.1	MNDA	myeloid cell nuclear differentiation antigen	2.5	0.036961

TC01001254.hg.1	S100A9	S100 calcium binding protein A9	2.48	0.001128
TC04000072.hg.1	S100P	S100 calcium binding protein P	2.38	0.000398
TC07000438.hg.1	NCF1B; NCF1	neutrophil cytosolic factor 1B pseudogene; neutrophil cytosolic factor 1	2.26	0.014863
TC07002399.hg.1	NCF1B	neutrophil cytosolic factor 1B pseudogene	2.22	0.007207
TC11002234.hg.1	MMP1	matrix metallopeptidase 1	2.14	0.01652
TC07003026.hg.1	NCF1C	neutrophil cytosolic factor 1C pseudogene	2.12	0.014792
TC07001516.hg.1	NCF1C	neutrophil cytosolic factor 1C pseudogene	2.03	0.014457
TC11003256.hg.1	LOC101928813	uncharacterized LOC101928813	2.02	0.025376
TC01003261.hg.1	S100A8	S100 calcium binding protein A8	1.96	0.002686
TC11003000.hg.1	H19	H19, imprinted maternally expressed transcript (non-protein coding)	1.86	0.00429
TC19000134.hg.1	MCEMP1	mast cell-expressed membrane protein 1	1.86	0.002984
TC01000510.hg.1	SMAP2	small ArfGAP2	1.84	0.00085
TC06004150.hg.1	FKBP5	FK506 binding protein 5	1.8	9.53E-07
TC11001833.hg.1	FABP5P7	fatty acid binding protein 5 pseudogene 7	1.79	0.004465
TC13000729.hg.1	FABP5P1	fatty acid binding protein 5 pseudogene 1	1.77	0.012524
TC08001365.hg.1	FABP4	fatty acid binding protein 4, adipocyte	1.75	0.001489
TC12001187.hg.1	A2M	alpha-2-macroglobulin	1.71	0.000088
TC01002736.hg.1	CFL1P3	cofilin 1 (non-muscle) pseudogene 3	1.7	0.02866
TC06000983.hg.1	ARG1	arginase 1	1.68	0.000108
TC04001267.hg.1	SULT1B1	sulfotransferase family 1B member 1	1.65	0.01606
TC04002645.hg.1	EMCN	endomucin	1.64	0.003133
TC04001366.hg.1	SPARCL1	SPARC like 1	1.63	0.000102
TC06003665.hg.1	CLIC5	chloride intracellular channel 5	1.62	0.03235
TC02000281.hg.1	EPAS1	endothelial PAS domain protein 1	1.59	0.002765
TC13000871.hg.1	IRS2	insulin receptor substrate 2	1.59	0.000514
TC02001986.hg.1	NAT8B	N-acetyltransferase 8B (GCN5-related, putative, gene/pseudogene)	1.58	0.01733
TC02000937.hg.1	TNFAIP6	tumor necrosis factor, alpha-induced protein 6	1.55	0.022854
TC11001273.hg.1	H19; MIR675	H19, imprinted maternally expressed transcript (non-protein coding); microRNA 675	1.54	0.007294
TC14000584.hg.1	IFI27	interferon, alpha-inducible protein 27	1.54	0.022195
TC09001473.hg.1	TXN	thioredoxin	1.52	0.014182
TC12002425.hg.1	IRAK3	interleukin 1 receptor associated kinase 3	1.52	0.000262
TC15002424.hg.1	NBEAP1	neurobeachin pseudogene 1	1.52	0.0484
TC0X001219.hg.1	GLA	galactosidase, alpha	1.51	0.003366
TC01000124.hg.1	RBP7	retinol binding protein 7, cellular	1.5	0.002233
TC03000630.hg.1	CSTA	cystatin A (stefin A)	1.5	0.011715
TC10001958.hg.1	RAB18	RAB18, member RAS oncogene family	1.5	0.046265

Supplemental Table 6. Differentially expressed genes. Genes down-regulated in patients with STEMI in the comparison between STEMI and Healthy Donors

Transcript Cluster ID	Gene Symbol	Description	Fold Change (linear) (M vs. H)	ANOVA p-value (M vs. H)
TC04002067.hg.1	CXCL8	chemokine (C-X-C motif) ligand 8	-9.74	0.033571

