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ORIGINAL ARTICLE

Integrative analysis of transcriptome-wide association study data and mRNA expression profiles identified candidate genes and pathways associated with atrial fibrillation

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Abstract

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia characterized by extensive structural, contractile and electrophysiological remodeling. The genetic basis of AF remained elusive until now. Transcriptome-wide association study (TWAS) was conducted by FUSION tool using gene expression weights of 7 tissues combined with a large-scale genome-wide association study (GWAS) dataset of AF, totally involving 8180 AF cases and 28,612 controls. Significant genes identified by TWAS were then subjected to gene ontology (GO) and pathway enrichment analysis. The genome-wide mRNA gene expression profiling of AF was compared with the results of TWAS to detect common genes shared by TWAS and mRNA expression profiling of AF. TWAS detected a group of candidate genes with P_{TWAS} values <0.05 across the seven tissues for AF, such as CMAH ($P_{TWAS}=3.15\times10^{-25}$ for whole blood), INCENP ($P_{TWAS}=1.77\times10^{-22}$ for artery aorta), CMAHP ($P_{TWAS}=4.57\times10^{-20}$ for artery aorta). Pathway enrichment analysis identified multiple candidate pathways, such as protein K48-linked ubiquitination (P value $=0.0124$), positive regulation of leukocyte chemotaxis (P value $=0.0046$) and fatty acid degradation (P value $=0.0295$). Further comparing the GO results of TWAS and mRNA expression profiling, 2 common GO terms were identified, including actin binding ($P_{TWAS}=0.0446$, $P_{mRNA}=7.00\times10^{-4}$) and extracellular matrix ($P_{TWAS}=0.0037$, $P_{mRNA}=3.00\times10^{-6}$). We detected multiple novel candidate genes, GO terms and pathways for AF, providing novel clues for understanding the genetic mechanism of AF.

Keywords Atrial fibrillation (AF) · Transcriptome-wide association studies (TWAS) · Genome-wide association studies (GWAS) · Gene expression profiling · Pathway

Lu Zhang and Li Liu contributed equally to this work.

Feng Zhang takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Introduction

Atrial fibrillation (AF) is a common sustained cardiac arrhythmia characterized by extensive structural, contractile and electrophysiological remodeling [1], with increased prevalence worldwide [2]. Symptomatic atrial fibrillation can seriously affect the quality of life, along with cardiac performance and functional status, even increase the risk of death. Evidence of familial clustering suggested that genetic factors contributed to the development of AF [3, 4] Genome-wide association studies (GWAS) have successfully identified thousands of genetic variants associated with complex diseases, including AF [5, 6].

Genome-wide association studies (GWAS) have successfully identified SNPs that are associated with many complex diseases or traits. Recent studies observed that the significant SNPs identified by GWAS were enriched in chromosomal non-coding regulatory regions such as enhancer elements,

DNase hypersensitivity regions and chromatin marks [7, 8]. It was reported that the non-coding regulatory variants [such as expression quantitative trait loci (eQTLs)] played important roles in the development of complex diseases. For example, enhancers are the core regulatory components of genome, which can positively regulate gene expression over a distance. It has been demonstrated that SNPs in enhancer regions can lead susceptibilities to activities. However, diseases associated with non-coding regulatory variants were easy to be missed in previous GWAS for the limited functional annotation.

Recently, integrative analysis of GWAS and non-coding regulatory loci information become more and more popular [9]. For instance, transcriptome-wide association study (TWAS) is a powerful tool for predicting the impact of cis-regulatory regions on expression and directly disease-associated genes [10]. The key idea of TWAS is to train multivariate eQTL models on reference expression panels, using these models to predict unobserved gene expression in large scale GWAS cohorts, and compute association statistics by regressing phenotype directly onto imputed gene expression. Multiple studies have demonstrated the power of TWAS to gain insights into the genetic basis of complex traits and diseases. For example, Lawrenson et al. performed a TWAS and identified multiple novel genes associated with high grade serous ovarian cancer (HGSOC).

In this study, we leveraged expression imputation from AF GWAS dataset to perform a transcriptome-wide association study (TWAS), considering seven tissues including artery tibia, artery coronary, artery aorta, heart left ventricle, heart atrial appendage, peripheral blood and whole blood. The AF associated genes identified by TWAS were then subjected to gene ontology and pathway enrichment analysis to explore which functional changes and biological pathway are enriched in the identified genes. The TWAS results were finally compared with the analysis results of mRNA expression profiling of AF to detect common genes, GO terms and pathways.

