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RESEARCH PAPERS

Quantitative test for COVID-19 in the cardiovascular process: *in silico* gene-target cluster evaluation

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Highlights

- Omics Data, Virtual Tools, NGS, and KEGG Identify Gene Clusters in COVID-19 Cardiac Damage
- "User-Friendly Virtual Tools Synthesize Data for Planning RT-qPCR Clusters in Research"
- "Next-Generation Sequencing, RNA-Seq, Crucial for Expression Profiles in Viral Infection"
- "Key Findings: Gene Clusters and Pathways Linked to COVID-19 Cardiac Damage and Disorders"
- "Potential Targets: Gene Overlap and Pathways for Mitigating COVID-19 Cardiac Damage"

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KEYWORDS
Omics;
KEGG canonical pathways; pre-and clinical cardiac evaluation;
SARS-CoV-2 pandemic;
laboratory medicine.

Abstract: The relative lack of clinical knowledge revealed by the most recent pandemic caused by SARS-CoV-2 has highlighted the need for molecular knowledge in 'omics disciplines and molecular databases, integrated with available and user-friendly virtual tools. The production of an RT-qPCR reaction cluster is a posteriori and must be planned based on the knowledge of several available sources: specialized literature and databases with laboratory medicine tools. However, the specificities of infection with the new virus would require the prior use and execution of techniques to obtain extensive expression profiles, such as next-generation sequencing (i.e., RNAseq). In this work, we cross-reference RT-qPCR and RNAseq resources from literature and the KEGG Disease Database (Kyoto Encyclopedia of Genes and Genomes) to provide comprehensive insights into COVID-19-related cardiovascular DEGs and gene-target relationships. Gene clusters were used to identify enriched pathways and compare the canonical metabolic pathways for COVID-19 cardiac quantitative evaluation. One hundred seventy-one genes were listed in 42 KEGG Disease entries, resulting in 194 enriched pathways, with seven annotated pathways showing statistically significant XD-scores. Some specific differentially expressed genes in transcriptional literature evaluations overlapped with the KEGG canonical cardiac processes. The KEGG Disease cardiovascular gene set showed six pathways linked to quantitative cardiac evaluation that are significantly enriched in COVID-19. Results indicated hypertrophic and dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, and cardiac muscle contraction. The presence of specific sets of genes with links between gene clusters, overlaps of genes and processes, and subsets of compartmentalization represent different possibilities for metabolic pathways and gene targets related to cardiac damage pre- and post-COVID-19 infection.

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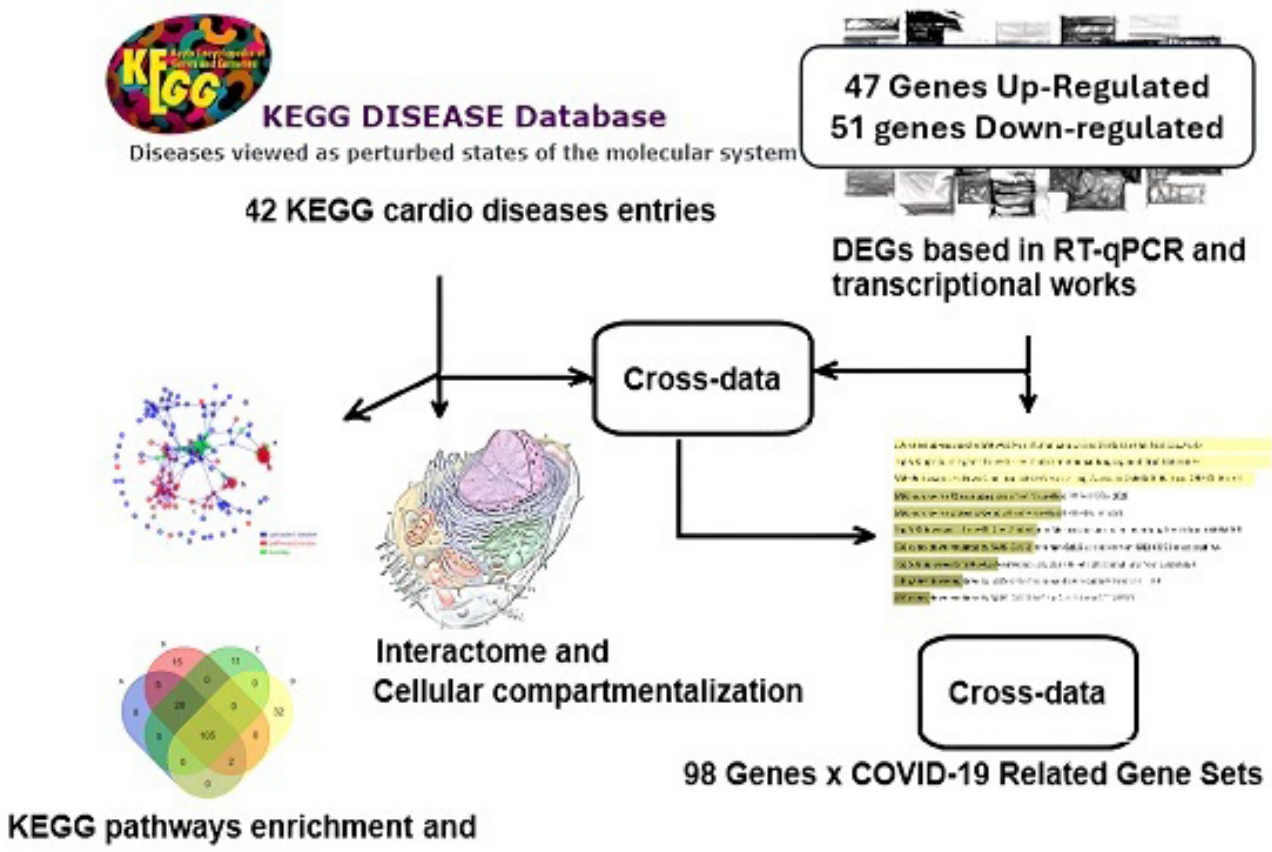
Graphical Abstract



Introduction

The relative lack of molecular and clinical knowledge revealed by the most recent pandemic caused by SARS-CoV-2 has highlighted unmistakable signs that the complexity of biological systems requires more research. A significant accumulation of molecular knowledge has occurred in the current scientific environment, involving 'omics disciplines and molecular databases, integrated with available and more user-friendly virtual tools. Despite the large amount of data used and accumulated, which has dramatically enriched basic research, the clinical application of this knowledge is complex, with the translational concept of technological transposition from "bench-to-bedside" (Singh et al., 2020). According to the National Cancer Institute (NCI) dictionary, clinical applications generate research using a fundamental approach: the "bench to bedside and back to the bench."

Given the complexity of the infection, the processes involved in the condition's evolution, and interfering pathologies, there are few clinical laboratory options in the biotechnological field. Thus, innovation in the characterization of diagnostic tests based on the signature of differentially expressed genes (DEGs) assembled in evaluation clusters applied to the RT-qPCR reaction is critical for optimizing the clinical response to SARS-CoV-2.

The scientific research necessary for understanding the complex metabolic-infectious processes linked to SARS-CoV-2, along with its relationships to comorbidities and multifactorial interactions, is still to be fully established. Nevertheless, we believe it may be one of the most promising biotechnological processes. This is a new field of Molecular Biology that integrates Systems Biology, the study of omics or post-genomics, Functional Genomics, Multifactorial Statistics, and Laboratory Medicine (Pertea, 2012). Computerized tools can integrate these and other disciplines and provide the foundations for understanding the metabolic processes involved in phenotypic characterization.