TC08000259.hg.1	SNORD13	small nucleolar RNA, C/D box 13	-4.64	0.037686
TC06001529.hg.1	SNORD117	small nucleolar RNA, C/D box 117	-3.61	0.032143
TC6_apd_hap1000080.hg.1	SNORD117	small nucleolar RNA, C/D box 117	-3.61	0.032143
TC6_cox_hap2000158.hg.1	SNORD117	small nucleolar RNA, C/D box 117	-3.61	0.032143
TC6_dbb_hap3000147.hg.1	SNORD117	small nucleolar RNA, C/D box 117	-3.61	0.032143
TC6_mann_hap4000135.hg.1	SNORD117	small nucleolar RNA, C/D box 117	-3.61	0.032143
TC6_mcf_hap5000135.hg.1	SNORD117	small nucleolar RNA, C/D box 117	-3.61	0.032143
TC6_qbl_hap6000150.hg.1	SNORD117	small nucleolar RNA, C/D box 117	-3.61	0.032143
TC6_ssto_hap7000130.hg.1	SNORD117	small nucleolar RNA, C/D box 117	-3.61	0.032143
TC10001589.hg.1	SNORA12	small nucleolar RNA, H/ACA box 12	-3.06	0.042686
TC03003185.hg.1	SCARNA7	small Cajal body-specific RNA 7	-2.8	0.024278
TC03001957.hg.1	SCARNA7	small Cajal body-specific RNA 7	-2.75	0.024879
TC09001056.hg.1	RMRP	RNA component of mitochondrial RNA processing endoribonuclease	-2.59	0.032926
TC05000231.hg.1	GZMA	granzyme A	-2.52	0.049644
TC16000936.hg.1	LOC100190986	uncharacterized LOC100190986	-2.37	0.036757
TC07001470.hg.1	POLR2J4	polymerase (RNA) II (DNA directed) polypeptide J4, pseudogene	-1.99	0.036326
TC12001222.hg.1	KLRC4-KLRK1; KLRK1; KLRC4	KLRC4-KLRK1 readthrough; killer cell lectin-like receptor subfamily K, member 1; killer cell lectin-like receptor subfamily C, member 4	-1.92	0.043814
TC20000241.hg.1	MIR644A	microRNA 644a	-1.86	0.045943
TC12001453.hg.1	SNORA2C; MIR1291	small nucleolar RNA, H/ACA box 2C; microRNA 1291	-1.85	0.026501
TC02001189.hg.1	SNORD70	small nucleolar RNA, C/D box 70	-1.84	0.019385
TC11000910.hg.1	SCARNA9; SCARNA9L	small Cajal body-specific RNA 9; small Cajal body-specific RNA 9-like	-1.79	0.041497
TC19002254.hg.1	ZNF667-AS1	ZNF667 antisense RNA 1 (head to head)	-1.73	0.032692
TC6_mann_hap4000159.hg.1	HLA-DRB4	major histocompatibility complex, class II, DR beta 4	-1.68	0.008239
TC17000327.hg.1	SNORD42A	small nucleolar RNA, C/D box 42A	-1.66	0.003801
TC01004068.hg.1	MIR3916	microRNA 3916	-1.64	0.028194
TC02001191.hg.1	SNORD11	small nucleolar RNA, C/D box 11	-1.62	0.005834
TC06003604.hg.1	TAP2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	-1.58	0.037581
TC02003892.hg.1	SCARNA5	small Cajal body-specific RNA 5	-1.56	0.021234
TC02001416.hg.1	SCARNA5	small Cajal body-specific RNA 5	-1.52	0.042713
TC05003305.hg.1	CD74	CD74 molecule, major histocompatibility complex, class II invariant chain	-1.51	0.019564
TC01005631.hg.1	TGFBR3	transforming growth factor beta receptor III	-1.5	0.040569
TC05000701.hg.1	EGR1	early growth response 1	-1.5	0.045454
TC09001390.hg.1	ANKRD18CP	ankyrin repeat domain 18C, pseudogene	-1.5	0.024815

Supplemental Table 7. DAVID analysis: identification of the biological process related to up-regulated genes in the comparison between patients with STEMI and Healthy Donors

Term	Genes in the category	P-Value	Benjamini
respiratory burst	NCF1; NCF1B; NCF1C	2,8E-4	6,8E-2
positive regulation of inflammatory response	S100A12; S100A8; S100A9; FABP4	3,2E-4	4,0E-2
superoxide metabolic process	NCF1; NCF1B; NCF1C	5,7E-4	4,8E-2
cytokine production	S100A8; S100A9; FABP4	9,0E-4	5,6E-2
defense response to fungus	S100A12; S100A8; S100A9	1,1E-3	5,2E-2
positive regulation of NF-kappaB transcription factor activity	S100A12; S100A8; S100A9; IRAK3	1,7E-3	7,0E-2
defense response to bacterium	CLEC4E; S100A12; S100A8; S100A9	2,3E-3	8,1E-2
chemokine production	S100A8; S100A9	3,6E-3	1,1E-1
neutrophil aggregation	S100A8; S100A9	3,6E-3	1,1E-1
neutrophil chemotaxis	S100A12; S100A8; S100A9	6,2E-3	1,6E-1
innate immune response	CLEC4E; S10012; S100A8; S100A9; NCF1	7,0E-3	1,6E-1
positive regulation of peptide secretion	S100A8; S100A9	7,1E-3	1,5E-1
sequestering of zinc ion	S100A8; S100A9	7,1E-3	1,5E-1
positive regulation of catalytic activity	NCF1; NCF1B; NCF1C	9,2E-3	1,8E-1

