Systems biology approaches investigating mitochondrial dysfunction in cyanotic heart disease: a systematic review

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eBioMedicine
2025;118: 105839
Published Online xxx
https://doi.org/10.1016/j.ebiom.2025.105839

Summary

Background Cyanotic congenital heart disease (CCHD) affects over 3 million individuals globally and can progress to heart failure. Mitochondrial dysfunction is well established in adult heart failure and is also a central feature of CCHD. CCHD cyanosis itself contributes to further mitochondrial dysfunction. Systems biology methods detail the epigenomic, transcriptomic, and metabolomic profile of biological samples. This systematic review highlights CCHD systems biology literature related to mitochondrial dysfunction.

Methods OVID/Medline was searched between January 2010 and June 2025. Studies implementing untargeted systems biology methods in CCHD tissue or plasma were included. Genes with differential expression between CCHD and unaffected controls were pooled and analysed using GO term functional enrichment for pathway analysis, transcription factor and kinase enrichment, and metabolic pathways.

Findings From 31 included studies (genomic: n = 5, epigenomic: n = 3, transcriptomic: n = 23, proteomic: n = 2, metabolomic: n = 3, lipidomic: n = 1), we identified 8 pathogenic/likely pathogenic single nucleotide polymorphisms, 73 differentially methylated genes, 4170 differentially expressed genes, 173 differentially expressed proteins between CCHD versus unaffected controls. Several genes involved in mitochondrial respiratory chain (NDUFV1, NDUFV2, NDUFA5, NDUFS3, COX5A, COQ7) were identified.

Interpretation CCHD pathogenesis and progression are associated with mitochondrial dysfunction through changes in metabolism, fission, and fusion.

Funding Vanier CIHR Scholarship, UHN Research Studentship, and Ontario Graduate Scholarship. Translational Biology and Engineering Program seed operating funds and research funding from the Heart and Stroke Foundation of Canada.

Keywords: Systems biology; Mitochondria; Cyanotic congenital heart disease; Multi-omics; Bioinformatics

Introduction

Cyanotic congenital heart diseases (CCHDs) are anatomic malformations (obstructive or mixing lesions) that expose patients both to the risk of cyanosis (arterial oxygen desaturation) as well as the functional consequences of heart failure. Obstructive lesions impede intracardiac blood flow, commonly consisting of: Tetralogy of Fallot (TOF), pulmonary valve stenosis or

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Research in context

Evidence before this study

Mitochondrial dysfunction has been widely implicated in adult heart failure and congenital heart disease. In adult heart failure, studies have demonstrated impaired oxidative phosphorylation and increased reactive oxygen species generation contribute to disease pathogenesis and progression. Congenital heart diseases, particularly cyanotic CHDs characterised by chronic hypoxia, experience bidirectional relationships with mitochondrial dysfunction: CCHD heart failure is exacerbated by mitochondrial dysfunction, while chronic hypoxia, itself, contributes to worsening of mitochondrial function.

Emerging therapies targeting mitochondrial reactive oxygen species and metabolism exist for adult heart failure, and integrative systems biology methods have been increasingly used for the identification of new prognostic and therapeutic targets. While systems biology has been applied previously to adult heart failure and ischaemic heart disease, applications to CCHD, specifically to examine mitochondrial dysfunction, has been limited.

Added value of this study

This systematic review comprehensively synthesises findings from multiple systems biology methods investigating mitochondrial dysfunction in CCHD. From analysing 31 studies reporting genomic, epigenomic, transcriptomic, proteomic, metabolomic, and lipidomic analyses in humans and animal models, we were able to integrate alterations to key mitochondrial components and processes in CCHD. Our analyses revealed several conserved mitochondrial differentially expressed genes across multiple omics platforms, including genes involved in the electron transport chain for mitochondrial respiration and mitochondrial dynamics, as well as changes to metabolic pathways for amino acid metabolism and fatty acid oxidation. Furthermore, we highlight the transcription factors HIF-1α and E2F1 as potentially implicated in mitochondrial adaptations to chronic cyanotic states seen in CCHD.