Material and method

GWAS of AF

A recently published large-scale GWAS dataset of AF was used here [11]. Briefly, 8,180 atrial fibrillation cases were recruited from BioBank Japan. The genotyping information of 28,612 controls was collected from population-based controls derived from the Tohoku Medical Megabank organization, the Japan Public Health Center-based Prospective study and the Japan Multi-institutional Collaborative Cohort Study. Individuals with a history of arrhythmia were excluded from the population-based controls. All researches were approved by Medical Ethical Committee, and the written informed consent was provided by each study subject or their relatives [11]. Case samples were genotyped using Illumina Human OmniExpressBeadChip kit and the InfiniumOmniExpressExomeBeadChip kit. For sample quality control, we excluded samples for which less than 98% of the SNPs were genotyped by performing analysis of identity-by-descent state to select unrelated samples. For quality control, the SNPs with call rate <0.99 and deviating from Hardy–Weinberg equilibrium in control samples were excluded. GWAS was performed by logistic regression analysis incorporating associated principal components, age and sex as covariates. Whole-genome imputation analysis was used to infer missing genotypes for markers not included on the SNP arrays. Detailed description of study subjects, genotyping, imputation, association analysis and quality control could be found in the published study [11].

TWAS of AF

FUSION software was applied here for tissue-related TWAS analysis [10]. Briefly, FUSION leveraged a set of reference individuals to measure both gene expression and genetic variation (SNPs), and then to impute the cis genetic component of expression into a much larger set of phenotyped individuals using their SNP genotype data. The imputed expression data can be viewed as a linear model of genotypes with weights based on the correlation between SNPs and gene expression in the training data while accounting for linkage disequilibrium (LD) among SNPs. FUSION uses pre-computed gene expression weights together with disease GWAS summary statistics to evaluate the association between target diseases and the expression levels of gene [13]. The pre-computed expression reference weights of different tissues were downloaded from the FUSION websites (https://gusevlab.org/projects/fusion/). For AF TWAS, we used 7 expression reference panels, including artery tibia, artery coronary, artery aorta, heart left ventricle, heart atrial appendage, peripheral blood and whole blood [13]. A TWAS *P* value was calculated for each gene.

Gene expression profile associated with AF

Differently expression genes in AF patients were driven from a mRNA expression profiling of AF [12]. This study was designed to pinpoint gene-expression changes in the cardiac transcriptome that are specifically associated with AF in the right atrial (RA) of patients with valvular heart disease (VHD). RA appendage (RAA) samples were obtained from 29 patients [11 patients with permanent AF and underlying VHD (AF-VHD), 7 patients in sinus rhythm with VHD and with no history of AF (SR-VHD) and 11 patients with CAD and no history of AF (SR-CTRL)] undergoing mitral/aortic

valve replacement or coronary artery bypass graft surgery. Study constructed a long-oligonucleotide microarray which were prepared in-house using 50-mer oligonucleotide probes (MWG Biotech). These oligonucleotides were obtained from the MWG Biotech human genome-wide microarray collection and had all been tested for specificity. 3863 genes that were represented on the microarray had been selected on the basis of evidence for involvement in normal or pathological cardiovascular functioning. This study was approved by the Committee for Ethics and was performed in adherence to the Declaration of Helsinki Guidelines. All participants gave their written informed consent before they entered the study [12]. Detailed description of study subjects, experimental design and quality control could be found in the published study [12].

Gene sets enrichment analysis

The AF related genes identified by TWAS and mRNA expression profiling were subjected to gene ontology (GO) and pathway enrichment analysis, implemented by the Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) [13]. A *P* value was calculated by DAVID for each GO terms and pathways. Finally, we compared the results of TWAS and mRNA expression profiling to screen out the common GO terms and pathways.

Results

TWAS analysis and Gene expression profiles analysis

A total of 734, 123 genes were analyzed in the TWAS of AF. TWAS identified 602 genes with $P_{TWAS} < 0.05$ across the seven tissues (Supplementary Table 1), such as CMAH ($P_{TWAS} = 3.15 \times 10^{-25}$ for whole blood), INCENP ($P_{TWAS} = 1.77 \times 10^{-22}$ for Artery aorta), CMAHP ($P_{TWAS} = 4.57 \times 10^{-20}$ for Artery aorta), UBE2H ($P_{TWAS} = 3.16 \times 10^{-14}$ for Peripheral blood), RPAP3 ($P_{TWAS} = 5.45 \times 10^{-14}$ for Peripheral blood) and STX3 ($P_{TWAS} = 3.90 \times 10^{-11}$ for Peripheral blood). Table 1 summarizes the top 30 AF associated genes identified by TWAS.

