Human Atrium Transcript Analysis of Permanent Atrial Fibrillation

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SUMMARY

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and is associated with increased risks of stroke and heart failure. However, the exact mechanisms of left atrium remodeling and AF-related biological behaviors are not completely understood.

The transcripts of left atrium in permanent atrium fibrillation patients ($n = 7$) were compared with those of healthy heart donors ($n = 4$) in sinus rhythm using Agilent 4x44K microarrays. Differently expressed genes were analyzed based on Gene Ontology and KEGG and Biocarta pathway analysis databases.

We identified 567 down- and 420 up-regulated genes in atrial fibrillation. The majority of the down-regulated genes participated in metabolic processes, particularly that for fatty acids. The most remarkable up-regulating effects were immune and platelet activation. In addition, atrial remodeling including structural, contractile, electrophysiological, neuro-hormone, and oxidant stress was also observed, suggesting various pathophysiology changes in fibrillating atrium. Nine AF closely related genes were validated by real-time RT-PCR.

Some AF specific genes were determined which may be a complement to the mechanism of left atrium remodeling. Metabolic changes and inflammation could promote or aggravate atrial fibrillation. (Int Heart J 2014; 55: 71-77)

Key words: Transcriptome, Remodeling, Metabolism, Inflammation

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, occurs in 1-2% of the general population and is associated with increased risks of stroke, heart failure, and even death.⁽¹⁾ Although epidemiological surveys have linked different risk factors to this poor prognosis,⁽²⁾ the exact mechanisms of increased risk of stroke and heart failure remain poorly understood. Current treatment strategies for AF are not yet completely satisfactory. Left atrium (LA) remodeling was emphasized to have a close relationship with AF,⁽³⁻⁵⁾ but the exact pathophysiological changes of LA structural and functional remodeling in the extracellular matrix compartment, cardiomyocytes, ionic channels, and neurohormonal expression⁽⁶⁾ have not yet been fully clarified.

Differential gene expression is an important driving force in the development, function, and pathology of multicellular organisms.⁽⁷⁾ Microarray analysis offers a high-throughput approach to the comprehensive study of differential genes and their functions. Previous microarray studies have confirmed some AF related genes,^(8,9) ventricular-like signature of fibrillating atrium,⁽¹⁰⁾ and metabolic adaption to persistent AF.⁽¹¹⁾ However, owing to the high heterogeneity of AF, all of the biological behavior changes of AF have not yet been completely clarified. Therefore, to screen for genes and pathways involved in the initiation and perpetuation of AF, we performed LA

whole-genome transcript analysis of patients with permanent AF and compared the results with healthy heart donor controls in sinus rhythm using human gene expression microarrays. AF related biological processes and pathways were identified based on Gene Ontology and pathway analysis databases.

METHODS

Human myocardium: Experiments were performed in LA muscle strips obtained from patients with a diagnosis of permanent AF ($n = 7$) with coronary artery disease undergoing LA radiofrequency ablation and mini incision coronary artery bypass grafting (CABG) surgery. Control LA samples were obtained from healthy heart donors ($n = 4$) in sinus rhythm whose hearts were not suitable to transplantation. AF was confirmed by electrocardiography, and the mean AF duration was 47.7 ± 25.0 months. Detailed demographic information is presented in Table I. AF samples (approximately $10.0 \times 5.0 \times 2.0$ mm) were taken from the LA posterior wall between the inferior and superior right pulmonary veins (adjacent to the atriotomy line)⁽¹²⁾ after performing the grafting. Donor hearts were arrested and transported in ice-cold, oxygenated cardioplegic solution. Samples were snap frozen in liquid N₂ and stored at

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Table I. Demographics of Atrial Fibrillation (AF) Patients and Sinus Rhythm (SR) Controls

Identifier	Age (Y)	Sex	Duration of AF (M)	EF (%)	LA diameter (mm)	Medication	CABG Grafts
AF A1	62	M	35	67	47	A,B	3
AF A2	51	M	24	71	56	B	3
AF A3	73	M	76	65	57	A,B	3
AF A4	72	F	49	70	43	A,B	3
AF A5	63	M	36	64	51	A,B	3
AF A6	60	M	88	58	62	A,B	3
AF A7	51	M	26	77	49	A,B	2
SR C1	33	M		70	39		
SR C2	45	F		65	46		
SR C3	53	M		64	41		
SR C4	46	F		73	49		

A indicates ACE-inhibitor; B, β -blocker; Y, years; and M, months.

