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Downregulation of Circulating UCA1 and APPAT Reveals Their Potential as Non-Invasive Biomarkers in Advanced Coronary Atherosclerosis

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Abstract

Background and Aims:

The study aimed to assess the expression levels of the APPAT and UCA1 in tissue and blood samples to clarify their potential roles in atherosclerosis pathogenesis.

Methods and Results

Coronary artery and left internal mammary artery (LIMA) tissue samples were obtained from patients who underwent coronary artery bypass grafting (CABG) (n = 30). In addition, whole blood samples were collected from both CABG patients and healthy controls (n = 30). Total RNA was isolated from tissue and blood samples, and the expression levels of APPAT and UCA1 were quantified using RT-qPCR. When blood-based expression levels were compared between the patient and control groups, both APPAT and UCA1 were significantly downregulated in the patient group ($p < 0.001$ and $p = 0.025$, respectively). In contrast, no statistically significant differences were observed in APPAT or UCA1 expression between coronary artery and LIMA tissue samples. However, UCA1 expression was significantly higher in LIMA tissues than in blood samples ($p = 0.005$). Moreover, coronary APPAT expression was significantly lower than blood APPAT expression in the patient group ($p = 0.0019$).

Conclusion

Based on the available data, we suggest that decreased expression levels of APPAT and UCA1 may be associated with the development of atherosclerosis. In addition, the higher expression of UCA1 in LIMA tissues compared with blood samples may reflect a potential role in resistance to atherosclerotic changes. Conversely, the lower expression of APPAT in coronary artery tissues compared with blood samples may be related to the progression of atherosclerosis. Nevertheless, further studies are required to elucidate the molecular mechanisms underlying these associations.

Introduction

Cardiovascular disorders, including myocardial ischaemia, myocardial infarction, atherosclerosis, heart failure, hypertension, and arrhythmias, are linked to elevated morbidity and mortality rates. Annually, around 17.9 million individuals globally die from cardiovascular diseases, representing 32% of total deaths (1). Atherosclerosis, one of the most deleterious chronic diseases characterized by lipid accumulation under the intima layer of arteries, eventually leading to narrowing of the lumen, forms the main pathological basis of cardiovascular diseases (2). Subclinical atherosclerosis is regarded as an early marker of atherosclerotic burden. Therefore, a better understanding of the disease at its early stages may contribute to the prevention of future cardiovascular morbidity and mortality (3). The risk factors for atherosclerosis include both acquired risk factors such as genetics, age, family history and

gender, as well as modifiable risk factors, including hyperlipidaemia, hypertension, smoking, diabetes, chronic kidney disease, obesity and inflammation (4).

Basic pathogenic mechanisms, including endothelial dysfunction, macrophage apoptosis, leukocyte recruitment and activation, impaired cholesterol metabolism, and vascular remodelling contribute substantially to the onset of atherosclerosis and the subsequent progression of vascular damage (5). In advanced cases of atherosclerosis, invasive interventions may be necessary to restore perfusion and regulate blood pressure. The invasive procedures encompass angioplasty, carotid endarterectomy, and coronary artery bypass grefting (CABG), a surgical intervention that utilizes venous or arterial blood vessels from other regions of the body to circumvent obstructed or constricted coronary arteries (6). It has been shown that some arteries such as coronary arteries, thoracic aorta, abdominal aorta, cerebral arteries, carotid arteries and renal arteries are susceptible to atherosclerosis, whereas arteries such as internal mammary artery (IMA), radial arteries and hepatic arteries are generally resistant to atherosclerosis in the same patient. Since the left or right IMA (LIMA or RIMA) and radial arteries are resistant to atherosclerosis, they are frequently used as grafts in CABG surgery (7).

Although once regarded as transcriptional “junk,” long non-coding RNAs (lncRNAs) are now recognized as RNA transcripts exceeding 200 nucleotides in length that do not encode functional proteins (8). These molecules regulate gene expression through diverse mechanisms, including transcriptional and post-transcriptional control, epigenetic modulation, translational regulation, and post-translational processes, mostly through interactions with DNA, mRNA, proteins, and microRNAs (miRNAs) (9–11). The roles of lncRNAs have been implicated in a range of diseases, including vascular development, various types of cancer, atherosclerosis, hypertension, kidney damage, diabetes and related complications (12). lncRNAs can directly regulate the expression of endothelial genes through transcriptional and/or post-transcriptional mechanisms, thereby influencing endothelial function under physiological conditions and contributing to endothelial dysfunction under pathological conditions (13).