Supplemental Table 8. DAVID analysis: identification of the biological processes related to down-

regulated genes in the comparison between patients with STEMI and Healthy Donors

Term	Genes in the category	P-Value	Benjamini
immune response	CXCL8; CD74; GZMA; HLA-DRB4; TGFBR3	4,4E-5	7,6E-3
T cell costimulation	KLRC4-KLRK1; KLRK1; HLA-DRB4	7,5E-4	6,3E-2
positive regulation of myeloid dendritic cell activation	KLRC4;KLRK1	1,6E-3	8,9E-2
negative regulation of natural killer cell chemotaxis	KLRC4;KLRK1	1,6E-3	8,9E-2
cellular response to lipopolysaccharide	CXCL8; KLRC4-KLRK1	2,0E-3	8,4E-2
signal transduction	CXCL8; CD74; KLRC4-KLRK1; KLRK1; HLA-DRB4	2,2E-3	7,3E-2
adaptive immune response	KLRC4-KLRK1; KLRK1; TAP2	2,6E-3	7,2E-2

Supplemental Table 9. Differentially expressed genes. Genes up-regulated in patients with STEMI in the comparison between STEMI and sCAD.

Transcript Cluster ID	Gene Symbol	Description	Fold Change (linear) (M vs. C)	ANOVA p-value (M vs. C)
TC01003260.hg.1	S100A12	S100 calcium binding protein A12	13.45	1.36E-07
TC12001178.hg.1	CLEC4E	C-type lectin domain family 4, member E	8.12	2.23E-07
TC01001624.hg.1	RGS2	regulator of G-protein signaling 2	5.69	0.000838
TC06003855.hg.1	VNN2	vanin 2	5.58	0.003255
TC15000441.hg.1	AQP9	aquaporin 9	5.17	0.00097
TC01001346.hg.1	MNDA	myeloid cell nuclear differentiation antigen	4.41	0.000166
TC07002399.hg.1	NCF1B	neutrophil cytosolic factor 1B pseudogene	3.62	0.000116
TC19002237.hg.1	FCAR	Fc fragment of IgA receptor	3.5	0.000117
TC06002121.hg.1	VNN2	vanin 2	3.49	0.003983
TC07000438.hg.1	NCF1B; NCF1	neutrophil cytosolic factor 1B pseudogene; neutrophil cytosolic factor 1	3.4	0.000126
TC07001738.hg.1	NAMPT	nicotinamide phosphoribosyltransferase	3.32	0.000972
TC01001254.hg.1	S100A9	S100 calcium binding protein A9	3.24	5.28E-07
TC21000717.hg.1	KCNJ15	potassium channel, inwardly rectifying subfamily J, member 15	3.23	0.015868
TC07003026.hg.1	NCF1C	neutrophil cytosolic factor 1C pseudogene	3.14	0.000376
TC21001069.hg.1	SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1	3.1	0.000003
TC04000072.hg.1	S100P	S100 calcium binding protein P	3.06	0.000005
TC13000100.hg.1	ALOX5AP	arachidonate 5-lipoxygenase-activating protein	2.81	0.002409
TC07001516.hg.1	NCF1C	neutrophil cytosolic factor 1C pseudogene	2.79	0.000364
TC01006308.hg.1	FCGR3B	Fc fragment of IgG, low affinity IIIb, receptor (CD16b)	2.77	0.000761
TC04001809.hg.1	ACSL1	acyl-CoA synthetase long-chain family member 1	2.72	0.000147
TC06001717.hg.1	TREM1	triggering receptor expressed on myeloid cells 1	2.72	0.008102
TC19001787.hg.1	FPR1	formyl peptide receptor 1	2.65	0.005323
TC19000676.hg.1	C5AR1	complement component 5a receptor 1	2.51	0.001633
TC01003261.hg.1	S100A8	S100 calcium binding protein A8	2.38	0.000008
TC02000978.hg.1	GCA	grancalcin, EF-hand calcium binding protein	2.36	0.000039
TC22000270.hg.1	NCF4	neutrophil cytosolic factor 4	2.36	0.000456
TC02000619.hg.1	IL1R2	interleukin 1 receptor, type II	2.33	0.000349
TC05002434.hg.1	MAP1B	microtubule associated protein 1B	2.33	0.001008
TC06003854.hg.1	VNN3	vanin 3	2.29	0.006736
TC01000510.hg.1	SMAP2	small ArfGAP2	2.28	0.000043
TC07000899.hg.1	MGAM	maltase-glucoamylase	2.21	0.007902
TC19000788.hg.1	FPR2	formyl peptide receptor 2	2.2	0.001012
TC12000309.hg.1	LRRK2	leucine-rich repeat kinase 2	2.19	0.00012
TC11002916.hg.1	SORL1	sortilin-related receptor, L(DLR class) A repeats containing	2.17	0.04713
TC09001187.hg.1	LINC01506	long intergenic non-protein coding RNA 1506	2.15	0.000086
TC04002623.hg.1	SPARCL1	SPARC like 1	2.13	0.021328
TC14002135.hg.1	SERPINA1	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	2.12	0.044958
TC01005928.hg.1	SELL	selectin L	2.06	0.01508
TC02002767.hg.1	CXCR1	chemokine (C-X-C motif) receptor 1	2.05	0.007321
TC06002120.hg.1	VNN3	vanin 3	2.05	0.005486
TC20000587.hg.1	RASSF2	Ras association (RalGDS/AF-6) domain family member 2	2.05	0.000091
TC03000846.hg.1	MME	membrane metallo-endopeptidase	2.03	0.000075
TC09000601.hg.1	TLR4	toll-like receptor 4	2.03	0.000035
TC12000295.hg.1	KIAA1551	KIAA1551	1.96	0.005074
TC16000983.hg.1	XPO6	exportin 6	1.96	0.003886