-80°C. The Ethics Committee of Chaoyang Hospital, Capital Medical University approved the study, and all patients and/or their relatives provided written informed consent to participate in the study. Detailed patient information is shown in Table I.

mRNA preparation, labeling and hybridization: Frozen left atrium samples (100 mg) were ground and homogenized and total RNA was isolated using TRIzol (Invitrogen) according to the manufacturer's instructions. RNA integrity was assessed using standard denaturing agarose gel electrophoresis. RNA quantity and purity were determined by spectrophotometry (NanoDrop ND-1000 spectrophotometer).

Sample labeling and microarray (Agilent Human 4x44K Gene Expression Microarrays, > 27,000 probes) hybridization were performed according to the Agilent one-color microarray-based gene expression analysis protocol. Briefly, 1 μ g of total RNA from each sample was linearly amplified and labeled with Cy3-dCTP. The labeled cRNAs were purified using an RNaseasy Mini Kit (Qiagen). The concentration and specific activity of the labeled cRNAs (pmol Cy3/ μ g cRNA) were measured by NanoDrop ND-1000. One μ g of each labeled cRNA was fragmented by adding 11 μ L 10 $\times$ blocking agent and 2.2 μ L of 25 $\times$ fragmentation buffer, then heated at 60°C for 30 min, and finally 55 μ L 2 $\times$ GE hybridization buffer was added to dilute the labeled cRNA. One hundred μ L of hybridization solution was dispensed into the gasket slide and assembled to the gene expression microarray slide. The slides were incubated for 17 hours at 65°C in an Agilent hybridization oven. The hybridized arrays were washed, fixed, and scanned using an Agilent DNA microarray scanner (part number G2505B). Microarray experiments were performed by Shanghai KangChen Biotech Company, Shanghai, China.

Data collection and normalization and differentially expressed gene screening: Agilent Feature Extraction software (version 10.7.3.1) was used to analyze the acquired array images. Quantile normalization and subsequent data processing were performed using the GeneSpring GX v11.5.1 software package (Agilent Technologies).

Given the relatively small number of samples, differentially expressed mRNAs were determined by the TwoClassDif method¹³ and subsequent Volcano Plot filtering. The default threshold of statistical significance was a fold change ≥ 2.0 and $P \leq 0.05$. Next, unsupervised hierarchical cluster analysis was conducted to ascertain the differentially expressed genes.

Gene ontology and pathway analyses based on microarray data: Differently expressed genes were grouped on the basis

of the Gene Ontology (GO) consortium (<http://www.geneontology.org/>). Meanwhile, pathway analyses were performed based on databases of KEGG (<http://www.genome.jp/kegg/>) and Biocarta (<http://www.biocarta.com/>). Fisher's exact test and the chi square test were used to classify the enrichment of both GO and pathway category. The P -value cut-off, which denotes the significance of GO item and pathway, was 0.05. Unsupervised hierarchical clustering was performed using Agilent GeneSpring GX software.

Validation by semiquantitative real-time RT-PCR: Expression patterns of 9 selected differentially expressed genes were validated by real-time RT-PCR (Applied Biosystems) using SYBR-Green (Applied Biosystems) as described previously.^{10,14} Primers were designed using the NCBI "Primer-BLAST" and the detailed list is presented in Table II. Because the calculated expression levels of samples were independent of the house-keeping gene, all gene expression was normalized to ribosomal protein RPL41.¹⁰ The results are presented as the mean of 3 independent experiments.

RESULTS

Overall differential gene expression profiles between permanent AF and sinus rhythm and prominent biological behaviors of AF: We identified 987 differentially expressed genes altogether, of which 420 and 567 were up- and down-regulated in AF, respectively (Figure 1A). Unsupervised hierarchical clustering (Figure 1B) showed a distinguishable gene expression profiling among AF and sinus rhythm samples. The top 10 up- and down-regulated mRNA are shown in Table III, which represented the most striking altered transcripts of AF.

