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# Transcriptomic Landscape of Human Left and Right Atria Associated with Atrial Fibrillation

## ABSTRACT

**Background:** Atrial fibrillation (AF) is the most common acquired cardiac rhythm disorder and has become a notable public health concern. Investigating the left versus right atrium (RA) transcriptome in AF is crucial because it provides insights into the gene expression changes that drive the molecular mechanisms underlying AF, potentially leading to targeted therapies and better patient outcomes. In this study, it is proposed that variances in the transcriptomic profiles between the human left atrium (LA) and RA, as well as alterations in molecular pathways, could offer potential targets for the onset and persistence of AF.

**Methods:** Here transcriptomes of LA and RA of patients (n = 31) undergoing mitral valve surgery were compared. Microarrays proceeded on the Affymetrix platform. Bioinformatic analyses were done on Partek Genomics Suite. Gene ontology, KEGG pathway, and functional enrichment analysis was conducted using differentially expressed genes on WebGestalt.

**Results:** Notably, transforming growth factor-β (TGF-β) and peroxisome proliferator-activated receptors signaling pathways were enriched commonly. Claudin 18, which encodes a tight junction transmembrane protein, was one of the most upregulated genes in LA. *PITX2* (paired like homeodomain 2) gene upregulation in LA is also involved in TGF-β signaling. Alongside the upregulation of TGF-β signaling, overexpression of extracellular matrix proteins like collagen, vitronectin, fibronectin, and thrombospondin also points out the cardiac fibrosis process preceded in LA, where AF originates.

**Conclusions:** In brief, comparisons of the AF-related transcriptomic landscapes of LA and RA propose targets for novel therapeutic and/or preventive strategies. This study highlights clinical evidence of genetic-based cardiac remodeling that could guide future therapeutic and preventive strategies.

**Keywords:** Atrial fibrillation, transcriptome profile, TGF-beta superfamily proteins, myocardial fibrosis

ORIGINAL INVESTIGATION

## INTRODUCTION

Atrial fibrillation (AF) is the most common acquired cardiac rhythm disorder and has become a notable public health concern, with approximately 44 million adults affected worldwide.[1] The prevalence of AF has been increasing due to the aging population, improved awareness, and advances in medical care.[1] Coronary artery disease, increasing age, diabetes mellitus, hypertension, heart failure, obstructive sleep apnea, smoking, excessive alcohol consumption, extremely active exercise, and inactive sedentary lifestyles are prominent risk factors for AF.[2] Atrial fibrillation is often asymptomatic until damaging consequences like stroke, systemic embolic events, or heart failure arise.[3] The life-threatening thromboembolic complications of AF can be significantly reduced by the use of oral anticoagulants, while carrying the risk of major bleeding.[4] So, risk assessment is critical due to the competing complications of AF.

Arrhythmia studies performed by implantable devices confirmed that approximately 80% of AF episodes were subclinical.[5] As AF is silent at the beginning, the first ~10 episodes are subclinically estimated, predicting the new-onset AF is crucial.[6] The need for certain and accurate biomarkers pointing to AF is still an important issue in the field of AF research.



**Günseli Çubukçuoğlu Deniz**

*Stem Cell Institute, Ankara University, Ankara, Türkiye*

**Corresponding author:**
Günseli Çubukçuoğlu Deniz
✉ gunselicubukcu@gmail.com

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This study has been registered in the ClinicalTrials.gov database under the accession number "ClinicalTrials.gov Identifier: NCT00970034.

We submitted the dataset of gene expression microarrays at GEO (Gene Expression Omnibus) open access database with accession number GSE115574.

