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Transcriptome data analysis of primary cardiomyopathies reveals perturbations in arachidonic acid metabolism

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Introduction: Cardiomyopathies are complex heart diseases with significant prevalence around the world. Among these, primary forms are the major contributors to heart failure and sudden cardiac death. As a high-energy demanding engine, the heart utilizes fatty acids, glucose, amino acid, lactate and ketone bodies for energy to meet its requirement. However, continuous myocardial stress and cardiomyopathies drive towards metabolic impairment that advances heart failure (HF) pathogenesis. So far, metabolic profile correlation across different cardiomyopathies remains poorly understood.

Methods: In this study, we systematically explore metabolic differences amongst primary cardiomyopathies. By assessing the metabolic gene expression of all primary cardiomyopathies, we highlight the significantly shared and distinct metabolic pathways that may represent specialized adaptations to unique cellular demands. We utilized publicly available RNA-seq datasets to profile global changes in the above diseases ($|\log_2FC| \geq 0.28$ and BH *adjusted p-val* 0.1) and performed gene set analysis (GSA) using the PAGE statistics on KEGG pathways.

Results: Our analysis demonstrates that genes in arachidonic acid metabolism (AA) are significantly perturbed across cardiomyopathies. In particular, the arachidonic acid metabolism gene *PLA2G2A* interacts with fibroblast marker genes and can potentially influence fibrosis during cardiomyopathy.

Conclusion: The profound significance of AA metabolism within the cardiovascular system renders it a key player in modulating the phenotypes of cardiomyopathies.

KEYWORDS

cardiomyopathies, GSA, metabolism, transcriptome, heart failure, arachidonic acid

1. Introduction

Primary cardiomyopathies, predominantly hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM) and arrhythmogenic cardiomyopathy (ACM) are a growing global burden on public health (1–6). Primary cardiomyopathies, on the whole, are genetic but tend to be influenced by environment and lifestyle (6–12). These heterogeneous heart muscle diseases lead to heart failure and sudden cardiac death (1, 6). Notably, metabolic impairment is vital in heart failure (13, 14). Generally, the heart meets its energy demand by utilizing fatty acids, glucose, amino acid, lactate and ketone bodies, but cardiomyopathies lead to severe metabolic perturbations (15–17). In cardiovascular research, metabolic explorations have provided new insights (18, 19). The role of metabolic genes and pathways has been explored in several cardiomyopathy studies to understand the pathophysiological changes involved in the progression of

cardiomyopathy (15–17, 20–22). These studies have reported a metabolic shift in energy sources during disease progression. HCM-focused transcriptome analysis showed down-regulated fatty acid metabolism (23). Similarly, in a DCM transcriptome analysis, mitochondrial dysfunction and oxidative phosphorylation pathways were significantly altered (24). Concurrently, multi-omics technologies have provided an opportunity to explore metabolic disruptions at a large scale. RNA-seq technique allows rapid measurement of global gene expression in the disease of interest. Further, it provides an indirect mechanism to assess metabolic alterations (25–27). Several computational algorithms incorporating transcriptome data and metabolic networks have been developed to assess the perturbation of biological pathways (28–30).

Over the years, oxidative phosphorylation, glucose and fatty acid metabolism have been highlighted exhibiting disturbances in individual cardiomyopathy studies. Albeit being important, individual cardiomyopathy-focused metabolic studies miss overall molecular patterns across cardiomyopathies. A comparative interpretation of the primary cardiomyopathies' metabolic alterations is crucial for a comprehensive understanding of the mechanisms of metabolic shifts. To the best of our knowledge, our study is the first to systematically explore metabolic correlations amongst primary cardiomyopathies (ACM, DCM, HCM and RCM).

This study aimed to identify shared metabolic perturbations across cardiomyopathies. We utilized gene expression profiles of primary cardiomyopathies: ACM, DCM, HCM and RCM, and donor samples. We carried out differential gene expression analysis of cardiomyopathy datasets. Further, we performed GSA on each dataset to investigate the metabolic alterations comprehensively. Apart from glycolysis and oxidative phosphorylation, the arachidonic acid (AA) metabolism pathway was significantly altered in all cardiomyopathy sets. Subsequently, we inferred the potential cell types in the candidate pathway using the snRNA-seq dataset. This analysis helped in the identification of marker genes for each cell type and the potential link between AA metabolism genes and cell type marker genes.

Earlier studies did not account for cross-comparison nor focused on arachidonic acid metabolism in depth. Arachidonic acid (AA) is a free fatty acid metabolized by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450) epoxygenase enzymes into biologically active fatty acid mediators (31). Through these mediators, AA participates in complex cardiovascular functions, including fibrosis (32, 33). COX, called Prostaglandin G/H synthases (PGHS), is attributed to synthesizing autoregulatory and homeostatic prostanoids. COX has two subtypes: COX-1 and COX-2, which convert AA to prostaglandin (PG)G₂ and PGH₂, respectively. These prostaglandins are further processed to various PGs such as PGD₂, PGE₂, PGF₂α, and prostacyclin (PGI₂) (34). PGD₂ protects against atherosclerosis and thrombosis by increasing vascular permeability and blood flow (35). LOXs, such as 5-LOX, 12-LOX, and 15-LOX, catalyze the dioxygenation of AA to their respective hydroperoxyeicosatetraenoic acids (HPETEs), such as 5-HPETE, 12-HPETE, and 15-HPETE. These HPETEs are then transformed into hydroxyeicosatetraenoic acids (HETEs), leukotrienes (LTs), and lipoxins (LXs). The 15-LOX pathway is reported to be involved in the development of atherosclerosis (36).

Epoxyeicosatrienoic acids (EETs) are metabolites produced from arachidonic acid (AA) through the action of CYP epoxygenases. These EETs are synthesized primarily by CYP2J₂, CYP2C₈, and CYP2C₉ enzymes in the human heart, liver, and endothelial cells (37). Among these enzymes, CYP2J₂ is noteworthy since it is the only human CYP2J₂ epoxygenase and is highly expressed in the heart and endothelium in particular (38). CYP2J₂ converts AA into four regioisomeric EETs, including 5,6-, 8,9-, 11,12-, and 14,15-EET (39). The 14,15-EET is released by the endothelium in BK-induced cardiodepression (40). Once formed, EETs are transformed into less active dihydroxyeicosatrienoic acids (DHETs) by the action of soluble epoxide hydrolase (sEH). Research has shown that EETs protect the heart against inflammation, endothelial dysfunction, cardiac remodeling, and fibrosis (41, 42). Our analysis indicates that AA enzymes are expressed in various cell types within the heart tissue, including fibroblasts, cardiomyocytes, smooth muscle cells, monocytes, macrophages, and mast cells. As a cardioprotective mechanism, the CYP2J₂ gene expression was upregulated in HCM and DCM phenotypes. Further PLA2G₂A gene among genes coding for enzymes of the phospholipase A₂ (PLA₂) superfamily was dysregulated in most cardiomyopathies under analysis. The phospholipase A₂ enzymes catalyze the endogenous production of AA mainly from cell membrane phospholipids (43). These findings highlight the importance of investigating the role of AA metabolism in cardiomyopathies. Overall, our study highlights novel and clinically valuable aspects of cardiomyopathies, with implications ranging from prognosis to therapeutic intervention.

