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ORIGINAL RESEARCH ARTICLE

# Role of lncRNAs in Remodeling of the Coronary Artery Plaques in Patients with Atherosclerosis

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## Abstract

**Introduction** Cardiovascular diseases (CVDs) are the leading cause of death worldwide according to World Health Organization (WHO) data. Atherosclerosis is considered as a chronic inflammatory disease that develops in response to damage to the vascular intima-media layer in most cases. In recent years, epigenetic events have emerged as important players in the development and progression of CVDs. Since noncoding RNA (ncRNAs) are important regulators in the organization of the pathophysiological processes of the cardiovascular system, they have the potential to be used as therapeutic targets, diagnostic and prognostic biomarkers. In this study long noncoding RNA (lncRNA) and mRNA gene expression were compared between coronary atherosclerotic plaques (CAP) and the internal mammary artery (IMA) which has the same genetic makeup and is exposed to the same environmental stress conditions with CAP in the same individual.

**Methods** lncRNA and mRNA gene expressions were determined using the microarray in the samples. Microarray results were validated by RT-qPCR. Differentially expressed genes (DEGs; lncRNAs and mRNAs) were determined by GeneSpring (Ver 3.0) [$p$ values $< 0.05$ and fold change (FC) $> 2$]. DAVID bioinformatics program was used for Gene Ontology (GO) annotation and enrichment analyses of statistically significant genes between CAP and IMA tissue.

**Results and Conclusions** In our study, 345 DEGs were found to be statistically significant ($p < 0.05$; FC $> 2$) between CAP and IMA. Of these, 65 were lncRNA and 280 were mRNA. Thirty-three lncRNAs were upregulated, while 32 lncRNAs were downregulated. Some of the important mRNAs are SPP1, CYP4B1, CHRDL1, MYOC, and ALKAL2, while some of the lncRNAs are LOC105377123, LINC01857, DIO3OS, LOC101928134, and KCNA3 between CAP and IMA tissue. We also identified genes that correlated with statistically significant lncRNAs. The results of this study are expected to be an important source of data in the development of new genetically based drugs to prevent atherosclerotic plaque. In addition, the data obtained may contribute to the explanation of the epigenetic mechanisms that play a role in the pathological basis of the process that protects the IMA from atherosclerosis.

**Key Points**

We present a group of coding and noncoding gene sets that have important effect on the formation or prevention of atherosclerotic plaques.

Novel therapeutic approaches that would involve these targets can be developed to prevent or cure the disease.

## 1 Introduction

World Health Organization (WHO) data indicate that 31% of the worldwide deaths stem from cardiovascular diseases (CVDs). The yearly death due to CVDs is around 17.5 million, and this number is expected to rise steadily. Within the European Union region the cost of CVDs is around 210 million euros, placing a heavy burden on the public health systems [1]. Due to increasing tendencies in risk factors for CVDs, including hypertension, diabetes, malnutrition, obesity, smoking, and lack of physical activities, CVDs are among the main health issues [2].

Atherosclerosis is the most common type of CVD. It is a vascular disease that has been characterized by endothelial activation and dysfunction, lipid accumulation, monocyte

Extended author information available on the last page of the article

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infiltration and differentiation, T-cell infiltration and activation, foam cell formation from macrophages, and fibrosis. Due to these characteristics, atherosclerosis is a complex disease that involves vascular, metabolic, and immune components during its development [3]. At the start of atherogenesis, extracellular lipid accumulation on the vascular wall stimulates endothelial deterioration. The monocytes migrate to the arterial walls as a result of lipid accumulation, transform into macrophages, and start expressing radical scavenger receptors on their surfaces. These radical scavenger receptors enable lipoprotein uptake, which leads to lipid accumulation in the macrophages and eventually the formation of foam cells. These cells on the vascular wall produce and secrete different cytokines and growth factors. These cytokines and growth factors lead to smooth muscle cell (SMC) migration and proliferation. Due to this inflammatory process the fibrous cap may get disrupted and plugged, and acute coronary syndromes (ACS) may develop [4].

Bypass surgery is a method that grafts blood vessels to the plugged coronary arteries to regulate the aberrant blood flow. These grafts enable novel paths for the flow of the blood to the heart bypassing the plugged coronary arteries. The internal mammary artery (IMA) is an artery that is protected from atherosclerosis despite the same genetic background and risk factors that it has been exposed to with CAP. Literature has suggested that grafts taken from the IMAs of patients whose coronaries have atherosclerotic involvement have the most compatible and ideal grafts in bypass surgeries based on histopathological, angiographical, and Doppler ultrasonic analysis [5]. Therefore we compared plaque-developing CAP tissue with non-plaque-developing IMA of the same individual.