APPAT lncRNA (LINC02969) was initially identified in circulation, and its expression level was found to be diminished in atherosclerotic plaques (14). APPAT is localised on chromosome 2 (2q11.2) and consists of 669 base pairs (15). Emerging evidence have suggested that APPAT may serve as a potential predictive biomarker for the development of atherosclerosis. APPAT is distributed in tunica media of the arterial wall, where vascular smooth muscle cells (VSMC)s are abundant, and is localized in the cytoplasm of VSMCs (15,16). This localization suggests that its biological function may be to act as a competing RNA for miRNA (14). Research suggests that APPAT may play a role in VSMC proliferation in cardiovascular diseases (17).

UCA1 lncRNA (LINC00178) was initially identified in a bladder cancer cell line (bladder transitional cell carcinoma) (18), and research on atherosclerosis has yielded inconsistent findings regarding the functions of this RNA. UCA1 is localized on the short arm of chromosome 19 (19p13.12) (19). It has been observed that ox-LDL, one of the parameters of atherosclerosis, increases UCA1 expression, while

suppression of UCA1 reduces oxidative stress and increases apoptosis (20). In addition, it has been stated that upregulation of UCA1 suppresses endothelial dysfunction (21).

This study aimed to investigate the expression profiles of the lncRNAs APPAT and UCA1 in blood and tissue samples and to assess their potential involvement in atherosclerosis. The ultimate goal of this study is to reduce the need for invasive treatment methods and to reduce mortality and morbidity from cardiovascular diseases through early diagnosis and early non-invasive treatment options.

Material and Methods

2.1. Study Population

An a priori power analysis was performed to estimate the required sample size. Assuming an effect size of 0.65, a statistical power of 80%, and a two-sided significance level of 0.05, the minimum sample size was calculated as 30 participants per group, including patients and controls. The analysis was performed using G*Power software (version 3.1.9.7). Volunteers aged 30–75 years who provided written informed consent were included in the study. All groups were matched in terms of age and sex. Patients admitted to the University of XXXXXXX, XXXXX, Department of XXXXXX, who were decided to underwent CABG based on clinical and laboratory evaluations were included in our study. Peripheral blood samples were obtained from the patients before surgery. During the surgical procedure, atherosclerotic coronary artery tissue samples (n = 30) and left internal mammary artery (LIMA) samples used as grafts (n = 30) were collected. Peripheral blood samples were also obtained from healthy individuals (n = 30) with no history of cardiovascular disease or other clinically evident disorders, as confirmed by clinical examination, and these individuals constituted the control group Laboratory data for both groups were retrieved from the hospital database. The study protocol was approved by the Hamidiye Scientific Research Ethics Committee of the University of Health Sciences, Türkiye (decision no. 2021/36 – 2). The study was conducted in accordance with the principles of the Declaration of Helsinki.

2.2. Total RNA Isolation and lncRNA Expression Levels

Total RNA was extracted from LIMA tissue and peripheral blood samples using the Quick-RNA™ Miniprep Plus Kit (Cat. No. R1057; Zymo Research, USA) according to the manufacturer's instructions. For atherosclerotic coronary artery tissue samples, total RNA isolation was performed using the Quick-RNA™ Microprep Kit (Cat. No: R1050, Zymo Research, USA). RNA concentration and purity were assessed using a nanophotometer (DeNovix DS-11, USA Qualified total RNA samples were reverse-transcribed into cDNA using the SensiFAST™ cDNA Synthesis Kit (Cat. No. BIO-65053; Bioline, USA) following the manufacturer's protocol. The expression levels of UCA1 and APPAT were then quantified by quantitative real-time polymerase chain reaction (qRT-PCR) using lncRNA-specific primers (Table 1). qRT-PCR analysis was performed using the SensiFAST™ SYBR® No-ROX Kit (Cat. No. BIO-98005; Bioline, USA) according to the manufacturer's instructions on a Bio-Rad CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., California, USA). Each sample was analyzed in duplicate. The

expression levels of UCA1 and APPAT were normalized to GAPDH, and relative expression levels and fold changes were calculated using the ΔCt and 2 − ΔΔCt methods, respectively, in accordance with the Livak method.