Implications of all the available evidence

Our review demonstrates that cyanotic congenital heart diseases likely exhibit changes to mitochondrial-associated genes and biological processes, particularly those involved with mitochondrial respiration for ATP production. These changes have been associated with disease progression, surgical outcomes, and heart failure risk. Existing pharmacological agents such as sildenafil and pioglitazone which have been shown to modulate mitochondrial function in other conditions may also be beneficial in improving outcomes for patients with CCHD. The findings from our review underscore the need for further mitochondrial research in CCHD and suggest systems biology strategies may be a powerful and efficient method to identify mitochondrial-targeted therapies for CCHD treatment.

atresia, tricuspid atresia, or hypoplastic left heart syndrome (HLHS). Mixing lesions are defined by oxygenated and deoxygenated blood mixing freely (e.g. truncus arteriosus, total anomalous pulmonary venous return, and transposition of great arteries). Among CCHD lesions, pulmonary/tricuspid atresia and HLHS are single-ventricle (SV) lesions, in which a ventricle is underdeveloped or incompletely septated. SV represents the highest risk category with the worst prognosis among all CCHDs, requiring three-step surgical Fontan track palliation. Regardless of type or severity, unrepaired CCHD is incompatible with short- or long-term life and can produce variable degrees of cyanosis. Overall, CCHDs comprise 25% of all congenital heart diseases (CHDs) worldwide and represents significant morbidity-mortality burden.[1]

Mitochondrial dysfunction in heart disease

Mitochondrial dysfunction is well-established in adult heart failure, with many emerging therapies targeting reactive oxygen species (ROS) stress pathways and metabolism.[2] Within the CHD population, mitochondrial dysfunction is also a central feature of heart disease.[3–7] Uniquely in CCHD, cyanosis itself further potentiates mitochondrial dysfunction. Thus, mitochondrial dysfunction likely plays a central role in CCHD pathophysiology.[5] Medical therapies for CCHD are extremely limited, and accurate prognostication of complex lesions remains challenging. There is a clear need to elucidate pathways (especially mitochondrial) and potential pharmacological targets impacted in CCHD pathogenesis, progression, and prognosis.

Systems biology in cardiovascular disease prognosis and therapeutics

Systems biology has enabled large-scale identification of prognostic and therapeutic targets through multiple 'omics' techniques to comprehensively profile biological samples.[8,9] Typical techniques involve nucleotide sequencing, microarray analysis, or mass spectrometry. Multiomic approaches are particularly ideal for polygenic conditions or those with complex genetic-environmental interplay, such as congenital heart disease (CHD).[10] Typically, candidate biomarkers identified by systems biology require orthogonal validation using additional targeted in vitro or in vivo experiments. Cardiovascular systems biology research focuses on adult heart failure, hypertrophic cardiomyopathy, aortopathy, and atherosclerosis.[8,9,11] Nevertheless, CHD systems biology data continue to emerge, suggesting differential gene expression and mRNA splicing,[12] altered regulation of inflammatory and metabolic

pathways,[13] and reduced mitochondrial function,[4] offering insights into complex cellular alterations occurring therein. This systematic review highlights CCHD systems biology literature with findings related to mitochondrial dysfunction.

Methods

Search strategy

OVID version of MEDLINE was searched systematically for studies relating to CCHD published between January 2010 and June 2025. We used Medical Subject Headings (MeSH) and keyword searches for congenital heart defects, mitochondria, mitochondrial dynamics, multiomics, gene ontology, metabolome, transcriptome, microRNAs, DNA methylation and transcription factors (full search syntax in Supplement). The protocol for this systematic review was not registered due to the methodological challenges specific to integrating basic science studies utilising systems biology approaches into a structured review framework. Specifically, the exploratory nature of this review synthesised data from multiple omics methods for mechanistic insight rather than assessing predefined clinical outcomes. We ensured transparency by clearly outlining our methods and decision-making process within the manuscript.