The human genome is composed of approximately 3300 Mb of nuclear DNA, 1% of which comprises protein-encoding sequences and 99% of which comprises non-protein-encoding sequences. The noncoding RNA (ncRNA) can be classified as long ncRNAs ($\geq$ 200 nt) and small ncRNAs ($\leq$ 200 nt) in accordance with the length of the RNA sequences. Long noncoding RNAs (lncRNAs) can be divided into three general groups: long intergenic noncoding (linc)RNAs, natural antisense transcripts (NATs), and intronic lncRNAs. In current literature, it is suggested that these lncRNAs include approximately 20,000 transcripts [6]. lncRNAs play an important role in the process of atherosclerosis, remodeling of endothelial cells, proliferation and migration of SMCs, inflammatory response of macrophages, and plaque formation. In recent years, it is thought that some lncRNAs can be used as therapeutic targets and prognostic markers for disease progression [7]. In our previous study ANRIL, MALAT1, and MIAT gene expression was found to be statistically significant between IMA and CAP tissue [8]. Another study revealed that lncRNA NEXN-AS1 plays a preventive role against atherosclerosis by affecting TLR4 oligomerization and NF-kB activity and monocyte activation in endothelial cells [9]

In our study, differentially expressed gene (DEG) expression levels were compared between the CAP and non-atherosclerotic IMAs of patients who had similar environmental stress conditions with similar genetic backgrounds. In this way, we present a group of coding and noncoding gene sets that have an important effect on the formation or prevention of atherosclerotic plaques. Novel therapeutic approaches involving these targets can be developed to prevent or cure the disease.

## 2 Materıals and Methods

### 2.1 Study Population

In our study, we perform microarray analysis on 32 samples consisting of 16 IMA and 16 CAP tissue samples that were taken from Sivas Cumhuriyet University Research Hospital Cardiovascular Surgery Department. The patients had bypass surgery, and the samples were taken from their IMA and CAP tissues on the basis of the ethical permissions (Sivas Cumhuriyet University Clinical Research Ethics Committee decision number: 2018–09/150 and 2018–04/18). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent form was obtained from all volunteers. Patients samples were taken by following the previously characterized protocols by coronary endarterectomy [10]. Coronary endarterectomy was applied to vessels with an outer diameter of at least 2.0 mm in which coronary vessels are completely occluded and bypassing would not be possible if endarterectomy was not performed during bypass. All the pathologic specimens were obtained from coronary endarterectomy materials. As reported in our previous papers (8; 10), the main indication of coronary endarterectomy was the presence of diffusely diseased coronary artery, not suited for distal grafting during coronary artery bypass graft (CABG) procedure. Histopathologic examination by expert pathologists confirmed the presence of atherosclerotic plaque, and then these tissues were further analyzed. Of note, diagnostic yield of fully calcific stenoses with complete obstruction was very low due to paucity of tissues. Mixed atherosclerotic plaques, mainly from left anterior descending or right coronary artery (circumflex in a few) along with significant diameter stenosis, yielded best tissue sampling results for this analysis. Tissue samples were taken into RNAlater (RNA-protective agents) to protect the RNA samples in these tissues.

### 2.2 Experimental Protocol

Tissue samples were stored at –80 °C in RNAlater up to homogenization and RNA isolation. RNA samples were extracted according to the protocol of RNeasy Mini Kit (Qiagen, cat. no. 217004). Before the isolation step, tissue samples were weighed as 40 mg with precision balance. They were homogenized with 700 µL TRIzol (ThermoFisher, cat. no. 15596026) in MagNa Lyser Green Beads (Roche, product no. 03358941001) twice at 7000*g* for 30 s (MagNA Lyser, Roche; cat. no. 03358976001). After each homogenization process the samples were kept at room temperature for 5 min. Finally, RNA was eluted using RNeasy Mini Kit. RNA concentration were measured using a flow meter (Qubit ver. 3.0) for RT-qPCR. RNA integrity number (RIN) value was determined with Bioanalyzer (Agilent 2100) for microarray experiments.

Microarray was performed by using SurePrint G3 Human Gene Expression v3 8x60K Microarray Kit (G4851C) (https://earray.chem.agilent.com/earray/). RNA samples were labeled and processed according to the manufacturer's recommended protocols. Total RNA (100 ng) was dephosphorylated using calf intestinal ALP. These samples were then heat denatured using dimethyl sulfoxide. Microarray analysis was composed of four steps: sample preparation, labeling, hybridization, and washing. All steps were performed according to the standard protocol recommended by Agilent Technologies. Finally microarray slides were scanned using microarray scanner (Agilent Microarray Scanner G2565A). Agilent Feature Extraction software (version 10.5.1) was used for image analysis.

### 2.3 RT-qPCR Validation

The standard protocols were followed to generate the cDNAs (A.B.T. cat. no. C03–01–05) from RNA samples. RT-qPCR was conducted for five statistically significant DEGs. These genes are SPP1, MMP12, KCNA3 (mRNA), IRX 1, and MALAT 1 (lncRNA). GAPDH was used as the endogenous control in RT-qPCR standard protocol was used for RT-qPCR (A.B.T. cat. no. Q03-02-01).