Table 1
Primer sequences

Gene	Primer sequences
*GAPDH*_F	5'-GTCTCCTCTGACTTCAACAGCG-3'
*GAPDH*_R	5'-ACCACCCTGTTGCTGTAGCCAA-3'
*APPAT*_F	5’-GCAGAGGCAGGTCACCAAC-3’
*APPAT*_R	5’-CAGGATGATTCGAGACCAGGA-3’
*UCA1*_F	5'-CTCTCCATTGGGTTCACCATTC-3'
*UCA1*_R	5'-GCGGCAGGTCTTAAGAGATGAG-3'

2.3. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 23.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using appropriate tests. Depending on the results of the normality analysis, Student’s t-test was used for parametric data, while the Mann–Whitney U test was used for non-parametric data. Pearson’s or Spearman’s correlation analysis was performed to evaluate the relationships between variables. Receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic performance of the potential biomarkers. All data are presented as mean ± standard deviation, and a p value < 0.05 was considered statistically significant.

Results

The participants of the study groups were equally distributed by age and gender. Among the patients from whom both tissue and blood samples were collected, 8 were female (26.7%) and 22 were male (73.3%). Among the control group from whom only blood samples were collected, 10 were female (33.3%) and 20 were male (66.7%) (p = 0.573). The mean age of participants in the patient group was 62.3 ± 9.42, whereas the mean age of participants in the control group was 59.40 ± 6.01 (p = 0.118). Laboratory parameters of the patient and control groups are presented in Table 2.

Table 2
Laboratory parameters in of the patient and control groups

Parameter	Patient	Control	*p* value
Glucose (mg/dL)	155.97 ± 70.18	98.10 ± 21.00	**< 0.001**
Uric acid (mg/dL)	5.81 ± 1.62	4.11 ± 0.93	**0.014**
Urea (mg/dL)	42.68 ± 16.38	28.79 ± 8.08	**< 0.001**
Creatinine (mg/dL)	0.90 ± 0.24	0.79 ± 0.16	0.091
Cholesterol (mg/dL)	200.41 ± 60.78	168.47 ± 31.58	0.074
HDL cholesterol (mg/dL)	38.36 ± 10.54	50.80 ± 14.00	**0.001**
LDL cholesterol (mg/dL)	119.23 ± 49.58	96.72 ± 29.50	0.13
Triglyceride (mg/dL)	234.55 ± 174.07	104.43 ± 49.34	**< 0.001**
Non-HDL cholesterol (mg/dL)	163.81 ± 63.02	117.67 ± 32.11	**< 0.005**
VLDL cholesterol (mg/dL)	47.43 ± 35.63	20.89 ± 9.87	**< 0.001**
Sodium (mmol/L)	139.03 ± 2.35	139.96 ± 2.62	0.183
Potassium (mmol/L)	4.64 ± 0.91	4.47 ± 0.38	0.507

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein.

When the expression levels of lncRNA UCA1 and APPAT were compared between coronary and LIMA tissue samples, both were found to be decreased levels in coronary tissue; however, these differences were not statistically significant ($p = 0.092$ and $p = 0.387$, respectively). Correlation analyses were also performed between expression levels and other parameters. A positive correlation was observed between lncRNA APPAT and UCA1 expression levels in both coronary and LIMA tissue samples ($r = 0.645$, $p < 0.01$ and $r = 0.846$, $p < 0.01$, respectively).

When the expression levels of lncRNA APPAT and UCA1 were compared between the patient and control groups, both were found to be significantly downregulated in the patient group (APPAT: $p = 0.025$; UCA1: $p < 0.001$). In the patient group, APPAT expression was decreased by 1.61-fold, while UCA1 expression was decreased by 5.33-fold compared with the control group (Fig. 1). A significant positive correlation was observed between APPAT and UCA1 expression levels in peripheral blood samples ($r = 0.427$, $p = 0.019$). In addition, APPAT expression levels showed a significant negative correlation with total cholesterol, LDL cholesterol, and non-HDL cholesterol levels ($p < 0.05$).

When UCA1 and APPAT expression levels were compared among coronary tissue, LIMA tissue, and peripheral blood samples in the patient group using the Kruskal-Wallis H test, significant differences were observed for both UCA1 and APPAT ($p = 0.028$ and $p = 0.005$, respectively). Post hoc analysis

revealed a significant difference in APPAT expression levels between coronary tissue and blood samples ($p = 0.019$), and in UCA1 expression levels between LIMA tissue and blood samples ($p = 0.005$).