Selection criteria

Studies were screened by two independent reviewers and discordances were resolved by a third independent reviewer, tabulating information against inclusion/exclusion criteria. The search returned 2448 results; case reports, duplicates, and studies that did not contain "mitochondria" or "metabolism" or "congenital" were excluded. 1675 studies remained, of which 1635 were English and 40 were non-English (31 Chinese, 4 Spanish, 3 French, 1 Russian). Papers were screened for title and abstract based on a pre-defined inclusion and exclusion criteria, using Google Translate for non-English papers. Studies were included if at least one systems biology analysis (epigenetic, genomic, transcriptomic, proteomic, metabolomic, or lipidomic) was conducted in humans, animals, and/or in vitro using cyanotic heart tissue, plasma, or urine. Studies that focused on validation or follow up experiments were also included if the experimental methods were untargeted. Studies that combined cyanotic and noncyanotic subgroups were also included for full-text analyses. Studies were excluded if they used targeted analyses only, focused on non-cyanotic lesions, or did not report mitochondrial findings. Because no study directly measured arterial saturation at the time of analysis, cyanotic heart disease was defined by the type of lesion anatomically, rather than confirmation of clinical cyanosis. After applying the inclusion and exclusion criteria for title and abstract review, 61 English studies and 9 non-English studies were selected for full-text review (Fig. 1). 6 non-English studies were not retrieved as full-text access was unavailable through our institution's library system or open-access sources. Attempts were made to obtain these articles through interlibrary loan but were unsuccessful, thus articles were excluded. A total of 31 studies were included in the final analysis, all of which are summarised in Supplementary Table S1.

Data extraction

Data extraction was completed by two independent reviewers. Included studies were reviewed to collate the following information from the main text and Supplementary files: systems biology analytical technique, biological specimen type analysed, use of human or animal sample sources, pathological group investigated, and sample number. Where reported, information on sex/gender of study participants was collected (Supplementary Tables S2–S8). For genomics studies, ethnicity information was also collected. Key findings were summarised from each study including differentially expressed genes, proteins, metabolites, lipids, and epigenetic changes, with p-values reported. Markers with differential expression between cyanotic cases and unaffected controls reported from genomic, epigenomic, transcriptomic, and proteomic studies were compiled into a pooled gene list. In cases where a complete list of all differentially expressed genes/proteins/metabolites/epigenetic changes were not available, only those discussed in text and available in Supplementary data files were included in the analysis.

Bias analysis

The quality of studies included in the systematic review was assessed with QUADOMICS criteria,[14] an adaptation of QUADAS to evaluate quality of studies adapted for omics-based diagnostic research. Studies were assessed by two independent reviewers and discordances resolved with a third independent reviewer.

Quantitative analysis

After filtering out duplicates, the pooled gene list analysed using GO term functional enrichment with PANTHER version 19.0 to determine biological functional consequences of differentially expressed genes (DEGs). Significantly DEGs and differentially expressed proteins were analysed individually, ranking results which passed the Fishers Exact Test, FDR <0.05 threshold by fold enrichment. The differentially expressed genes collected from transcriptomic studies were further analysed using the X2KWeb transcription factor and kinase enrichment algorithm (https://maayanlab.cloud/X2K/) which infers upstream regulators (ie: kinases and transcription factors (TFs)) using X2KWeb, which integrates data on TF-target protein relationships, protein–protein interaction (PPI)