2. Materials and methods

2.1. Data acquisition and preprocessing

Transcriptomic raw data comprising RNA-seq, single-nucleus RNA-seq (snRNA-seq) and microarray for cardiomyopathy studies were acquired from the European Nucleotide Archive (ENA) database (<https://www.ebi.ac.uk/ena/browser/>) and Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). Search terms like “hypertrophic cardiomyopathy”, “dilated cardiomyopathy”, “restrictive cardiomyopathy”, and “arrhythmogenic cardiomyopathy” were searched against these databases to obtain the RNA-seq and microarray dataset results. Each result was manually reviewed and considered for inclusion if (1) the disease samples in the study indicated heart tissues from the cardiomyopathy patients, and (2) control samples came from non-failing heart patients. We focused on the studies that were supported in our R-analysis pipeline. We considered seven publicly available cardiomyopathy transcriptomic data, including five RNA-seq (SRP125284, SRP125595, SRP052978, SRP186138 and SRP061888) and two microarray datasets (GSE29819, GSE36961) using these inclusion criteria. One single-nucleus RNA sequencing (snRNA-seq) dataset (GSE183852) with dilated cardiomyopathy (DCM) was selected to explore cell type expression of candidate genes due to the cell annotation in the

supplementary files of the original dataset (44). The details of the transcriptome datasets are shown in **Supplementary Table S1**.

2.2. Data processing and differential expressed genes (DEGs)

Previously described and curated raw data were downloaded and reprocessed to ensure uniform processing and normalization of each study. RNA-seq and microarray studies were processed using independent pipelines. In the RNA-seq pipeline, custom shell scripts were used to download data. Salmon (v1.5.2) (a fast and bias-aware quantification tool) was utilized to align and quantify samples using NCBI human reference transcriptome (Gencode v38) (45). The count data output of Salmon quantification was used for differential gene expression analysis between disease and donor samples. This analysis was performed using the DESeq2 (v1.26.0) package in R (v 3.6.3) (46). It uses the negative binomial distribution to model a statistical analysis for differential gene expression. It also normalizes samples automatically. Wald *t*-test was applied to the distribution (47). To control the false discovery rate (FDR), the resultant *p*-values were adjusted using Benjamini and Hochberg's test (BH) correction (48). Genes with *adjusted p* < 0.1 and $|\log_2FC| \geq 0.28$ were assigned as being differentially expressed. Likewise, microarray datasets were processed with the limma (v3.42.2) package (49). A linear model was constructed between disease and control samples, and the empirical Bayes statistical method was utilized to obtain the significant genes. BH correction was used to obtain the *adjusted p* value. The snRNA-seq expression stored as the R object (44) was processed and analyzed using the R package "Seurat" (version 3.2.3) (50).

2.3. Sample variability and study consistency

Principal component analysis (PCA) and t-distributed stochastic neighbor embedding (t-SNE) were applied to assess the sample variability across datasets. Only RNA-seq datasets were explored for testing. These datasets were normalized using the "DESeq" method in the R package. The R function "prcomp" was used to perform PCA. On the other hand, the "Rtsne" function was utilized to perform t-SNE. Principal component and t-SNE plots, Venn diagrams, and heatmap plots were prepared using the ggplot2 R package (version 3.3.5) and the Matplotlib package in Python 3.

2.4. Gene set analysis (GSA)

The gene set analysis was carried out on the gene expression data to identify significantly perturbed pathways in each study. We utilized *p*-values from the differential expression analysis for all genes in individual datasets for this analysis. A ranking was generated based on these *p*-values, representing the input for Gene-level statistics (gene expression). The Kyoto Encyclopedia

of Genes and Genomes (KEGG) pathways list file was downloaded from KEGG (<https://www.kegg.jp>). Parametric analysis of gene set enrichment (PAGE) was employed for GSA analysis using the Piano (version 2.2.0) package in R (51, 52). The PAGE method uses the mean of the gene-level statistics of a gene set (a particular pathway in this case) and corrects for the background, represented by all gene-level statistics. The cumulative normal distribution is used to estimate the PAGE gene-level statistics significance. Heatmap of pathways, volcano plots and bar charts were plotted using Matplotlib and Seaborn packages in Python 3.

2.5. Identification and screening of significant pathways

Pathways with PAGE statistics *p*-values < 0.1 were considered significant. Among many altered pathways like glycolysis and oxidative phosphorylation, arachidonic acid metabolism was chosen as the candidate for further analysis due to its significance in all primary cardiomyopathies. To show that the AA metabolism perturbation was consistent in all datasets rather than only individual studies, we used microarray data to validate its role in ACM, DCM and HCM independently. We could not find any microarray dataset for RCM, so it was not considered. Gene set analysis of these microarray studies was performed using the sorted Gene-level statistics (GSA) as previously described. Genes differentially regulated in AA metabolism were mapped to the KEGG mapper using the online server: Pathview Web (<https://pathview.uncc.edu/>) (53).

2.6. Finding DEGs, cell types annotation in arachidonic acid metabolism and cell-specific marker genes

Finally, to explore AA metabolism genes, AA genes were screened with a cut-off value of *adjusted p* < 0.1 and $|\log_2FC| \geq 0.28$. To understand the expression of these genes in different cell types of heart tissue, gene expression and cell phenotypes were considered from an snRNA-seq study (GSE183852) (44). In this, control samples were filtered to identify the expression of screened AA metabolism genes in the donor heart cell types. Further, marker genes were identified in each cell type. The "FindMarkers" function in the Seurat package was used to accomplish this task with the cut-off values of *adjusted p* < 0.1, minimal percentage > 0.1, and $\log_2FC \geq 0.58$. These marker genes were utilized to select the cell types significantly enriched within cardiomyopathies DEGs. The GSA method PAGE was employed to evaluate the significance of the DEGs against the cell-specific marker genes in the Piano (version 2.2.0) package in R (51, 52). Functional enrichment of cell-specific marker DEGs was performed using the online tool "g:Profiler" (<https://biit.cs.ut.ee/gprofiler/>) (54).

2.7. Identification of dysregulated marker genes interactions in the cell types

For human interactome data, PPI data (HPRD, MINT, IntAct) along with protein complex and kinase substrate data (CORUM, Phosphositeplus) were obtained from our previous study (55). Then, AA metabolism genes and cell type marker genes were mapped to the human interactome, and a subnetwork consisting of these genes was constructed. Network visualization was executed in Gephi (version 0.9.3).

3. Results

3.1. Gene expression profile in primary cardiomyopathies

We identified and selected seven studies fitting our inclusion criteria (see **Supplementary Table S1** and Materials and methods), consisting of arrhythmogenic cardiomyopathy, dilated

cardiomyopathy, hypertrophic cardiomyopathy and restrictive cardiomyopathy samples. Analyses were performed using 4 RNA-seq, one single-nucleus RNA-seq (snRNA-seq) and two microarray datasets. We performed sample variability analysis on normalized RNA-seq studies. The PCA and t-SNE analysis revealed variability in the samples of different datasets. Attributes like demography, genetic differences and tissue biopsies influence the above variability (**Figures 1A,B**). Since each dataset independently consists of control and disease samples, these variations can be ignored. For the RNA-seq differential gene expression, the negative binomial generalized linear model was used (see Materials and methods). We employed the empirical Bayes method on the generalized linear model for the microarray datasets DEGs. This analysis led to the identification of 4,158, 5,822, 3,048 and 1,655 DEGs in ACM, DCM, HCM and RCM RNA-seq studies. We screened KEGG metabolic genes amongst these DEGs and found 527, 644, 346 and 150 differentially regulated metabolic genes (see **Figure 1C**). Gene expression results indicate that DCM gene expression differed from other primary cardiomyopathies (see **Figure 1D**).