To systematically identify biological connections of differentially expressed genes and identify new pathways associated with AF, we performed GO and KEGG and Biocarta pathway analysis. Down-regulation was particularly noted for metabolism processes, as 6 terms of the top 10 significant down-regulated biological processes (BP) were processes about metabolism (Figure 2A). Consistent with the GO results, pathway analysis also showed a down-regulation of metabolism, particularly remarkable for fatty acids and propanoate metabolism (Figure 2C), exemplified by the reduction of the key lipid uptake enzyme ACSL3 (acyl-CoA synthetase long-chain family member 3),¹⁵ ACADL (acyl-CoA dehydrogenase, long), and ADH (alcohol dehydrogenase) gene family. In addi-

Table II. Oligonucleotides Used for Real-Time RT-PCR of Nine Selected Genes

Gene	Sequence of primer	Annealing temperature
CA14	Forward:5'- TTGAGGTGGGTGAGACTAAG -3'	60°C
	Reverse:5'- AGGTGAAGGGAGGCACT -3'	
RyR2	Forward:5'- GAGTGGCTCAAGTGGGT -3'	60°C
	Reverse:5'- TGTCTATGGTGCCGTCTA -3'	
DLK1	Forward:5'- TGGGCACTGTGGGTATCGTCT -3'	60°C
	Reverse:5'- TTCCGCAGCATGTGGTTGTAG -3'	
MYH7	Forward:5'- TGTCACCGTCAACCCTTAC -3'	60°C
	Reverse:5'- CGGGCTCTGGTCCTTCTT -3'	
CTGF	Forward:5'- GCGGCTTACCGACTGGA -3'	60°C
	Reverse:5'- ACAGGCGGCTCTGCTTC -3'	
IL6	Forward:5'- CCTTCGGTCCAGTTGCCTTCT -3'	60°C
	Reverse:5'- GCCTCTTTGCTGCTTTCACA -3'	
PF4	Forward:5'- CACTGTGGTCGCCTTCG -3'	60°C
	Reverse:5'- CAGCGTGGCTATCAGTTGG -3'	
HSPA2	Forward:5'- CCAACGACCAGGGCAATC -3'	60°C
	Reverse:5'- GCCAGTGTTCATATCCGACT -3'	
NPPB	Forward:5'- TCCCACAGGTGTCTGGAAGT -3'	60°C
	Reverse:5'- GCAGGGTGTAGAGGACCATTTT -3'

Figure 1. Heat map (A) and unsupervised hierarchical clustering (B) of all differently expressed genes of patients with permanent atrial fibrillation compared with healthy heart donors in sinus rhythm. “Red” indicates high relative expression; “green” low relative expression, and “blue” low relative expression.

tion, fatty acid synthesis was also down-regulated, as evidenced by the reduction of ACACB (acetyl-CoA carboxylase beta) and FASN (fatty acid synthase). Furthermore, the metabolism of some amino acids such as cysteine, methionine, and tyrosine was down-regulated (Figure 2A). In GO directed graphs where an annotated gene moves from more general to more specific genes, the most specific metabolic effect was down-regulation of the triglyceride metabolic process, regulation of primary metabolic processes, and positive regulation of cellular metabolic processes (Figure 2B). In the case of up-regulated BP (Figure 3A), the most significant BP was the response to stress, which reflected the essence of AF pathophysiology. The immune process played a considerable part in the pathological process, which confirmed the important role of

inflammation in the initiation and perpetuation of AF.¹⁶⁾ The corresponding up-regulation inflammation factors such as IL-6 (interleukin-6)¹⁷⁾ and TNF (tumor necrosis factor)¹⁸⁾ have been reported to be linked with the clinical outcomes of AF. In addition, there was also evidence for platelet activation, exemplified by the up-regulated PF4 (platelet factor 4) and F5 (coagulation factor V) which mediate the blood coagulation cascade. Inflammatory factors and mediators could also confer a pro-thrombotic state through platelet activation.¹⁹⁾ Meanwhile, up-regulated inflammation was also demonstrated in pathway analysis (Figure 3C), exemplified by cytokine-cytokine receptor interactions and chemokine signal pathways. These inflammation pathways initiated leukocyte trafficking and activation and triggered downstream pathways with respect to the establishment and perpetuation of AF. The complement and coagulation cascade pathway also confirmed the relationship between inflammation and thrombogenesis. In an up-regulated GO directed graph (Figure 3B), immune response and platelet activation were proved to be the most specific BP terms.