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Atrial fibrillation causes structural and electrical remodeling and is determined by rapid and uncoordinated atrial electrical conduction activity. Although several genes and signaling pathways are reported to be associated with cardiac remodeling and AF, the need to clarify underlying pathophysiologic molecular mechanisms is still there.[7]

Numerous molecular pathways like inflammation, myocardial fibrosis, calcium handling abnormalities, oxidative stress, electrical, structural, and contractile remodeling are substantial underlying pathophysiologic mechanisms of AF.[8,9] The TGF-β (transforming growth factor-β) signaling pathway and ECM (extracellular matrix) activation participates in cardiac fibrosis process which have influence on AF initiation and maintenance.[10]

We hypothesized that variations in the transcriptomic profile of atrial tissue and changes in the molecular pathways in both the left atrium (LA) and right atrium (RA) might present promising targets for understanding the development and maintenance of AF. The establishment of biomarker panels with the capability of diagnostic and prognostic signs in human AF would be groundbreaking. Variations in gene expression configurations between the LA and RA of the human heart may be linked to the progressive pathophysiology of AF. This study provides leftness and rightness transcriptional frameworks that may guide the generation of biomarker panels before AF initiation and progression. The findings may provide fresh enlightenment on the mechanisms underlying AF and identify novel targets for the treatment of AF.

## METHODS

### Human Atrial Tissue Samples

Patients with chronic severe primary mitral regurgitation scheduled for mitral valve repair or replacement surgery were eligible to participate the study. The Local Ethics Committee approved study protocol which conformed to the ethical standards of the 1975 Declaration of Helsinki. Details on patient demographics and clinical characteristics are provided in Supplementary Table 1. All patients provided signed informed consent prior to undergoing surgery. Tissue samples from the LA and RA were collected from patients with persistent AF (n = 15) and those in sinus rhythm (SR) (n = 16) during mitral valve surgery. Persistent AF was characterized as AF that extended beyond 7 days, including episodes terminated by cardioversion, whether by medication or by direct current cardioversion, after ≥ 7 days. In this analysis, there are 3 principal comparisons: LA versus RA tissues in conditions of only SR, only AF, and combined SR and AF. Artificial intelligence-assisted technology was not used during the preparation of this work.

## HIGHLIGHTS

- Human left and right atrium transcriptome comparisons highlight cardiac fibrosis were dominant in LA suggesting AF mostly originates in LA.
- Extracellular matrix proteins were highly expressed in LA.
- *CLDN18* gene which encodes a tight junction protein, was the most upregulated gene in LA.
- *HAMP* gene which is the main regulator of iron metabolism by controlling iron influx through ferroportin, was the most downregulated gene in LA.

### Comparisons of Gene Expression Profiles

Biopsies of both the right and left atrial appendages were collected while accessing the RA before initiating heart-lung bypass and during the closure of the left atrial appendage while on heart-lung bypass. Microdissection technique was performed for preparing human atrium tissues as described previously.[11] Subsequently, tissues were promptly rapid-freeze in liquid nitrogen during the surgical procedure and preserved in −80°C freezer until subjected to analysis.

Total RNA from atrial tissues were extracted using MELT™ Total Nucleic Acid Isolation System (P/N AM1983 Ambion, Life Technologies, Thermo Fisher Scientific, Illinois, USA). Agilent 2100 Bioanalyzer RNA 6000 PicoChip was used to assess the quality and quantity of total RNA (Agilent Technologies Inc., USA), addition to NanoDrop® ND-1000 (NanoDrop Technologies, Wilmington, DE, USA) UV/Vis spectrometer and electrophoretic gel seperation.

For each human atrium tissue sample, 500 ng of isolated total RNA was used to produce amplified and biotinylated cRNA using the GeneChip® 3′ IVT Express Kit (P/N 901229 Affymetrix Inc., Santa Clara, CA, USA). Subsequently, 15 µg of the fragmented and biotin-labeled cRNA was hybridized to Human Genome U133 Plus 2.0 Affymetrix GeneChips (P/N 900467 Affymetrix Inc., Santa Clara, CA, USA) which analyze 47 401 transcripts from 38 572 human genes via 54 120 probesets. The arrays were then washed and stained at GeneChip® Fluidics Station 450, and finally, scanned with the GeneChip® Scanner 3000 7G, following the standard Affymetrix protocols. The gene expression microarray dataset is available in the open-access Gene Expression Omnibus database under the accession number GSE115574.