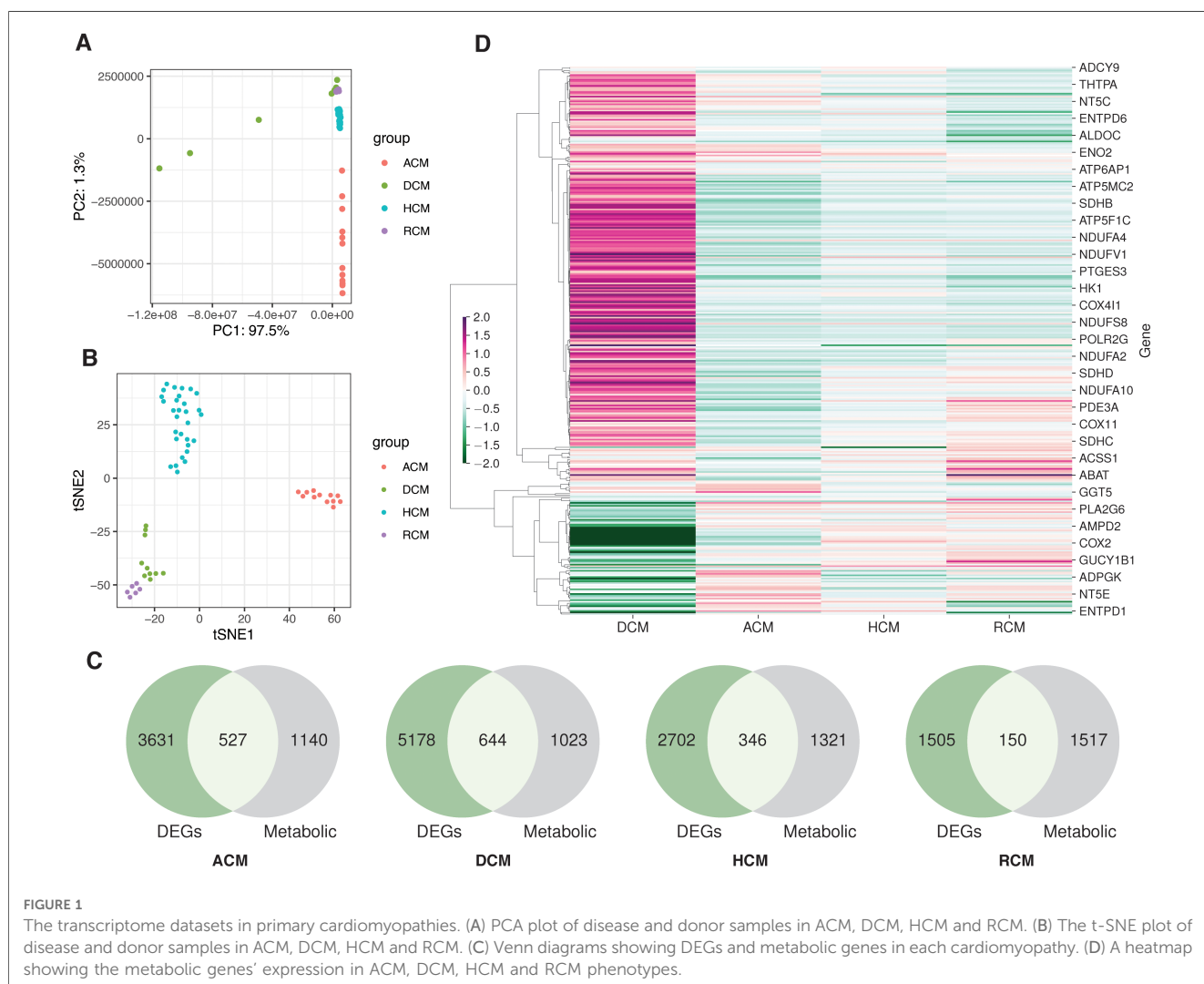


FIGURE 1

The transcriptome datasets in primary cardiomyopathies. (A) PCA plot of disease and donor samples in ACM, DCM, HCM and RCM. (B) The t-SNE plot of disease and donor samples in ACM, DCM, HCM and RCM. (C) Venn diagrams showing DEGs and metabolic genes in each cardiomyopathy. (D) A heatmap showing the metabolic genes' expression in ACM, DCM, HCM and RCM phenotypes.

3.2. The metabolic pathways are significantly dysregulated in cardiomyopathies

To further understand the role of metabolic genes in cardiomyopathies, we performed gene set analysis (GSA) on RNA-seq and microarray studies. The *p-values* from the

differential expression analysis of each dataset were ranked to estimate the gene-level statistics (see Materials and methods). The KEGG pathway metabolic gene signature was used to identify the dysregulated pathways. The GSA revealed widespread alterations of glycolysis/gluconeogenesis, TCA cycle, oxidative phosphorylation, and riboflavin, thiamine, purine and arachidonic acid metabolism (see **Figure 2**).

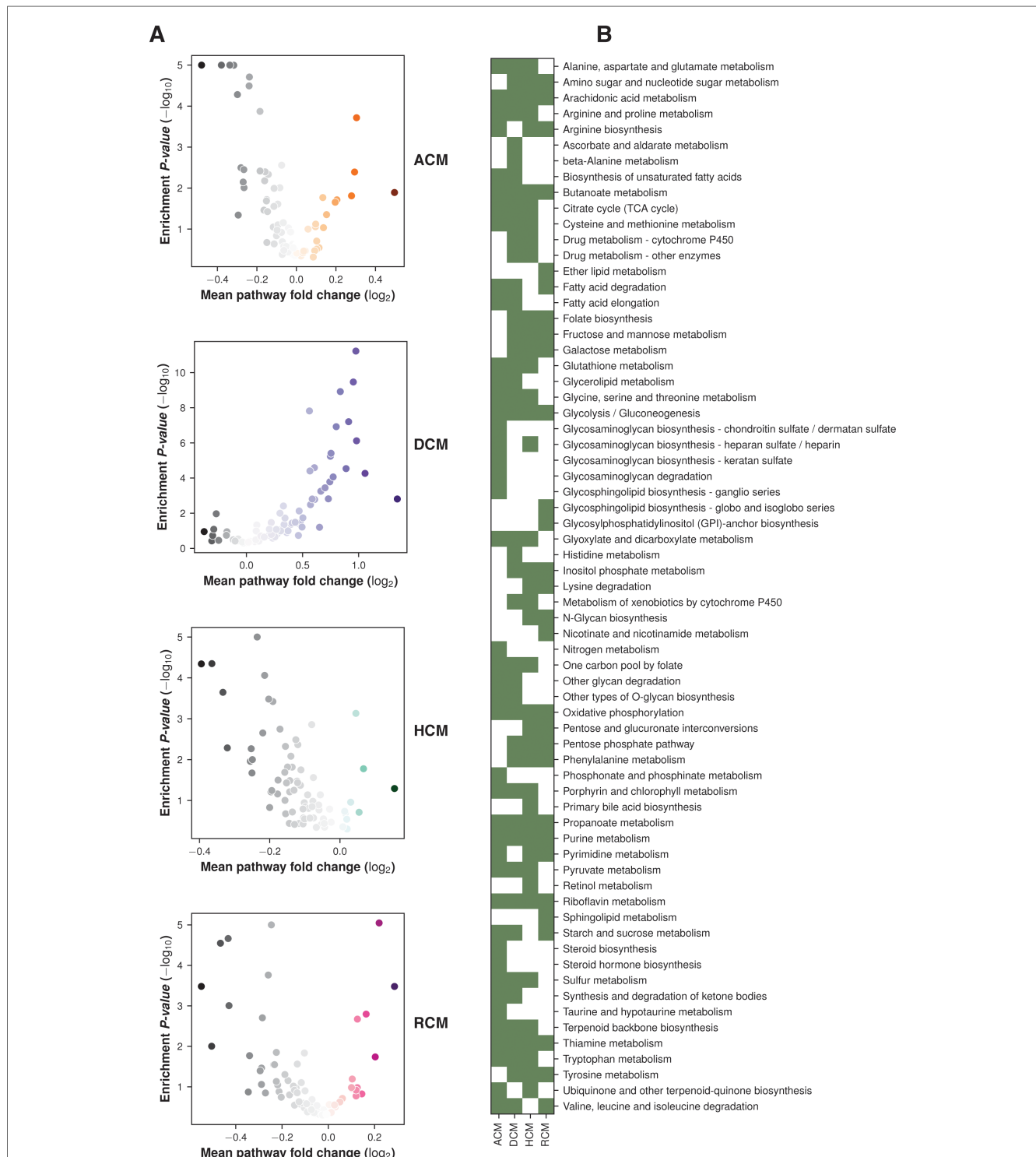


FIGURE 2 GSA enrichment of KEGG pathways in primary cardiomyopathies. (A) Volcano plots showing pathways enrichment and fold change in primary cardiomyopathies. (B) Heatmap showing major pathway perturbation in ACM, DCM, HCM and RCM phenotypes.