Table 1 List of top 30 candidate genes identified by TWAS for AF

Gene	Chromosome	*P* value	Tissue
RP1-228P16.1	12	2.98×10^{-30}	Artery tibia
CMAH	6	3.15×10^{-25}	whole blood
INCENP	11	1.77×10^{-22}	Artery aorta
CMAHP	6	4.57×10^{-20}	Artery aorta
UBE2H	7	3.16×10^{-14}	Peripheral blood
RPAP3	12	5.45×10^{-14}	Peripheral blood
RAPGEF3	12	3.46×10^{-12}	whole blood
STX3	11	3.90×10^{-11}	Peripheral blood
ANLN	7	8.87×10^{-10}	Artery tibia
FBN2	5	1.07×10^{-9}	whole blood
ASAH2B	10	4.92×10^{-9}	Artery tibia
AC073834.3	2	8.50×10^{-9}	Heart left ventricle
CALHM2	10	1.01×10^{-8}	Peripheral blood
INCENP	11	5.82×10^{-8}	Artery tibia
CARNS1	11	1.97×10^{-7}	Heart left ventricle
ASAH2B	10	1.98×10^{-7}	Heart left ventricle
INCENP	11	2.06×10^{-7}	Heart atrial appendage
CASC10	10	2.58×10^{-7}	Artery tibia
PCDHGB7	5	3.11×10^{-7}	Artery aorta
TMEM18	2	4.58×10^{-7}	Heart left ventricle
AC092159.2	2	5.15×10^{-7}	Heart left ventricle
SNX1	15	5.82×10^{-7}	Artery tibia
AGPS	2	7.71×10^{-7}	Peripheral blood
CDKN2B	9	8.58×10^{-7}	Peripheral blood
HM13	20	1.05×10^{-6}	Peripheral blood
CD52	1	1.08×10^{-6}	Heart left ventricle
ASAH2B	10	1.42×10^{-6}	Artery aorta
TMSB10	2	1.93×10^{-6}	Whole blood
NOC3L	10	2.08×10^{-5}	Heart left ventricle
AC073834.3	2	2.68×10^{-6}	Artery aorta
ASB8	12	3.94×10^{-6}	Peripheral blood

Gene sets enrichment analysis

GO enrichment analysis of the genes identified by TWAS results were shown in Table 2. DAVID detected 34 GO terms, such as ER to Golgi transport vesicle (GO:0030134, *P* value = 0.0120), protein K48-linked ubiquitination (GO:0070936, *P* value = 0.0124) and positive regulation of leukocyte chemotaxis (GO:0002690, *P* value = 0.0046). For pathway enrichment analysis of the genes identified by TWAS, DAVID detected 8 candidate pathways for AF, such as fatty acid degradation (hsa00071, *P* value = 0.0295), endocytosis (hsa04144, *P* value = 0.0440), arginine and proline metabolism (hsa00330, *P* value = 0.0409) (Table 3).

The GO and pathways enrichment analysis results of mRNA expression profiling of AF were summarized in Supplementary Table S2 and Table S3. Further comparing the GO enrichment analysis results of TWAS and mRNA expression profiling, we identified 2 common GO terms, namely actin binding (GO:0003779, $P_{TWAS} = 0.0446$, $P_{mRNA} = 7.00 \times 10^{-4}$) and extracellular matrix (GO:0031012, $P_{TWAS} = 0.0037$, $P_{mRNA} = 3.00 \times 10^{-6}$).

Table 2 Gene ontology enrichment analysis results of AF associated genes identified by TWAS