Structural and functional remodeling in fibrillating left atrium:

GO and pathway analysis also confirmed some differently expressed genes participating in LA remodeling, such as the down-regulated adherens junction pathway which controls tissue architecture and cell polarity. This pathway could explain the LA dilation and fibrosis, exemplified by the down-regulation of profibrotic growth factor FGFR1 (fibroblast growth factor receptor 1).¹⁰⁾ In up-regulated BP, structural remodeling was also identified, exemplified by the up-regulation of morphogenesis and development of anatomical structure. There was also evidence for structure and function remodeling as a pathway in cardiac muscle contraction, and the TGF- β signaling pathway and ECM-receptor interaction were also up-regulated. Specifically, a shift from fast α - to β -myosin heavy chain, encoded by MYH7, reflected the adaption of left atrial to chronic overload induced by AF, but structural changes were affected by paid the price of depressed contraction velocity⁵⁾ and compliance, which consequentially brought about the impaired LA function. Atrial fibroblast forms through Ang II? induced TGF- β pathway activation, an important profibrotic pathway, and subsequent connective tissue growth factor

Table III. The Top 10 Differently Expressed Genes Between Atrial Fibrillation and Sinus Rhythm

Symbol	Gene name	P	Fold change value
CA14	carbonic anhydrase XIV	1.8E-08	-8.6
DLK1	delta-like 1 homolog	5.3E-07	-10.6
HLF	hepatic leukemia factor	7.2E-06	-3.1
MID1IP1	MID1 interacting protein 1	1.2E-05	-2.8
HOXA4	homeobox protein Hox-1D	2.5E-05	-2.5
SCGB1D1	secretoglobin, family 1D, member 1	2.7E-05	-10.2
GABBR2	gamma-aminobutyric acid (GABA) B receptor, 2	4.0E-05	-5.4
EPN3	epsin 3	7.9E-05	-3.8
ADRBK2	adrenergic, beta, receptor kinase 2	8.2E-05	-3.2
ZMAT4	zinc finger, matrin-type 4	1.3E-04	-2.2
NPPB	natriuretic peptide B	5.6E-06	+30.0
HSPA2	heat shock 70kD protein 2	1.6E-05	+3.3
HLA-B	major histocompatibility complex, class I, B	8.6E-05	+2.6
RBCK1	RanBP-type and C3HC4-type zinc finger containing 1	9.2E-05	+6.6
CTGF	connective tissue growth factor	1.2E-04	+6.9
VWA1	von Willebrand factor A domain containing 1	1.3E-04	+2.4
MYOF	Fer-1-like 3, myoferlin	1.6E-04	+2.3
PF4	platelet factor 4	1.8E-04	+3.1
ABTB2	ankyrin repeat and BTB (POZ) domain containing 2	2.0E-04	+3.6
NCRNA00152	long intergenic non-protein coding RNA 152	2.2E-04	+3.2

– indicates down-regulation; and +, up-regulation.

Figure 2. **A:** Top 10 down-regulated biological process terms based on Gene Ontology (GO) analysis. **B:** GO directed acyclic graph of top 10 down-regulated biological terms and their parent annotations. Terms in red represented the top 10 terms. **C:** Top 8 down-regulated pathways based on KEGG and Biocarta databases. Enrichment score equals $-\lg P$ value, where P represents the significance testing value.

(CTGF) expression.²⁰ These combined with the down-regulated adherens junction pathway form the basis of atrial fibroblasts, which reflected the morphogenetic changes in anatomical structure (Figure 3B). We also found evidence of association in pathways involved in viral myocarditis and hypertrophic cardiomyopathy, which displayed an analogous pathophysiology with AF.