### 2.4 Statistical and Bioinformatic Analysis

All statistic and bioinformatic analysis of the study population was performed between the CAP and IMA tissues. Microarray results were analyzed by GeneSpring GX 10.0 Software (Agilent). Microarray expression data were normalized by quantile methods. We used Bonferroni correction for multiple comparisons. Gene Ontology (GO) analysis of mRNAs was performed using DAVID bioinformatics software [GO term $p$ values $< 0.05$ and a false discovery rate (FDR) $< 0.05$]. GO analysis determined the enrichment of DEGs including biological processes (BPs) and molecular functions (MFs). Also, the target genes of statistically significant DEGs were submitted to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database for pathway analysis across groups. The RT-qPCR results were uploaded onto the "Data Analysis Center" (https://www.qiagen.com/de/shop/genes-and pathways/data-analysis-center-overview-page/), and the analysis was performed according to the ΔΔCq method. The $p$ value was calculated on the basis of the Student's $t$-test for each gene studied in CAP and IMA. $p < 0.05$ was considered statistically significant.

## 3 Results

In this study, 32 tissues (CAP and IMA) of 16 patients were used for microarray analysis. All patients (100%) in the study group had at least one bypass surgery. The average age of the study group was 62 years. In terms of sex distribution, 13 of 16 individuals were male and 3 were female. Ninety percent of the study group had hyperlipidemia, and 80% had hypertension. Eight individuals have a previous history of myocardial infarction (MI) and diabetes. Thirteen individuals have a smoking habit. Median of high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol, and triglyceride was 39, 144, 190, and 254, respectively. According to the New York Heart Association (NYHA) classification, nine patients were in class 3, while seven of them were in class 2. Median of both right and left coronary artery stenosis was 80%.

When the CAP and IMA groups were compared, 439 genes specifically expressed only in the CAP, 2170 genes were specific to the IMA, and the expression of 13,047 overlapping genes was detected in both tissue samples (Fig. 1A). A Scatter plot of the DEGs between CAP and IMA is shown in Fig. 1B. In our study, 345 DEGs were significantly different between CAP and IMA [$p < 0.05$; fold change (FC) $> 2$]. Sixty-five DEGs were lncRNAs, and 280 were mRNAs. Thirty-three lncRNAs were upregulated, whereas 32 lncRNAs were downregulated (Table 1). While some mRNAs that had the highest FC are SPP1, CYP4B1, CHRDL1, MYOC, and ALKAL2, lncRNAs that had the highest FC are LOC105377123, LINC01857, DIO3OS, LOC101928134, KCNA3, KMT2E-AS1, KRT18P55, LOC729966, SFTA1P, HOTAIRM1, and MALAT1 (Table 1). In addition, genes with the highest FC were correlated with differentially expressed lncRNAs (Table 2).

We performed GO analysis to identify the BP (Fig. 2A, D), MF (Fig. 2B, E pathways and KEGG pathways (Fig. 2E, F) associated with statistically significant downregulated and upregulated genes. DAVID 6.8 Bioinformatics software (https://david.ncifcrf.gov/tools.jsp date of access: 20 April 2022) (GO $p$ values $< 0.05$ and FDR

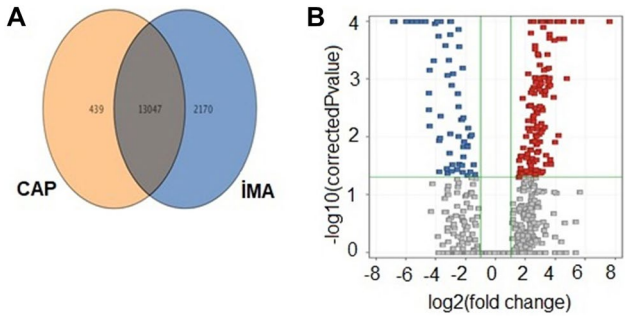


**Fig. 1.** Venn diagram showing number of genes overlapped in CAP and IMA (**A**). Scatter plot of differentially expressed genes between CAP and IMA (**B**). The total number of genes expressed in CAP and IMA was 13486 and 15,217, respectively. A total of 439 genes were expressed only in CAP, whereas 2170 genes were detected only in IMA (**A**). Spots above the horizontal midline represent statistically significant DEGs. FC < 1 and FC > 1 represent the downregulated and upregulated significant DEGs, respectively. *FC* fold change (**B**)

< 0.05) was used for GO analysis of the statistically significant DEGs. Expected mRNA and lncRNA targets were grouped as BP and MF for GO analysis. Wnt signaling pathway and endothelial cell activation (Fig. 3A) as well as positive regulation of macrophage fusion (Fig. 2D) were some of the significant GO terms in terms of BP. Some significant GO terms of downregulated and upregulated genes in terms of MF were heparin binding (Fig. 2B) and scavenger receptor activity (Fig. 2E). KEGG pathways such as MAPK signaling pathway were detected in connection to the downregulated genes (Fig. 2C). CD4 receptor binding and scavenger receptor activity were some of the KEGG pathways associated with the upregulated genes (Fig. 2F). Also in this study we detected mRNAs with high correlation of lncRNAs. Statistically significant five lncRNAs correlated with mRNAs are presented in Table 2.

