Identification of disturbed pathways in heart failure based on Gibbs sampling and pathway enrichment analysis

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ABSTRACT. We identified disturbed pathways in heart failure (HF) based on Gibbs sampling combined with pathway enrichment analysis. A total of 396 Markov chains (MCs) (gene count >5) were obtained. After Gibbs sampling, six differentially expressed molecular functions (DEMFs) (possibility ≥0.8) were obtained. As statistical analysis was performed on the number of individual differentially expressed genes (DEGs), we found that there were 137 DEGs with frequency of occurrence ≥2 in the DEMFs. Pathway enrichment analysis showed that these 137 DEGs were enriched in eight significant pathways under the condition of $P < 0.001$. The five most significant pathways were: the calcium signaling pathway ($P = 9.08E-19$), arrhythmogenic right ventricular cardiomyopathy ($P = 5.66E-13$), cardiac muscle contraction ($P = 8.04E-13$), hypertrophic cardiomyopathy ($P = 2.55E-12$), and dilated cardiomyopathy ($P = 7.30E-12$). In conclusion, this novel

method for identifying significant pathways in HF based on Gibbs sampling combined with pathway enrichment analysis was suitable. We predict that several altered pathways (such as the calcium signaling pathway and dilated cardiomyopathy) may play important roles in HF and are potentially novel predictive and prognostic markers for HF.

Key words: Heart failure; Gibbs sampling; Bayesian inference; Markov chain Monte Carlo; Molecular functions

INTRODUCTION

Heart failure (HF) is one of the leading causes of death and hospitalization worldwide (Setoguchi et al., 2007). The clinical syndrome of HF is the result of heterogeneous myocardial or vascular diseases, and is defined as the inability to maintain blood circulation throughout the body (McMurray et al., 2014). Although survival after HF diagnosis has improved, HF still has a poor prognosis and 30-40% of people diagnosed with HF die within one year (Taylor et al., 2014).

Several biomarkers are associated with the development of HF: ST2 (Weinberg et al., 2003), growth differentiation factor 15 (GDF-15) (Anand et al., 2010), TNF-α (Moe et al., 2004), pentraxin 3 (PTX3) (Norata et al., 2009) and AAV1/SERCA2a (Zsebo et al., 2014) have gained interest as biomarkers in HF. The gene expression profiling that reveals potential prognostic biomarkers associated with the progression of HF has been identified (Maciejak et al., 2015). However, there is still a lack of understanding about the mechanism underlying HF. That knowledge would facilitate the customization of novel treatment strategies for the disorder and uncover potential therapeutic targets to slow HF progression to improve the quality of life and reduce mortality.

Genes are tightly regulated to execute the proper biological functions of a cell in order to respond to internal or external perturbations (Kostka and Spang, 2004). Therefore, their expression variations during the disease deterioration process are causally associated with phenotype changes. In general, differentially expressed genes (DEGs) can provide clues about the pathogenesis of destructive pathogen (Liu et al., 2013). However, DEGs alone may lead to false positives because some genes show significant expression differences due to certain stimuli, but are not related to the pathogenic pathway (MacFarlane and Singh, 2006). Therefore, whole-level analysis is necessary and pathway analysis has become the first choice for gaining insight into the underlying biology of DEGs and proteins (Goeman and Bühlmann, 2007). Gibbs sampling, a Markov chain Monte Carlo (MCMC) algorithm for obtaining a sequence of observations that are approximated from a specified multivariate probability distribution (Walsh, 2004), can be used to identify differentially expressed molecular functions (DEMFs).

Therefore, in this paper, Gibbs sampling combined with pathway analysis was applied to identify disturbed pathways in HF. To achieve this, firstly the gene expression profile of HF was acquired and preprocessed. Secondly, the genes were transformed to Markov chains (MCs) to conduct Gibbs sampling. Gibbs sampling was then performed to identify the DEMFs. Finally, pathway enrichment analysis for the genes associated with the DEMFs was carried out based on Database for Annotation, Visualization and Integrated Discovery (DAVID), so as to better understand the underlying mechanism of HF.

MATERIAL AND METHODS

Data acquisition and preprocessing

The preprocessed gene expression profile of HF was downloaded from the ArrayExpress database (http://www.ebi.ac.uk/arrayexpress/), with accession number E-GEOD-59867 (Maciejak et al., 2015). It was found on the A-AFFY-141-Affymetrix GeneChip Human Gene 1.0 ST Array [HuGene-1_0-st-v1] Platform, and comprised 80 samples (46 normal controls and 34 HF samples). The microarray data and annotation files were downloaded for further analysis. The gene expression profile at the probe level was converted to the gene symbol level, and the duplicated symbols were wiped off; 18977 gene symbols were obtained for further analysis.