Real-time RT-PCR analysis: We selected 9 genes screened by microarray analysis. CA14 (carbonic anhydrase XIV) is an important monitor of cytosolic pH. Sarcoplasmic reticulum (SR) RyR2 (ryanodine receptor 2) functions as a channel that supplies Ca^{2+} to cells. DLK1 (Delta-Like 1 Homolog) is one kind of growth regulator. CTGF is an important regulatory fac-

Figure 3. **A:** Top 10 up-regulated biological process terms based on GO. **B:** GO directed acyclic graph of top 10 up-regulated biological terms and their parent annotations. Terms in dark blue represented the top 10 terms. **C:** Top 8 up-regulated pathways based on KEGG and Biocarta databases. Enrichment score equals $-\lg P$ value, where P represents the significance testing value.

tor of atrial fibrosis. IL6 is an inflammation factor. PF4 is a cytokine that mediates the blood coagulation cascade. HSPA2 encodes one member of human Heat Shock 70kD Protein 2 (HSP70-2). NPPB is the precursor of B-type natriuretic peptide (BNP). These AF related genes were confirmed by real-time RT-PCR analysis (Table IV).

DISCUSSION

LA structure and function remodeling in AF: To maintain homeostasis against external stressors, tissues and organs adjust accordingly and form new phenotypes, such as LA remodeling to AF stressors. LA remodeling refers to a wide range of structural changes including cardiomyocytes, extracellular ma-

Table IV. Validation of Differently Expressed Genes observed in microarray analysis with RT-PCR

Gene	Genbank Accession	Regulation	Microarray fold-change	PCR fold-change	Validation status
CA14	NM 012113	down	8.6	4.3	+
RyR2	NM 001035	down	2.5	2.3	+
DLK1	NM 003836	down	10.6	3.6	+
MYH7	NM 000257	up	2.8	3.4	+
CTGF	NM 001901	up	6.9	5.6	+
IL6	NM 000600	up	2.3	3.6	+
PF4	NM 002619	up	3.1	2.6	+
HSPA2	NM 021979	up	3.3	4.5	+
NPPB	NM 002521	up	30.0	10.6	+

PCR results represent the mean of three independent experiments.

trix (ECM), and ionic channels, and subsequent functional remodeling.

To adapt for permanent AF induced volume overload, LA structural proteins changed and atrial fast α -myosin heavy chain switched to ventricular contractile β -phenotype, exemplified by up-regulated MYH7. Moreover, in the top-ten differently expressed genes (Table III), some cardiomyocyte specific growth factors and transcription factors were abnormally expressed. DLK1 encodes one kind of growth regulator, and Anisimov *et al*⁽²¹⁾ found similar expression profiles between DLK1 and some other known cardiac transcription factors such as GATA4, Hand1, and Mx1 in the development phase of embryonic stem cell to cardiomyocytes, thereby determining the differentiation dependent role of DLK1. Hepatic leukemia factor (HLF) is an important member of the PAR bZip protein family which controls the circadian clock and makes organisms adapt their physiology and behavior to environmental changes. Wang, *et al*⁽²²⁾ found PAR bZip knockout mice developed cardiac hypertrophy and left ventricular dysfunction, thus verifying the cardiovascular modulation effect of HLF. Home box (HOX) genes play a central role in the development process of the specification of some organs. Mutant mice lacking the entire HOXA, one cluster of HOX genes, displayed a spectrum of postnatal cardiac and respiratory defects.⁽²³⁾ Though the specific regulation mechanisms of DLK1, HLF, and HOXA4 are unknown, their down-regulated expression levels were inextricably related to permanent AF and may reflect the dedifferentiation and maladaptation state of LA cardiomyocytes to AF stressors.

The main pathological change of ECM remodeling is atrial fibrosis. The compensatory elevated Ang II and downstream like TGF- β signaling pathway⁽¹⁰⁾ activate LA fibrosis. CTGF plays an important role in structural remodeling of atrial fibrosis through mediation by angiotensin II induced regulation of lysyl oxidase.⁽²⁴⁾ Up-regulated CTGF represented the elevated signal transduction state in which left atrial myocardium developed collagen cross-linking and interstitial fibrosis. The interaction between ECM and cardiomyocytes facilitated the adaption to AF and at the same time constitutes electrical remodeling substructure rendering LA more vulnerable to AF development.⁽⁶⁾