COVID-19 infection results in a higher immune-metabolic demand that can facilitate cardiac complications. The role of inflammation and secondary organ involvement was primarily caused by further pulmonary damage, subsequent hypoxemia, and additional cardiovascular stress, leading to systemic inflammation that injures distant organs (Guo et al., 2020). Patients with underlying cardiovascular disease are at greater risk of severe complications from COVID-19 (Bansal, 2020). Cardiac complications of COVID-19 have shown acute onset heart failure (Ranard et al., 2020), myocardial infarction (Italia et al., 2021), myocarditis (Madjid et al., 2020), and cardiac arrest (Marwaha et al., 2024). SARS-CoV-2 directly affects the cardiovascular system via a cytokine storm, which may play a role in coronary plaque instability, as previously observed with SARS-CoV (Channappanavar & Perlman, 2017; Tsui et al., 2005). Patients infected with SARS-CoV or SARS-CoV-2 typically present with lymphopenia

(Zhu et al., 2020; Huang et al., 2020), a condition associated with atherosclerosis development and adverse cardiovascular outcomes in SARS-CoV (Núñez et al., 2009) and SARS-CoV-2 (Vinciguerra et al., 2020).

The biotechnological challenges presented by the pandemic are numerous: a) currently, the clinician's decision regarding coronavirus treatment depends only on the infection test or, if unavailable, on the standard assessment of the patient, and b) pre-existing comorbidities (such as diabetes, hypertension, cardiovascular diseases, etc.) affect the clinical evolution of those infected by the coronavirus, and often the prognosis depends exclusively on the existence of updated medical records or the decision of the clinician. Using both RT-qPCR and RNAseq is essential in biotechnological research, as it combines the advantages of both methods to fully understand gene expression.

This paper aims to present the use of DEG datasets to develop potential quantitative tests for genetic cardiovascular complications in COVID-19 by analyzing gene-target interactions and spatial organization in cardiac cells. Overall, the methodology involves a comprehensive in silico exploration using bioinformatics to explore and produce molecular genetic cross-data. The biotechnological approaches – bioinformatic tools applied to RT-qPCR and RNAseq datasets – were used to analyze gene expression patterns and pathway enrichment, providing insights into the molecular mechanisms underlying cardiac complications, uncovering molecular mechanisms, and identifying potential therapeutic targets for COVID-19-related cardiac diseases.

Methods

This work demonstrated user-friendly virtual tools and molecular databases to obtain gene clusters, identify enrichment pathways, and compare canonical metabolic pathways for COVID-19 cardiac quantitative evaluation. Genes obtained from 42 KEGG cardiovascular disease entries were listed from the Kyoto Encyclopedia of Genes and Genomes (KEGG - KEGG Disease subdivision entries (Kanehisa & Goto, 2000). Figure 1 shows a pipeline summary for all studied interactions and the results obtained for an in-silico gene target cluster evaluation.

This entry proceeded to gene enrichment and interactome analysis using the EnrichNet approach (enrichnet.org), accessed in Mar/2024 (Glaab et al., 2012). Networks of the obtained gene set were constructed using the EnrichNet online tool. Furthermore, these gene clusters were analyzed for enrichment and in literature to compile the canonical pathways for COVID-19 cardiac quantitative evaluation and biotechnology purposes. Using QIAGEN Ingenuity Pathway Analysis (IPA) resources, we performed comparison and spatial compartmentalization of cardiac markers, gene-target interactions pathway analysis, canonical pathways, overlapping pathways, pathway import, and scoring methods.

An initial cross-data was manually obtained from DEG lists based on RT-qPCR and next-generation sequencing (i.e., RNAseq) or transcriptional works, producing 194 enriched pathways with seven annotated pathways that had statistically significant XD-scores. To focus on the COVID-19 cardiac

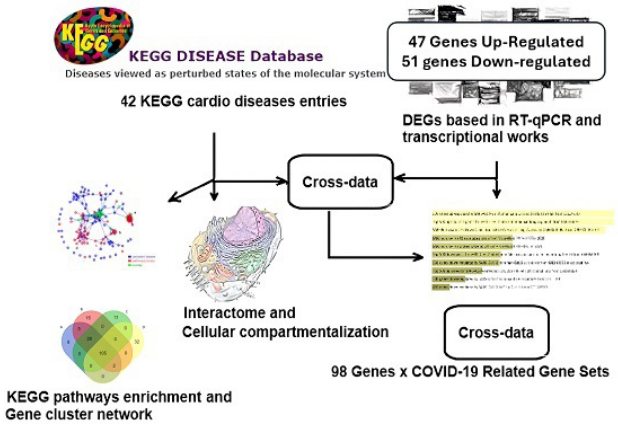


Figure 1. Pipeline schematic resume for all studied interactions *in silico* gene-target cluster evaluation. Search Analytical comparison: genes were listed from *Kyoto Encyclopedia of Genes and Genomes* (KEGG Disease subdivision) entries were cross-data with DEGs obtained in cardiac transcriptional literature works. The software enrichment phase results in three sets of analysis: gene list (cross-data) and more analysis: Interactome with Cellular compartmentalization and Enrichment evaluation.

genes, a second cross-data analysis was performed with the up- and down-regulated genes from the initial data, both used to focus on COVID-19-related gene sets, resulting in a second cross-data. To facilitate this, the EnrichR tool was used in standard mode (Chen et al., 2013). The molecular gene library used was "COVID-19_Related_Gene_Sets_2021," a collection of gene sets related to COVID-19 research, accessed in April 2024.

Statistics for the gene set's network-based similarity score/significance overlaps with the pathway were measured using a Fisher test (XD-Score/Fisher) (Glaab et al., 2012; Dennert et al., 2008). The similarity was presented as an XD-score. The higher the XD-score value, the higher the similarity, indicating an increased possibility of a KEGG pathway being enriched with genes. To validate the criteria of the XD-score, the classical overlap-based Fisher test was used to calculate the significance score (q-value) via the EnrichNet tool, and linear regression analysis between the q-value and XD-score was performed. An XD-score lower than the threshold value of 0.79, corresponding to a q-value of 0.05, indicated significance. Results are shown in parentheses, with the gene set's network-based similarity score/significance overlaps with the pathway measured by a Fisher test (XD-Score/Fisher) (Glaab et al., 2012; Dennert et al., 2008).

Results and discussion

Cardiomyopathies pathways for evaluation in COVID-19

For the obtained results, 171 protein-coding genes were listed in 42 KEGG Disease entries, producing 194 enriched pathways, with seven annotated pathways showing statistically significant XD-scores. The results are grouped into two tables. Table 1 presents a KEGG database of disease

entries and the respective pathways for human cardiovascular diseases. Table 2 shows the differentially expressed genes obtained from cardiac transcriptional studies.

Thus, the evaluation of metabolic pathways of interest for the study of cardiac alterations during the infection process and the evolution of the SARS-CoV-2 infection can be derived from analyzing transcriptomes already produced by research and evaluating canonical pathways assessed in databases. Table 1 shows some genes obtained from the first method.

Different research approaches can provide additional insights, leading to the identification of other metabolic pathways of interest for assessment. Cultured cardiac myocytes (Chen et al., 2019), transgenic models (Marian & Roberts, 2001), knock-out animal models (Spitler et al., 2017), and pharmacological studies (Dewey et al., 2016) have not only revealed critical molecules involved in hypertrophic signaling but have also highlighted the redundancy within the hypertrophic signaling cascade. Based on the genes described in this set, genes validated with RT-qPCR, protein immunoprecipitation assays, or other identification methods were compared with the genes found in the KEGG Disease Database (Table 2), resulting in a genetic set commonly used as biomarkers, some of which are exceptionally linked to the SARS-CoV-2 condition and its evolution.

The list of genes with cross-referencing (Table 3) compares Tables 1 and 2. In the search for biomarkers, these genes play diverse roles in cardiovascular health, including markers of myocardial injury. In Table 3, it is possible to visualize the functions of these markers, as understanding their functions and interactions can provide insights into the pathology of cardiovascular diseases, especially in the context of cardiac complications related to COVID-19. Some of these major complications are discussed in several studies that address cardiovascular diseases in COVID-19: The mortality risk associated with acute cardiac damage was higher in patients with advanced age (Heidecker et al., 2008), diabetes mellitus (Haşlak et al., 2020), chronic pulmonary disease (Guan et al., 2020), or a prior history of cardiovascular disease (Guo et al., 2020; SHI et al., 2020). In a report on 138 patients with COVID-19 hospitalized in Wuhan (Hubei Province, China), 64 (46.4%) had one or more coexisting medical conditions, primarily cardiovascular or cerebrovascular (Wang et al., 2020). Hypertension was present in 31.2%, diabetes in 10.1%, and cardiovascular disease in 14.5% of patients (Zhu et al., 2020). In a SARS study of cardiovascular complications in 121 patients, 71.9% of patients developed persistent tachycardia, including 40% who had persistent tachycardia during outpatient follow-up. Additionally, 50.4% of patients developed sustained asymptomatic hypotension during hospitalization; one patient required inotropic support; 14.9% of patients developed transient bradycardia, and 10.7% developed transient cardiomegaly without signs or symptoms of heart failure. The case-fatality rate for patients with underlying cardiovascular disease was higher (10.5%) compared to those with chronic respiratory disease (6.3%) (Hulot, 2020).