Oxidative phosphorylation metabolism, a mitochondrial-associated process, was significantly downregulated in ACM, HCM and RCM but not in DCM. It was upregulated in the DCM phenotype. Similarly, another primary energy pathway, glycolysis/gluconeogenesis metabolism, was also downregulated in ACM, HCM and RCM and upregulated in DCM. Nucleotide-specific purine metabolism was downregulated in all phenotypes except DCM. In addition, the pathway related to the amino acid arginine biosynthesis was significantly upregulated in DCM but significantly downregulated in other primary cardiomyopathies. Pathway related to co-factor metabolism, riboflavin was upregulated in DCM but downregulated in ACM, HCM and RCM. We found this surprising; it is possible that impact on metabolic pathways in DCM may vary compared to HCM or other cardiomyopathies due to its hypo-contractile nature. While some studies have indicated a downregulation of oxidative phosphorylation in DCM (56), a previous study by Verdonschot et al. reported an upregulated oxidative phosphorylation in DCM caused by truncating titin variants (TTNtv) (57). Interestingly, inositol phosphate metabolism

was upregulated in HCM and RCM but downregulated in DCM. Our analysis also indicated significant downregulation of fatty acid precursor, arachidonic acid metabolism. Surprisingly, it was downregulated in the HCM and RCM phenotypes but upregulated in the ACM and DCM phenotypes (see Figure 3). A previous study highlighted that arachidonic acid (AA) induces mitochondrial depolarization in isolated myocytes by a lipoxygenase (LOX)-dependent mechanism and that such depolarization might contribute to arrhythmogenesis (58). ACM's pathological feature results from the replacing the myocardium with fibrous and fatty (fibro-fatty) tissue (59). In light of this, AA metabolism requires further investigation. As observed in the literature, major metabolic pathways are disrupted in almost all cardiomyopathies, either upregulated or downregulated, depending upon the nature of cardiomyopathy (60). Our results concluded that DCM displayed opposite trends compared to the other primary cardiomyopathies in critical metabolic pathways, such as glycolysis/gluconeogenesis, oxidative phosphorylation, riboflavin, thiamine, and purine metabolism.

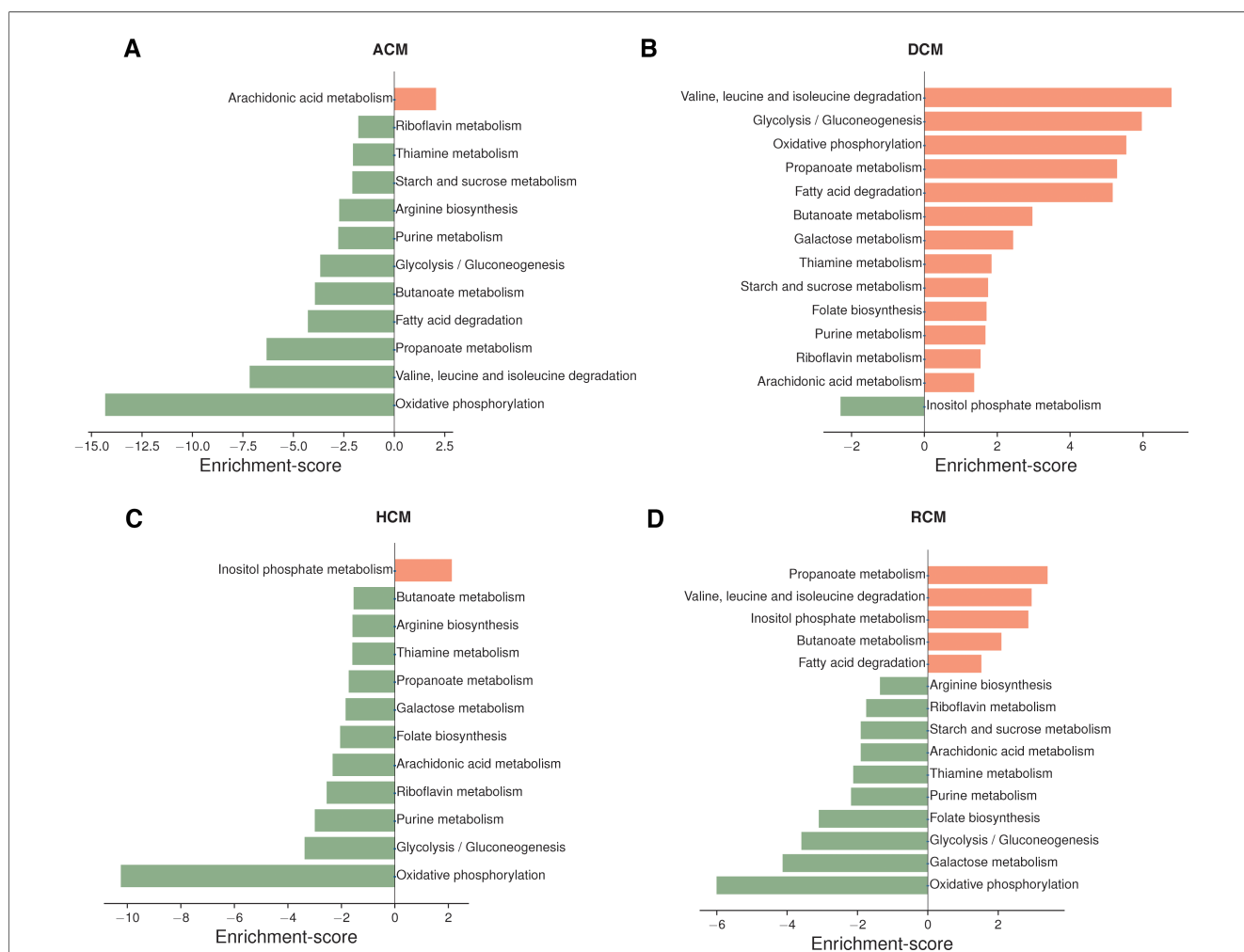


FIGURE 3 Top pathways enriched in ACM, DCM, HCM and RCM phenotypes (A–D). Pathways were ranked based on enrichment scores calculated by the PAGE. Pathways enriched with up-regulated genes are marked in saffron color, and those enriched with down-regulated genes are highlighted in green.

