

Combined metabolic, phenomic and genomic data to prioritize atrial fibrillation-related metabolites

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Abstract. Metabolites in atrial fibrillation (AF) were characterized to further explore the molecular mechanisms of AF by integrating metabolic, phenomic and genomic data. Gene expression data on AF (E-GEOD-79768) were downloaded from the EMBL-EBI database, followed by identification of differentially expressed genes (DEGs) which were used to construct gene-gene network. Then, multi-omics composite networks were constructed. Subsequently, random walk with restart was expanded to a multi-omics composite network to identify and prioritize the metabolites according to the AF-related seed genes deposited in the OMIM database, the whole metabolome as candidates and the phenotype of AF. Using the interaction score among metabolites, we extracted the top 50 metabolites, and identified the top 100 co-expressed genes interacted with the top 50 metabolites. Based on the FDR <0.05, 622 DEGs were extracted. In order to demonstrate the intrinsic mode of this method, we sorted the metabolites of the composite network in descending order based on the interaction scores. The top 5 metabolites were respectively weighed potassium, sodium ion, chitin, benzo[a]pyrene-7,8-dihydrodiol-9,10-oxide, and celebrex (TN). Potassium and sodium ion possessed higher degrees in the subnetwork of the entire composite network and the co-expressed network. Metabolites such as potassium and sodium ion may provide valuable clues for early diagnostic and therapeutic targets for AF.

Introduction

Atrial fibrillation (AF), a highly prevalent heart disorder with a significant genetic component (1), is regarded as the most common sustained arrhythmia in clinical practice

worldwide, which can result in heart failure and represents an important risk factor for ischemic stroke, thereby leading to significant morbidity and mortality (2-4). A higher rate of adverse outcomes for the elderly are connected with AF. According to literature, arrhythmia has been demonstrated to arise because of the interaction between genetic and acquired risk factors (5). However, the molecular pathogenesis of AF was not well elucidated, leading to the need of exploring the precise mechanisms of this disease as well as developing novel treatments. Extraction of novel biomarkers for AF is critically important to the understanding and future prevention of this disease.

Metabolites have been regarded as the eventual response of biological systems to the changes of inheritance or environment, whose level directly reflect the physiological status of the body (6). Consequently, selecting disease-associated metabolites is very important for enhancing clinical diagnosis (7,8). Of note, functionally associated metabolites and genes are tended to infer to phenotypically similar disorders. Notably, 'omics' data, for example, metabolic, phenomic and genomic, will offer valuable information for identification of disease risk candidate metabolites. From a biological perspective, a biological system is expressed as a multi-omics network. It is a natural method to combine gene, metabolite and phenotype data to establish a composite network for detecting disease-related metabolites, and this combination approach can offer accurate information (9,10). Nevertheless, few attempts have been made to explore the possible involvement of cardiac metabolism in AF and the underlying pathways remain poorly understood.

In the present study, with the aim of further clarifying metabolic processes in the progression of AF and to confirm whether metabolic derangements may exert important functions in contributing to the arrhythmia occurrence, human atrial tissues obtained from the matched cohorts in sinus rhythm (SR) were analyzed to identify and prioritize candidate metabolites by constructing a composite network that combined metabolic, phenomic, and genomic data. Specifically, microarray data selection (E-GEOD-79768) and differentially expressed genes (DEGs) identification were first conducted. Then, a multi-omics composite network by combining six data sets was established. Next, we mapped the AF-related seed genes and known metabolites to the composite network, following

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by the prioritization of candidate metabolite related to AF according to the global functional relations of the composite network using random walk with restart (RWR). Via using the interaction score among metabolites, we extracted the top 50 metabolites and identified the top 100 co-expressed genes interacted with the top 50 metabolites.

Materials and methods

Acquisition of data set and pretreatment of the original data. The genechip data on AF (E-GEOD-79768) (11) were downloaded from the EMBL-EBI database according to the platform of A-AFFY-44 - Affymetrix GeneChip Human Genome U133 Plus 2.0 [HG-U133_Plus_2]. E-GEOD-79768 included 26 samples including data from patients with AF (n=14), and SR samples as control (n=12).

Subsequently, the original data were pre-processed based on a series of steps including background correction, quantile normalization, perfectmatch/mismatch correction, and mediapolish summarization, and transformation of probe IDs into human gene symbols.

Identification of DEGs. As reported, the change of gene expression reflects the propensity of a given disease. In the present study, detecting DEGs between two groups was performed using Student's t-test. Then, we utilized the multiple test to correct the original P-values based on Benjamini-Hochberg (12) using false discovery rate (FDR). DEGs were screened out when the FDR was <0.05. These DEGs were used to establish a gene network.