Identifying molecular functions (MFs)

To implement the Gibbs sampling, the data set must be transformed to a data set with functional class expression measurements (Quiroz-Zárate et al., 2015). The Gene Ontology (GO) project (http://www.geneontology.org/), which is freely available for community use in the annotation of genes, gene products, and gene sequences, can provide structured, controlled vocabularies and classifications that cover several domains of molecular and cellular biology (Gene Ontology Consortium, 2004). Moreover, the GO MF terms represent activities rather than entities (molecules or complexes) that perform actions, and do not specify where, when, or in what context the action takes place (Ashburner et al., 2000). Therefore, the gene function of the preprocessed data was classed via the GO project, and all of the genes were distributed to the MFs. Only MFs from GOs with at least five genes were considered for further analysis. All the MFs were then converted into MCs using the MCMCDataSet function of the BAGS package.

Posterior inference

After having transformed all the MFs into MCs, their posterior inferences were defined to identify probability distributions of MFs for HF (Moradkhani et al., 2012). The Bayesian framework, which is implemented using the MCMC method, not only provides the marginal and joint posterior distributions of all the unknown parameters, but also presents a convenient means of incorporating parameter uncertainty into predictive inferences (Neuwald, 2014). Given the observations, $y = (y_1, \ldots, y_n)$, the posterior distribution of β, $\pi(b|y)$ is given by:

$$\pi(\beta|y) \propto L(y|\beta)\, p(\beta)$$

Where $p(\beta)$ is the prior distribution of β and $L(y|\beta)$ is the likelihood function written as:

$$L(y|\beta) = p^n\,(1-p)^n exp\left\{-\sum_i \rho_p(y_i - x_i'\beta)\right\}$$

Gibbs sampling

To implement Gibbs sampling, we needed to define the empty objects in which the posterior samples of the parameters of interest were to be kept. In the present research, Gibbs 2 function of the BAGS package provided the MCs for the parameters of interest that would form their posterior distribution, and this function was used to obtain the differentially expressed gene sets among the normal control and disease groups (Quiroz-Zárate et al., 2013). First of all, an empty Gibbs sampling set comprising a k-dimensional ($k = 10{,}000$) random vector was defined. Secondly, N samples ($N =$ MFs identified above) were put into the empty Gibbs sampling set. Thirdly, k cycles of randomly fixed k-1 elements of the vector, were extracted from the remaining element to gain a new sample set. Finally, N cycles of the second step were applied to obtain new MCs. The possibility of an MF appearing k times, or the posterior value of an MF was calculated according to the following formula:

Possibility = average P value of the MFs (from 2000 to 10,000) x 250 / (10,000-2000 + 1)

Identifying DEMFs

After obtaining the posterior values of the MFs, a possibility value of 0.8 was selected as the cut-off criteria, and the MFs with possibility values ≥0.8 were regarded as DEMFs. To further investigate the properties of the DEMFs, the gene compositions of the DEMFs were studied, and genes involved in the DEMFs were considered DEGs. Statistical analysis was then performed on the number of individual DEGs. The genes that appeared ≥2 times were considered critical DEGs and were chosen for in-depth analysis.

Pathway enrichment analysis of the DEGs

The Kyoto Encyclopedia of Genes and Genomes (KEGG) represents an effort to link genomic information with higher order functional information by computerizing current knowledge of cellular processes and standardizing gene annotations (Kanehisa and Goto, 2000). In this study, the KEGG database was used to investigate the enrichment analysis of DEGs involved in the occurrence and development of HF. The DAVID database (Huang et al., 2007) was used to perform the KEGG pathway enrichment analysis. Pathways with P values < 0.001 and gene counts >5 were selected according to the expression analysis systematic explorer (EASE) test implemented in DAVID (Wang and Simon, 2011).