3.3. Arachidonic acid metabolism is perturbed in primary cardiomyopathies

We focused on arachidonic acid metabolism from previous results due to its involvement in all cardiomyopathies and its less studied nature in cardiomyopathies. We chose independent studies from microarray datasets to validate AA metabolism disruption in the ACM, DCM, and HCM human heart tissues. After GSA on validation datasets, we observed that ~76% of KEGG pathways enriched in the original RNA-seq datasets were also significantly enriched in microarray sets. In the validation set, too, AA was dysregulated across ACM, DCM and HCM phenotypes (see **Supplementary Figure S1**). As referred in the previous result that arachidonic acid (AA) induces mitochondrial depolarization via lipoxygenase (LOX), which may be attributed to arrhythmogenesis (58). Therefore, we focused on DEGs to further understand the molecular players in AA metabolism. We identified 25 AA metabolism DEGs in primary cardiomyopathies (see **Table 1**). Genes like *AKRIC3*, *CYP2J2*, *EPHX2*, *LTC4S*, *PLA2G2A*, *PLA2G5*, *PTGDS* and *PTGIS* were particularly interesting (see **Figure 4** and **Supplementary Figure S2**). The aldo/keto reductase superfamily protein-coding gene *AKRIC3* was downregulated in DCM and HCM but upregulated in ACM. A recent work suggests that *AKRIC3* might be involved in the process of ferroptosis in cardiac myocytes and may act as a biomarker for Acute Myocardial Infarction (AMI) (61). The

cytochrome P450 superfamily protein-coding gene *CYP2J2* was upregulated in ACM, DCM and HCM phenotypes. *CYP2J2* coding for cytochrome P-450 2J2 is reported to be implicated in hypertension and coronary artery disease (CAD) (62). The epoxide hydrolase family protein-coding gene *EPHX2* encoding sEH was upregulated in DCM and RCM but downregulated in ACM. This gene was shown to be associated with heart failure in a rat model of heart failure (63). The MAPEG family protein-coding gene *LTC4S* was downregulated in HCM while being upregulated in ACM and DCM. Nobili et al. highlighted that *LTC4S* antagonists could protect against a hypoxic heart (64). The phospholipase A2 family (PLA2) protein-coding gene *PLA2G2A* was upregulated in DCM and downregulated in HCM and RCM. At the same time, the PLA2 family protein-coding gene *PLA2G5* and glutathione-independent prostaglandin D synthase enzyme coding-protein *PTGDS* were downregulated in ACM, HCM and RCM. Phospholipase A2 (PLA2) enzyme is attributed to as a risk factor for coronary heart disease (65). The enzyme coded by *PTGDS* catalyzes the conversion of prostaglandin H2 to prostaglandin D2. It is recognized as a circulating marker for cardiovascular injuries and the severity of CAD (66). Lastly, the cytochrome P450 superfamily protein-coding gene *PTGIS* was upregulated in ACM, while being downregulated in DCM and HCM (see **Figure 5A**). The endogenous expression of the *PGIS* gene product is reported to have a potentially protective effect against hereditary pulmonary arterial hypertension (HPAH) (67). In summary, AA genes are significantly dysregulated in primary cardiomyopathies.

TABLE 1 A list of differentially expressed genes in arachidonic acid (AA) metabolism in arrhythmogenic, dilated, hypertrophic and restrictive cardiomyopathies.

Gene	AA metabolism DEGs (log2FC)			
	ACM	DCM	HCM	RCM
<i>AKRIC3</i>	0.7539	-0.9029	-0.3240	NA
<i>ALOX5</i>	0.7306	NA	NA	NA
<i>CBR1</i>	NA	0.5106	NA	NA
<i>CBR3</i>	0.6172	NA	NA	NA
<i>CYP2J2</i>	NA	1.6690	0.5889	NA
<i>CYP2U1</i>	0.3272	-0.6522	0.3382	NA
<i>EPHX2</i>	-0.4213	0.7662	NA	0.6280
<i>GGT1</i>	NA	1.1862	NA	-0.8778
<i>GGT5</i>	1.0935	NA	-0.4816	NA
<i>GPX1</i>	NA	NA	-0.4593	NA
<i>GPX3</i>	NA	1.2371	-0.4969	NA
<i>GPX7</i>	0.3851	NA	NA	NA
<i>GPX8</i>	0.6545	NA	NA	NA
<i>LTC4S</i>	0.5660	1.174	-0.6461	NA
<i>PLA2G2A</i>	NA	2.1743	-1.3455	-1.1266
<i>PLA2G4A</i>	0.4587	NA	NA	NA
<i>PLA2G4C</i>	NA	0.8315	NA	NA
<i>PLA2G5</i>	-0.2836	NA	-0.3504	-0.5976
<i>PLA2G6</i>	0.4439	-0.6529	NA	NA
<i>PTGDS</i>	-1.0987	NA	-0.5926	-0.9405
<i>PTGES</i>	NA	NA	NA	-1.3769
<i>PTGES2</i>	NA	1.2104	NA	NA
<i>PTGES3</i>	-0.3304	0.4085	NA	NA
<i>PTGIS</i>	0.7085	-1.3175	-0.8043	NA
<i>TBXAS1</i>	0.4671	NA	-0.9969	NA

3.4. Arachidonic acid metabolism regulators cell types and marker genes in these cells

Next, we performed meta-analysis of the expression of the previous 25 AA metabolism genes in heart cell types. We utilized the cell types annotated in the human heart from an earlier single-nucleus RNA sequencing (snRNA-seq) study (GSE183852) (44). These cells were grouped into 15 clusters. Earlier mentioned genes *AKRIC3*, *CYP2J2*, *EPHX2*, *LTC4S*, *PLA2G2A*, *PLA2G5*, *PTGDS* and *PTGIS* were predominantly expressed in cardiomyocytes, epicardium, smooth muscle, endocardium, pericytes and fibroblasts (see **Figure 5B**). Inflammation remains a key player in heart failure (HF) pathogenesis, in both acute and chronic HF. Our analysis revealed multiple immune and inflammation inducing cell types were associated with AA metabolism. Studies have highlighted the role of AA metabolism in inflammation (39, 68). Interestingly, genes common in up to two phenotypes were expressed in more immune cells like mast cells, macrophages and monocytes. The lipoxygenase gene family member *ALOX5*, the glutathione peroxidase family member *GPX1* and the cytochrome P450 superfamily member *TBXAS1* belong to this category. The glutathione peroxidase family members *GPX3*, *PLA2G5* and *PTGIS* were expressed in a significant number of fibroblasts (greater than 50%). The presence of *AKRIC3* and *LTC4S* was negligible in all the major cell types of the heart.

T/NK cells, cardiomyocytes, endocardium, endothelium, epicardium, fibroblast, macrophages, mast cells, myocytes, pericytes and smooth muscles were further explored for marker gene identification. A few AA metabolic genes were present in multiple cell types but were left out as marker genes in all cell types as per the marker definition strategy. *PLA2G2A* gene is such an example that was expressed in fibroblast and epicardium. However, due to its higher expression in the epicardium, it was missed as a marker in fibroblast. Once the marker genes in the above cell types were identified, GSA was carried out on these genes set to inquire enrichment of these markers within upregulated or downregulated genes of ACM, DCM, HCM and RCM (see **Figure 5C, Supplementary Tables S2–S5**). The cardiomyocytes marker genes were enriched within upregulated genes of DCM, HCM and RCM (84, 68, 49) but within downregulated genes of ACM (76) (enrichment *p-values* < 0.1 and gene *adjusted p* < 0.1 & $|log_2FC| \geq 0.28$). In comparison, marker genes of fibroblasts were enriched within upregulated genes of ACM and RCM (63, 20) and within downregulated genes of DCM and HCM phenotypes (enrichment *p-values* < 0.1 and gene *adjusted p* < 0.1 & $|log_2FC| \geq 0.28$). Moreover, the marker genes of pericyte and endocardium were enriched within upregulated genes of ACM and RCM but within downregulated genes of DCM. Likewise, the smooth muscle marker genes were enriched within upregulated genes of ACM and RCM only. In this, a total of 21 and 16 marker genes were found to be upregulated (enrichment *p-values* < 0.1 and gene *adjusted p* < 0.1 & $|log_2FC| \geq 0.28$). The marker genes of the epicardium, macrophages and monocytes were enriched within downregulated genes of DCM, HCM and RCM and opposite in ACM phenotype. The T/NK cells and mast cell marker genes were enriched in the upregulated genes of ACM but enriched within downregulated genes of DCM. Lastly, endothelium marker

genes were enriched within upregulated genes of DCM (62) only (enrichment *p-values* < 0.1 and gene *adjusted p* < 0.1 & $|log_2FC| \geq 0.28$). Overall, expression of AA genes in heart tissue cell types and enrichment analysis on marker genes of these cell types demonstrates that AA metabolic genes are expressed in cardiomyocytes, fibroblast and immune cells, and a large proportion of cell type marker genes are dysregulated in all primary cardiomyopathies.