Constructing multi-omics composite network. A composite network was constructed by combining six data, which could be denoted by six networks, namely gene, phenotype, metabolite, phenotype-gene association, gene-metabolite association network, as well as phenotype-metabolite association network.

Gene network construction. In the present study, all protein-protein interactions (PPIs) of human having combine scores (1,048,576 interactions) were downloaded from the STRING to build the original PPI network. Following removal of the duplicated PPIs, and transformation of proteins into gene symbols, 1,515,370 highly correlated gene-gene interactions among 16,785 genes were identified to construct the background PPI network (combine-score of edges <0.8). Subsequently, the intersection between the 16,785 genes in the background PPI network and DEGs was extracted to construct the informative gene-gene network.

Metabolite network construction. First, a total of 4,994 human metabolites were collected from KEGG, HMDB, Reactome, MSEA (13) and SMPDB (14). Subsequently, metabolite-metabolite associations of human and their corresponding confidence scores were extracted from STITCH (15), where the 4,994 human metabolites were covered. Finally, a total of 3,764 human metabolites and 74,667 human metabolite-metabolite interactions were obtained.

Phenotype network construction. In the phenotype-phenotype similarity associations (16), there are 5,080 phenotypes and

similarity scores across them. The majority of recorded human phenotypes were included in these phenotypes. Based on the phenotype-phenotype similarity associations, we constructed a phenotype network.

Gene-metabolite association network construction. With the goal of extracting human gene-metabolite interactions, we downloaded the chemical and gene associations of human and the corresponding confidence scores from STITCH. After discarding the metabolites that were not included in the metabolite network mentioned above and removing the genes that were not involved in the above gene network, overall 192,763 gene-metabolite interactions were obtained among 12,342 genes as well as 3,278 metabolites.

Phenotype-gene association network construction. We got the phenotype-gene interactions according to the OMIM database. Following removing the phenotypes not appearing in the phenotype network and the genes not covered in the gene network, 2,603 gene-phenotype associations were found between 1,715 genes and 1,886 phenotypes. The weighted score was determined as 1 for every phenotype-gene interaction.

Phenotype-metabolite association network construction. We obtained the phenotype-metabolite associations from the HMDB. Analogously, a total of 664 associations between 388 metabolites and 149 phenotypes were reserved after filtration. Moreover, the weighted score was defined as 1 for every phenotype-metabolite interaction.

Establishment of a multi-omics composite network. To identify and prioritize the potential metabolite, the six networks mentioned above were merged into one weighted composite network. Specific steps were as described in Yao *et al* (17).

Prioritization of candidate metabolite relying on the multi-omics composite network. The corresponding seed genes of AF were *ABCC9*, *GJA5*, *KCNA5*, *KCNE2*, *KCNJ2*, *KCNQ1*, *NPPA*, *NUP155*, *SCN1B*, *SCN2B*, and *SCN5A*, which were deposited in the database of OMIM. Next, we mapped these seed genes and known disease metabolites to the multi-omics composite network.

In an attempt to obtain the candidate metabolite prioritization from the weighted composite network, the RWR method was expanded to the multi-omics composite network (18). Then, we scored the candidate metabolites and ranked these candidate metabolites based on distance proximity. Based on the interaction score, we identified the top 50 metabolites which were defined as the AF-prioritized metabolites.

Then, based on the top 50 metabolites, co-expressed genes interacted with the top 50 metabolites were identified, and then, we analyzed these co-expressed genes. According to the score distribution, the top 100 co-expressed genes were extracted.

The subnetwork on the top 50 metabolites obtained from the composite network, and the co-expressed network were constructed. Furthermore, degree analyses were performed for these two networks to further identify several important AF-related metabolites.

Table I. The top 20 differentially expressed genes (DEGs).

Gene symbols	FDR	Gene symbols	FDR
<i>LBH</i>	9.23E-06	<i>IDH3A</i>	1.73E-04
<i>PPP4C</i>	7.52E-05	<i>RNF141</i>	1.85E-04
<i>NOL6</i>	7.48E-05	<i>TOMM22</i>	2.14E-04
<i>AGK</i>	1.25E-04	<i>TSPAN12</i>	2.78E-04
<i>DEDD</i>	1.37E-04	<i>PDK1</i>	2.92E-04
<i>THOC6</i>	1.55E-04	<i>SAFB</i>	3.20E-04
<i>COL21A1</i>	1.61E-04	<i>LRIF1</i>	3.21E-04
<i>CAPZA2</i>	1.65E-04	<i>INPP4A</i>	3.26E-04
<i>FLI1</i>	1.69E-04	<i>ZBTB43</i>	3.32E-04
<i>KLHL12</i>	1.71E-04	<i>SETD5</i>	3.39E-04

FDR, false discovery rate.