RESULTS

Identifying differentially expressed MFs and DEGs

In this paper, Gibbs sampling was used to analyze the expression profile of HF. The BAGS package was applied to analyze DEMF identification. By setting the threshold value of the gene count to >5, we obtained 396 MFs in total. After obtaining the posterior value of the MFs by Gibbs sampling, and choosing a threshold value of possibility of 0.8, as shown in Figure 1, we obtained six DEMFs as follows: inositol or phosphatidylinositol phosphatase activity (possibility = 0.96), lipoprotein binding (possibility = 0.93), small conjugating protein binding (alfa.pi = 0.85), gated channel activity (possibility = 0.85), receptor activity

(possibility = 0.82), and ion channel activity (possibility = 0.82). Statistical analysis on the number of individual DEGs revealed that 137 DEGs had a frequency of occurrence ≥2 in the DEMFs, and those 137 DEGs were extracted for further analysis.
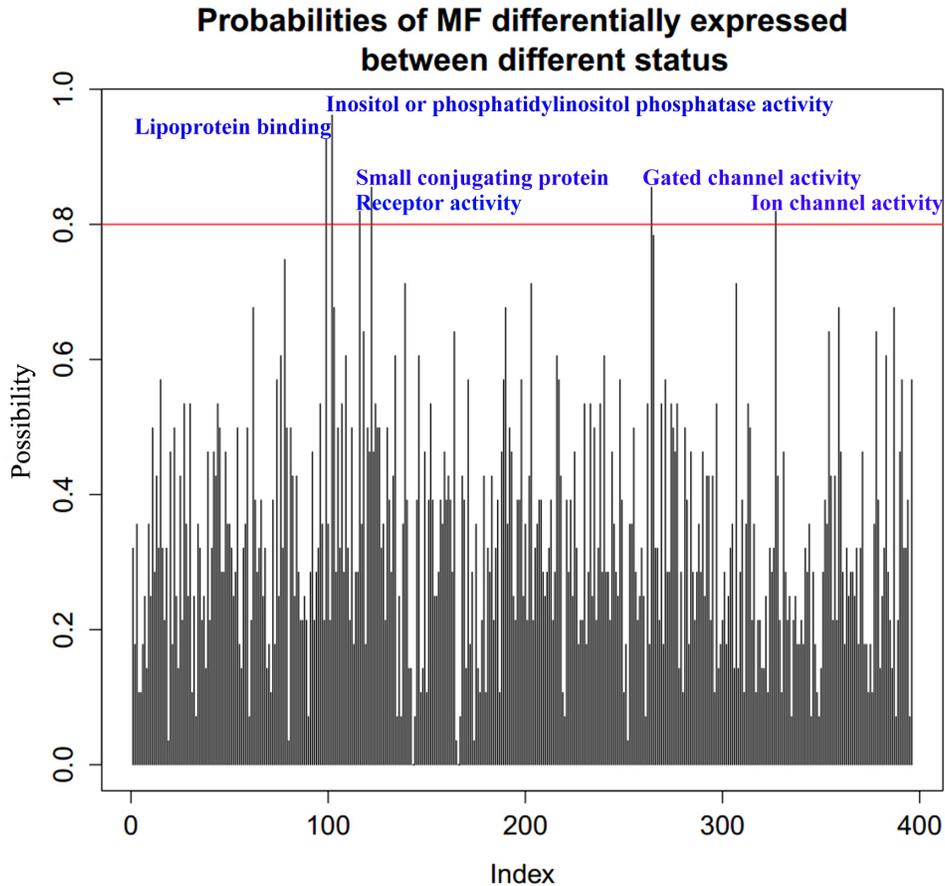

Figure 1. Probabilities for 396 molecular functions (MFs). The horizontal ordinate represents the MFs, and the vertical coordinate represents the posterior value of the MFs.

Pathway enrichment analysis

To gain further insights into the function of the DEGs, DAVID was applied to identify the significantly disturbed KEGG pathways. By setting the threshold value of the P value to < 0.001 and the gene count to >5, the results showed that 137 DEGs were enriched in eight significant pathways (Table 1). The most significant five pathways were the calcium signaling pathway (P = 9.08E-19), arrhythmogenic right ventricular cardiomyopathy (ARVC) (P = 5.66E-13), cardiac muscle contraction (P = 8.04E-13), hypertrophic cardiomyopathy (HCM) (P = 2.55E-12), and dilated cardiomyopathy (P = 7.30E-12).

Table 1. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the differentially expressed genes (DEGs) (P < 0.001).