Receiver operating characteristic (ROC) curve analysis was conducted to assess the diagnostic performance of blood APPAT and UCA1 ΔCt values. Blood APPAT ΔCt demonstrated an area under the curve (AUC) of 0.669. The optimal cutoff value was 3.76, corresponding to a sensitivity of 66.7% and a specificity of 56.7%. For blood UCA1 ΔCt, the ROC analysis yielded an AUC of 0.762, with an optimal cutoff value of 8.465, a sensitivity of 80.0%, and a specificity of 70.0%. The combined ROC analysis of blood APPAT and UCA1 ΔCt values resulted in an AUC of 0.776, with a cutoff value of 0.550, a sensitivity of 70.0%, and a specificity of 80.0% (Fig. 2).

Discussion

Atherosclerosis, characterized by thickening and hardening of the arterial walls, is a chronic inflammatory process linked to substantial morbidity and mortality and recognized as a major contributor to myocardial infarction and stroke (22). Despite optimal control of cholesterol levels, approximately 20% of patients experience recurrent myocardial infarction within three years after the initial clinical event, highlighting the need for complementary therapeutic approaches aimed at reducing the morbidity and mortality associated with atherosclerosis (23). Despite significant advances in atherosclerosis research, current treatment strategies remain insufficient for the effective management of the disease. It has been reported that existing therapies fail to prevent up to 70% of adverse clinical events. Timely detection of coronary atherosclerosis is therefore essential for reducing the risk of plaque rupture and sudden cardiac death. It has been found that increased endothelium cell turnover, disruption of gap junctions, inflammation, and oxidation play a vital role in the onset of endothelium cell dysfunction, vascular disorders, and atherosclerosis. During the formation of atherosclerotic plaques, media-resident VSMCs undergo a phenotypic switch from a contractile toward a synthetic phenotype, followed by proliferation and directed migration into the intima, thereby significantly contributing to disease progression (16,24). Damage to the endothelium layer in arterial branches results in diminished eNOS expression and elevated nuclear factor kappa B (NF-κB) expression, which facilitate thereby facilitating LDL infiltration, platelet aggregation, leukocyte adherence, and an inflammatory response. LDL infiltration increases the accumulation of monocytes in the injured region of the arterial wall and stimulates a chronic inflammatory response, thereby inducing atherosclerotic plaque formation (25–27). Numerous studies have demonstrated that lncRNAs are important functional regulators in endothelial cells, macrophages, and VSMCs (28). For this reason, lncRNAs appear to be important regulators involved in the pathogenesis of atherosclerosis. Studies have shown that abnormal VSMC proliferation and migration are common pathophysiological mechanisms in many cardiovascular diseases, such as atherosclerosis and restenosis after angioplasty (29). Numerous lncRNAs have been shown to be associated with atherosclerosis as well as with the proliferation and migration of VSMCs. A previous study reported that ANRIL, MALAT1, MEG3, APPAT, NEXN, GAS5, lincRNA-p21, CHROME, and lncRNA n342419 exhibit atheroprotective properties, whereas H19, MIAT, TUG1, and NEAT1 are considered atherogenic lncRNAs (26,30).

APPAT, which has been detected in the blood of healthy individuals, patients with angina pectoris, and those who have suffered a myocardial infarction, has been suggested to have potential utility as a non-invasive biomarker for the early diagnosis of cardiovascular diseases due to its correlation with the development of atherosclerosis. Previous studies have reported decreased APPAT expression in severely stenotic coronary artery tissues (16,31). The potential mechanism of APPAT is attributed to its effect on VSMC phenotype change by signaling specific miRNAs such as miR-647 and miR-135 (8). In a study where ox-LDL was applied to human VSMCs, it was observed that APPAT was downregulated in the ox-LDL-treated group compared to the control group, and that it was further downregulated as the concentration of applied ox-LDL increased. In the same study, they concluded that APPAT may be involved in maintaining the VSMC phenotype during disease progression and may thereby influence the severity of coronary artery stenosis, particularly the stability of vulnerable plaques (16). In two different studies, it was demonstrated that APPAT is downregulated and its target miR-328a is upregulated in plasma and whole blood samples from patients with acute MI (32,33). In breast cancer, APPAT, typically upregulated, has been shown to promote cell proliferation, migration, and invasion, whereas its inhibition suppresses these processes. Wang et al. demonstrated that APPAT regulates cell proliferation, migration, and invasion in breast cancer by modulating the miR-328a/plakophilin-1 (PKP1) axis, with PKP1 also acting as a regulator of the Wnt signaling pathway (34). In another study involving patients with primary hypertension, fibroblast growth factor 5 (FGF5) gene and protein expression levels were found to be upregulated, and FGF5 genetic variants were positively associated with primary hypertension (35). In a subsequent study, Meng et al. demonstrated that APPAT downregulates its target gene fibroblast growth factor 5 (FGF5) through miR-647 (31). Additionally, miR-647 has been shown to promote the proliferation and migration of oxidized low-density lipoprotein (ox-LDL)-treated vascular smooth muscle cells by modulating the PTEN/PI3K/Akt signaling pathway. In another study, miR-647 was reported to regulate atherosclerosis-related cell proliferation, migration, invasion, and inflammatory responses via the circRNA Circ_0000345 (36). In summary, APPAT regulates various signaling pathways through miR-647, and considering the role of miR-647 in atherosclerosis development, the downregulation of APPAT in circulation, as supported by the literature, may play a role in the pathogenesis of atherosclerosis. In our study, APPAT expression was significantly downregulated in peripheral blood samples from patients who underwent CABG compared with the control group. These findings suggest that APPAT may exert a protective role in the development of atherosclerosis. However, although APPAT expression was lower in coronary tissue samples than in LIMA tissues, this difference did not reach statistical significance. Furthermore, when APPAT expression levels were evaluated across blood, coronary tissue, and LIMA samples within the patient group, APPAT expression was found to be significantly lower in coronary tissue compared with blood. This observation suggests that APPAT may be closely associated with the development and progression of atherosclerosis in coronary tissue, particularly in regions where atherosclerotic lesions are already established.