Spatial co-location for cardiac evaluation and enrichment in COVID-19 gene-target

The relationship between the most common target markers for cardiac diseases is present and involves cell spatial compartmentalization (Figure 2). A complex network of second messengers, protein kinases, enzymes, and other molecular features shows impressive interactions within myocyte cell components (Fatkin & Graham, 2002). The nucleus contains cardiomyocyte differentiation factors, such as BMP receptors, cardiogenic factors, and especially transcription factors. Cardiac transcription factors coordinate inducible gene expression, are required for the molecular basis of the genetic program, and are natural targets for biotechnological purposes.

After the nucleus, the cytoplasm and membranes contain enzymes, transmembrane receptors, transporters, growth factors, and ion channels. The extracellular space contains soluble molecules. This physical separation allows the temporal regulation of various cellular processes, co-localization of pathways, and signal transmission between adjacent molecules (Minerath et al., 2019). Signalosomes within the cardiac myocyte are formed by clusters of discrete multi-molecular complexes, a mechanism for enhancing hypertrophic signal transduction efficiency. These signalosomes can alter gene and protein expression, including changes in cell size and chamber remodeling (Negro et al., 2008).

The spatial co-location has been a focus for over 30 years to explain how various G protein-coupled receptors achieve specificity despite converging on a ubiquitous messenger, cyclic adenosine monophosphate (cAMP) (Zhang et al., 2020). cAMP is a signaling messenger produced in response to cellular receptor stimulation, and in the heart, cAMP is responsible for regulating contraction (Lefkimmiatis & Zaccolo, 2014). The compartmentalization of cAMP production, such as in transverse tubules (T-tubules) and caveolae, is part of cAMP's spatial confinement in cardiomyocytes, as exemplified by beta-adrenergic receptor signaling (Bhogal et al., 2018).

Cardiomyocyte beta2-adrenergic receptors (beta-ARs) provide an inotropic influence on heart failure (PRKAG - Protein Kinase AMP-Activated Non-Catalytic Subunit Gamma 2), CACNA1C (Calcium Voltage-Gated Channel Subunit Alpha1 C), and RYR2 (Ryanodine receptor 2) (Figure 2). Components of the beta2-AR signaling complex compartmentalize into restricted membrane subdomains in adult rat cardiomyocytes (Rybin et al., 2003). Kinase anchoring proteins (AKAP9 – A-Kinase Anchoring Protein 9 – Figure 2) promote the termination of cAMP signals by phosphodiesterases and protein phosphatases, integrating signaling pathways (Skroblin et al., 2010). The Bone Morphogenetic Proteins (BMPs), as shown in Figure 2, are the PRDM16 (PR/SET Domain 16), GATA4 (GATA Binding Protein 4), NPPA (Natriuretic Peptide A), and MYH7 (Myosin Heavy Chain 7) genes. BMPs and their receptors are regulators of embryonic patterning and organogenesis, essential for cardiovascular structure and function by recruiting pathways (Morrell et al., 2016). The enriched gene set for cardiovascular diseases showed 155 genes from hsa04020: Calcium signaling pathway (Figure 3), including the Calmodulin gene (Calm1). Calmodulin is a highly conserved Ca2+ sensor protein in eukaryotic cells with no intrinsic enzymatic function. The calmodulin regulatory process involves activating Ca2+-sensitive enzymes such as CaMKII and calcineurin. This calcineurin-dependent pathway has been shown to play a crucial role in cardiac development and the adult cardiac hypertrophic response (Wilkins & Molkentin, 2004).

Table 1. KEGG Database Diseases entries and respective pathways of human cardiovascular diseases.

Kegg Entry	Name	Pathways	References
H00292	Hypertrophic cardiomyopathy	hsa05410	(Marian & Roberts, 2001)
H00293	Arrhythmogenic right ventricular cardiomyopathy	hsa05412	(Soor et al., 2009)
H00294	Dilated cardiomyopathy	hsa05414	(Luk et al., 2009)
H01219	Restrictive cardiomyopathy	hsa04260	(Parvatiyar et al., 2010)
H00295	Viral myocarditis	hsa05416	(Dennert et al., 2008)
H01216	Left ventricular noncompaction	hsa04260	(Posch et al., 2010)
H00546	Atrial septal defect		(Maslen, 2004)
H00547	Atrioventricular septal defect		
H00549	Tetralogy of Fallot	hsa04330	(Lin et al., 2010)
H00550	Complete transposition of the great arteries		(Asadollahi et al., 2013)
H01786	Congenitally corrected transposition of the great arteries		(Hornung & Calder, 2010)
H00553	Congenital supravalvar aortic stenosis		(Hickey et al., 2008)
H00555	Char syndrome		(Satoda et al., 2000)
H00654	Barth syndrome	hsa00564	(Finsterer & Stöllberger, 2008)
H00669	Naxos disease	hsa05412	(Protonotarios & Tsatsopoulou, 2004)
H02094	Carvajal syndrome		(Finsterer & Stöllberger, 2008)
H00720	Long QT syndrome	hsa04261 hsa04921	(Zareba & Cygankiewicz, 2008)
H00725	Short QT syndrome		(Zareba & Cygankiewicz, 2008)
H02091	Jervell and Lange-Nielsen syndrome	hsa04261	(Schwartz et al., 1997)
H00728	Brugada syndrome	hsa04010 hsa04020 hsa04260 hsa04270 hsa05410 hsa05412 hsa05414	(Giudicessi et al., 2012)
H00729	Sick sinus syndrome		(Satoda et al., 2000)
H00730	Familial idiopathic ventricular fibrillation		(Napolitano & Priori, 2006)
H00731	Atrial fibrillation	hsa04270 hsa04261	(Tsai et al., 2008)
H00918	Double-outlet right ventricle		(Obler et al., 2008)
H00939	Darsun syndrome		(Boztug & Klein, 2011)
H01019	Catecholaminergic polymorphic ventricular tachycardia	hsa04020 hsa04261 hsa04260	(Liu et al., 2007)
H01154	Wolff-Parkinson-White (WPW) syndrome	hsa04910 hsa04920 hsa05410	(Fragakis et al., 2007)
H01263	Progressive cardiac conduction defect (PCCD)		(Kruse et al., 2009)
H01632	Angina pectoris		(Aronow, 2003)
H01729	Premature ventricular complexes		(Saurav et al., 2015)
H01730	Myocardial infarction		(Thygesen et al., 2012)
H01736	Persistent truncus arteriosus		(Heathcote et al., 2005)
H01783	Ebstein anomaly		(Digilio et al., 2011)

Table 1. Continued...

Kegg Entry	Name	Pathways	References
H01785	Tricuspid atresia		(Sarkozy et al., 2005)
H01787	Univentricular heart		(Khairy et al., 2007)
H01802	Pulmonary atresia with intact ventricular septum		(Bakhru et al., 2017)
H01803	Pulmonary atresia with a ventricular septal defect		(Murthy et al., 2010)
H01868	Mitral valve prolapse		(Freed et al., 1999)
H01926	Ventricular septal defect		(Peng et al., 2010)
H02122	Chronic atrial and intestinal dysrhythmia	hsa04114]	(Chetaille et al., 2014)
H02125	Cardiac conduction disease with dilated cardiomyopathy		(Wang et al., 2020)
H02269	Familial ventricular tachycardia		(Zuberi et al., 2010)

Table 2. Differential expression genes obtained in cardiac transcriptional works. The most genes presented were qPCR e/or WB process validated.