TC15001719.hg.1	BCL2A1	BCL2-related protein A1	1.95	0.000364
TC01006030.hg.1	ZNF281	zinc finger protein 281	1.93	0.000161
TC11002344.hg.1	AMICA1	adhesion molecule, interacts with CXADR antigen 1	1.93	0.039132
TC02000623.hg.1	IL18RAP	interleukin 18 receptor accessory protein	1.92	0.001899
TC04001267.hg.1	SULT1B1	sulfotransferase family 1B member 1	1.91	0.000008
TC16001773.hg.1	LITAF	lipopolysaccharide-induced TNF factor	1.89	0.000134
TC22001185.hg.1	RPL23AP82	ribosomal protein L23a pseudogene 82	1.89	0.040122
TC12002425.hg.1	IRAK3	interleukin 1 receptor associated kinase 3	1.88	0.000058
TC02000937.hg.1	TNFAIP6	tumor necrosis factor, alpha-induced protein 6	1.87	0.000006
TC06004150.hg.1	FKBP5	FK506 binding protein 5	1.87	5.97E-07
TC01001090.hg.1	TXNIP	thioredoxin interacting protein	1.85	0.003333
TC07000459.hg.1	NCF1	neutrophil cytosolic factor 1	1.85	0.000187
TC12002520.hg.1	PLXNC1	plexin C1	1.85	0.025561
TC01000124.hg.1	RBP7	retinol binding protein 7, cellular	1.84	0.000047
TC06000983.hg.1	ARG1	arginase 1	1.84	0.000016
TC06002863.hg.1	OGFRL1	opioid growth factor receptor-like 1	1.84	0.007888
TC11002123.hg.1	PAK1	p21 protein (Cdc42/Rac)-activated kinase 1	1.84	0.002744
TC15000864.hg.1	IQGAP1	IQ motif containing GTPase activating protein 1	1.84	0.012224
TC02001292.hg.1	CXCR2	chemokine (C-X-C motif) receptor 2	1.81	0.000105
TC02004161.hg.1	EIF2AK2	eukaryotic translation initiation factor 2-alpha kinase 2	1.8	0.001449
TC01000462.hg.1	AGO4	argonaute RISC catalytic component 4	1.78	0.004653
TC16000870.hg.1	LITAF	lipopolysaccharide-induced TNF factor	1.78	0.000316
TC01002287.hg.1	PADI2	peptidyl arginine deiminase, type II	1.77	0.000015
TC01003500.hg.1	SELL	selectin L	1.77	0.012469
TC10000333.hg.1	RNA5SP316	RNA, 5S ribosomal pseudogene 316	1.73	0.005712
TC13000871.hg.1	IRS2	insulin receptor substrate 2	1.73	0.000107
TC16000766.hg.1	MSRB1	methionine sulfoxide reductase B1	1.73	0.000051
TC01000261.hg.1	CDA	cytidine deaminase	1.72	0.000002
TC10000293.hg.1	ALOX5	arachidonate 5-lipoxygenase	1.72	0.003936
TC11001833.hg.1	FABP5P7	fatty acid binding protein 5 pseudogene 7	1.71	0.038894
TC13000642.hg.1	LCP1	lymphocyte cytosolic protein 1 (L-plastin)	1.71	0.005125
TC03000630.hg.1	CSTA	cystatin A (stefin A)	1.7	0.00088
TC19001180.hg.1	HSPC102	uncharacterized LOC105372274	1.7	0.007931
TC05000336.hg.1	MAP1B	microtubule associated protein 1B	1.69	0.001559