UCA1 functions as a competing endogene (ceRNA) that regulates multiple signaling pathways associated with the development and pathogenesis of atherosclerosis, influencing biological processes including apoptosis, cell proliferation, migration, invasion, and drug resistance in various diseases (37).

In the literature, the potential roles of UCA1 have been predominantly investigated in cancer, while its involvement in several pathways relevant to atherosclerosis has also been identified. In their study, Wang et al. demonstrated that UCA1 contributes to immune escape in gastric cancer cells by sponging miR-193a and miR-214, thereby upregulating the expression of programmed cell death ligand 1 (38). Another study indicated that the upregulation of UCA1 may result in the repression of the p27 protein and its targets, thereby leading to the inhibition of cell growth and the induction of apoptosis (39). Conversely, another study demonstrated that miR-1 inhibits cell growth, induces apoptosis, and reduces cell motility in bladder cancer by downregulating UCA1 expression (40). The transforming growth factor-β (TGF-β) signaling pathway can also induce the expression of UCA1-associated miRNAs such as miR-1 and miR-203a (41). Additionally, it has been stated that the upregulation of UCA1 suppresses endothelial dysfunction (21). In another study, the predictive performance of UCA1 for acute myocardial infarction was evaluated using ROC curve analysis. Although UCA1 demonstrated significant sensitivity and specificity for acute myocardial infarction, its diagnostic performance was inferior to that of established biomarkers such as troponin and cardiac creatine kinase (17,42). Previous studies have demonstrated that UCA1 suppresses VSMC proliferation by acting as a miR-26 sponge, thereby relieving inhibition of the PTEN pathway (8,29). In addition, UCA1 has been shown to attenuate cardiomyocyte injury by reducing apoptosis through activation of the PI3K/Akt signaling pathway (43). Only a limited number of studies have investigated the association between UCA1 and cardiovascular injury or disease. One study reported that UCA1 is reactively induced following ischemia/reperfusion injury, and that adenovirus-mediated overexpression of UCA1 confers significant myocardial protection by suppressing endoplasmic reticulum stress and reactive oxygen species-induced apoptosis (44). Additionally, another study demonstrated that UCA1 exhibits a trapping effect on miR-873–5p and miR-143 in hypoxia-induced cell lines, thereby playing a protective role against cardiac damage (45–47). UCA1 provides protection against H_2O_2-induced apoptosis in cardiac myocytes (17). UCA1 negatively regulates miR-1, miR-203a, miR-16, miR-216b, miR-193a-3p, and miR-143 by acting as a sponge and plays a role in invasion, proliferation, and migration processes in various types of cancer (48). Among these, miR-1 and miR-16 have been shown to be potential risk factors for secondary cardiovascular events (49). Similarly, miR-143 has been reported to be a potential specific biomarker for atherosclerosis (50). UCA1 lncRNA has been shown to be downregulated in plasma samples from patients with myocardial infarction and heart failure and has been proposed as a potential biomarker for predicting MI (15,42). In a 2023 study, Chen et al. reported that UCA1 expression was upregulated in the plasma of patients with atherosclerosis, whereas miR-132, which is proposed to be a target of UCA1, was downregulated (51). UCA1 has been shown to exert a sponge effect on miR-26a, regulating the proliferation and migration of VSMCs and thereby alleviating the proliferation of VSMCs in response to atherosclerosis (29,52). Zhang et al. demonstrated that UCA1 mitigates damage by regulating apoptosis in cardiomyocytes post-MI through the PI3K/Akt signaling pathway (53). Our investigation revealed that UCA1 expression levels were markedly downregulated in the blood of patients with atherosclerosis compared to the control group. These results suggest that UCA1 may play a protective role in the development of atherosclerosis. However, in lesion-containing coronary samples, although UCA1 expression was lower compared to healthy LIMA samples, it was not statistically significant. Additionally, when UCA1