Reference	Condition	Regulation	Genes
(Meng et al., 2019)	Hypertrophic cardiomyopathy	Up	EID1, GNG2, EMP2, TIMM8B, TMEM158, IGF1, DUSP18, MESDC1, COX14, GM10136, MRPS21, RPL41, MRPS2, SLC35E3, GM6166, ABRACL, CCDC50, TBC1D24, RPP25L, GM24336, KLF13, MRPL50
		Down	MRPL34, LIX1L, RDH14, SLC38A1, ABHD17C, JRK, VAPB, UFM1, PIP4K2B, PTX3, TMEM177, CD276, RP24-236E2.1, SNX3, APH1B, PFN2, TIMP2, FAM63B, ABI3, SLN
(Spitler et al., 2017)	Hearth failure	Up	FATP, FABP3, CPT1A, CPT1B, CPT2, ACADM, ACADVL, CS, CKMT2, PPARGC1A, PPARGC1B, POARA, ESRRA
Data not show	Minerath et al.	Up	NPPA, NPPB, ACTA1, TNNT1
(Bos et al., 2020)	Obstructive Hypertrophic Cardiomyopathy	Up	ACE2, SFRP1, RASL11, CENPA, APOA1, HS.576694, SMOC2, PROS1, FRZB, HSPA2
		Down	SERPINA3, RASD1, S100A9, S100A9, MT1X, CEBPD, ZFP36, MTIM, TUBA3D, TUBA3E
(Haywood et al., 2020)	Ventricular arrhythmia	Up	BRD4, TP53, EIF4G2, CTNNB1, HDAC6, TGFB1, HOXC6, H1F1A, ERK1/2, IKBKG, HTT, CEBPA, TP73, TGFBR1, TSC2, FAZ, CTNNA1, RB1, EOMES
		Down	

Different combinations are presented in Figure 3 to enrich the cardiovascular disease KEGG analysis in the gene interactions that form multi-molecular complexes interacting with a particular biological significance. The KEGG Disease Database revealed six significantly enriched pathways in the cardiovascular gene set. Figure 3 shows four of them, which are more closely related to cardiac quantitative evaluation purposes. The results identified hypertrophic and dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, and cardiac muscle contraction (Meng et al., 2019). Two other significant pathways identified were hsa05330: Allograft rejection (0.9943/3.9e-02) and hsa05416: Viral myocarditis (0.99433/4.4e-05). Non-significant pathways identified were hsa04940: Type I diabetes mellitus (0.8336/4.8e-02), hsa00020: Citrate cycle (TCA cycle) (0.1693/1.0e+00), and hsa04020: Calcium signaling pathway (01488/2.3-01). In parentheses, the gene set's network-based similarity score/significance overlaps with the pathway measured by a Fisher test (XD-Score/Fischer) (Glaab et al., 2012; Dennert et al., 2008).

The results showed that KEGG canonical genes (206 elements) showed four interactions. In parentheses, the

Table 3. Cross-data genes list compares Tables 1 (KEGG Database Diseases entries and respective pathways of human cardiovascular disease) and 2 (Differential expression genes obtained in cardiac transcriptional works).

Gene	Name	Importance	References
TNN1	*Troponin 1 gene-one cardiac*	COVID-19 feature was high levels of troponin 1. Troponin 1 protein is encoded by a multigene family whose members are expressed differently in several muscles Thus, elevated troponin suggests a myocardial injury, increasing the diagnosis differential in interstitial pneumonia and heart failure	Marian & Roberts (2001), Guo et al. (2020)
ACE2	*Angiotensin Converting Enzyme 2 gene*	Membrane-bound zinc metallopeptidase involved in angiotensin cleavage. ACE2-expressing cells indirectly affect the immune response to SARS-CoV-2 in the myocardium and vessels. ACE2 is highly expressed in the lungs and heart and is localized to macrophages, vascular endothelium, smooth muscle, and myocytes.	Chiodo et al. (2020)
TGFß1 and TP53	*Transforming Growth Factor Beta 1 Tumor* and *Protein P53*	Signaling pathways are involved in increased fibrosis, and activated TP53 signaling was demonstrated in the heart tissue DCM patients with VT	Araya et al. (2006)
TF53	*Tumor Protein P53 gene*	encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate target genes' expression, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or metabolism changes. AKT Pathway, Apoptosis Pathway, MAPK Pathway, and mTOR Pathway (Adams et al., 2000).	
IGF1	*Insulin-Like Growth Factor 1*	This gene is structurally and functionally related to insulin but has a much higher growth-promoting activity. It may be a physiological regulator of [1-14C]-2-deoxy-D-glucose (2DG) transport and glycogen synthesis in osteoblasts. It may also possibly have a role in synapse maturation. It acts as a ligand for IGF1R and initiates the PI3K-AKT/PKB and the Ras-MAPK pathways. It binds to integrins ITGAV: ITGB3 and ITGA6: ITGB4. MAPK1/ERK2 and AKT1	Gibala et al. (2009)
SLC8A1	*Solute Carrier Family 8 Member A1*	It mediates the exchange of Ca (2+) against Na(+) ions across the cell membrane, contributing to the regulation of cytoplasmic Ca(2+) levels and Ca(2+)-dependent cellular processes	Alves et al. (2020)
TFB1 and TFB2	*Transforming Growth Factor Beta 1* and *Insulin-Like Growth Factor 2*	This gene encodes a ligand of the TGF-beta (transforming growth factor-beta) superfamily proteins. This family binds various TGF-beta receptors, leading to the recruitment and activation of SMAD family transcription factors. The mature peptide may also form heterodimers with other TGFB family members.	Dobaczewski et al. (2011)
TNNA1	*Catenin Alpha 1*	The gene encodes a member of the catenin family of proteins, which plays an essential role in cell adhesion by connecting cadherins to the actin filaments inside the cell. The encoded mechanic sensing protein contains three vinculin homology domains and changes in response to cytoskeletal tension	Protonotarios & Tsatsopoulou (2004)