### Microarray Data Analysis

CEL format files containing the raw data were uploaded to Partek® Genomic Suite® (PGS, V7.0, St. Louis, MO, USA). The probe-level data underwent preprocessing and transformation using the robust multiarray analysis algorithm. To identify differentially expressed transcripts, researchers set fold change thresholds of ± 1.5 and established a significance level with $P < .05$. Additionally, researchers took notice of $q$ values, calculated as adjusted $P$ values, utilizing the optimized false discovery rate (FDR). A step-up FDR approach was applied to the $P$-values derived from linear contrasts to determine a cutoff for identifying genes that were significantly differentially expressed. A significance threshold for FDR was set at $q < 0.05$. Furthermore, unsupervised hierarchical clustering was employed to explore the relationships between the LA and RA groups (Figure 1). Lists of differentially expressed genes (DEGs) between LA vs RA comparisons can be found in Supplementary Table 2.

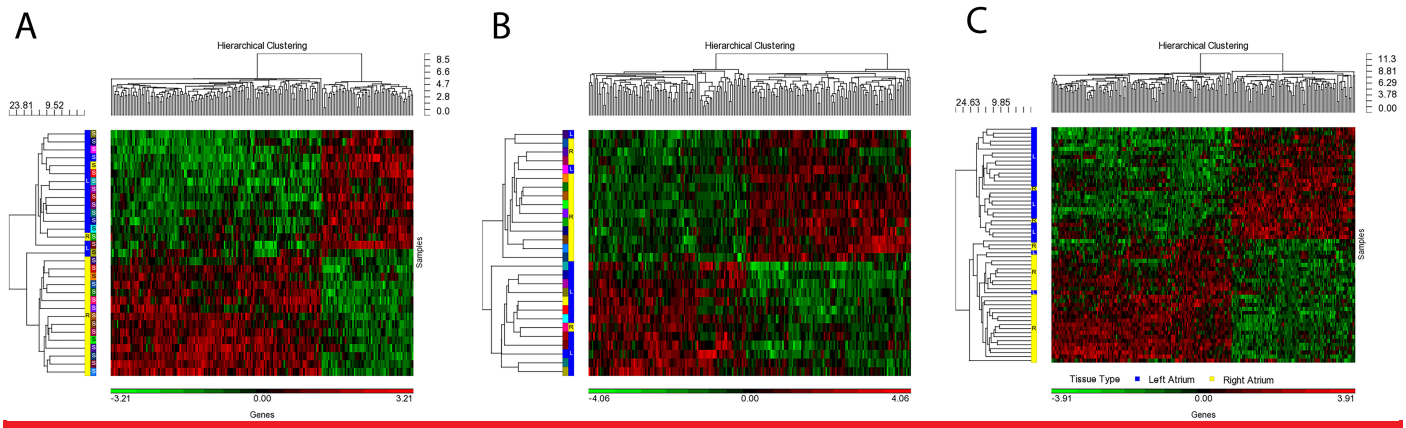


**Figure 1. Heatmaps from hierarchical clustering of differentially expressed genes between LA and RA groups. Differentially expressed genes between A. sinus rhythm tissues B. atrial fibrillation tissues C. all tissues together (sinus rhythm + atrial fibrillation).**

### Differential Gene Expression Analysis

Functional enrichment analysis was conducted by using DEGs. Differentially expressed genes identified by their differential regulation between the LA and RA groups (Affy probeset IDs), were analyzed using the WebGestalt (WEB-based Gene SeT AnaLysis Toolkit, http://www.webgestalt.org/) software. This tool was utilized for the annotation, enrichment, and visualization of the coding genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were carried out using the WebGestalt toolkit.[12] The functional enrichment analysis was performed using an overrepresentation analysis method,[7] with multiple test corrections applied through the Benjamini–Hochberg procedure to maintain a FDR threshold of 0.05.[13]