expression levels were examined in the patient group across blood, coronary, and LIMA tissues, it was observed that UCA1 expression was higher in LIMA tissue compared to blood. This supports the notion that LIMA possesses mechanisms that protect against atherosclerosis and is resistant to it. Additionally, the higher UCA1 expression in LIMA tissue compared to blood suggests that it may play a role in the pathogenesis of protective mechanisms.

Most lncRNA expression studies to date have been conducted in cell lines and animal models. In the present study, coronary artery and LIMA tissues obtained from human patients during CABG surgery were analyzed. The limited number of human tissue-based studies in this field underscores the novelty of our work, and our findings provide a valuable contribution to the existing literature. In vivo modulation of lncRNA expression holds promise as a new therapeutic strategy for modifying atherosclerosis or vascular remodeling. Increasing evidence from studies also suggests that lncRNAs play important roles in cancer, neurological diseases, and cardiovascular diseases. In recent years, numerous studies have investigated the role of lncRNAs in the pathogenesis of cardiovascular disease. Given the substantial frequency and mortality associated with cardiovascular diseases, it is crucial to understand the molecules involved in their pathophysiology and the signaling networks they stimulate. This may enhance the development of more accurate and appropriate diagnostic techniques, the identification of more effective prognostic biomarkers, and the discovery of novel targets for treatment (54). Despite technological advances, there remains a substantial unmet need for non-invasive biomarkers that enable the early diagnosis and potential therapeutic targeting of atherosclerosis before the onset of myocardial infarction, ischemia, or other vascular complications. Given the critical roles of lncRNAs in both physiological and pathological processes, they represent promising candidates as biomarkers in this context.

Limitations

The primary limitation of the current study is that it is a single-center study; more studies with larger sample sizes from multiple centers are needed to validate the study results on a broader scale. This study is a preliminary study, and larger studies are planned. Considering that lncRNAs have potential roles in the pathophysiology of many diseases, including cardiovascular diseases, the lack of studies on the lncRNAs UCA1 and APPAT we studied has created an opportunity to fill the gap in the literature and highlight the need for further research in this area.

Declarations

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CRediT authorship contribution statement

Conceptualization: S.K., K.K. and H.H.P. Data curation: S.K., M.K., S.A., M.S. Formal analysis: S.K. and K.K. and B.E. Methodology: S.K., M.K., S.A., M.S., K.K. and BE. Project Administration: S.K. and H.H.P. Supervision: S.K. and H.H.P. Writing – original draft: S.K. Writing – review and editing: S.K., K.K.

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Data Availability

No datasets were generated or analysed during the current study.

Competing interests

The authors declare no competing interests.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study received ethical approval from the Hamidiye Scientific Research Ethics Committee of University of Health Sciences, Türkiye (decision no. 2021/36-2).

Author Statement

The authors assert that this manuscript is original and has not been previously published. This work is not under consideration for publication in any other journal. All authors listed have reviewed and approved the final version of the manuscript, and no eligible contributor has been omitted. The authors have reached a consensus regarding the sequence of their names in the listing. The Corresponding Author will serve as the primary contact throughout the editorial and publication process. They will also oversee the communication among all co-authors, ensuring discussions about manuscript changes and final proof approval are effectively managed.