TC21000167.hg.1	KCNJ15	potassium channel, inwardly rectifying subfamily J, member 15	1.69	0.008323
TC01006192.hg.1	EGLN1	egl-9 family hypoxia-inducible factor 1	1.68	0.0031
TC02004970.hg.1	MXD1	MAX dimerization protein 1	1.68	0.001508
TC01003240.hg.1	S100A11	S100 calcium binding protein A11	1.67	0.00049
TC01003616.hg.1	NCF2	neutrophil cytosolic factor 2	1.67	0.000343
TC08000402.hg.1	UBXN2B	UBX domain protein 2B	1.67	0.000054
TC12001269.hg.1	PLBD1	phospholipase B domain containing 1	1.67	0.00783
TC07002222.hg.1	CREB5	cAMP responsive element binding protein 5	1.66	0.016183
TC12000130.hg.1	CLEC4D	C-type lectin domain family 4, member D	1.66	0.000837
TC19001241.hg.1	ADGRE3	adhesion G protein-coupled receptor E3	1.65	0.018173
TC02002637.hg.1	STK17B	serine/threonine kinase 17b	1.62	0.001018
TC19000885.hg.1	FCAR	Fc fragment of IgA receptor	1.62	0.000478
TC02000237.hg.1	QPCT	glutaminyl-peptide cyclotransferase	1.6	0.000079
TC08000487.hg.1	LY96	lymphocyte antigen 96	1.6	0.001759
TC12000730.hg.1	PLXNC1	plexin C1	1.6	0.02873
TC01006307.hg.1	FCGR3A	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)	1.59	0.004299
TC20000363.hg.1	MMP9	matrix metallopeptidase 9	1.58	0.000585
TC10001958.hg.1	RAB18	RAB18, member RAS oncogene family	1.57	0.004852
TC14001036.hg.1	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	1.56	0.025697
TC19001640.hg.1	PGLYRP1	peptidoglycan recognition protein 1	1.56	0.000068
TC01001738.hg.1	CR1	complement component (3b/4b) receptor 1 (Knops blood group)	1.55	0.031704
TC04000856.hg.1	SAP30	Sin3A associated protein 30kDa	1.55	0.000312
TC12001368.hg.1	H3F3C	H3 histone, family 3C	1.55	0.000386
TC12001187.hg.1	A2M	alpha-2-macroglobulin	1.54	0.003505
TC02000432.hg.1	DYSF	dysferlin	1.53	0.000451
TC09001473.hg.1	TXN	thioredoxin	1.53	0.001651
TC12001027.hg.1	GLT1D1	glycosyltransferase 1 domain containing 1	1.53	0.003599
TC12001842.hg.1	HAL	histidine ammonia-lyase	1.53	0.001073
TC04001366.hg.1	SPARCL1	SPARC like 1	1.51	0.004446
TC10000360.hg.1	UBE2D1	ubiquitin conjugating enzyme E2D 1	1.51	0.000725
TC17001341.hg.1	EVI2B	ecotropic viral integration site 2B	1.51	0.034717
TC02001911.hg.1	PELI1	pellino E3 ubiquitin protein ligase 1	1.5	0.008062
TC14000070.hg.1	RNASE6	ribonuclease, RNase A family, k6	1.5	0.040643
TC16000812.hg.1	MEFV	Mediterranean fever	1.5	0.011693
TC16000879.hg.1	CPPED1	calcineurin-like phosphoesterase domain containing 1	1.5	0.028903

