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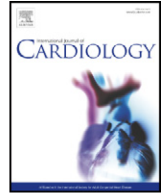

Differential left-to-right atria gene expression ratio in human sinus rhythm and atrial fibrillation: Implications for arrhythmogenesis and thrombogenesis☆

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ABSTRACT

Background: Atrial fibrillation (AF) causes atrial remodeling, and the left atrium (LA) is the favored substrate for maintaining AF. It remains unclear if AF remodels both atria differently and contributes to LA arrhythmogenesis and thrombogenesis. Therefore, we wished to characterize the transcript profiles in the LA and right atrium (RA) in sinus rhythm (SR) and AF respectively.
Methods: Paired LA and RA appendages acquired from patients receiving cardiac surgery were used for ion-channel- and whole-exome-based transcriptome analysis. The ultrastructure was evaluated by immunohistochemistry.
Results: Twenty-two and twenty ion-channels and transporters were differentially expressed between the LA and RA in AF and SR, respectively. Among these, 15 genes were differentially expressed in parallel between AF and SR. AF was associated with increased LA/RA expression ratio in 9 ion channel-related genes, including genes related to calcium handling. In microarray, AF was associated with a differential LA/RA gene expression ratio in 309 genes, and was involved in atherosclerosis-related signaling. AF was associated with the upregulation of thrombogenesis-related genes in the LA appendage, including P2Y12, CD 36 and ApoE. Immunohistochemistry showed higher expressions of collagen-1, oxidative stress and TGF-β1 in the RA compared to the LA.
Conclusions: AF was associated with differential LA-to-RA gene expression related to specific ion channels and pathways as well as upregulation of thrombogenesis-related genes in the LA appendage. Targeting the molecular mechanisms underlying the LA-to-RA difference and AF-related remodeling in the LA appendage may help provide new therapeutic options in treating AF and preventing thromboembolism in AF.
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1. Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia. AF causes atrial remodeling and makes it more difficult to achieve rhythm control [1–7]. Studies and clinical practice have indicated that the left (LA) and right (RA) atrium have different susceptibilities for maintaining AF. In paroxysmal AF, the pulmonary vein-LA junctions are the important targets for ablation, whereas the remaining atria are mostly neglected [8]. When the decision is reached to ablate persistent AF, either by catheter or surgical MAZE ablation, LA is much more extensively targeted than RA, which is consistent with the common view from prior studies that the LA is the favored substrate for maintaining AF [9,10]. It has been demonstrated in humans and animal studies that during AF, the high-frequency reentrant sources (rotors) are more commonly

Abbreviations: AF, atrial fibrillation; ApoE, apolipoprotein E; CD36, cluster of differentiation 36; DHE, dihydroethidium; ESTs, expressed sequence tag; IPA, Ingenuity Pathway Analysis; IP3R1, inositol 1,4,5-trisphosphate receptor type 1; LA, left atrium; P2RY12, purinergic receptor P2Y, G-Protein coupled, 12; qPCR, quantitative polymerase chain reaction; RA, right atrium; RyR2, ryanodine receptor 2; ROS, reactive oxidative species; SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2; SR, sinus rhythm; TGF-β1, transforming growth factor-β1.

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located in the LA than RA [11]. Firstly, complex fractionated atrial electrograms (CFAÉ) were reported to be more extensively located in the LA [12], and secondly, it was also shown that the dominant frequency of AF was mostly localized in the LA, thus resulting in LA-to-RA fibrillatory conduction [13].

Several studies have compared the characteristics of LA and RA by transcript profiles, ultrastructure and cellular electrophysiology, all of which suggest that LA is the driver in maintaining AF [14–21]. However, these studies have been limited to specific transcripts, animal models, small study population or samples without distinguishing AF from SR. A recent RNA sequencing study has compared human LA and RA and has shown limited LA-to-RA gene expression difference related to ion-channels and other accessory proteins, which was likely due to the small samples of the study. Another possibility is that the relatively low level of ion-channel gene expression, when using a whole-genomic approach, may make it difficult to distinguish altered expression from background noise [7].

At this point, it is unclear whether AF causes differential remodeling in both atria and contributes to LA arrhythmogenesis and thrombogenesis in the LA appendage. In this study, we therefore set out to characterize the expression profiles of the both atria from patients with SR or AF separately, by using a whole-exomic microarray and by highlighting cardiac ion channel and transporter subunits. Our results show that AF was associated with the differential LA-to-RA gene expressions, which may help improve our understanding of LA arrhythmogenesis.