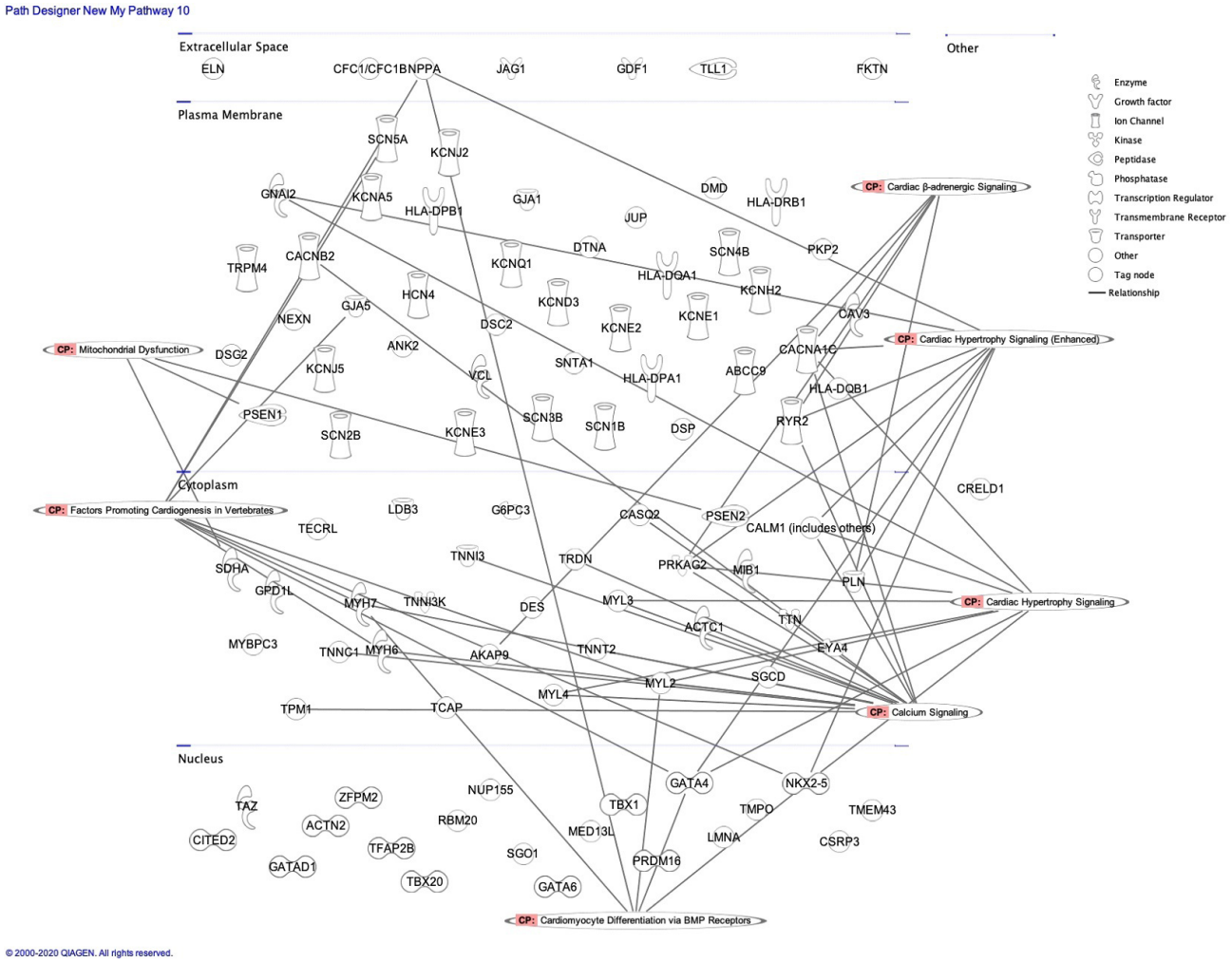


Figure 2. KEGG gene-target compilation of cardiovascular diseases in differential cell compartments. CP: Canonical pathways. The subtitles show the molecular classifications.

gene set's network-based similarity score/significance overlaps with the pathway measured by a Fisher test (XD-Score/Fischer) (Glaab et al., 2012). Genes not clustered showed dispersed and disconnected patterns. The uploaded dataset is shown in blue, forming the base of the molecular interaction. The subgroups were labeled a- to e- clusters. Overlapping pathway genes are shown in green. The reference pathways described here are presented in the red profile. In the hypertrophic cardiomyopathy KEGG disease pathway (Figure 3A), four subset clusters are well-characterized: a – Calcium signaling, b – AMPK (AMP-activated protein kinase genes) process, c – TGFB (Transforming Growth Factor-Beta) process, and d – Integrins genes. The dilated cardiomyopathy KEGG disease pathway (Figure 3B) does not present the c – TGFB sub-group as well-characterized. Arrhythmogenic cardiomyopathy (Figure 3C) did not show b and c clusters but has the d- Integrins and the new e – cluster: arrhythmogenic Right Ventricular Cardiomyopathy, specific for 11 specific gene sets. The 3C interactome revealed these three subsets but did not show the c – TGFB as a well-distinguished subset.

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular disease. HCM is a highly complex and heterogeneous disease concerning the number of associated mutations, severity of the phenotype, symptom burden, and the risk of complications such as heart failure and sudden death (Ho, 2012). Calcium signaling and the AMP-activated protein kinase (AMPK) signaling networks (a – and b – clusters) broadly regulate numerous aspects of cell biology. There is significant overlap in the downstream consequences of calcium and AMPK signaling (Dunn & Munger, 2020). Endogenous TGFB (c – cluster) is one of the most pleiotropic and multifunctional peptides. It plays an essential role in the pathogenesis of cardiac fibrotic and hypertrophic remodeling and in a wide variety of biological processes, immune, and inflammatory responses (Dobaczewski et al., 2011). Integrins (d – cluster) functions as cell surface receptors for the profibrotic TGF-β activation (Nishimura, 2009) and the homeostasis of the pulmonary epithelial-mesenchymal trophic unit (Araya et al., 2006). Arrhythmogenic cardiomyopathy (Figure 3C) specific genes: LEF1 (Lymphoid Enhancer Binding Factor 1), CTNNA2 (Catenin Alpha 2), ACTN1 (Actinin Alpha 1), TCF7L1 (Transcription Factor 7 Like 1), TCF7L2 (Transcription Factor 7 Like 2), CDH2 (Cadherin 2), CTNNA1 (Catenin Alpha 1), CTNNA3 (Catenin Alpha 3), CTNNB1 (Catenin Beta 1), TCF7 (Transcription Factor