## RESULTS

### Comparisons of Transcriptomes of LA versus RA and Differentially Expressed Genes

Transcriptome analysis of human LA versus RA using only AF patient tissues, only SR patient tissues, and all tissues together have been done by microarray. Partek Flow Gene Expression Analysis performed one-way analysis of variance (ANOVA) and finds genes that vary across all samples based on a single factor, which was tissue type. The gene list contains 293 genes that passed the specified criteria and varied across all samples (n = 31) on tissue type with FDR (step-up) <0.05 in only SR tissues. Then LA versus RA contrast was added to create a differentially expressed gene list between LA and RA tissues. One hundred ninety-eight genes passed the specified criteria that have any change in LA relative to RA with FDR < 0.05 and fold changes <–1.5 or >1.5 in only SR tissues. The same criteria were used for comparisons between LA versus RA in AF and all (AF + SR) tissues. Differentially expressed Affy probeset identification numbers and directions that indicate DEGs are summarized in Table 1. Some of genes were represented with 2 or more probeset IDs in the dataset (Supplementary Table 2). A fold change >1.5 and $P < .05$ were required for differences to be considered statistically significant. Gene Ontology annotations showed that various pathways are differentially regulated between LA and RA tissues including biological processes, cellular components, and molecular function terms (Figure 2). Gene Ontology terms of SR, AF, and together DEGs were unambiguously mapped to 167, 175, and 197 unique entrezgene IDs, respectively. The number of genes on DEGs overlapped with the annotated genes in the GO Slim terms from biological processes reflected by red bar plots, cellular components reflected by blue bar plots and molecular function ontologies reflected by green bar plots (Figure 2).

### Gene Ontology and Pathway Enrichment Analysis

The TGF-β signaling pathway and the PPAR (peroxisome proliferator-activated receptors) signaling pathway were enriched in 3 DEGs (Supplementary Data 1). In all 3 analyses of the DEGs between LA versus RA, certain genes were enriched. SMAD6 (SMAD family member 6), SMAD7 (SMAD family member 7), SMAD9 (SMAD family member 9), BMP5 (bone morphogenetic protein 5), BMP10 (bone morphogenetic protein 10) and PITX2 were among the genes engaged in the TGF-β signaling pathway. While SMAD6, SMAD7, SMAD9, and BMP10 were downregulated, PITX2 and BMP5 were upregulated in LA versus RA of all 3 comparisons. LPL (lipoprotein lipase) and SCD5 (stearoyl-CoA desaturase 5) were associated with the PPAR signaling pathway. In the PPAR signaling pathway, LPL showed an increase in expression while SCD5 exhibited a simultaneous decrease. CLDN18 (claudin 18) was the most upregulated gene and NTM (neurotrimin) was the most downregulated gene in all comparisons between LA versus RA (Supplementary Data 1).

Other pathways enriched with DEGs were the Wnt signaling pathway, ECM-receptor interaction, cell adhesion molecules (CAMs), and the Rap1 signaling pathway. WNT5A (Wnt family member 5A) and one of its protein receptor FZD2 (frizzled

**Table 1. Differentially Expressed Gene Numbers and Directions between LA vs RA in Sinus Rhythm Tissues, Atrial Fibrillation Tissues and all Tissues Together**

| | SR Tissues | AF Tissues | All (SR + AF) Tissues |
|---|---|---|---|
| LA upregulated | 60 | 98 | 92 |
| LA downregulated | 138 | 102 | 134 |