When these genes were further studied by RT-qPCR, the results were in the same direction with microarray findings (Fig. 3 and Table 3). While SPP1 (FC 7.54), MMP12 (FC 4.35), and KCNA3 (FC 2.18) were upregulated, IRX1 (FC −2.43) and MALAT1 (FC −3.47) were downregulated in CAP compared with IMA (Fig. 3 and Table 3).

**Table 1.** Statistically significant DEGs as a result of CAP–IMA tissue group comparison

| mRNA | Fold change | Regulation | lncRNA | Fold change | Regulation |
|---|---|---|---|---|---|
| SPP1 | 260.62 | Up | LOC105377123 | 22.98 | Up |
| CYP4B1 | 157.37 | Down | LINC01857 | 21.93 | Up |
| CHRDL1 | 138.11 | Down | DIO3OS | 20.19 | Down |
| MYOC | 122.77 | Down | LOC101928134 | 12.98 | Down |
| ALKAL2 | 77.16 | Down | KCNA3 | 10.55 | Up |
| IRX1 | 61.79 | Down | KMT2E-AS1 | 9.87 | Down |
| FBLN1 | 55.73 | Down | KRT18P55 | 9.04 | Up |
| MMP12 | 55.37 | Up | LOC729966 | 8.22 | Down |
| CAPN6 | 55.31 | Down | SFTA1P | 8.20 | Up |
| MLC1 | 38.79 | Up | HOTAIRM1 | 7.29 | Down |
| FOS | 37.58 | Down | MALAT1 | 7.15 | Down |
| FAM180B | 37.14 | Down | LINC00426 | 7.05 | Up |
| KCNK2 | 32.49 | Down | LINC00603 | 7.02 | Down |
| THSD4 | 31.91 | Down | LOC100506725 | 6.11 | Up |
| HOXA6 | 31.54 | Down | PCED1B-AS1 | 6.00 | Up |
| ASPA | 29.88 | Down | RPS16P5 | 5.61 | Down |
| CRABP1 | 29.57 | Down | LINC00607 | 5.29 | Up |
| NR4A3 | 29.38 | Down | LOC100506100 | 5.13 | Up |
| HOXA6 | 29.19 | Down | lnc-FRK-1 | 5.01 | Down |
| S100P | 26.95 | Down | LOC102724849 | 4.54 | Up |
| APOC2 | 26.31 | Up | DUXAP9 | 4.31 | Up |
| TREM2 | 21.45 | Up | LINC01546 | 4.11 | Up |
| SLAMF8 | 20.37 | Up | lnc-PUM2-1 | 4.10 | Down |
| GJA3 | 19.82 | Up | LOC101926963 | 3.91 | Up |
| SFRP1 | 19.79 | Down | LINC01091 | 3.89 | Up |

All DEGs are in Supplementary File 1

**Table 2.** The highest correlated genes with up- and downregulated lncRNAs

| lncRNA (up) | Correlated gene | Correlation value | lncRNA (down) | Correlated gene | Correlation value |
|---|---|---|---|---|---|
| | CAPN6 | −0.963 | | NRN1 | 0.964 |
| | HOXA6 | −0.956 | | CYP4B1 | 0.959 |
| LOC105377123 | CATSPER1 | 0.941 | DIO3OS | PDE1C | 0.957 |
| | HOXA10 | −0.933 | | HOXA7 | 0.949 |
| | DUSP1 | −0.931 | | IRX1 | 0.947 |
| | HAVCR2 | 0.9595 | | MALAT1 | 0.941 |
| | MSR1 | 0.959 | | FAM180B | 0.938 |
| LINC01857 | MMP12 | 0.950 | KMT2E-AS1 | FBLN1 | 0.936 |
| | ABCG1 | 0.949 | | CCNL1 | 0.935 |
| | GALNT6 | 0.944 | | CHRDL1 | 0.934 |
| | C15orf48 | 0.909 | | IRX1 | 0.948 |
| | IL17B | −0.886 | | FBLN1 | 0.946 |
| LINC02244 | APOC2 | 0.884 | HOTAIRM1 | CAPN6 | 0.939 |
| | SCUBE3 | −0.882 | | KCNK2 | 0.935 |
| | GARNL3 | −0.879 | | KRT18P55 | − 0.934 |
| | LTB | 0.954 | | KCNK2 | 0.966 |
| | C4orf48 | 0.945 | | CCNL1 | 0.959 |
| KCNA3 | GATA3 | 0.944 | MALAT1 | CAPN6 | 0.948 |
| | DOCK8 | 0.944 | | GUCA2B | 0.946 |
| | NLRC3 | 0.943 | | FBLN1 | 0.945 |
| | TBX1 | −0.925 | | BTC | 0.930 |
| | LOC105377123 | 0.917 | | SYTL4 | 0.920 |
| ANK3 divergent transcript | ALKAL2 | −0.898 | lnc-FRK-1 | ADRA1B | 0.910 |
| | IRX1 | −0.894 | | THSD4 | 0.900 |
| | KRT18P55 | 0.890 | | GARNL3 | 0.890 |