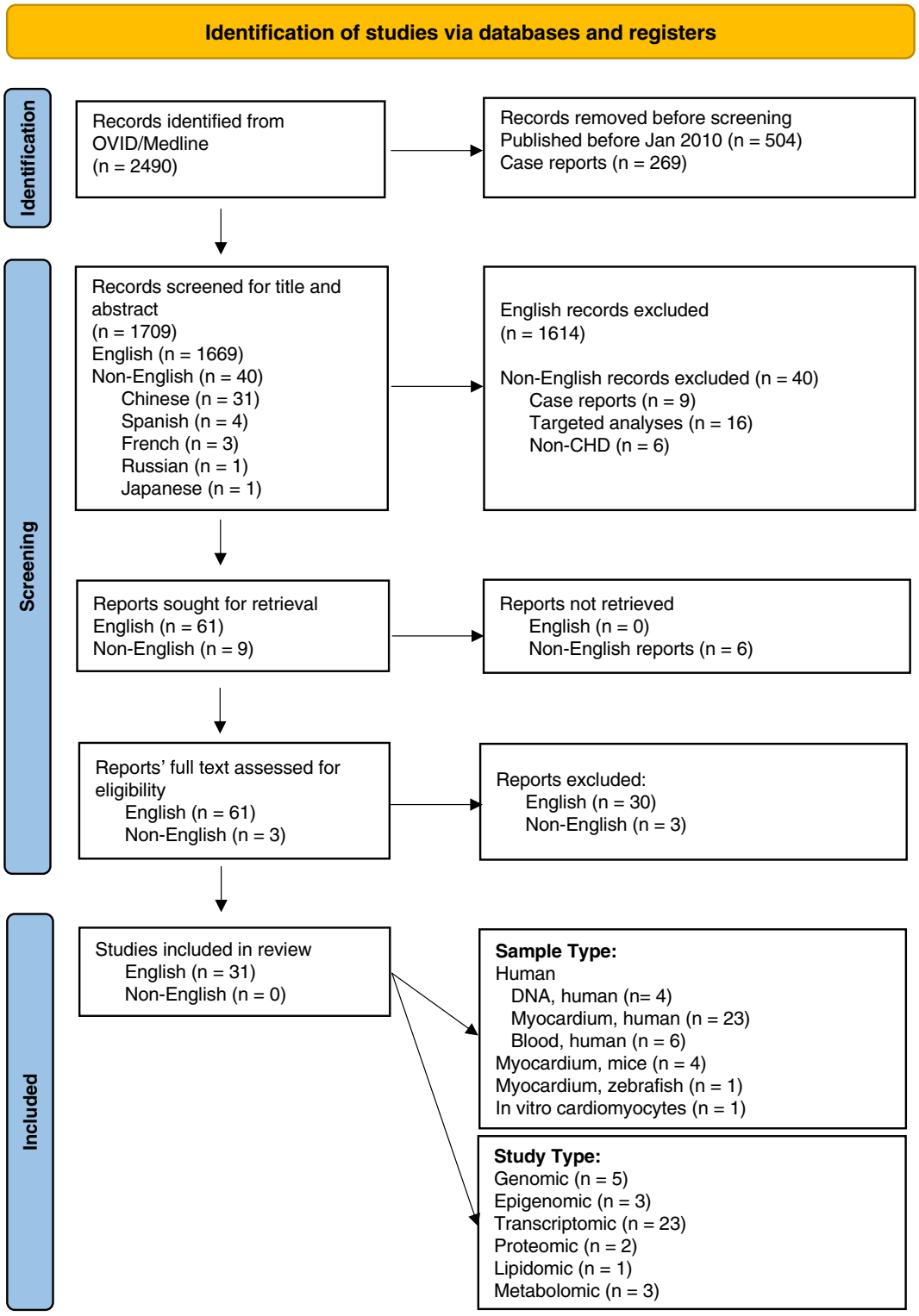

***Fig. 1:* Flow diagram of search strategy. From OVID/Medline,** 2448 records were identified and 1675 records were screened for title and abstract. Of these, 61 records were assessed with full text for eligibility, and a total of 31 studies were included in this review.

networks, and kinase-substrates.[15] Data were analysed and retrieved from X2KWeb using the default TF and kinase enrichment algorithm. Metabolomics and lipidomics data were analysed using MetaboAnalyst version 6.0. Metabolite and lipid set size was determined based on fold-enrichment and two metabolite sets are connected by an edge if the number of their shared metabolites was over 25% of combined metabolite sets.