Name	*P* value	Fold Enrichment
GO:0005737 ~ cytoplasm	2×10^{-5}	1.3692
GO:0031012 ~ extracellular matrix	0.0037	2.5426
GO:0002690 ~ positive regulation of leukocyte chemotaxis	0.0046	11.5528
GO:0005739 ~ mitochondrion	0.0089	1.5348
GO:0006607 ~ NLS-bearing protein import into nucleus	0.0093	9.0413
GO:0030027 ~ lamellipodium	0.0101	3.0239
GO:0030134 ~ ER to Golgi transport vesicle	0.0120	8.2705
GO:0052650 ~ NADP-retinol dehydrogenase activity	0.0122	17.2080
GO:0070936 ~ protein K48-linked ubiquitination	0.0124	5.5306
GO:0005730 ~ nucleolus	0.0173	1.6309
GO:0033628 ~ regulation of cell adhesion mediated by integrin	0.0180	14.1784
GO:0008237 ~ metallopeptidase activity	0.0202	3.8240
GO:0008235 ~ metalloexopeptidase activity	0.0216	12.9060
GO:0006606 ~ protein import into nucleus	0.0250	4.4817
GO:0016805 ~ dipeptidase activity	0.0252	11.9132
GO:0005634 ~ nucleus	0.0280	1.1715
GO:0050660 ~ flavin adenine dinucleotide binding	0.0351	4.0331
GO:0007010 ~ cytoskeleton organization	0.0358	2.5832
GO:0033365 ~ protein localization to organelle	0.0368	9.7477
GO:0071344 ~ diphosphate metabolic process	0.0380	51.9876
GO:0035803 ~ egg coat formation	0.0380	51.9876
GO:0001147 ~ transcription termination site sequence-specific DNA binding	0.0383	51.6239
GO:0003948 ~ N4-(beta-*N*-acetylglucosaminyl)-L-asparaginase activity	0.0383	51.6239
GO:0006886 ~ intracellular protein transport	0.0386	2.2029
GO:0042622 ~ photoreceptor outer segment membrane	0.0388	9.4867
GO:0016491 ~ oxidoreductase activity	0.0408	2.3231
GO:0043161 ~ proteasome-mediated ubiquitin-dependent protein catabolic process	0.0423	2.3049
GO:0003779 ~ actin binding	0.0446	2.0427
GO:0005856 ~ cytoskeleton	0.0447	1.8837
GO:0030864 ~ cortical actin cytoskeleton	0.0452	5.0008
GO:0045022 ~ early endosome to late endosome transport	0.0458	8.6646
GO:0051496 ~ positive regulation of stress fiber assembly	0.0463	4.9512
GO:0000226 ~ microtubule cytoskeleton organization	0.0474	3.6611
GO:0016477 ~ cell migration	0.0482	2.4180

Table 3 Pathway enrichment analysis results of AF associated genes identified by TWAS

Category	Name	*P* value	Fold enrichment
BIOCARTA	Mechanism of gene regulation by peroxisome proliferators via PPARa(alpha)	0.0083	5.7870
KEGG_PATHWAY	hsa00071:Fatty acid degradation	0.0295	5.8709
KEGG_PATHWAY	hsa04146:Peroxisome	0.0382	3.8903
KEGG_PATHWAY	hsa04666:Fc gamma R-mediated phagocytosis	0.0396	3.8440
KEGG_PATHWAY	hsa00330:Arginine and proline metabolism	0.0409	5.1664
KEGG_PATHWAY	hsa05130:Pathogenic *Escherichia coli* infection	0.0430	5.0651
KEGG_PATHWAY	hsa04144:Endocytosis	0.0440	2.2528
KEGG_PATHWAY	hsa00340:Histidine metabolism	0.0478	8.4234

Discussion

The cellular and molecular basis of AF has been a field of enormous interest over the past few years. And recent studies have shown genetic factors contribute greatly to the development of AF. Genome-wide association studies (GWAS) of AF was also conducted and identified several candidate genetic variants for AF. However, recent studies demonstrated the importance of non-coding regulatory loci in the development of human complex diseases. The roles of non-coding regulatory loci in the mechanism of AF remains unclear. To further explore the expression associated genes with AF, we performed a large scale integrative analysis of transcriptome-wide association study and mRNA expression profiles for AF, which had the potential to provide novel insight into the molecular mechanism of AF.

TWAS identified several candidate genes for AF, such as CD14 and PPFIA4. CD14 encodes protein ISA surface antigen preferentially expressed on monocytes/macrophages. It cooperates with other proteins to mediate the innate immune response to bacterial lipopolysaccharide and acts via MyD88, TIRAP and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response. Recent studies indicated that activated inflammatory cells and inflammatory mediators such as the MCP-1, TNF-a, IL-2, IL-6, IL-8 and CRP [14, 15] might confer a proarrhythmic state by promoting endothelial damage in patients after cardiac surgery [16]. A study reported that rapid atrial activation in AF resulted in a calcium overload in atrial myocytes, often leading to cell apoptosis and inducing a low-grade inflammatory response [17]. Inflammation of the atrial myocardium, associated with monocyte activation, might contribute to the formation of AF [16]. Frustaci et al. detected the inflammation process might modify the cell death, increase the atrial fibrosis, and change the atrial conduction properties, which are related to mechanoelectricalre modelling [18].