All correlated genes are in Supplementary File 3

## 4 Discussion

CVDs were previously a major problem only for the western part of the world, whereas nowadays they have become a global problem with the increasing life standards. Atherosclerosis is the main cause of many diseases, including but not limited to myocardial infarction, arrhythmias, ischemic cardiomyopathy, paralysis, and peripheral artery disease [11]. Although we have more knowledge about the disease mechanism of atherosclerosis, we are still at the initial stages of the detection of effective and low-cost epigenetic targets for therapeutic purposes.

To have a better understanding of the development of atherosclerosis, deciphering the epigenetic factors involved in the process will be vital to developing novel drug targets or therapy regimens [12]. Khyzha and his colleagues (2017) suggested that in vitro human cell culture models will be beneficial to determining the genes that are involved in the development of atherosclerosis. In addition, they suggested that finding ncRNA-based therapeutic agents that will target the epigenetic pathways involved in the CVDs in tissue and cell culture studies will be crucial to developing better therapy methods against CVDs [12].

lncRNAs have been shown to play major roles in these pathways [13]. Disturbed lncRNA expression has been shown to affect the effector molecules in certain pathways that have roles in the development of CVDs. Therefore, determining the functional lncRNAs in CVDs will be informative for the development of novel therapy approaches. lncRNAs affect the cellular changes in the vascular walls and therefore have crucial roles in atherosclerosis [14]. Previous studies have shown that interaction of the inflammatory processes with the cardiac risk factors leads to the development of the cardiovascular disorders [15]. lncRNAs are considered as one of the main players in the regulatory pathways that play role in the homeostasis of the vascular walls and in the development of the atherosclerosis if they are not functioning properly. Therefore, they can be efficient therapeutic targets. In our study, microarray analysis was used for CAP tissue samples that are part of the plaque- and the non-plaque-forming tissues from patients with atherosclerosis during bypass surgery.