Regarding electrical remodeling, the destruction of calcium homeostasis plays a key role in forming and maintaining AF. In normal hearts, Ca^{2+} enters cells through L-type Ca^{2+} channels and Ca^{2+} release is induced through sarcoplasmic reticulum (SR) RyR2 (ryanodine receptor 2). In AF cardiomyocytes, however, hyperphosphorylation of RyR2 increases its

sensitivity to Ca^{2+} and induces greater Ca^{2+} leak, which in combination with the subsequent larger Na^{+} - Ca^{2+} -exchanger current and increased diastolic Ca^{2+} -voltage coupling cause AF-promoting atrial delayed after-depolarization activity.⁽²⁵⁾ In our study, the decrease in RyR2 in AF was more than two-fold greater than that in SR. Though we did not test phosphorylation of RyR2, the decreased RyR2 expression suggested RyR2-mediated SR Ca^{2+} leak which facilitated cardiac arrhythmogenesis. Moreover, the down-regulation of L-type Ca^{2+} channel transcripts and sarcoplasmic Ca^{2+} -ATPase⁽¹⁰⁾ form the basis of electrical remodeling. Furthermore, cardiomyocyte membrane CA14, an important monitor of cytosolic pH, was notably down-regulated. CA14 together with anion exchanger 3 constitute a $\text{Cl}^{-}/\text{HCO}_3^{-}$ exchange system to dispose of CO_2 and regulates pH within cardiac myocytes.⁽²⁶⁾ The down-regulation of CA14 reflected the reduced $\text{Cl}^{-}/\text{HCO}_3^{-}$ exchange activity and increased intracellular pH. Although this is the result of AF-induced cardiac contraction up-regulation, changes in pH directly affect cardiac contractility and increase muscle tension. Thus reflects the mechanism of "AF begets AF".⁽³⁾

Some neurohormones are activated in response to AF stressors. Although it is difficult to differentiate these factors predisposing to AF self from the pathology resulted from AF, identifying their expression may result in the development of new therapeutic strategies and permit early treatment. Apart from the aforementioned Ang II, N-terminal pro-B-type natriuretic peptide coded by NPPB, the precursor of B-type natriuretic peptide (BNP), was up-regulated. In addition to the endocrine role in controlling salt and water balance, BNP could maintain cardiac structural stability through its inhibition of both cardiomyocyte hypertrophy and cardiac fibroblast activation,⁽²⁷⁾ and recently BNP was also shown to have a favorable cardiometabolic function like increasing fatty acid metabolism.⁽²⁸⁾ Based on structural and the following metabolic remodeling results, dramatically elevated NPPB represented the decompensation state of permanent AF. Compensatory increased BNP was thought to be closely related to the increased risk of AF.⁽²⁹⁾

Human left atrium metabolism in AF: Metabolism changes in AF reflect both changes in LA remodeling induced by AF and underlying heart disease substrates. The metabolism of fibrillating atrial tissue is generally thought to be in a hypermetabolic state. The increased energy demand may originate from ion transport processes like Ca^{2+} handling and cardiomyocyte contraction and high LA wall stress. Being different from glycolysis of early proliferating cardiomyocytes, fatty acid β -oxidation is the main energy metabolism form of terminally

differentiated one.³⁰⁾ However, in permanent AF, fatty acid metabolism including uptake, synthesis and oxidation were all down-regulated, exemplified by the decrease of some key enzymes in fatty acid metabolism such as ACSL3, ACADL, ADH, ACACB, and FASN. Being compensatory, the increased energy demand is achieved through glycolysis, as demonstrated by the increased lactate production in coronary arteriovenous in early stage of AF³¹⁾ and some up-regulated transcripts of glycolytic enzymes in permanent AF.¹⁰⁾ Mayr, *et al*¹¹⁾ found a rise in the levels of β -hydroxybutyrate, some ketogenic amino acids, and 3-oxoacid transferase, a key enzyme in ketone body metabolism, in fibrillating atrial tissues using nuclear magnetic resonance spectroscopy, thereby demonstrating a negative feedback of ketone bodies on glucose metabolism in AF. The switch of metabolism from fatty acids to glucose and feedback of ketone bodies reflected the metabolic compensation of LA faced by hypermetabolic AF stressor. Structure remodeling like the switch of α -MHC to the more energy-saving β -isoform in fibrillating atrium may be due to the demand of high metabolism.¹⁰⁾ Actually, when suffering from stressors such as hypertrophic stress and overloaded pressure, cardiac tissue is inclined to return to the fetal gene program to survive,³²⁾ just as we observed the switch of metabolism, myosin heavy chain, and natriuretic peptide. However, in the long run, maladaptation will inevitably result in some unfavorable consequences. In our study, compensatory increased BNP neither increased fatty acid metabolism, nor inhibited cardiomyocyte hypertrophy or cardiac fibroblast activation. Furthermore, Mayr, *et al* found that more than 80% of discordant metabolism patients developed AF after cardiac surgery.¹¹⁾ Thus, we concluded that dysfunction of metabolism regulation also plays a role in the perpetuation of AF.