Another notable gene is PPFIA4, which encodes a member of the liprin (LAR: leucocyte antigen related, protein-tyrosine phosphatase-interacting protein) protein family [19]. Although liprin is expressed in the heart, the association between PPFIA4 and cardiac disease has not been reported before. A previous study discovered that PPFIA4 protein could bind to the leukocyte common antigen-related family receptor protein tyrosine phosphatases that play key roles in synapse maturation and regulation [20]. A recent study showed the potential involvement of axon guidance in the pathogenesis of AF [21]. Accumulated evidence supported the association between neural crest cell development and atrial fibrillation. For instance, cardiac ganglia which innervate the conduction system components of the heart, are mostly derived from the neural crest [22]. Noteworthy, some molecules, such as the semaphorins, which have a role in axon guidance, have been shown to be closely related to the neural crest and cardiovascular patterning [22]. Therefore, there may be a chance that PPFIA4 is involved in the development of atrial fibrillation through affecting the function of axons. Further biological studies are required to confirm our findings.

Pathway enrichment analysis detected several candidate biological pathways for AF, for instance fatty acid degradation. Owais el. Observed a positive association between plasma free fatty acids (FFA) and the risk of AF via a prospective cohort of 4,175 men and women, who aged ≥ 65 years and from the Cardiovascular Health Study [23]. A previous study reported that subjects who developed any arrhythmia had higher mean peak FFA levels than those who did not [24]. Paolisso et al. Also observed a positive association between plasma FFA concentration and the incidence of ventricular premature contractions among non-insulin dependent diabetic patients [25]. Researchers have demonstrated an increased incidence of ventricular arrhythmia following injection of saturated fatty acids in animal models [26]. However, evidence related to the detailed mechanisms by how FFA lead to cardiac arrhythmias has been lacking. A study reported that the mechanism may involve the production of lysophospholipids from a breakdown of membrane lipids and acylcarnitine from circulating FFA [27]. In addition, FFA may inhibit Na^+, K^+, ATPase pump with subsequent increase in intracellular sodium and calcium [28] that may predispose to arrhythmias.

Additionally, we also detected 2 peroxisome related biological pathways associated with AF, including KEGG Peroxisome pathway and BIOCARTA Mechanism of Gene Regulation by Peroxisome Proliferators via PPARa (alpha) pathway. Lin et al. observed that the concentration of peroxisome proliferator-activated receptor-γ(PPAR-γ) receptor protein was decreased in AF patients compared with healthy controls [29]. Chen et al. reported that PPAR-γmRNA expression was decreased in AF patients with hypertension as compared with controls [30]. Previous studies also found that PPAR-γactivation had favorable effects on AF. For example, Shima no et al. observed that pioglitazone suppressed arrhythmogenic atrial structural remodeling and AF promotion, suggesting that PPAR-γagonists may be a potential therapeutic agent for human AF [31]. Gu et al. observed that pioglitazone improved the preservation of sinus rhythm and reduced the reablation rate in patients with paroxysmal AF after catheter ablation [32].

It is well known that genetic variants can influence complex traits by modulating gene expression [33, 34]. Although the relationships between gene expression levels and traits can be investigated through association scans using the individuals with both gene expression and traits data [35], the measurement methods are easy to be hampered by multiple confounding factors. Consequently, it is usually difficult to detect the gene expression-trait

associations, especially the genes with minor effects. Examining the overlap of gene expression related genetic variants (eQTLs) with the trait-associated variants identified by GWAS, is also likely to miss expression-trait associations of small effect. In this study, we conducted TWAS via integrating genetic and transcriptional variation to identify genes expression associated with complex traits. It is capable of capturing the cis-SNP signal and does not require any individual SNP genotype data. So our approach can be seen as a supplement to GWAS by identifying the association of expression-trait that are not well interpreted by individual tagging SNPs.

Two limitations should be noted in our study. First, we leveraged expression imputation from AF GWAS dataset to perform a TWAS of AF. The variants influencing diseases that are independent of cis-gene expression will not be identified by TWAS. Second, the number of genes which can be accurately imputed is still limited by the training cohort size and the quality of the training data. Further studies with large samples and biological are needed to confirm our findings and clarify the potential mechanism of identified genes and pathways involved in the development of AF.

To the best of our knowledge, this is the first integrative study in AF combining TWAS and gene expression profiling dataset. The great power of TWAS can provide novel clues for understanding the complex pathogenesis of AF. However, our study results should be interpreted with caution. Further functional biology studies are needed to confirm our findings and reveal the potential roles of identified genes and pathways in the development of AF.

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Author contributions LZ and LL drafted the manuscript, FZ designed the study. MM, SC, YZ, HH, XL and PL performed the statistical analyses. YW, BC, CL, MD, QX and YD provided feasible advice on data analysis and drafting manuscript. All authors read and approved the final manuscript. All authors discussed the results and commented on the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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