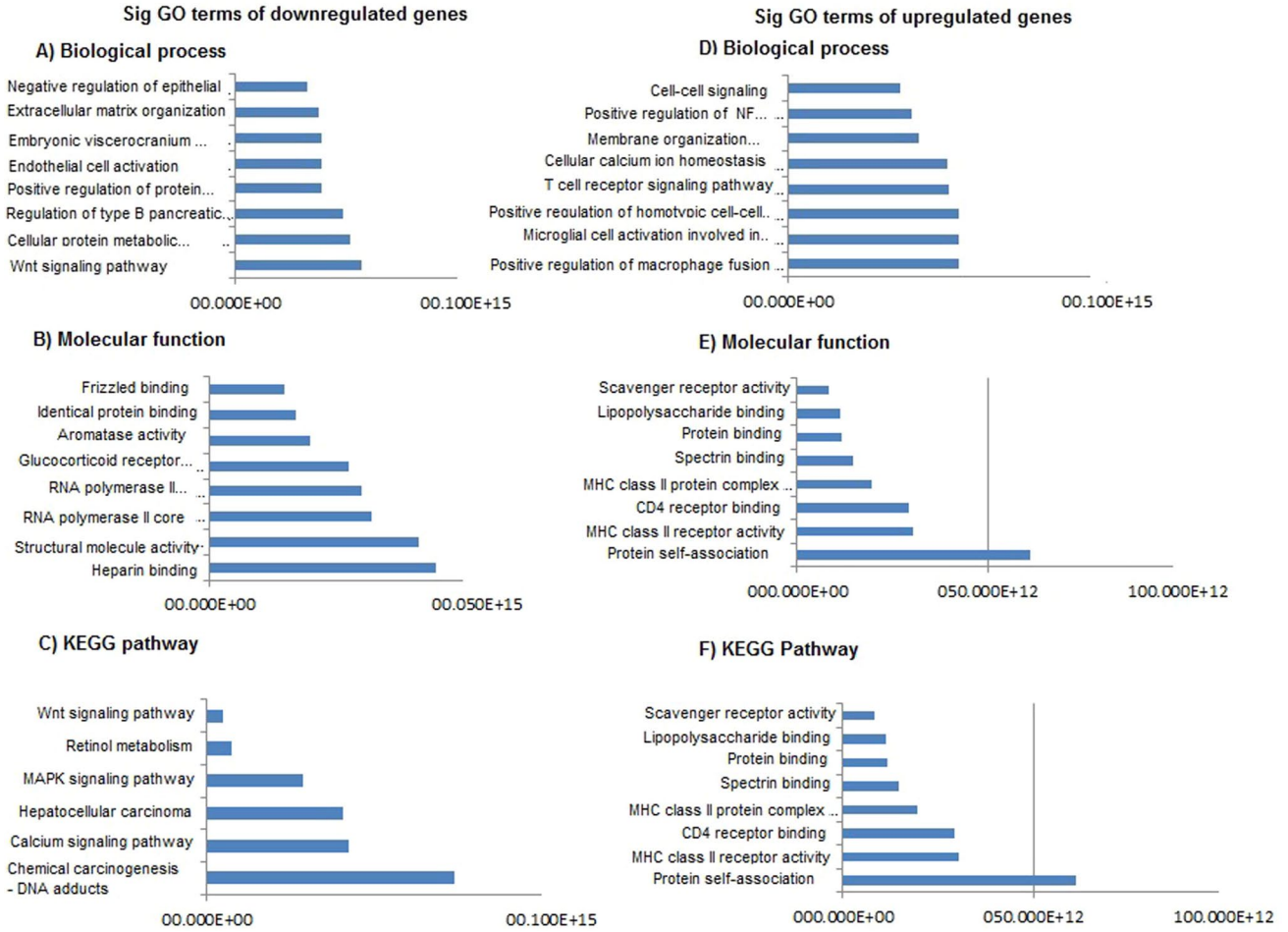


**Fig. 2.** Gene Ontology analysis of the DEGs. Functional annotations of differentially expressed genes in terms of BP (**A**, **D**) and MF (**B**, **E**) and KEGG pathway (**C**, **F**) in CAP and IMA. *BP* biological process, *KEGG* Kyoto Encyclopedia of Genes and Genomes, *MF* molecular function, *sig* significant. All significant pathways are in Supplementary File 2

According to the microarray analysis, SPP1 gene had the highest fold change in CAP compared with IMA (Table 1). Previous studies have suggested that SPP1 is an integrin binding N-linked glycoprotein involved in atherosclerotic inflammation. A microarray study focusing on abdominal aortic plaques of tissues from patients with atherosclerosis indicated that SPP1 was higher in their serum compared with the healthy counterparts [16]. This protein is correlated with the biomarkers that have been used as indicators of some CVDs. Compared with normal tissues, plaque regions have higher SPP1 protein levels [16]. In our study, we found that SPP1 gene expression was the most upregulated in the plaque regions compared with the non-plaque-forming tissues. Our results for the upregulation of this gene are compatible with the previous findings of another study on SPP1 and atherosclerosis [16]. Moreover, we also found that CAP had higher expression of matrix metalloproteases (MMPs) compared with IMA. The literature supports the roles of MMPs in the development of atherosclerosis. Especially MMP12 is considered as a drug target in atherosclerosis due to its involvement in the development of atherosclerosis [17]. Functional polymorphisms in MMP1, MMP2, MMP3 MMP7, MMP9, MMP12, and MMP13 genes have been associated with coronary artery disease [18]. Moreover, there is a study suggesting MMP genotyping to determine the prognosis of the patients during the treatment [18]. Our study also suggested the importance of MMPs for the development of the plaques in patients with atherosclerosis. There is a high accumulation of macrophages in atherosclerotic plaques. The most important reason for foam cell formation is macrophage cell accumulation [19]. Therefore, it is expected to be high in plaque but not in IMA. Also, other tissue-specific factors may be effective in this process [20]. One of the reasons that macrophage-specific genes are highly upregulated is pathological differences between CAP and IMA tissues. However, there may be other factors accompanying this process. Another recent study reveals that MMPs were found to be an important parameter for the