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Figures

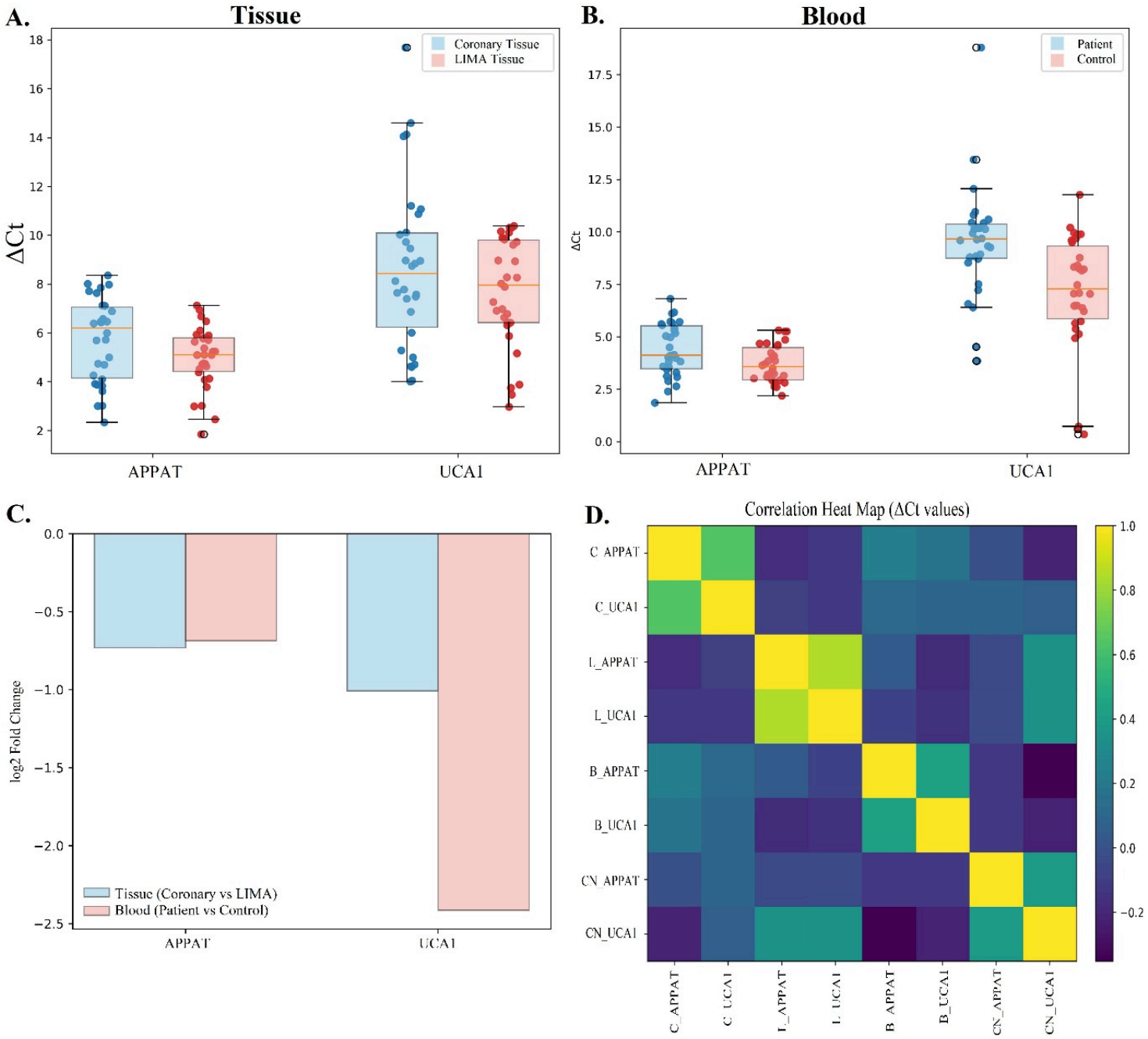


Figure 1

(A) Tissue expression analysis: APPAT and UCA1 expression levels in coronary artery tissue (C) and left internal mammary artery (LIMA; L) samples were evaluated using ΔCt values. Data are presented as mean ± standard deviation (SD), displayed as boxplots with individual data points overlaid. No statistically significant differences were observed between coronary and LIMA tissues for either APPAT ($p = 0.092$) or UCA1 ($p = 0.388$) (independent samples t-test). (B) Blood expression analysis: APPAT and UCA1 ΔCt values were compared between patient blood (B) and control blood (CN) samples. Data are presented as mean ± SD. Both APPAT ($p = 0.025$) and UCA1 ($p = 0.001$) showed statistically significant differences between patient and control groups (Mann-Whitney U). (C) Relative expression changes (Fold Change): Relative expression changes of APPAT and UCA1 are shown as $\log_2$ fold change ($\log_2$FC)

values, calculated using the $2^{-\Delta\Delta Ct}$ method. LIMA tissue and control blood samples were used as reference groups. Negative log_2FC values indicate downregulation relative to the reference condition. D) Correlation heat map: Correlation heat map demonstrating relationships between ΔCt values of APPAT and UCA1 across tissue and blood samples (Spearman correlation test). Color intensity indicates the strength and direction of the correlation. As ΔCt values inversely reflect expression levels, negative correlations correspond to opposite expression patterns.