Supplemental Table 10. Differentially expressed genes. Genes down-regulated in patients with STEMI in the comparison between STEMI and sCAD.

Transcript Cluster ID	Gene Symbol	Description	Fold Change (linear) (M vs. C)	ANOVA p-value (M vs. C)
TC09001698.hg.1	RNU6ATAC	RNA, U6atac small nuclear (U12-dependent splicing)	-3.09	0.038323
TC15002175.hg.1	KRTAP6-1	keratin associated protein 6-1	-2.08	0.014975
TC17001015.hg.1	ATP2A3	ATPase, Ca++ transporting, ubiquitous	-1.96	0.046252
TC09001056.hg.1	RMRP	RNA component of mitochondrial RNA processing endoribonuclease	-1.94	0.043824
TC01005458.hg.1	TAL1	T-cell acute lymphocytic leukemia 1	-1.91	0.021614
TC15000870.hg.1	FURIN	furin (paired basic amino acid cleaving enzyme)	-1.82	0.016446
TC17002123.hg.1	MAP2K3	mitogen-activated protein kinase kinase 3	-1.79	0.011628
TC17001885.hg.1	UNC13D	unc-13 homolog D (C. elegans)	-1.66	0.036061
TC05003305.hg.1	CD74	CD74 molecule, major histocompatibility complex, class II invariant chain	-1.65	0.005547
TC22001326.hg.1	MYH9	myosin, heavy chain 9, non-muscle	-1.65	0.040705
TC11002092.hg.1	ARRB1	arrestin, beta 1	-1.64	0.0468
TC11000374.hg.1	CD82	CD82 molecule	-1.63	0.002587
TC15002610.hg.1	PKM	pyruvate kinase, muscle	-1.63	0.016613
TC19000009.hg.1	BSG	basigin (Ok blood group)	-1.63	0.03403
TC01004503.hg.1	PRKACB	protein kinase, cAMP-dependent, catalytic, beta	-1.62	0.011993
TC02001303.hg.1	VIL1	villin 1	-1.61	0.005089
TC19000029.hg.1	HMHA1	histocompatibility (minor) HA-1	-1.61	0.023424
TC15002368.hg.1	FURIN	furin (paired basic amino acid cleaving enzyme)	-1.59	0.015616
TC19001553.hg.1	TGFB1	transforming growth factor beta 1	-1.56	0.036929
TC19001291.hg.1	FKBP8	FK506 binding protein 8	-1.54	0.032919
TC17000297.hg.1	MAP2K3	mitogen-activated protein kinase kinase 3	-1.53	0.013912
TC08002364.hg.1	MYBL1	v-myb avian myeloblastosis viral oncogene homolog-like 1	-1.52	0.022026
TC15000872.hg.1	MAN2A2	mannosidase, alpha, class 2A, member 2	-1.52	0.024624
TC19000373.hg.1	GATAD2A	GATA zinc finger domain containing 2A	-1.51	0.0131

Supplemental Table 11. DAVID analysis: identification of the biological processes related to up-regulated genes in the comparison between patients with STEMI and sCAD

Term	Genes in the category	P-Value	Benjamini
innate immune response	CLEC4D; CLEC4E; S100A12; S100A8; S100A9; CR1; EIF2AK2; LY96; MSRB1; NCF1C; NCF2; PGLYRP1; TLR4; TREM1	6,6E-7	4,8E-4
inflammatory response	CXCR1; CXCR2; MEFV; S100A12; S100A8; S100A9; TNFAIP6; C5AR1; FPR1; FPR2; IL18RAP; LY96; TLR4	1,2E-6	4,3E-4
cellular defense response	CXCR2; C5AR1; LY96; MNDA; NCF1; NCF2	2,1E-5	5,1E-3
neutrophil chemotaxis	CXCR2; S100A12; S100A8; S100A9; C5AR1; TREM1	3,0E-5	5,6E-3
respiratory burst	NCF1; NCF1B; NCF1C; NCF2	5,6E-5	8,1E-3
positive regulation of NF-kappaB transcription factor activity	NFKB1A; S100A12; S100A8; S100A9; EIF2AK2; IRAK3; TLRK4	9,4E-5	1,1E-2
superoxide metabolic process	NCF1; NCF1B; NCF1C; NCF2	1,7E-4	1,8E-2
immune response	FCAR; FCGR3A; FCGRB; AQP9; C5AR1; IL1R2; IL18RAP; NCF4; PGLYRP1; TLR4	4,9E-4	4,4E-2

response to lipopolysaccharide	S100A8; C5AR1; EIF2AK2; IRAK3; LY96; PELI1; TLR4	5,4E-4	4,3E-2
leukocyte migration	C5AR1; FPR1; FPR2; MMP9; SELL; TREM1	5,8E-4	4,1E-2

Supplemental Table 12. DAVID analysis: identification of the biological processes related to down-regulated genes in the comparison between patients with STEMI and sCAD.