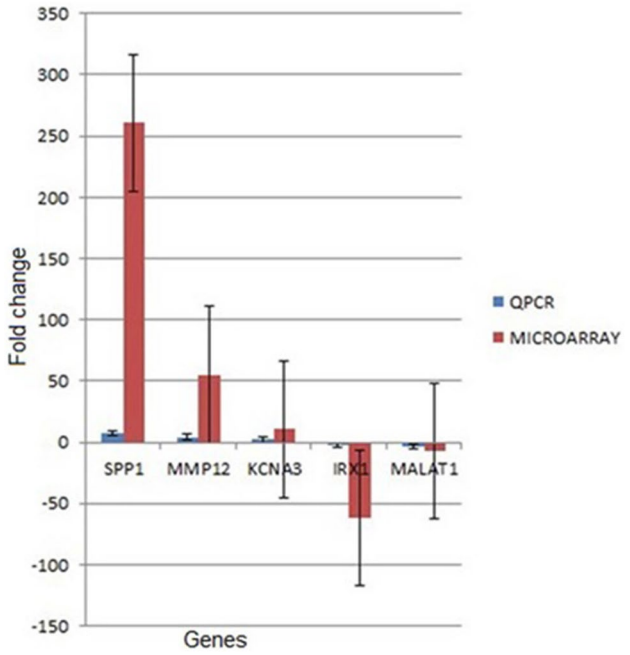


**Fig. 3.** Validation of microarray data by RT-qPCR. RT-qPCR results and microarray data of selected DEGs in CAP compared to IMA. Vertical axis represents the FC ($log_2$ transformed) of DEGs. FC < 1 and FC > 1 represent the downregulated and upregulated significant DEGs, respectively. *FC* fold change, *RT-qPCR* quantitative real-time polymerase chain reaction

development of the atherosclerosis [21]. Our results also suggest the importance of MMP family members in the development of atherosclerotic plaques.

In our study, we compared DEG expression levels between the CAP and non-atherosclerotic IMAs of patients who had similar environmental stress conditions with similar genetic backgrounds. We found that CSF1R and MMP12 were highly upregulated in CAP.

It is possible that the differentially expressed mRNAs and lncRNAs we have identified are just a reflection of arteries with and without an atherosclerotic plaque, especially the high accumulation of macrophages. Apolipoprotein C2 (APOC2) is another gene whose expression was statistically significant upregulated in CAP compared with IMA (Table 1). A lipidomics and proteomics study suggested that two important modulators (APOC2 and APOC3) in lipoprotein lipase activity are associated with the development of CVDs [22]. Compared with IMA, CAP tissues had 21.45-fold increase in the expression of TREM2 gene (Table 1). Studies on TREM2 have suggested a crucial role in macrophage function and inflammatory factor expression [23]. TREM2 is highly expressed in macrophages that play an important role in cholesterol metabolism and oxidative phosphorylation pathways [24]. Our study also suggested that TREM2 is important for plaque formation.

**Table 3.** RT-qPCR validation results

| Gene | FC | FR | *p* value |
|---|---|---|---|
| SPP1 | 7.56 | 7.56 | 0.01 |
| MMP12 | 4.35 | 4.35 | 0.03 |
| KCNA3 (lncRNA) | 2.18 | 2.18 | 0.03 |
| IRX 1 | 0.41 | −2.43 | 0.03 |
| MALAT 1 (lncRNA) | 0.29 | −3.47 | 0.02 |

*FC* fold change, *FR* fold regulation

SLAMF8 was upregulated in CAP compared with IMA (Table 1). A previous study suggested its role in reactive oxygen species' modulation and inflammation [25]. In our study, we also found that SLC14A1 gene expression was 19.13-fold higher in CAP compared with IMA (Supplementary File 1). Previous studies have suggested that protein product activity of this gene played a role in the apoptotic pathways of phagocytes [26]. Other genes found to be statistically significantly different were C15ORF48, TIFAB, IL1, TNF, and CXCR6 (Supplementary File 1). These genes are related to the immune system and inflammatory responses [27; 28].

CYP4B1 was downregulated in CAP compared with IMA (Table 1). To the best of our knowledge, our study is the first to report the downregulation of the expression level of this gene in the development of CVDs. CHRDL1, MYOC, FBLN1, and CAPN6 were also downregulated (Table 1). These genes have been associated with lipid metabolism and inflammation [29]. FOS-1 was another downregulated gene in CAP compared with IMA (Table 1). This gene has been associated with matrix remodeling, prostaglandin synthesis, glycolysis, and cholesterol biosynthesis [30]. KCNK2 was another downregulated gene in CAP (Table 1). Previous studies have suggested that its absence leads to cardiac problems and atrial fibrillation in mice [31]. THSD4 was downregulated in the plaques (Table 1), and it was shown to play an important role in innate aortic aneurysms [32]. HOXA6 was also downregulated in CAP (Table 1), and it plays an important role in the suppression of cell proliferation by inhibiting the PI3K/Akt/ERK pathways [33]. SFRP-1, HOXA10, WNT11, and IRX1 were also downregulated in CAP (Table 1). Our findings are in accordance with the findings of a previous study [34].