3.5. Network analysis reveals association of key arachidonic acid metabolism regulator *PLA2G2A* and fibroblast marker genes

We next performed in-depth analysis of the significantly enriched marker genes within DEGs of primary cardiomyopathies. Gene ontology analysis of cardiomyocytes and fibroblast cell type enriched marker genes revealed biological processes associated with muscle contraction and extracellular matrix organization, confirming the relevance of these markers (see **Supplementary Figures S3–S5**). The AA metabolism DEGs and cell type enriched marker genes were mapped to human interactome to understand their underlying association (see Materials and methods). Cardiac fibrosis is a significant player in cardiomyopathies. Therefore, we examined fibroblast cell types in this analysis. The fibroblast cell type dysregulated marker genes were associated with the *PLA2G2A* gene of AA metabolism in ACM, DCM and HCM (see **Figure 6 and Supplementary Figures S6–S8**). In the RCM phenotype, the interaction was missing, possibly due to much fewer overall DEGs in RCM. Network analysis of dysregulated HCM marker genes revealed that AA gene *PLA2G2A* interacts with *DCN*, which interacts with *FN1*, *COL4A4*, *SLIT2*, *EGFR*, *GSN*, *COL1A2*, *ROBO1*, *COL4A1*

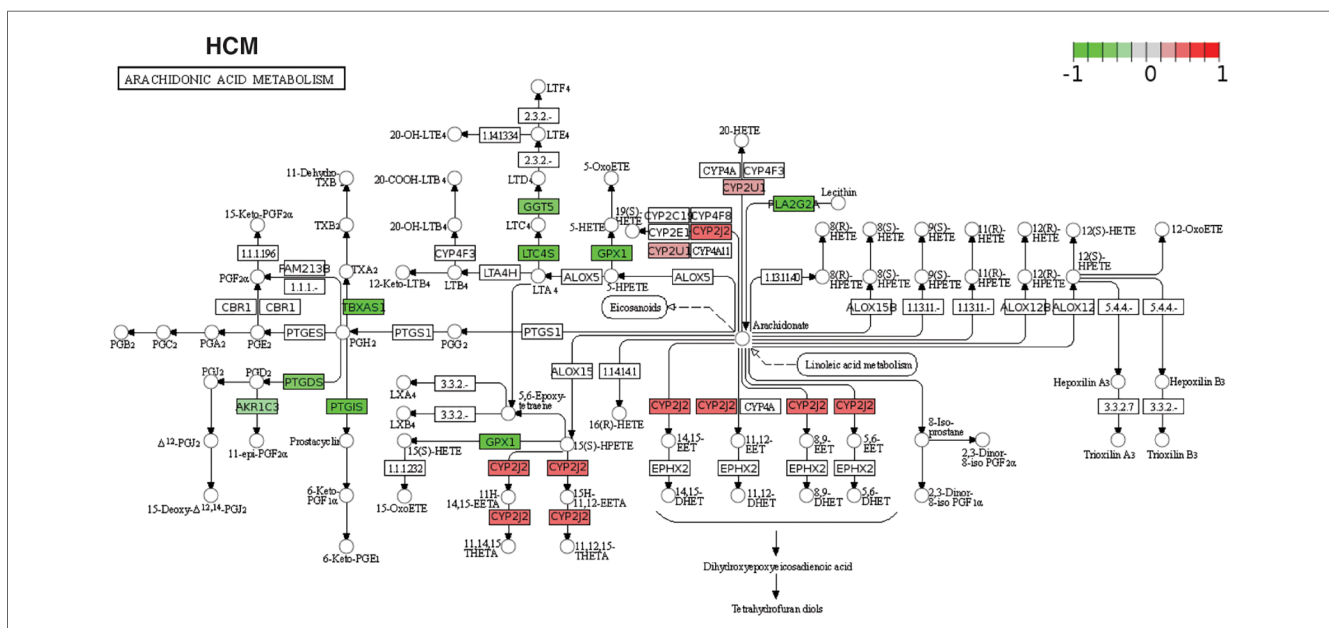
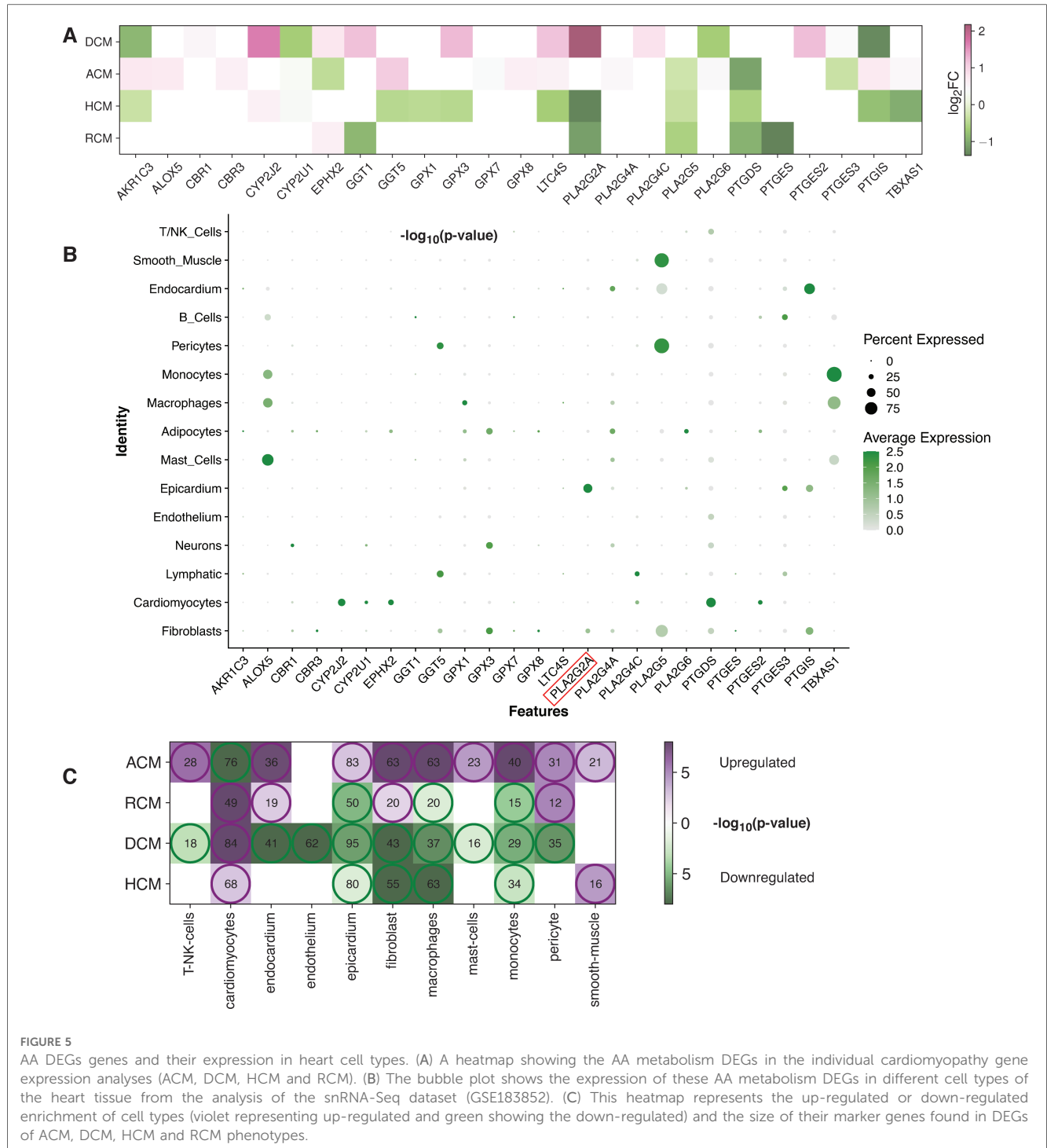