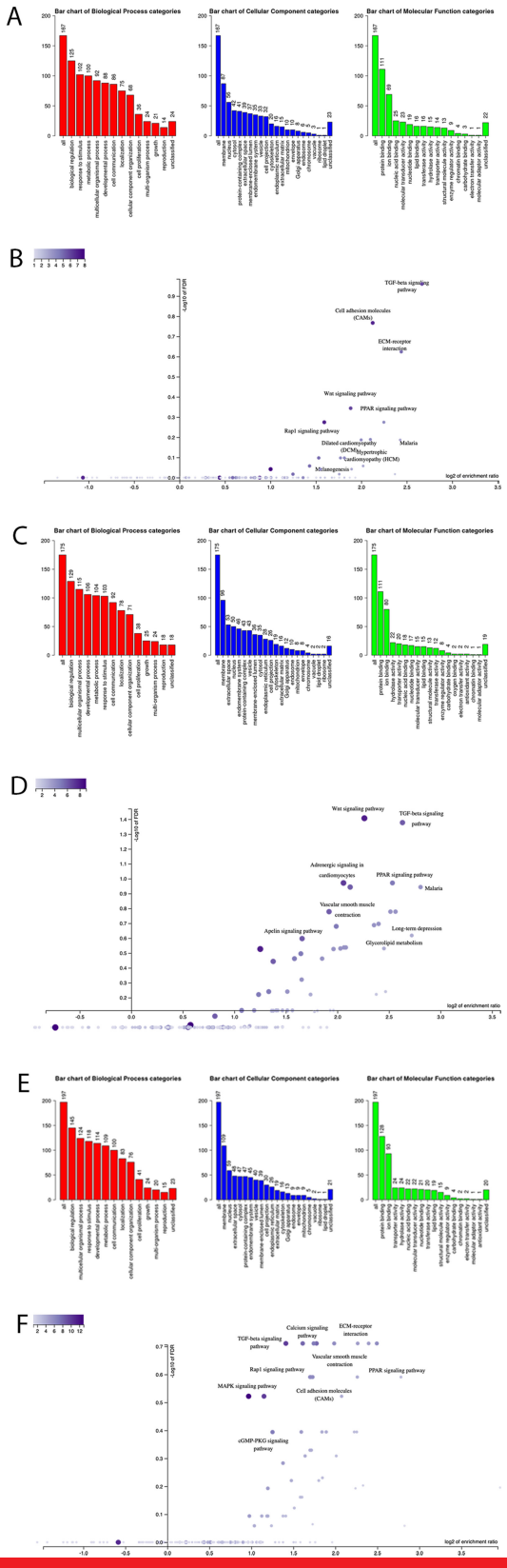


**Figure 2. Gene Ontology Slim summaries including biological process, cellular components and molecular functions and pathway enrichment results as volcano plots of differentially expressed genes between A. B. sinus rhythm tissues C. D. atrial fibrillation tissues E. F. all tissues together (sinus rhythm + atrial fibrillation).**

class receptor 2) gene expressions were both reduced in LA, while SFRP1 (secreted frizzled related protein 1) was upregulated. Wnt signaling was negatively regulated in LA tissues in all patients. COL4A6 (collagen type IV alpha 6 chain, CD36 (thrombospondin receptor), THBS4 (thrombospondin 4), LAMA4 (laminin subunit alpha 4), FBN2 (fibrillin 2), and VTN (vitronectin) gene expressions were significantly accelerated in LA tissues, meaning ECM protein accumulation was higher.

One of the CAMs was CLDN18, which was the most upregulated gene in the data. Moreover, ALCAM (activated leukocyte cell adhesion molecule), L1CAM (L1 cell adhesion molecule), NRXN3 (neurexin 3) were differentially expressed CAMs between LA and RA. Among all, the most upregulated gene in RA was HAMP (hepcidin antimicrobial peptide), which encodes a protein that functions to maintain iron homeostasis in cells.

## DISCUSSION

The regional gene expression profiles in patients' atriums could be a foresight for AF progression. Numerous research investigations have revealed that there are atrial cardiomyocytes within the pulmonary vein sleeves, displaying heightened pacemaker activity.[14] These cells have the potential to function as ectopic foci that can initiate single or multiple re-entry circuits. Atrial fibrillation is usually originates from spontaneous ectopic beats from a pulmonary vein-LA intersection pacemaker region.[15] Lone, persistent and permanent are different forms of AF and are promoted by single or multiple wave/circuit re-entries caused by ectopic beats. Key factors contributing to the re-entry mechanism include; structural remodeling driven by atrial fibrosis and left atrial dilation.

One of the main contributors to AF progression and maintenance is atrial fibrosis. Atrial fibrosis can lead to structural changes in the atriums, and structural remodeling is another subject for AF. Atrial fibrosis plays a role in converting uniformly activated atrial myocardium into a fragmented and branching tissue, increasing its vulnerability to multiple wavelet re-entry.[16] The main characteristics of atrial fibrosis are the protein accumulation of ECM and increased activity of cardiac fibroblasts. When atrial structural remodeling has started to compensate the arrhythmia atrial premature complexes arise as a trigger for AF by multiple wavelet re-entry.[17]

Transforming growth factor-β is a cytokine secreted by fibroblasts and epithelial cells in a pleiotropic fashion. The TGF-β superfamily of proteins regulates growth, development, and differentiation of cells. The results of the study showed that the TGF-β signaling pathway was significantly and highly regulated between LA and RA in all comparisons. Recently, a few studies published that myocardial fibrosis could be alleviated by molecules targeting the TGF-β signaling pathway.[18-21] The inhibitory smads SMAD 6 and 7 inhibit the TGF-β signaling which promotes fibrosis by inducing the proliferation and activation of cardiac fibroblasts. BMP10 is a peptide growth factor from the TGF-β superfamily of proteins, which is known for its cardioprotective effect.