Term	Genes in the category	P-Value	Benjamini
activation of MAPK activity	CD74; ARRB1; MAP2K3	5,0E-3	8,7E-1
leukocyte migration	CD74; BSG; MYH9	6,7E-3	7,4E-1
positive regulation of transcription, DNA-templated	MYBL1; TAL1; MAP2K3: TGFB1	1,4E-2	8,5E-1
positive regulation of ERK1 and ERK2 cascade	CD74; ARRB1; TGFB1	1,4E-2	7,6E-1
positive regulation of cell migration	FURIN; TGFB1; VIL1	1,5E-2	7,1E-1
platelet formation	TAL1; MYH9	1,8E-2	7,0E-1
positive regulation of histone acetylation	ARRB1; TGFB1	1,9E-2	6,7E-1
positive regulation of protein complex assembly	TAL1; TGFB1	2,0E-2	6,4E-1
ATP biosynthetic process	PKM; TGFB1	2,9E-2	7,3E-1
positive regulation of cell division	TAL1; TGFB1	4,7E-2	8,5E-1

Supplemental Table 13. Perfomance of classification of each putative-gene signatures using kNN algoritm

Genes	#gene	#missclassification	Sensitivity	Specificity
F.C.2 p0001	26	0.15	0.862	0.831
F.C.2 p001	38	0.15	0.885	0.786
F.C. 2 p005	46	0.179	0.875	0.751
F.C. 2.5 p0001	16	0.144	0.872	0.834
F.C. 2.5 p001	22	0.183	0.85	0.775
F.C. 2.5 p005	24	0.199	0.849	0.736
F.C 1.5 p005	138	0.177	0.846	0.792

Supplemental Table 14. List of genes in the comparison between patients with STEMI and sCAD using a fold change of 2 and a p.value ≤ 0.01

Transcript Cluster ID	Gene Symbol
TC01003260.hg.1	S100A12
TC12001178.hg.1	CLEC4E
TC01001624.hg.1	RGS2
TC06003855.hg.1	VNN2
TC15000441.hg.1	AQP9
TC01001346.hg.1	MNDA
TC07002399.hg.1	NCF1B
TC19002237.hg.1	FCAR
TC06002121.hg.1	VNN2
TC07000438.hg.1	NCF1B;

	NCF1
TC07001738.hg.1	NAMPT
TC01001254.hg.1	S100A9
TC07003026.hg.1	NCF1C
TC21001069.hg.1	SAMSN1
TC04000072.hg.1	S100P
TC13000100.hg.1	ALOX5AP
TC07001516.hg.1	NCF1C
TC01006308.hg.1	FCGR3B
TC04001809.hg.1	ACSL1
TC06001717.hg.1	TREM1
TC19001787.hg.1	FPR1
TC19000676.hg.1	C5AR1
TC01003261.hg.1	S100A8
TC02000978.hg.1	GCA
TC22000270.hg.1	NCF4
TC02000619.hg.1	IL1R2
TC05002434.hg.1	MAP1B
TC06003854.hg.1	VNN3
TC01000510.hg.1	SMAP2
TC07000899.hg.1	MGAM
TC19000788.hg.1	FPR2
TC12000309.hg.1	LRRK2
TC09001187.hg.1	LINC01506
TC02002767.hg.1	CXCR1
TC06002120.hg.1	VNN3
TC20000587.hg.1	RASSF2
TC03000846.hg.1	MME
TC09000601.hg.1	TLR4

Supplemental Table 15. List of genes in the comparison between patients with STEMI and sCAD using a fold change of 2 and p.value ≤ 0.01 and annotation with Panther Family/Subfamily

Gene ID	Gene Name/ Gene Symbol	Panther Family/ Subfamily
CLEC4E	C-type lectin domain family 4 member E;CLEC4E;ortholog	C-TYPE LECTIN DOMAIN FAMILY 4 MEMBER E (PTHR22802:SF274)
S100A9	Protein S100-A9;S100A9;ortholog	PROTEIN S100-A9 (PTHR11639:SF110)
AQP9	Aquaporin-7;AQP7;ortholog	AQUAPORIN-7 (PTHR43829:SF15)
S100A12	Protein S100-A12;S100A12;ortholog	PROTEIN S100-A12 (PTHR11639:SF104)
TREM1	Triggering receptor expressed on myeloid cells 1;TREM1;ortholog	TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS 1 (PTHR19357:SF4)