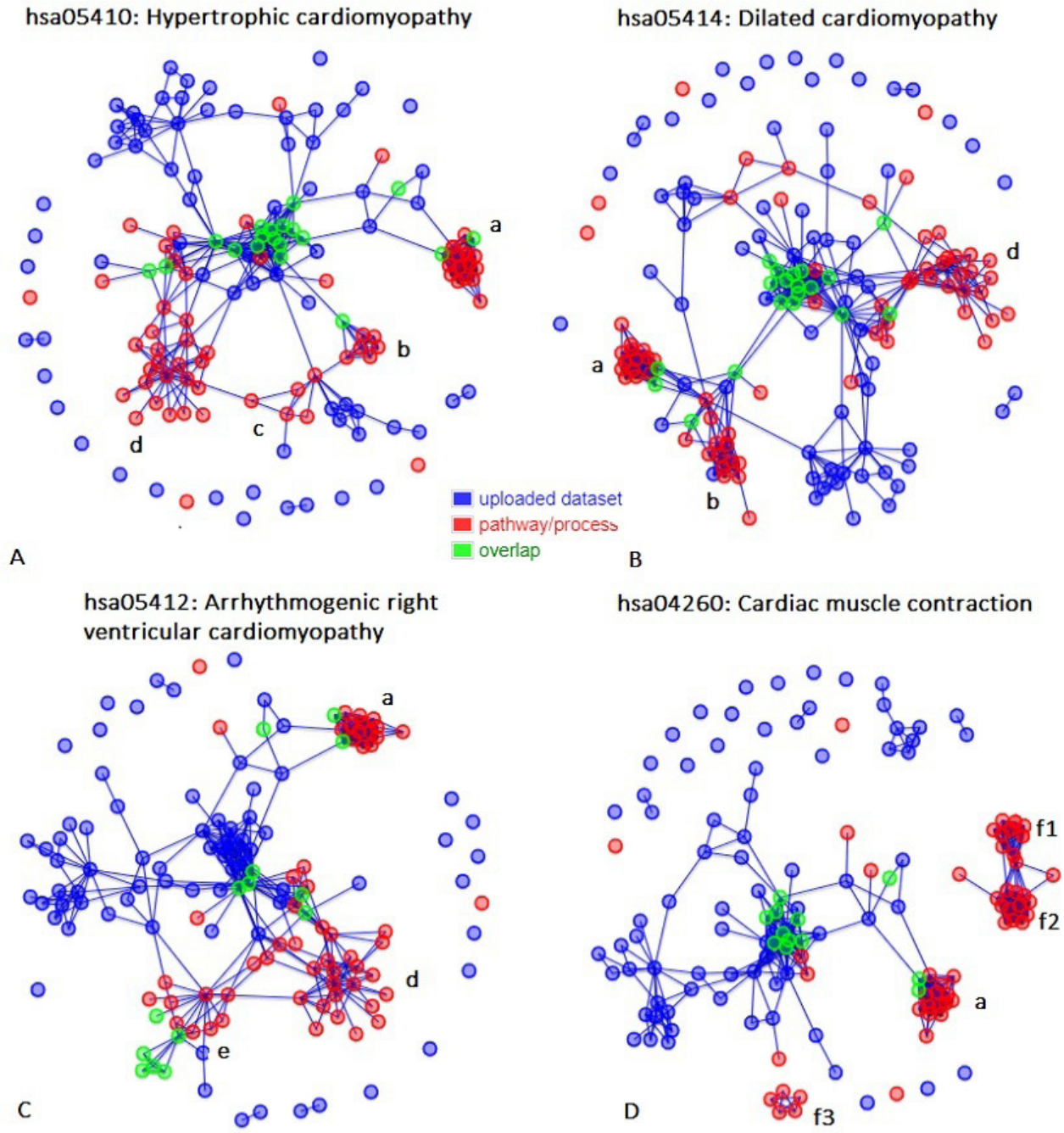


Figure 3. Four main significant KEGG human cardiovascular canonical pathways based on enrichment analysis tools. A - hsa05410: Hypertrophic cardiomyopathy (2.0339/ 2.2e-20), B – hsa05414: Dilated cardiomyopathy (1.8577/6.4e-20), C – hsa05412: Arrhythmogenic right ventricular cardiomyopathy (1.6693/1.2e-13), D – hsa04260: Cardiac muscle contraction (1.5836/2.4e-11). Gene subsets: a – Calcium signaling, b – AMPK process, c – TGFB, d – Integrins, e – ARVC, f – METC, f1 – f3 – METC gene subsets. In the parenthesis, the gene set's network-based similarity score/ significance overlaps between the pathway measured by a Fisher-test (XD-Score/Fischer)(Glaab et al., 2012).

7), LEF1 (Lymphoid Enhancer Binding Factor 1), are essential nuclear mediators of canonical Wnt/β-catenin signaling, which controls cardiac proliferation and differentiation in several stages of cardiac development (Maron et al., 2006).

The Cardiac muscle contraction KEGG Disease pathway (Figure 3D) showed a unique interactome with more than three f- subsets distinguished, belonging to METC – Mitochondrial Electron Transport Chain: 3D – f1: UQCR (Ubiquinol-Cytochrome C Reductase) genes and MT-CYB (Mitochondrially Encoded Cytochrome C Oxidase I); Figure 3D – f2 represents Cytochrome C oxidase (Cytochrome c oxidase – COX) genes, the terminal enzyme of the mitochondrial respiratory chain, and CACNG6 (Calcium Voltage-Gated Channel Auxiliary Subunit Gamma 6) and CACNG7 (Calcium Voltage-Gated Channel Auxiliary Subunit Gamma 7) genes, which regulate the trafficking and gating properties of AMPA-selective glutamate receptors (AMPARs) (Song et al., 2018). 3D – f3: ATPase family of P-type cation transport ATPases, specifically the subfamily of Na+/K+-ATPases, an integral membrane protein responsible for establishing and maintaining the electrochemical gradients of Na and K ions across the plasma membrane (Parvatiyar et al., 2010). The cardiac muscle contraction pathway revealed a unique interactome involving key mitochondrial components, such as UQCR and MT-CYB genes in the electron transport chain, COX genes for respiratory function, and CACNG6/7 for calcium channel regulation. Additionally, Na+/K+-ATPases maintain electrochemical gradients critical for cardiac muscle function.

The cardiovascular gene groups through the KEGG Disease Database revealed six enriched pathways, four of which are explicitly associated with cardiac function and important for assessing cardiovascular health. These pathways, such as HCM, DCM, ARVC, and cardiac muscle contraction, are important in causing heart disease and functional impairment, especially during stressful situations like viral infections. The findings emphasize how the cardiovascular system is vulnerable to malfunction when exposed to viral pathogens such as SARS-CoV-2, causing cardiomyopathies and associated complications. In addition, two other important pathways were discovered: allograft rejection and viral myocarditis. Although not directly linked to primary heart-related issues, these pathways indicate immune-triggered reactions and inflammation that may worsen cardiovascular problems in COVID-19 patients. It is noteworthy that viral-induced cardiac damage is highly relevant in the case of SARS-CoV-2 infection due to viral myocarditis (hsa05416). However, less important pathways like Type I diabetes mellitus, the citrate cycle (TCA cycle), and calcium signaling indicate metabolic processes that, although crucial for general well-being, showed decreased significance in this cardiovascular-centered study. These results highlight the importance of focusing on cardiac pathways when developing treatment strategies. The gene enrichment overlap from the enrichment is shown in Figure 4, illustrating the pathway proximity. There is significantly more proximity for AxB, and that of CxD is more distant from the first three pathways, with 32 specific pathway genes. To further explore the interconnections between these pathways, a Venn diagram can illustrate the gene overlap, providing a visual representation of shared and distinct genes among hypertrophic, dilated, arrhythmogenic cardiomyopathies, and cardiac muscle contraction. Identifying these overlapping genes may reveal common molecular targets for therapeutic interventions across various forms of heart disease.

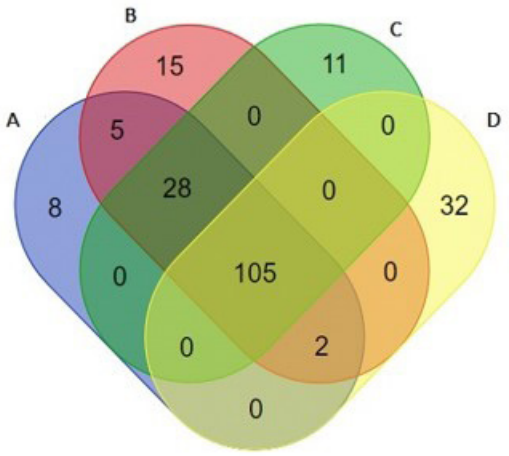
Quantitative genic expression for cardiovascular COVID-19 evaluation

RNAseq provides quantitative data that can be analyzed to define specific gene signatures related to particular tissues, the evolution of the disease, response to therapy, pathogen infection, or other conditions such as placental tissue subjected to insulin and obesity (Lassance et al., 2015), chronic inflammatory skin disease (Coates et al., 2019), pancreatic cancer (Müller et al., 2015), prostate cancer, and chemotherapy (Buttarelli et al., 2019). This method does not require specific probes for genes or species, detects new transcripts, gene fusions, alternative splicing, SNP variants, and indels (small insertions or deletions), and identifies genes with low expression, rare abundance, or small fold changes (regulated “up” or “down” genes). It currently requires expertise in laboratory medicine but does not require being a developer. Techniques based on omics are evolving as new practices are incorporated. Therefore, two main points are considered: detecting the transcripts and the subsequent analysis of the results, characterizing the main canonical metabolic pathways. Several proposals for enrichment analysis exist, with the primary objective being to qualify and quantify the genes of interest.

By combining these two powerful techniques, RT-qPCR and RNAseq, in cross-data results, we can present new clinical and biotechnological applications. Results from the pandemic demonstrate that SARS-CoV-2 levels can reach an impressive 2.5 copies/well of viral RNA in some kits (Okamaoto et al., 2020). The advantages obtained from the precision of RT-qPCR, along with its relative ease of use, combined with RNAseq’s effectiveness in covering gene expression and enrichment evaluation, make them highly complementary. RNAseq, like other molecular techniques, has both advantages and disadvantages. It encompasses the detection of various molecules, new transcripts, and their expression capacity. None of these techniques can replace the other; both are effectively complementary. In this work, we cross-reference the extensive resources of RT-qPCR and RNAseq in literature and KEGG Disease Database to provide comprehensive insights into COVID-19-related cardiovascular DEGs and gene relations. RT-qPCR offers high precision in quantifying specific RNA molecules, making it ideal for validating and quantifying known transcripts. At the same time, RNAseq allows for the discovery of novel transcripts and a broader analysis of gene expression patterns across the genome.

The cross-data from genes obtained in cardiac transcriptional studies found in COVID-19 related gene sets are shown in Table 4.