Lately, a couple of α-myosin heavy chain-Bmp10 transgenic mice studies showed that BMP10 promotes cardiomyocyte survival and prevents cardiac adverse remodeling through diminuting cardiomyocyte apoptosis and myocardial fibrosis.[22] Conversely, the PITX2 gene was overexpressed in LA in the study, consistent with other studies that analyzed left atrial PITX2 expression. PITX2 encodes an evolutionarily conserved homeodomain transcription factor and involves the left-right asymmetric development of the embryo and cardiac development. In humans, PITX2 has 3 isoforms; PITX2a and PITX2b are alternative splicing variants that originate from the same upstream promoter, while PITXc is generated from a distint intergenic promoter. The PITX2c variant is primarily expressed in the human heart, especially in the LA.[23,24] Genome-wide association studies showed that the *PITX2* gene is located in proximity to the familial AF locus on chromosome 4q25 in the human genome.[25] As expected, *PITX2* gene expression was more overexpressed in LA tissues in the SR group than the AF group in the dataset. This result also supported that reduction in *PITX2* gene expression causes a shortening of atrial action potential duration and vulnerability to AF.[26]

Additionally, the ECM-receptor interaction pathway points out cardiac fibrosis, which is associated with excessive deposition of ECM proteins in the atrial myocardium. As a result of fibrosis, structural remodeling occurs in response to pressure overload starting in the LA where AF originates. ECM proteins, including collagen, vitronectin, fibronectin, and thrombospontin were overexpressed in LA then RA suggesting fibrosis progress starts earlier in LA.

Hepcidin antimicrobial peptide is the main regulator of iron metabolism by controlling iron influx through ferroportin.[27] The expression of HAMP is regulated by one of the TGF-β superfamily of proteins, BMP6. Likewise, *HAMP* gene upregulation in RA and the *HCN4* (hyperpolarization activated cyclic nucleotide gated potassium channel 4) gene, which encodes an ion channel that causes spontaneous depolarization, were upregulated in RA in the study. Both *HAMP* and *HCN4* genes were upregulated in RA cardiomyocyte cells in a recent study consistent with the results.[28] The protein encoded by the *HCN4* gene is necessary for cardiac pacemaker activity, and the mutations in this gene have been associated with atrial arrhythmia. HCN4 downregulation in LA was probably associated with AF progression.

Peroxisome proliferator-activated receptors signaling pathway was enriched with DEGs between LA vs RA groups in the study. Peroxisome proliferator-activated receptors are a class of ligand-dependent transcriptional regulators and are members of the nuclear receptor superfamily. Peroxisome proliferator-activated receptors function in fatty acid catabolism and oxidation in the heart. Cells in heart tissue, especially cardiomyocytes, have a high demand for energy. The majority of energy in the heart is derived from glucose and fatty acids through mitochondrial metabolic processes. Lipolysis of triglyceride-rich lipoproteins to fatty acids by LPL found on the endothelial cell surface of the coronary lumen is considered the predominant source of energy for cardiac contraction.[29] Metabolic syndrome, diabetes mellitus, and obesity are important risk factors for AF. As the severity of obesity and diabetes mellitus escalates, the heart experiences significant alterations like increased oxidative stress and triglyceride accumulation in response to the abundance of mitochondrial fatty acid from adipose tissue through regulation with LPL.[30]

The accumulation of immune cells and proteins that trigger inflammation in heart tissue and the circulatory system is linked to AF. Several inflammatory markers and mediators, including C-reactive protein (CRP), tumor necrosis factor (TNF)-α, interleukin (IL)-2, IL-6, and IL-8 have been associated with the occurrence or progression of AF.[31] However, Marcus et al[32] showed that past AF episodes do not influence CRP or IL-6 levels, but AF present at the time of blood sampling does lead to elevated levels of these markers. They also found that AF causes inflammatory cytokines to accumulate in the heart. Additionally, higher CRP and TNF levels appear to be associated with increased AF severity and persistence. The role of inflammation in the various stages of atrial remodeling in AF is still not fully understood due to the complexity of AF's underlying mechanisms.[33] While inflammatory markers have been recognized as important predictors in AF, more research is needed to determine their value beyond traditional clinical and echocardiographic risk factors.