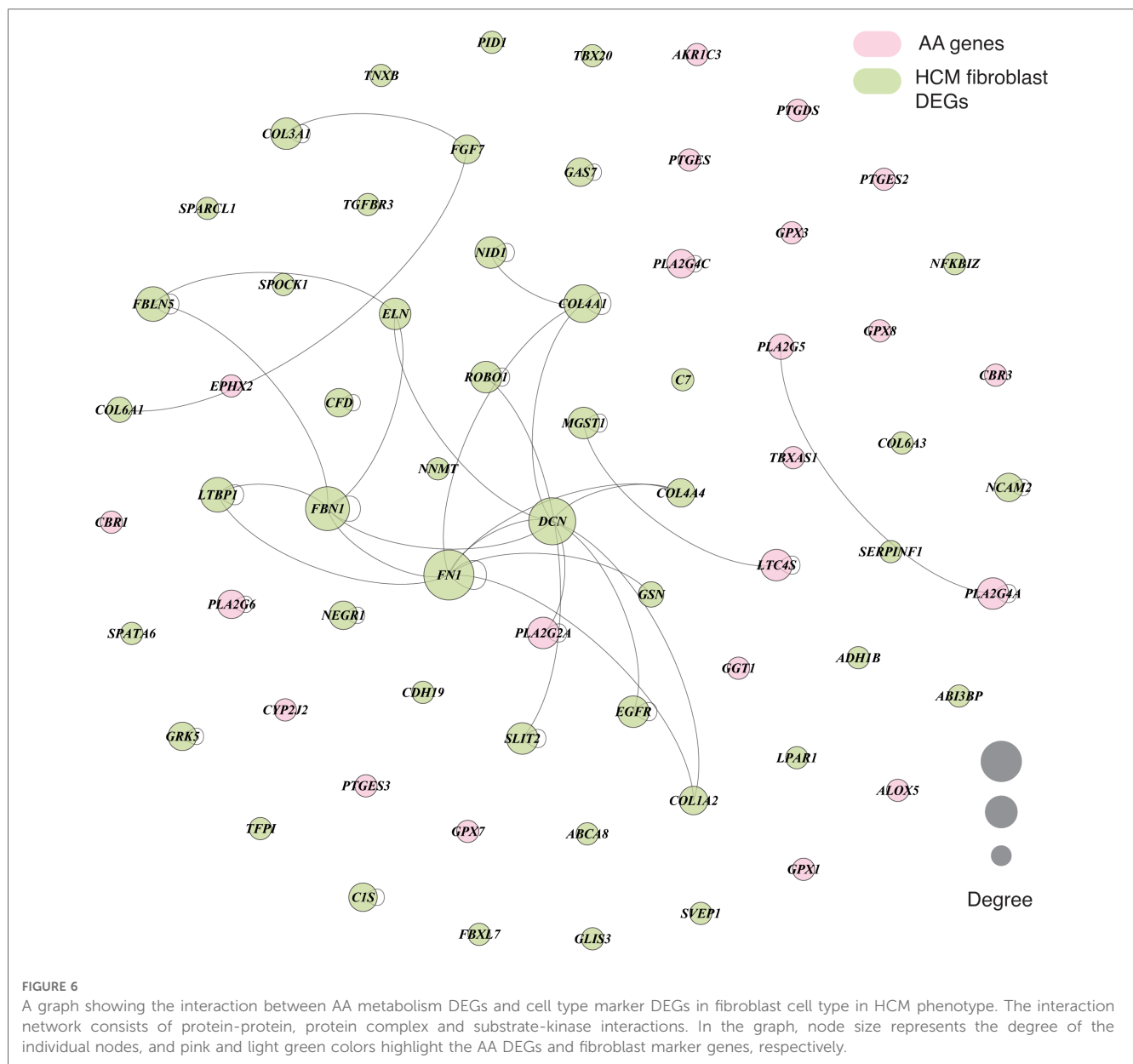
FIGURE 4
The KEGG pathway map of AA metabolism DEGs. Differentially expressed AA metabolism genes are mapped to the KEGG pathway map in individual studies (in this case, HCM). The red color on the map represents the up-regulated genes, and green marks the down-regulated genes in the study.



and *ELN* that are primarily involved in extracellular matrix (ECM) organization and heart development. Similarly, DCM-specific dysregulated marker genes network uncovered *PLA2G2A* interaction with *DCN*, which was directly linked to *FBN1* and *COL4A1*. These genes are primarily involved in ECM organization and anatomical structure morphogenesis. Collectively, network analysis demonstrates that the phospholipase A2 family gene *PLA2G2A* influences fibroblasts and may be involved in fibrosis during cardiomyopathy.

4. Discussion

Primary cardiomyopathies like ACM, DCM, HCM and RCM are heterogenous heart muscle diseases with poor prognosis, and are a leading cause of sudden cardiac death (6–12). The current study accomplishes GSA on the transcriptome profile to understand metabolic pathways perturbation across primary cardiomyopathies. Among the GSA-enriched KEGG metabolic pathways between cardiomyopathy and normal samples,



glycolysis/gluconeogenesis, oxidative phosphorylation, nucleotides metabolism, amino acid biosynthesis, cofactor metabolism, and fatty acid precursor arachidonic acid metabolism were statistically significant. Previous studies have reported oxidative phosphorylation, glycolysis and fatty acid metabolism shift in individual cardiomyopathies (23, 24). In this work, we carried out an integrated analysis of AA metabolic alteration across cardiomyopathies (see Figure 7).

Arachidonic acid (AA) metabolism is an important mediator of cardiovascular processes such as fibrosis and inflammation. However, its role in cardiomyopathies is less explored (31, 32). Arachidonic acid is a free fatty acid that gets transformed into biologically active mediators by COX, LOX, and CYP450 epoxygenase enzymes (31). These mediators allow AA to participate in complex cardiovascular functions (32). Our study highlighted the statistically significant dysregulation of AA

metabolism in all primary cardiomyopathies. This was also found to be consistent in the validation set. Further, AA metabolic DEGs in our study were expressed in heart tissue cell types like cardiomyocytes, fibroblasts, epicardium, endocardium, smooth muscle, and pericytes and immune cells such as macrophages, monocytes and mast cells at the single-cell level. Our analysis showed that the AA metabolic genes *ALOX5* and *TBXAS1* were present in macrophages, monocytes and mast cells. However, these were not significant in a majority of cardiomyopathies. These results point to a potential role of AA metabolism in modulating fibrosis and inflammation in cardiomyopathies.