LINC01857 was upregulated in CAP (Table 1), and it has been associated with insulin resistance, apoptosis, and inflammation [35]. lncRNAs LINC02244, KCNA3, and KRT18 were also upregulated in CAP (Table 1), and these are associated with cancer development, cell proliferation, and apoptosis [36, 37]. lncRNA WFDC21P was downregulated in CAP compared with IMA (Table 1). It has important functions in cell proliferation and metabolism [38]. lncRNA KRT18P55 is a novel lncRNA, and in our study its

expression was statistically significantly different (Table 1). Its effect on the pathways is not well known, but it has been associated with cancer development [39]. Other upregulated lncRNAs were associated with cancer, autophagy, apoptosis, inflammation, and metabolism [40; 8]. In our previous study, we selected ANRIL and MIAT, which are thought to be associated with atherosclerosis. We determined expression level of these lncRNAs using the RT-qPCR method. However, in this current study, the DEGs in CAP and IMA were determined by the microarray method. According to our results, ANRIL and MIAT were not found among the DEGs. There are two reasons for this difference: the use of different methods and the sample size.

KEGG pathway analysis results suggested that there were differences in the pathways that play important roles in inflammation, metabolism, cell survival, and cell proliferation when CAP and IMA were compared (Fig. 2C,F). In a previous study some downregulated proteins were involved in extracellular matrix organization and vascular smooth muscle cytoskeleton [41]. Extracellular matrix organization was found to be an important downregulated gene target pathway in terms of biological process in our study (Fig. 2A). It is found that the activation of Wnt signaling is negatively correlated with atherosclerotic severity [42]. The Wnt signaling pathway, one of the important biological processes (Fig. 2A), and the KEGG pathway (Fig. 2C) were involved in downregulated gene expression in our study. Several studies have suggested that Wnt1, Wnt2, and Wnt3a mediate a direct link between canonical Wnt signaling and VSMC proliferation in the neointimal formation of atherosclerosis [43]. MAPK signaling that targeted downregulated genes was found to be another important pathway in the present study (Fig. 2C). It is known that native and/or modified LDL, such as ox-LDL, is an important inducer of the MAPK signaling pathway in early-stage atherosclerosis [44]. In addition, a previous study reported that MAPK signaling might be responsible for endothelial cell migration, which is observed with atherosclerotic lesion formation [45]. Binding of ox-LDL to atherogenic receptors results in a rapid increase in intracellular ROS generation. Increased ROS causes inflammation by activating the NF-κB signaling pathway. This pathway was found to be an upregulated biological process in our study (Fig. 2D). When correlation studies were performed, lncRNA LOC105377123 was highly correlated with CAPN6, HOXA6, CATSPER1, HOXA10, and DUSP1 genes in CAP (Table 1). When this lncRNA was investigated in the literature, no studies suggested its interaction with the listed genes. Functional analysis will further elucidate its role in the development of atherosclerosis by possibly regulating the expression of the above-listed genes. The functional properties of lncRNAs are not fully known and will continue to be investigated. In this study, mRNAs with correlated expression of important lncRNAs were detected in CAP tissue.

A major limitation is the limited sample size in our study. This sample size was due to the limited budget opportunities and laboratory facilities. In addition, the fact that male individuals are in the majority in the sample is considered a limitation, although atherosclerosis is more common in males. Furthermore, detailed studies on plaques have not been performed, and only atherosclerotic plaques have been confirmed.

Our results mostly overlap with the previous findings. Moreover, in this study, we detected novel mRNA genes and ncRNAs that could lead to the development of CVDs. However, further functional studies are needed to identify new therapeutic targets and treatment options.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s40291-023-00659-w.

## Declarations

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**Conflict of Interest** Serdal Arslan, Nil Özbilüm Şahin, Burcu Bayyurt, Öcal Berkan, Mehmet Birhan Yılmaz, Mehmet Aşam, and Furkan Ayaz declare that they have no conflicts of interest that might be relevant to the contents of this manuscript."

**Ethics Approval** In our study, we performed microarray analysis on 32 samples that consisted of 16 IMA and 16 CAP tissue samples taken from Sivas Cumhuriyet University Research Hospital Cardiovascular Surgery Department. The patients had bypass surgery, and the samples were taken from their IMA and CAP tissues on the basis of the ethical permissions (Sivas Cumhuriyet University Clinical Research Ethics Committee decision number: 2018-09/150 and 2018–04/18). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent form was obtained from all volunteers.

**Consent (Participation and Publication)** Not applicable.

**Author Contributions** S.A., N.Ö.Ş., and B.B. performed experiments; S.A., N.Ö.Ş., B.B., and F.A. prepared figures; S.A., N.Ö.Ş., B.B., F.A., and M.B.Y. edited and revised manuscript; S.A., N.Ö.Ş., B.B., and F.A. drafted manuscript; S.A., N.Ö.Ş., and B.B. analyzed data; S.A., M.B.Y., and Ö.B. conceived and designed research; M.B.Y., Ö.B., and M.A. performed sample collection and analyzed demographic and clinical parameters.

**Code Availability** Not applicable.

**Data Availability Statement** Data is available upon reasonable request from the corresponding authors.

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