Cell adhesion molecules are transmembrane glycoproteins that connect cells to each other or attach ECM through ECM proteins. L1 cell adhesion molecule gene expression is reduced in LA in all comparisons. Interestingly, a recent AF and valvular heart disease patient study published that L1CAM was a risk indicator for AF independently.[34] They showed that the L1CAM plasma level was substantially decreased in AF patients as opposed to SR controls.

Claudins are principal components of tight junctions and mediate cell-cell adhesion, controlling ion and small-molecule flux between cells and the ECM. The CLDN18 was the most upregulated gene in LA in the study. Claudin 18 gene is expressed in the lung alveolar epithelium and regulates postnatal lung development.[35] The association of CLDN18 gene expression with AF in the heart is a novel finding, as it's linked to gastric and esophageal adenocarcinomas.[36] This gene is expressed in the stomach widely and in the heart minimally.

The Wnt signaling pathway is a master regulator of cardiac development as well as regulating fibrotic progression. It has been shown that the Wnt signaling pathway contributes to AF-induced atrial structural and electrical remodeling.[37] Additionally, the Wnt proteins function in fatty acid and glucose metabolism, affecting mitochondrial biogenesis in the heart and increasing reactive oxygen species. Lately, a study showed that inhibition of the canonical Wnt signaling cascade caused mitochondrial dysfunction and cardiomyocyte failure; reactivation of the Wnt/β-catenin pathway protected mitochondria.[38] The genes involved Wnt signaling pathway were differentially expressed between RA and LA, meaning this pathway underlying AF pathophysiology shows

variability in RA and LA depending on the AF onset time or tissue characteristics. Functional enrichment of Wnt signaling genes reveals progression of fibrosis is also different in LA and RA.

Lately published review concludes that AF is linked to the reduced activity of signaling pathways that mediate cellular responses to external signals, such as *MAPK1* (mitogen-activated protein kinase 1) gene and Wnt signaling by RNA sequencing studies, which could greatly enhance understanding of AF electropathology.[39] This study supports that Wnt signaling was negatively regulated in LA compared to RA meaning AF progression is further in LA. Additionally they have found that Rap1, MAPK, cardiac muscle contraction pathways were upregulated in AF patient's atriums likewise upregulation in LA tissues.

### Study Limitations

Transcriptome profiling provides an overview of genome-wide mRNA expression. However, changes in translation, functional protein structures, protein-protein interactions, and regulatory processes cannot be predicted solely from gene expression studies. Proteomics and analysis of protein interactions will help to improve the results. Additionally, studying with human/animal tissues is another significant limitation doing global gene expression profiling. Leaving out all metobolic processes affecting gene expressions can't be overcome working on tissue biopsies. Researchers tried to minimize the variables that could affect the molecular pathways by selecting isolated patients with only degenerative mitral valve disease, regardless of sex difference and similar criteria. The most interference on atrial tissue gene expression was atrial pressure/volume stressors of chronic mitral valve insufficiency the endication of patient's cardiovascular surgery independently their rhythm status. In the future an in vitro AF model can be used to exclude the interfering molecular processes on global gene expression profiling.

## CONCLUSION

In summary, this study provides insights into the transcriptomes of both the LA and RA associated with AF, emphasizing the importance of pinpointing specific targets for a comprehensive understanding of disease progression. All those results might be helpful in better understanding the regionally critical pathophysiologic mechanisms of AF.

All those DEGs and pathways indicate that AF progression is different in LA and RA. This study provides evidence that the transcriptomic landscapes of LA and RA exhibit profound differences in the progression of AF. Data is limited to the sample size and to the moment of each patient's operation date when atrial tissue sampling was done. In future cell/organ cultures, atrial modeling should be used to follow the time-lapse progression of AF. In addition, these results have identified target genes and pathways, and this study can enlighten future studies on novel therapeutic and/or preventive strategies. Disclosures: The author has no financial disclosures that would be a potential conflict of interest with the current manuscript.