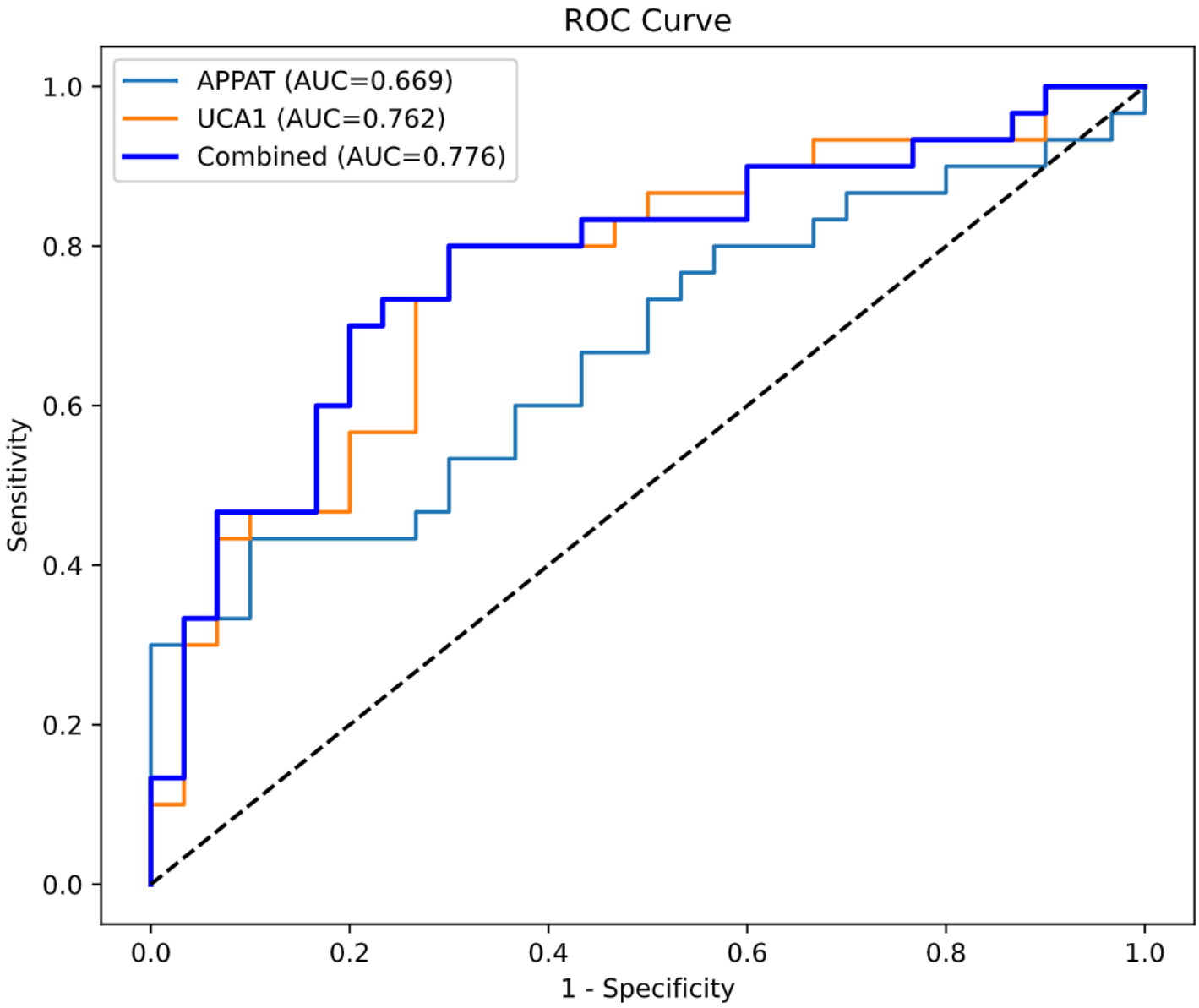


Figure 2

ROC curves of blood APPAT and UCA1 ΔCt values for the discrimination of patients and controls.