This list focuses on the genome-wide literature and transcriptional sets of genes in the Enrichr libraries. ACE2, SERPINA3 (Serpin Peptidase Inhibitor, Clade A Alpha-1 Antiproteinase), ZFP36 (Ring Finger Protein), SFRP1 (Secreted Frizzled-Related Protein 1), RPL41 (Ribosomal Protein L41), CEBPD (CCAAT Enhancer Binding Protein Delta), CTNNB1, PTX3 (Pentraxin 3), and TP53 (Tumor Protein P53) genes form a group that shows a notable increase in activity in human



Names	total	elements
A B C D	105	TMPO PRDM16 CACNA2D4 KCNJ2 NPPA VCL CACNA1C MYH6 MYL4 CACNA1D KCNJ5 LMNA MYBPC3 NUP155 ZFPM2 LDB3 CASQ2 CITED2 CACNG3 CACNG6 NKX2-5 PSEN1 JUP TTN CACNA2D1 SGOL1 AKAP9 CACNA1F CACNG7 SCN2B KCNH2 HLA-DRB1 KCNE1 PSEN2 DSP SCN1B CAV3 PRKAG2 ANK2 KCND3 TNNI3 MYL2 TCAP ATP2A2 HCN4 MED13L KCNE3 ELN ACTC1 DES CALM1 CACNB4 CACNA1S TNNI3K GJA1 TAZ CACNB1 GNAI2 KCNE2 SCN4B CALM2 CACNG1 DTNA DSG2 GPD1L DMD JAG1 TFAP2B CRELD1 MYH7 TNNT2 RYR2 PKP2 ACTN2 MIB1 DSC2 GJA5 SLC8A1 PLN TNNC1 CACNG4 HLA-DQA1 FKTN TRDN EYA4 SCN5A HLA-DQB1 SDHA TBX20 KCNA5 GATA4 CACNB3 CACNB2 SNTA1 KCNQ1 CACNG2 MYL3 CACNG8 CSRP3 TPM1 TBX1 GATA6 SCN3B SGCD ABCC9
A B C	28	ACTB ITGA7 ITGA5 EMD ITGB8 LAMA2 ITGA11 ITGA2 ITGB1 SGCB ITGB6 SGCG DAG1 ACTG1 ITGA2B ITGAV ITGA9 ITGA1 ITGA3 ITGA8 ITGB5 ITGB4 ITGB3 ITGA6 SGCA ITGB7 ITGA4 ITGA10
A B D	2	TPM2 TPM4
A B	5	IGF1 TGFB2 TNF TGFB1 TGFB3
A	8	ACE PRKAA2 PRKAG3 PRKAB2 PRKAA1 IL6 PRKAB1 PRKAG1
B	15	ADCY7 PRKACB ADRB1 ADCY3 ADCY8 ADCY5 ADCY9 ADCY4 ADCY2 PRKACA PRKACG ADCY6 PRKX GNAS ADCY1
C	11	ACTN4 LEF1 CTNNA2 ACTN1 TCF7L1 TCF7L2 CDH2 CTNNA1 CTNNA3 CTNNB1 TCF7
D	32	FXYD2 ATP1A4 COX7A2L COX5A UQCR11 UQCRQ ATP1A3 COX8A COX7C UQCRFS1 UQCRC1 COX6C COX4I2 UQCRB COX7A2 MT-CO2 SLC9A1 ATP1A2 CYC1 COX6B1 UQCR10 MT-CYB UQCRH MT-CO3 COX7B UQCRC2 MT-CO1 COX5B ATP1A1 COX4I1 ATP1B1 COX6A1

List names	number of elements	number of unique elements
A	148	148
B	155	155
C	144	144
D	139	139
Overall number of unique elements		206

Figure 4. Venn Diagram of genes overlap. A - hsa05410: Hypertrophic cardiomyopathy, B – hsa05414: Dilated cardiomyopathy, C – hsa05412: Arrhythmogenic right ventricular cardiomyopathy, D – hsa04260: Cardiac muscle contraction. List of gene names.

Table 4. Cross-data from genes obtained in cardiac transcriptional works founded in COVID-19 Related Gene Sets.

COVID-19 Related Gene Sets 2021	Genes of Table 2	p-Value*
500 genes up-regulated by SARS-CoV-2 in human Lung Organoid cells at 24 hpi from GSE148697	ACE2 (*Angiotensin-Converting Enzyme 2*), SERPINA3 (*Serpin Peptidase Inhibitor, Clade A (Alpha-1 Antiproteinase*), ZFP36 (*Ring Finger Protein*), SFRP1 (*Secreted Frizzled Related Protein 1*), RPL41 (*Ribosomal Protein L41*), CEBPD (*CCAAT Enhancer Binding Protein Delta*), CTNNB1 (*Catenin Beta 1*), PTX3 (*Pentraxin 3*), TP53 (*Tumor Protein P53*)	0.0004235
Top 500 upregulated genes for SARS-CoV-2 infection in human lung organoids from GSE148697	ACE2, SERPINA3, ZFP36, SFRP1, RPL41, CEBPD, CTNNB1, PTX3, TP53	0.0004235
SARS Perturbation 76 Down Genes from GEN3VA Mouse Lung; Accession: GSE68820 Platform: GPL7202 Entry 1	CKMT2 (*Creatine Kinase, Mitochondrial 2*), FABP3 (*Fatty Acid Binding Protein 3*), NPPA (*Natriuretic Peptide A*), S100A9 (*S100 Calcium Binding Protein A9*)	0.0004855
SARS coronavirus P2 envelope protein from Virus-Host PPI P-HIPSTer 2020	CTNNB1, BRD4	0.002040
SARS coronavirus protein E (gene: E) from Virus-Host PPI P-HIPSTer 2020	CTNNB1, BRD4	0.002040
Top 500 down genes for SARS-CoV-2 infection in Mesocricetus auratus hamster lung Day 14 from GSE162208	CKMT2, CPT1A, ACADVL (*Acyl-CoA Dehydrogenase Very Long Chain*) FABP3, SLN (*Sarcolipin*), PTX3, CPT1B (*Carnitine Palmitoyltransferase 1B*) SNX3 (*Sorting Nexin 3*)	0.002440
500 genes down-regulated by SARS-CoV-2 in human Calu3 cells at 4h from GSE148729 mock totalRNA	*TIMM8B* (*Translocase Of Inner Mitochondrial Membrane 8 Homolog B*), EID1 (*EP300 Interacting Inhibitor Of Differentiation 1*), ACE2, RPL41, MRP521 (*Mitochondrial Ribosomal Protein S21*), ABRACL (*ABRA C-Terminal Like*) IKBKG (*Inhibitor of Nuclear Factor Kappa B Kinase Regulatory Subunit Gamma*)	0.002509

COVID-19 Related Gene Sets 2021	Genes of Table 2	p-Value*

Table 4. Continued...

COVID-19 Related Gene Sets 2021	Genes of Table 2	p-Value*
Top 500 up genes for SARS-CoV-2 infection Day 21 in ferret right cranial lung from GSE160824	SNX3 (*Sorting Nexin 3)* TIMM8B (*Translocase Of Inner Mitochondrial Membrane 8 Homolog B*) FABP3, CTNNB1, ACADM (*Acyl-CoA Dehydrogenase Medium Chain*), ABAD17C (*Abhydrolase Domain Containing 17C, Depalmitoylase*) MRPL34 (*Mitochondrial Ribosomal Protein L34*)	0.003246
500 genes down-regulated by SARS-CoV-2 in human liver organoids from GSE151803	ESRRA (*Estrogen Related Receptor Alpha*), CEBPA (*CCAAT Enhancer Binding Protein Alpha*) CPT2 (*Carnitine Palmitoyltransferase 2*) RDH14 (*Retinol Dehydrogenase 14*) MRPL34 (*Mitochondrial Ribosomal Protein L34*) RPP25L (*Ribonuclease P/MRP Subunit P25 Like*) HDAC6 (*Histone Deacetylase 6*)	0.004243
499 genes down-regulated by SARS-CoV-2 in Calu-3 cells from GSE148729	SNX3 (*Sorting Nexin 3*) EID1 (*EP300 Interacting Inhibitor Of Differentiation 1*) RBPL41 (*Ribosomal Protein L41*) MRPL50 (*Mitochondrial Ribosomal Protein L50*) MRPS21 (*Mitochondrial Ribosomal Protein S21*) ABRACL (*ABRA C-Terminal Like)*	0.005391

*Independent probability of any gene belongs to the gene set. This assumes a binomial distribution (Fisher Test).