2. Methods

2.1. Human tissue samples

The specimens of atrial appendages were obtained from patients receiving surgery for mitral valve or coronary artery disease. The research protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and passed the review of the Chang-Gung Memorial Medical Foundation Institutional Review Board. Written informed consent was obtained from all participating patients. Patients with AF presented persistent AF known more than 6 months, and patients with SR had no evidence of AF clinically without use of any anti-arrhythmic drugs.

2.2. RNA isolation and cardiac ion channel and transporter gene expression analysis

Total RNA was isolated from LA and RA appendages with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and was further purified and concentrated by using the WelPrep tissue RNA array (Welgene, Taipei, Taiwan). The quality and quantity of total RNA were analyzed by using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The mRNAs of interest were further analyzed with assay provided by NanoString Technology as previously described elsewhere [22]. Briefly, 100 ng mRNA of each sample was hybridized with the designed Reporter CodeSet and Capture ProbeSet (according to the manufacturer's instructions; NanoString Technologies, Seattle, WA, USA) for direct labeling of mRNAs of interest, which include genes encoding human cardiac ion-channels and transporters shown in Fig. 1, with molecular barcodes without the use of reverse transcription or amplification. The hybridized samples were recovered and counted with the NanoString nCounter. The resulting counts were corrected by subtracting the mean value of the negative control from the raw counts obtained for each mRNA. The corrected raw data were finally normalized to the housekeeping genes.

2.3. Microarray analysis

Gene expression profiles in tissue RNA were analyzed using human U133 Plus2 GeneChip (Affymetrix, Santa Clara, CA, USA), following the manufacturer's protocol as described elsewhere [23]. The differential LA-to-RA gene expression ratios between AF and SR were selected with supervised (unpaired t test with a false discovery rate < 5%) methods and displayed with unsupervised (hierarchical clustering) methods.

2.4. Pathway analysis

Genes of interest were imported into Ingenuity Pathway Analysis (IPA; Ingenuity System, Redwood City, CA, USA) for pathway analysis. Fisher's exact test was applied by IPA to examine the likelihood that the association between genes of interest and related pathways are not due to random association.

2.5. Quantitative polymerase chain reaction

Total RNA was extracted by using the TRIzol reagent, and quantitative polymerase chain reaction (qPCR) was performed. The oligonucleotide sequences that were used are shown in Supplemental Table 5. Glyceraldehyde 3-phosphate dehydrogenase mRNA was used as the internal control.

2.6. Confocal immunohistochemistry

Immunohistochemistry and quantitative assessment were performed on tissue slides as previously described [24]. Immunohistochemical analyses were performed using confocal microscopy (Leica TCSSP2, Wetzlar, Germany) with collagen-1 and TGF-β1 (Santa Cruz, Dallas, TX, USA) as primary antibodies, followed by Cy3 (red; Chemicon, Temecula, CA, USA) conjugated secondary antibodies. Nuclei were visualized by 40,6-diamidino-2-phenylindole staining. The expression of target proteins was calculated entire average fluorescence intensity as protein-occupied area in the tissue. For each analysis, at least 5 random fields were chosen with >30 observations in each. Oxidative stress from reactive oxidative species (ROS) in the tissues was measured with fluorescent dye (dihydroethidium [DHE]) and was detected using confocal microscopy. Samples were preincubated with 10 μmol/L of DHE for 30 min. DHE was excited at 543 nm with an argon laser, and emission at 605 nm was recorded. Two-dimensional images (512 × 512 pixels) were acquired and analyzed with MetaMorph software (Universal Imaging Corp, West Chester, PA, USA).

2.7. Western blotting

Proteins were extracted and processed as described elsewhere [24]. Briefly, tissue specimens were lysed in lysis buffer. After the proteins were separated using sodium dodecylsulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes, the membranes were incubated overnight at 4 °C with primary antibodies against PYR2, ApoE (Abcam, Cambridge, MA, USA), CD36 (Millipore, Temecula, CA, USA), Kv11.1 (Bioworld, St Louis Park, MN, USA), Kv1.5 (Proteintech, Rosmont, IL, USA) and GAPDH (Santa Cruz, Dallas, TX, USA), followed by anti-rabbit and anti-mouse IgG (Sigma, St Louis, MO, USA) conjugated secondary antibodies. Signals were detected by electrochemiluminescence and quantified by densitometry. Immunoreactive signal bands were in the linear range and expressed relative to GAPDH.

2.8. Statistical analysis

For patient characteristics, mean ± standard deviation for continuous variables and count and percent for categorical variables were used. Mean ± standard error for continuous variables was used for the others. Fisher's exact test was used for category comparisons, and the unpaired Student's *t*-test was applied for the two-group continuous variable comparisons. For cardiac ion channel and transporter analysis,

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