Table II. The composite network.

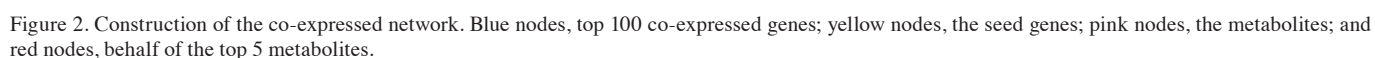
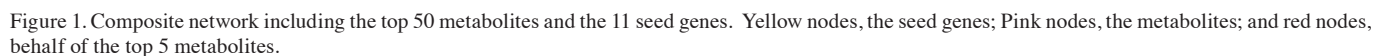
Statistics of the composite network	Nodes	Edges
Gene-gene network	571	4,254
Metabolite-metabolite network	3764	74,667
Phenotype-phenotype network	5080	10,140,046
Gene-metabolite association network	571	3,763
Phenotype-gene association network	5080	2,600
Phenotype-metabolite association network	537	664
Sum	9415	10,227,292

Results

DEG identification and construction of multi-omics composite network. In the present study, we used DEGs to comprise the gene nodes of the composite network, thus, we first identified the DEGs between the two groups. Based on the FDR <0.05, a total of 622 genes were extracted as DEGs. The top 20 DEGs are shown in Table I. This method identified and prioritized the disease-related metabolites via combining multi-omics information. In the present study, we first established a multi-omics composite network by merging information derived from the genome, phenome, and metabolome. There were three kinds of nodes (metabolite, gene, and phenotype) and six kinds of interactions (gene-gene, phenotype-phenotype, metabolite-metabolite, phenotype-gene, gene-metabolite, and phenotype-metabolite) in the multi-omics composite network. In this network, there were 9,415 nodes and 10,227,292 edges (Table II).

Table III. The top 50 metabolites.

Metabolite ID	Metabolite name	Score
813	Potassium	0.004268
923	Sodium ion	0.002251
24139	Chitin	0.001765
41322	Benzo[a]pyrene-7,8-dihydrodiol-9,10-oxide	0.001509
2662	Celebrex (TN)	0.001131
5957	Triphosadenine (DCF)	0.001094
3676	Dentipatch (TN)	0.001053
935	Nickel	0.001025
187	Acetylcholine	0.000897
1775	Dilantin (TN)	0.000829
2554	Tegretol (TN)	0.000805
5816	Adrenalin (TN)	0.000798
1103	Spermine	0.000772
105024	L-selenomethionine	0.000758
945	Nitric oxide	0.000753
3348	Telfast (TN)	0.000735
888	Magnesium ion	0.000725
24755493	PIPP	0.000711
4913	Procainamide (INN)	0.000709
3446	Neurontin (TN)	0.00064
5505	Orinase (TN)	0.00063
5284627	Topamax (TN)	0.000603
2337	Parathesin (TN)	0.000546
4946	Propranolol (TN)	0.000536
5202	Serotonin	0.000535
439155	S-adenosylhomocysteine	0.000521
2812	Lotrimin (TN)	0.000515
89594	Habitrol (TN)	0.000484
4914	Procaine (INN)	0.000483
24798682	1-Radyl-2-acyl-sn-glycero-3-phosphocholine	0.00048
5839	Aldosterone	0.00047
5354618	Caesium	0.000439
5280961	Genistein	0.000429
10114	Hidermart (TN)	0.000426
441074	Conquinine	0.000409
6022	Adenosine 5'-diphosphate	0.000395
444795	Tretinoin (TN)	0.00038
1548943	Capsaicin (JAN/USP)	0.000367
3121	Depakene (TN)	0.000364
702	Dehydrated ethanol	0.000361
5819	Forthyron (TN)	0.000342
172198	Delivert (TN)	0.000327
60961	Adenocard (TN)	0.000327
94715	Glucuronic acid	0.000317
5994	Prometrium (TN)	0.0003
2519	Caffeine (USP)	0.000293
65064	Epigallocatechin 3-gallate	0.000273
8980	Benadryl (TN)	0.000271
5546	Dyrenium (TN)	0.000267
2719	Chloroquine (USP/INN)	0.000264