**Ethics Committee Approval:** The study protocol received approval from the Local Ethics Committee of Ankara University "14 Aug 2008/136-3994". The study protocol upgraded with the "30 Jan 2012/02-57-12" decision number.

**Informed Consent:** Written informed consent was obtained from the patients who agreed to take part in the study.

**Peer-review:** Externally and internally peer-reviewed.

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**Declaration of Interests:** The author have no conflict of interests to declare.

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**Supplementary Table 1. Patient Demographics and Hemodynamics**

| Characteristic | Atrial Fibrillation (n = 15) | Sinus Rhythm (n = 16) | *P* |
|---|---|---|---|
| Age, years mean ± SD (min-max) | 67.0 ± 13.0 (28-80) | 59.3 ± 11.6 (33-82) | .098 |
| Sex, male n (%) | 4 (26.6) | 5 (33.3) | .8 |
| BSA, $m^2$ mean ± SD (min-max) | 1.81 ± 0.17 (1.39-2.05) | 1.87 ± 0.17 (1.66-2.31) | .341 |
| Smoking, n (%) | 7 (46.6) | 8 (53.3) | .9 |
| COPD, n (%) | 5 (33.3) | 4 (26.6) | .8 |
| Diabetes mellitus, n (%) | 3 (20) | 4 (26.6) | .20 |
| CVD, n (%) | 4 (26.6) | 1 (6.6) | .134 |
| Hypertension, n (%) | 11 (73.3) | 4 (26.6) | .015* |
| Hyperlipidemia, n (%) | 8 (53.3) | 5 (33.3) | .148 |
| Total Cholesterol, mg/dL, mean ± SD | 185.8 ± 7.1 | 185.8 ± 7.6 | .999 |
| LDL Cholesterol, mg/dL, mean ± SD | 116.5 ± 5.6 | 115.7 ± 6.3 | .93 |
| HDL Cholesterol, mg/dL, mean ± SD | 44.4 ± 4.2 | 39.5 ± 1.9 | .35 |
| LVEDD, mm, mean ± SD | 54.6 ± 6.9 | 54.3 ± 12 | .927 |
| LVESD, mm, mean ± SD | 34.7 ± 6.2 | 35.5 ± 9.4 | .748 |
| DBP, mmHg, mean ± SD | 86.8 ± 11.4 | 89 ± 9.6 | .07 |
| LVEF, % mean ± SD | 54.4 ± 8.1 | 51.5 ± 7.6 | .247 |
| Systolic PAP, mmHg, mean ± SD | 54.7 ± 9 | 43 ± 16 | .008* |
| LA diameter, mm, mean ± SD | 55.9 ± 6.2 | 47.7 ± 6.3 | > .0001* |
| NYHA, > Class 2 n (%) | 10 (66.6) | 9 (60) | .3 |
| Tricuspid regurgitation, > 2, n (%) | 6 (40) | 2 (13.3) | > .0001* |
| Euroscore mean ± SD | 5.8 ± 3.5 | 3.8 ± 2.4 | .051 |
| Logistic Euroscore, % mean ± SD | 8.2 ± 8.1 | 4.1 ± 4.8 | .07 |
| ***Medication*** | | | |
| Antihyperlipidemic, n (%) | 7 (46.6) | 7 (46.6) | 1 |
| Digitalis, n (%) | 5 (33.3) | 2 (13.3) | .184 |
| Ca channel blocker, n (%) | 5 (33.3) | 0 (0) | .014* |
| Beta blocker, n (%) | 11 (73.3) | 10 (66.6) | .621 |
| ACE/ARB inhibitors, n (%) | 9 (60) | 8 (53.3) | .224 |

Data presented as mean ± SD.
*indicates significantly different *P* values. *P* < .05 Student's *t*-test for paired comparisons.
ACE; angiotensin-converting enzyme, ARB; angiotensin II receptor blockers, BSA; body surface area, COPD; chronic obstructive pulmonary disease, CVD; cerebrovascular disease, DBP; diastolic blood pressure, LA; left atrium, LVEDD; left ventricular end diastolic diameter, LVEF; left ventricular ejection fraction, LVESD; left ventricular end systolic diameter, PAP; pulmonary artery pressure, NYHA; New York Heart Association.

**Supplementary Table 2. Sinus Rhythm Tissues**