ID	Term	Count	P value
hsa04020	Calcium signaling pathway	23	9.08E-19
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	14	5.66E-13
hsa04260	Cardiac muscle contraction	14	8.04E-13
hsa05410	Hypertrophic cardiomyopathy (HCM)	14	2.55E-12
hsa05414	Dilated cardiomyopathy	14	7.30E-12
hsa04010	MAPK signaling pathway	18	1.19E-09
hsa04080	Neuroactive ligand-receptor interaction	16	4.37E-08
hsa04930	Type II diabetes mellitus	6	1.34E-04

DISCUSSION

HF is a cardiovascular disease characterized by abnormalities in ventricular function, specifically left ventricular ejection caused by injuries to the heart structure (Duan et al., 2015). Meta-analysis has been used to identify DEGs and the biological processes associated with changes in gene expression in disease (Ning et al., 2015). The traditional methods for identifying DEGs are mainly based on linear models of a microarray data package (Gentleman et al., 2004) or significance analysis of microarrays (Tusher et al., 2001). Traditionally, the analysis of HF has been conducted by identifying the DEGs in the myocardium of HF patients using human whole genomic oligonucleotide microarray-assisted pathway analysis (Wu et al., 2009). In the present paper, Gibbs sampling, an MCMC algorithm that is based on Bayesian theory, was applied to identify DEMFs, to determine disrupted genes and pathways in HF. This was a novel application of the method for studying HF.

By applying Gibbs sampling to research the gene expression profile of HF, we successfully identified six DEMFs. Furthermore, statistical analysis was performed on the number of individual DEGs, and 137 key DEGs were identified. Moreover, by performing KEGG pathway enrichment analysis on the 137 key DEGs, we obtained eight significant pathways (P < 0.001), and the most significant was the calcium signaling pathway (P = 9.08E-19). To better understand the relationship between the occurrence and development of HF and the most significant pathway, we have included an in-depth discussion, as follows.

Ionized calcium (Ca^{2+}) is the most common signal transduction element in cells ranging from bacteria to specialized neurons. Unlike many other second-messenger molecules, Ca^{2+} is required for life, yet prolonged high intracellular Ca^{2+} levels lead to cell death (Clapham, 2007). Furthermore, Ca^{2+}, via the activation of various kinases and phosphatases, acts on Ca^{2+}-dependent transcription pathways (Lompré et al., 2010). Ca^{2+}-dependent signaling is highly regulated in cardiomyocytes and determines the force of cardiac muscle contraction. There is clear evidence that alterations in sarcoplasmic reticulum (SR) Ca^{2+} cycling are involved in the impaired contractile performance of the failing heart (Hasenfuss, 1998), and the role of a pathological diastolic SR Ca^{2+} leak via calstabin2-depleted leaky ryanodine receptor 2 (RyR2) has been recognized as an important contributor to altered Ca^{2+} handling in HF (Wehrens et al., 2005). Currently, two new therapeutic strategies for HF are being tested in clinical trials: (a) fixing the leak in RyR2 channels with a novel class of Ca^{2+}-release channel stabilizers called Rycals; and (b) increasing the expression of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase to improve SR Ca^{2+} reuptake with viral-mediated gene therapy (Marks, 2013). In the present study, by performing analysis on the HF gene expression profile using Gibbs sampling

combined with KEGG pathway enrichment analysis, we found that calcium signaling ($P = 9.08E-19$) was the most significant pathway, which indirectly illustrated the applicability of the method.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) ($P = 5.66E-13$), cardiac muscle contraction ($P = 8.04E-13$), hypertrophic cardiomyopathy (HCM) ($P = 2.55E-12$), and dilated cardiomyopathy ($P = 7.30E-12$) are recognized as significant pathways in the occurrence and development of HF, and further discussions were conducted on the relationship between HF and these pathways. ARVC is a structural cardiomyopathy of genetic origin, and is characterized by structural and functional abnormalities of the right ventricle, ranging from regional wall motion abnormalities and aneurysms to global dilation and dysfunction, with or without left ventricular involvement (Corrado et al., 2011). Idiopathic dilated cardiomyopathy is a form of HF defined by the presence of left ventricular dilatation and left ventricular systolic dysfunction in the absence of an obvious etiology, such as coronary artery disease, hypertension, valvular disease, or congenital defect (Jefferies and Towbin, 2010). Dilated cardiomyopathy is a major cause of systolic HF and the main indication for heart transplantation (Villard et al., 2011). Gadolinium cardiovascular magnetic resonance can exclude the presence of left ventricular dysfunction related to coronary artery disease in heart failure (McCrohon et al., 2003).

In conclusion, this novel method for identifying significant pathways in HF based on Gibbs sampling combined with KEGG pathway enrichment analysis was suitable. We predict that several altered pathways (such as the calcium signaling pathway and dilated cardiomyopathy) may play important roles in HF and are potentially novel predictive and prognostic markers for HF. However, there were some limitations to our study. First, the preprocessed gene expression profile of HF was downloaded from an existing database rather than obtained by ourselves. Secondly, further experimental verification should be conducted to verify our results.

Conflicts of interest

The authors declare no conflict of interest.

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