Cardiomyopathies are generally characterized by cardiac fibrosis (69). Cardiac fibrosis results from the dysregulation of the balance between the synthesis and degradation of extracellular matrix (ECM) proteins (69, 70). It has been suggested that fibrosis primarily manifests in the interstitial space

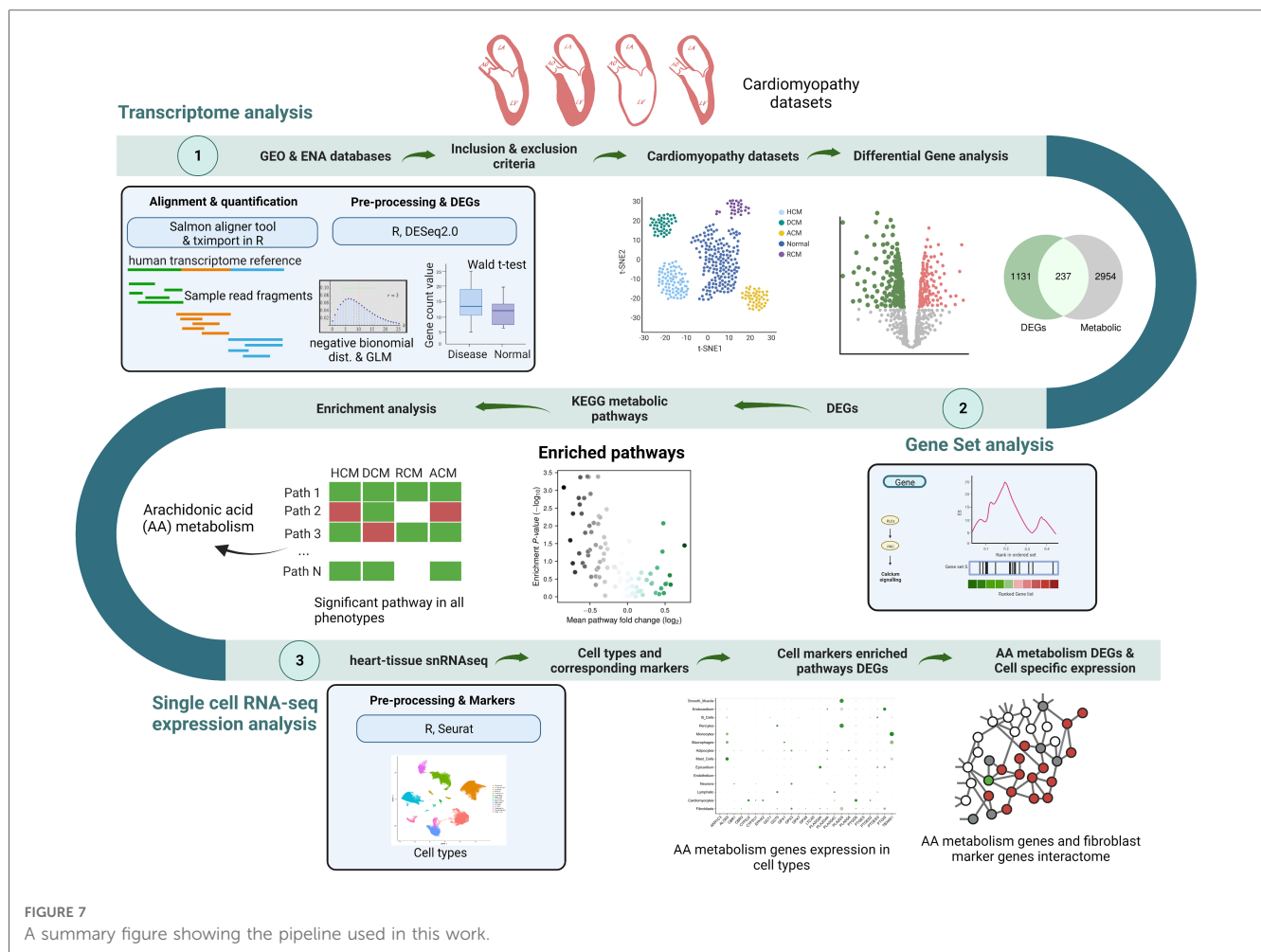


FIGURE 7 A summary figure showing the pipeline used in this work.

in pressure-induced cardiac remodeling without cardiomyocyte deletion. Whereas volume overload-induced cardiac remodeling leads to a significant reduction in cardiomyocytes, and fibrosis is triggered, especially in myocardial infarction (MI) (39). Fibroblast cells synthesize the ECM proteins and get involved in tissue repair and remodeling after injury in heart tissues during cardiomyopathies (69–71). We investigated whether AA metabolism genes were associated with dysregulated fibroblast marker genes. Our analysis showed the interaction between the AA metabolism gene *PLA2G2A* and the fibroblast marker gene *DCN*, which was linked to many other fibroblast marker genes. Fibroblast marker genes’ gene ontology enrichment analysis confirmed their role in ECM organization. Decorin (*DCN*) belongs to chondroitin sulfate proteoglycan and has been shown to exhibit antifibrotic effects in the mouse model (72). In short, our analysis demonstrated that AA metabolism genes interact with the ECM proteins, such as decorin and others, directly or indirectly and might influence cardiac fibrosis in primary cardiomyopathies.

Epoxyeicosatrienoic acids (EETs) are metabolites that arise from arachidonic acid (AA) through the activity of CYP epoxygenases. Successive studies have revealed that EETs protect the heart against inflammation, cardiac remodeling, endothelial dysfunction, and fibrosis (41, 73). EETs have been reported to be

nitric oxide (NO) independent vasodilators *in vivo* (74). Other analyses suggest that EETs act as an endothelium-derived hyperpolarizing factor (EDHF), inducing vasodilation of vascular smooth muscle by activating Ca²⁺-activated K⁺ channels (75). CYP2J2, CYP2C8, and CYP2C9 enzymes are major sources of synthesis of EETs (37). Our analysis also showed upregulation of *CYP2J2* gene expression in DCM as well as HCM. Notably, *CYP2J2* is the only human *CYP2J2* epoxygenase and is highly expressed in the heart and endothelium (38). *CYP2J2* catalyzes AA into four regioisomeric EETs, including 5,6-, 8,9-, 11,12-, and 14,15-EET (39). It will be worthwhile to explore *CYP2J2* further in cardiomyopathies.

This study has limitations due to heterogeneity in samples aggregated from different transcriptome studies. Sequencing methodology, environment and sequencing depth are the main challenges in an integrative analysis. Quantification of RCM RNA-seq reads was missing for many genes due to less coverage of these genes. Next, this study only demonstrates statistically perturbed pathways, important DEGs and heart cell types and their interactions in each cardiomyopathy through computational analysis. Experimental validation in an *in vitro* or *in vivo* system was not performed due to the limitation of resources. Lastly, ACM, DCM, HCM and RCM are highly heterogeneous diseases. Thus, their classification may vary substantially in the population.

Regardless of the limitations, the current study provides new insights into cardiomyopathy research. This work recognizes the arachidonic acid metabolism as a potential regulator of all major primary cardiomyopathies. Apart from this, the study also demonstrates the expression of AA metabolism genes in major heart-specific cell types and immune cells, facilitating a better interpretation of its potential roles in the disease. The interaction between the products of AA gene *PLA2G2A* and fibroblast marker gene *DCN* could present a promising therapeutic target to regulate cardiac fibrosis.

5. Conclusion

Arachidonic acid metabolism is perturbed in primary cardiomyopathies. AA metabolism genes are expressed in most heart cell types and immune cells, influencing the immune activation and cardiac fibrosis. The association of the dysregulated AA gene *PLA2G2A* with fibroblast marker gene *DCN* may be an important factor related to fibrosis.

Data availability statement

Existing datasets are available in a publicly accessible repository: Publicly available datasets were analyzed in this study. This data can be found here: http://caps.ncbs.res.in/download/cardiomyo_transcriptome.

Author contributions

RS and PC conceptualized the study. PC integrated data, performed data analysis and visualization. PC wrote the manuscript and RS wrote the final draft of manuscript. All authors contributed to the article and approved the submitted version.

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In memoriam

This paper is dedicated to and in the memory of our late collaborator, Prof. N Srinivasan.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2023.1110119/full#supplementary-material>.

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