However, in AF patients with coronary artery disease substrate, the dissimilarities in angiopathy and concomitant medication like β -blockers make metabolism more complicated. At the ischemia cardiomyocyte level, β -blockers can improve myocardial metabolism and decrease myocardial glucose uptake.³³⁾ The pharmacological effects of β -blockers of reducing heart contraction and coronary blood flow decrease myocardial oxygen consumption and metabolism. This may explain our results of the down-regulation of metabolism.

Inflammation and thromboembolism in AF: Some inflammation factors and mediators are closely related to the initiation, perpetuation, and prognosis of AF. In our study, the expression of IL-6 in fibrillating LA was more than two-fold that in sinus rhythm. IL-6, as a pleiotropic cytokine, has various biological effects such as stimulating the synthesis of fibrinogen and C reactive protein (CRP), and endothelial cell activation and damage.¹⁹⁾ In the Heart and Soul Study, elevated serum IL-6 in CAD patients was proven to be closely associated with AF.¹⁷⁾ Because IL-6 could be produced not only by immune-related cells but also by endothelial cells and cardiomyocytes, it is difficult to identify the source of this inflammation factor. The fact that elevated IL-6 emerged in LA tissue or in serum seems to be paradoxical, and it may just demonstrate the heterogeneity of AF possessing both local and systemic inflammation behaviors. Moreover, inflammation factors like IL-2, IL-8, and CRP were also tightly linked to AF.¹⁶⁾

In our study, we observed up-regulated PF4 and von Willebrand factor (vWF) and platelet activation state, which suggested a hypercoagulable state. PF4, released from activated

platelets, binds and neutralizes heparin and plays a vital role in coagulation. vWF, synthesized in endothelial cells and megakaryocytes, mediates the adhesion of platelets to sites of vascular perturbation and acts as a carrier protein for coagulation factor VIII. In view of the over-expression of IL-6, we can conclude the positive correlation between inflammation and thromboembolism. Actually, other inflammation factors also possess thrombotic induction effects.¹⁶⁾ Based on this, inflammation could influence the prognosis of AF. Inflammatory cells and factors had also the potential effects of resulting in AF structure remodeling like apoptosis and fibrosis.¹⁶⁾ Although the question of inflammation initiating AF or just the consequence of AF has not reached a consensus due to controversial reports, the increasing evidence of a close relationship between inflammation and AF make the inhibition of inflammation the new therapeutic target of AF.

An oxidant stress response was also found in LA upon AF stressor exposure. NAD(P)H oxidase is the main source of atrial superoxide production in AF. Nitric oxide synthases (NOS) contributed significantly to a basal- and NAD(P)H-stimulated superoxide release.³⁴⁾ NAD(P)H plays a critical role in fibrillating atrium oxidative injury.³⁵⁾ Heat Shock 70 kD Protein 2 (HSP70-2), one member of the human HSP70 family encoded by HSPA2, is widely thought to possess a cardioprotective function towards oxidative stress. The intracellular HSP70 level was demonstrated to be negatively correlated with atrial fibrillation.³⁶⁾ In our study, up-regulation of both oxidative NOS1 and protective HSPA2 represented a compensatory state of LA to the oxidant stress brought about by an AF stressor. Their imbalance will certainly have the consequences of cardiomyocyte injury and remodeling.

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