FCAR	Immunoglobulin alpha Fc receptor;FCAR;ortholog	IMMUNOGLOBULIN ALPHA FC RECEPTOR (PTHR11738:SF125)
FPR2	N-formyl peptide receptor 2;FPR2;ortholog	N-FORMYL PEPTIDE RECEPTOR 2 (PTHR24225:SF39)
MME	Macrophage metalloelastase;MMP12;ortholog	MACROPHAGE METALLOELASTASE (PTHR10201:SF207)
ALOX5AP	Arachidonate 5-lipoxygenase-activating protein;ALOX5AP;ortholog	ARACHIDONATE 5-LIPOXYGENASE-ACTIVATING PROTEIN (PTHR10250:SF25)
TLR4	Toll-like receptor 4;TLR4;ortholog	TOLL-LIKE RECEPTOR 4 (PTHR24365:SF494)
MNDA	Myeloid cell nuclear differentiation antigen;MNDA;ortholog	MYELOID CELL NUCLEAR DIFFERENTIATION ANTIGEN (PTHR12200:SF20)
SMAP2	Bromodomain-containing protein 8;BRD8;ortholog	BROMODOMAIN-CONTAINING PROTEIN 8 (PTHR15398:SF8)
NCF4	Neutrophil cytosol factor 4;NCF4;ortholog	NEUTROPHIL CYTOSOL FACTOR 4 (PTHR10663:SF249)
VNN2	Vascular non-inflammatory molecule 2;VNN2;ortholog	VASCULAR NON-INFLAMMATORY MOLECULE 2 (PTHR10609:SF22)
S100A8	Protein S100-A8;S100A8;ortholog	PROTEIN S100-A8 (PTHR11639:SF111)
LRRK2	Leucine-rich repeat serine/threonine-protein kinase 2;LRRK2;ortholog	LEUCINE-RICH REPEAT SERINE/THREONINE-PROTEIN KINASE 2 (PTHR23257:SF669)
FCGR3B	Low affinity immunoglobulin gamma Fc region receptor III-B;FCGR3B;ortholog	LOW AFFINITY IMMUNOGLOBULIN GAMMA FC REGION RECEPTOR III-A-RELATED (PTHR11481:SF78)
FPR1	fMet-Leu-Phe receptor;FPR1;ortholog	FMET-LEU-PHE RECEPTOR (PTHR24225:SF41)
MME	Neprilysin;MME;ortholog	NEPRILYSIN (PTHR11733:SF163)
MGAM	Maltase-glucoamylase, intestinal;MGAM;ortholog	MALTASE-GLUCOAMYLASE, INTESTINAL (PTHR22762:SF95)
VNN3	Vascular non-inflammatory molecule 3;VNN3;ortholog	VASCULAR NON-INFLAMMATORY MOLECULE 3 (PTHR10609:SF20)
MAP1B	Microtubule-associated protein 1B;MAP1B;ortholog	MICROTUBULE-ASSOCIATED PROTEIN 1B (PTHR13843:SF15)
S100P	Protein S100-P;S100P;ortholog	PROTEIN S100-P (PTHR11639:SF103)
AQP9	Aquaporin-9;AQP9;ortholog	AQUAPORIN-9 (PTHR43829:SF8)
RASSF2	Ras association domain-containing protein 2;RASSF2;ortholog	RAS ASSOCIATION DOMAIN-CONTAINING PROTEIN 2 (PTHR22738:SF16)
CXCR1	C-X-C chemokine receptor type 1;CXCR1;ortholog	C-X-C CHEMOKINE RECEPTOR TYPE 1-RELATED (PTHR10489:SF858)
GCA	Grancalcin;GCA;ortholog	GRANCALCIN (PTHR10183:SF356)
SMAP2	Stromal membrane-associated protein 2;SMAP2;ortholog	STROMAL MEMBRANE-ASSOCIATED PROTEIN 2 (PTHR23180:SF355)
SAMSN1	SAM domain-containing protein SAMSN-1;SAMSN1;ortholog	SAM DOMAIN-CONTAINING PROTEIN SAMSN-1 (PTHR12301:SF13)
IL1R2	Interleukin-1 receptor type 2;IL1R2;ortholog	INTERLEUKIN-1 RECEPTOR TYPE 2

		(PTHR11890:SF39)
NCF1B	Putative neutrophil cytosol factor 1B;NCF1B;ortholog	NEUTROPHIL CYTOSOL FACTOR 1-RELATED (PTHR15706:SF14)
C5AR1	C5a anaphylatoxin chemotactic receptor 1;C5AR1;ortholog	C5A ANAPHYLATOXIN CHEMOTACTIC RECEPTOR 1 (PTHR24225:SF31)
NCF1C	Putative neutrophil cytosol factor 1C;NCF1C;ortholog	NEUTROPHIL CYTOSOL FACTOR 1-RELATED (PTHR15706:SF14)
NCF1	Neutrophil cytosol factor 1;NCF1;ortholog	NEUTROPHIL CYTOSOL FACTOR 1-RELATED (PTHR15706:SF14)
NAMPT	Nicotinamide phosphoribosyltransferase;NAMPT;ortholog	NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (PTHR43816:SF1)
ACSL1	Long-chain-fatty-acid--CoA ligase 1;ACSL1;ortholog	LONG-CHAIN-FATTY-ACID--COA LIGASE 1 (PTHR43272:SF3)
RGS2	Regulator of G-protein signaling 2;RGS2;ortholog	REGULATOR OF G-PROTEIN SIGNALING 2 (PTHR10845:SF218)
LINC01506		

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