Prioritization of the risk metabolites of AF. There are 11 disease-related genes related to AF in OMIM, including *ABCC9*, *GJA5*, *KCNA5*, *KCNE2*, *KCNJ2*, *KCNQ1*, *NPPA*, *NUP155*, *SCN1B*, *SCN2B*, and *SCN5A*, which were extracted and defined as seed genes. Nevertheless, no known disease metabolite data on AF were deposited in HMDB. In our work, the whole metabolome as candidates, the phenotype of AF and the 11 disease genes were utilized as seeds. With the goal of illustrating the intrinsic mode of this method, we sorted the metabolites of the composite network in descending order based on the interaction scores. The top 50 metabolites were dissected, as shown in Table III. The top 5 metabolites were respectively potassium (score = 0.004268), sodium ion (score = 0.002251), Chitin (score = 0.001765), Benzo[a]pyrene-7,8-dihydrodiol-9,10-oxide (score = 0.001509), and Celebrex (TN) (score = 0.001131). A subnetwork was detected from the whole composite network in which the top 50 metabolites were included (Fig. 1). Based on the degree analysis for the entire composite network, we found that potassium had the highest degree (degree = 42), and sodium ion also possessed the higher degree (degree = 39).

Subsequently, we identified the co-expressed genes which interacted with the top 50 metabolites based on the score ranking. Based on the defined condition, we identified the top 100 genes, and the co-expressed network of the top 100 genes and the top 50 metabolites are shown in Fig. 2. After degree analysis for the co-expressed network, 4 metabolites possessed the degree >50, including adenosine 5'-diphosphate (degree = 58), magnesium ion (degree = 57), potassium (degree = 52), and sodium ion (degree = 52).

Discussion

AF is the most common cardiac arrhythmia, but the molecular metabolites remain undefined. To the best of our knowledge, the present study was the first to compare the expression of metabolites between both atria in AF and SR. Based on the results, we found that the top metabolites of potassium and sodium ion had the highest degrees in the composite network and co-expressed network.

Potassium is reported to be connected with a higher risk of cardiovascular disease, for example, ventricular arrhythmias and cardiac arrest (19). Many studies have suggested that increased inward-rectifier K⁺ current (IK1) is a prominent feature of atrial electrical remodeling (20-22). Moreover, AF is a final common endpoint of atrial remodeling (23). Olesen *et al* (24) demonstrated that enhanced potassium current increases AF susceptibility and Linz *et al* (25) demonstrated that blocking the activated atrial potassium currents inhibits AF in a pig model of obstructive apnea. Accordingly, we infer that the changes of potassium during AF might contribute to the self-perpetuating nature of the arrhythmia.

Another metabolite of sodium ion also had the higher degree in our study.

Sodium current is responsible for the early fast depolarization upstroke of the cardiac action potential (26). Reduced sodium current has been demonstrated to shorten the refractory period, and then to create a substrate for re-entry, thereby contributing to AF susceptibility (27,28). Importantly, decreased sodium current may slow the electric conduction,

and electrical remodeling plays important roles in the development of AF (29). Moreover, AF-related mutations have been found in sodium ion channel subunits, for example, *SCN5A*, *SCN1B*, and *SCN2B* (30). Thus, we speculate that sodium ion is highly associated with the progression of AF.

Our work is the first to implement the analysis on AF by identifying the metabolites through systematically integrating multi-data including metabolic, phenomic, and genomic information. This is the main strong advantage of our work. We successfully detected several significant metabolites according to this computational method. Nevertheless, in the process of our study, we carried out an in-depth analysis using extremely well-matched but small size samples. The evaluation of the influence of metabolism underlying AF progression will need further exploration based on larger independent data sets. Moreover, our study merely fixed on analysis based on the bioinformatics, but the findings were not proven by the experiments. Further, this work is based on the gene expression in transcriptional level, not protein level. Consequently, further validation studies and functional experiments are required to confirm the significance of these initial discoveries. Despite these limitations, we believe that the prioritized metabolites can provide researchers valuable information for focusing research efforts to explore the molecular mechanisms of disease, and extracting potential bio-signatures for diagnosis and treatment of AF.

In a nutshell, metabolites such as potassium and sodium ion might be potential biomarkers for AF. Moreover, these metabolites might provide worthy clues for early diagnostic and therapeutic targets for AF.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZTY and JMH designed the study, revised the figures and drafted the manuscript. WLL, JWL and KZ acquired and analyzed the data, and prepared the figures. All authors read and approved the final manuscript.

Ethics' approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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