lung organoid cells following infection with SARS-CoV-2 for 24 hours. ACE2 is recognized as the viral receptor, while TP53 is a well-known gene that helps prevent tumors, showing a robust cellular response to viral invasion (Chiodo et al., 2020). CKMT2 (Creatine Kinase, Mitochondrial 2), FABP3 (Fatty Acid Binding Protein 3), NPPA (Natriuretic Peptide A), and S100A9 (S100 Calcium Binding Protein A9) are genes downregulated in response to SARS perturbation in a mouse lung model, reflecting how gene expression in mouse models can mirror the response seen in human cases (Bos et al., 2020). CTNNB1 (Catenin Beta 1) and BRD (Bromodomain Containing) genes are related to the interaction of the SARS-CoV-2 P2 envelope protein, highlighting viral-host protein-protein interactions. CTNNB1 is involved in cellular signaling, while BRD4 is associated with transcription regulation, both of which may be important in the virus's hijacking of the host cell machinery (Haywood et al., 2020). CKMT2, CPT1A (Carnitine Palmitoyltransferase 1A), ACADVL (Acyl-CoA Dehydrogenase Very Long Chain), FABP3, SLN (Sarcolipin), PTX3, and CPT1B (Carnitine Palmitoyltransferase 1B) refer to genes downregulated in hamster lungs after 14 days of SARS-CoV-2 infection. These genes are involved in metabolic processes (CPT1A, CPT1B) and immune response (PTX3), indicating metabolic disruption due to the virus (Bos et al., 2020; Chiodo et al., 2020). SNX3 (Sorting Nexin 3), TIMM8B (Translocase of Inner Mitochondrial Membrane 8 Homolog B), EID1 (EP300 Interacting Inhibitor of Differentiation 1), ACE2, RPL41, MRPS21 (Mitochondrial Ribosomal Protein S21), ABRACL (ABRA C-Terminal Like), and IKBKG (Inhibitor of Nuclear Factor Kappa B Kinase Regulatory Subunit Gamma) are significantly downregulated in human lung cells (Calu3) early after infection for four hours. The downregulation of ACE2 here may indicate a protective or defensive response by the cell after the virus binds to it. The second list found in these results includes: SNX3, TIMM8B, FABP3, CTNNB1, ACADM (Acyl-CoA Dehydrogenase Medium Chain), ABAD17C (Abhydrolase Domain Containing 17C, Depalmitoylase), and MRPL34 (Mitochondrial Ribosomal Protein L34). This set describes upregulated genes in ferret lungs infected with SARS-CoV-2 after 21 days. FABP3 and CTNNB1 are involved in metabolism and signaling, highlighting the continued cellular response to long-term infection. The gene list ESRRA (Estrogen Related Receptor Alpha), CEBPA (CCAAT Enhancer Binding Protein Alpha), CPT2 (Carnitine Palmitoyltransferase 2), RDH14 (Retinol Dehydrogenase 14), MRPL34, RPP25L (Ribonuclease P/MRP Subunit P25 Like), and HDAC6 (Histone Deacetylase 6) refers to genes related to metabolic regulation (CPT2, ESRRA) and cellular growth (HDAC6) that are downregulated in human liver organoids, suggesting liver function impairment during SARS-CoV-2 infection. Lastly, SNX3 (Sorting Nexin 3),

EID1, RPL41, MRPL50 (Mitochondrial Ribosomal Protein L50), MRPS21, and ABRAC (ABRA C-Terminal Like) are part of a gene set focusing on the downregulation of ribosomal proteins (RPL41, MRPL50) and others, indicating a suppression of protein synthesis machinery in human lung cells.

Gene suppression in human lung cells (e.g., SNX3 – Sorting Nexin 3, RPL41) and liver organoids (e.g., ESRRA, HDAC6) further demonstrates how the virus impairs protein synthesis and metabolism, impacting organ function and potentially leading to severe clinical outcomes. These molecular changes provide insights into how SARS-CoV-2 disrupts both immune responses and cellular homeostasis across different tissues. Given the significant role of these genes in SARS-CoV-2 infection, a natural next area of inquiry is how COVID-19 infection impacts cardiac health. The interplay between genes like TP53 and PTX3, both involved in cellular defense and immune response, and cardiac conditions is crucial. Could their heightened expression during infection contribute to inflammation or cardiovascular complications in COVID-19 patients (Haywood et al., 2020). Furthermore, metabolic genes such as CPT1A, CPT1B, and ACADVL, which are downregulated in response to the virus, are critical for energy production in the heart (Spitler et al., 2017). How might their suppression lead to energy deficits in cardiac cells, potentially contributing to heart failure or arrhythmias? BRD4 and CTNNB1, key players in transcriptional regulation, could also be involved in the remodeling processes within heart tissues post-infection (Haywood et al., 2020). Is there a connection between these molecular disruptions and the increased incidence of myocarditis or long-term cardiac issues observed in post-COVID patients. Moreover, with the observation that ACE2 serves as the viral entry point, and given its central role in regulating blood pressure and heart function, how does its upregulation during COVID-19 infection impact patients with pre-existing heart conditions (D'Cruz et al., 2020). Could this viral engagement exacerbate conditions like hypertrophic cardiomyopathy or heart failure? These questions highlight the need for further investigation into how SARS-CoV-2 infection interacts with cardiac gene regulation, potentially leading to long-term cardiovascular complications (Marian & Roberts, 2001).

In summary, the group of genes listed in Table 4 reflects distinct responses to SARS-CoV-2 infection in various biological models, emphasizing their roles in viral entry, immune response, and metabolic disruption. In human lung organoid cells, ACE2, the recognized receptor for SARS-CoV-2, and TP53, a key tumor suppressor, show upregulation after 24 hours of infection, indicating a strong cellular defense mechanism. Genes such as SERPINA3, ZFP36, and PTX3 also participate in this heightened response, which likely reflects broader inflammatory or immune activation. In contrast, downregulated genes, like CKMT2 and FABP3, identified in mouse models, and CPT1A and CPT1B in hamster lungs, suggest significant metabolic disruption and changes in energy pathways. These downregulations are consistent with viral perturbation affecting normal cellular function. Additionally, proteins like BRD4 and CTNNB1, involved in transcriptional regulation and cellular signaling, play crucial roles in host-virus interactions, as seen in their interaction with SARS-CoV-2 envelope proteins (Wilkins & Molkentin, 2004; Haywood et al., 2020).

The integration of data from KEGG disease entries and differential expression studies provides a comprehensive view of the molecular underpinnings of cardiovascular diseases. The involvement of these genes in critical pathways associated with cellular stress, apoptosis, fibrosis, and metabolic regulation suggests that these processes are central to the pathogenesis of cardiac conditions. Understanding these pathways could lead to more targeted therapies that address specific molecular mechanisms involved in cardiovascular disease progression. For instance, targeting TGF-beta signaling could help manage fibrosis in heart failure and could be relevant in treating COVID-19-related cardiovascular complications.

Conclusion and prospects

The advent of the cardiac 'omics approach has led to considerable progress in understanding the mechanisms of phenotype relationships and their interaction with COVID-19 infection. The interaction between transcriptome tools and RT-qPCR research could serve as the basic process for applying gene clusters and conducting qualitative and quantitative evaluations of COVID-19 disease and cardiovascular damage at different stages. Genes obtained from the KEGG human database were used to illustrate the possibilities for enriching gene sets for the quantitative evaluation of cardiac COVID-19 infection or effects. Critical genes such as TNN1, ACE2, and TP53 play roles in myocardial injury, immune response, and cellular regulation. Understanding these gene interactions offers valuable insights into managing COVID-19-related cardiac diseases and potential therapeutic targets. The presence of specific groups of genes with links between gene clusters, gene overlaps and processes, and subsets of compartmentalization highlights various possibilities of metabolic pathways and gene targets for cardiac damage both pre- and post-COVID-19 infection. These cross-data genes play essential roles in various biological processes, including inflammation, cardiac function, and immune response, all of which are particularly relevant during viral infections.

Conflict of interests

The authors reported no potential conflict of interest.

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