Statistics

Statistical tests were conducted automatically by PANTHER version 19.0 and X2KWeb databases version 1.0 using Fisher's Exact Test with False Discovery Rate (FDR) correction. Fisher's Exact Test is a common statistical test used in bioinformatics, particularly useful when dealing with small sample sizes where expected counts might be low. It identifies cellular components or pathways which may be over-represented in a gene set compared to a reference set. Statistical significance was determined at FDR $p < 0.05$ with Benjamini correction.

Gene ontology analysis of Cellular Components (CC)

The gene list from genomics, epigenomics, transcriptomics, and proteomics was then segregated by species and submitted into PANTHER version 19.0 for gene ontology (GO) cellular component analysis (GO Ontology database https://doi.org/10.5281/zenodo.14083199 Released 2024-11-03; GO cellular component complete), Fisher's Exact Test was used to determine statistical significance, filtering for results with FDR $p < 0.05$. Human DEGs annotated with GO CC "mitochondrion" (GO:0005739; "mitoDEGs") were selected and compared for species conservation against mouse, rat, and zebrafish mitoDEGs. Human mitoDEGs were cross-referenced back to the omics study type from which they were reported (genomics, epigenomics, transcriptomics, proteomics).

Gene ontology analysis of Biological Processes (BP)

Species-segregated gene lists were submitted into PANTHER version 19.0 for GO biological processes analysis (GO Ontology database https://doi.org/10.5281/zenodo.14083199 Released 2024-11-03; PANTHER GO-Slim Biological Process). Fisher's Exact Test was used to determine statistical significance, filtering for results with FDR $p < 0.05$. Post FDR-filtering, results were ranked by fold enrichment.

Ethics

No ethical approval was sought for the conduct of this systematic review and meta-analysis because no primary data collection involving human or animal subjects took place. All data and analyses presented in this study are based on previously published peer-reviewed research.

Role of funders

All authors declare that funding sources were not involved in study design; collection, analysis, and interpretation of the data; in writing of the report; and in the decision to submit the paper for publication.

Results

Description of included studies

Of the 31 included studies in Fig. 1, full study details are summarised in Supplementary Tables S1–S8. The majority employed a single systems biology method: 74%, n = 23 transcriptomic, metabolomic: n = 3, epigenomic: n = 3, proteomic: n = 2, genomic: n = 5, lipidomic: n = 1. Only three were multiomic.[4,16,17] Most investigated human samples (myocardium: 74%, n = 23; DNA: 13% n = 4; blood: 16%, n = 5). One study investigated a heterogeneous cohort of CHD including non-cyanotic CHD; however nearly 400 patients in the cohort had cyanotic CHD and thus the study met the inclusion criteria for our analysis.[18]

Bias analysis

The least fulfilled QUADOMICS criteria were around detailed description of procedures and timing of biological sample collection with respect to clinical/physiological factors (criterion 4a; 14 studies fulfilled criteria) and diagnostic/treatment procedures (criterion 4b; 19 studies) (Supplementary Figure S1). Most studies did not clearly state whether results were interpreted with blinding to the reference standard (in the context of our study, the condition of interest; criterion 12). As all studies included in this systematic review were phase 1–2, criterion 2 and 14 evaluating cohorts' representativeness to patients in clinical practice, and interpretation of results were not applicable.

Functional enrichment analysis

We identified 4170 differential genes from 23 transcriptomic studies, 173 from two proteomic studies, 72 differentially methylated genes from three epigenetic studies, and 2273 genes with unique single-nucleotide polymorphisms (SNPs) from five genomic studies. A list of all significant biomarkers with associated significance values can be found in Supplementary Tables S2–S8. Pathway analysis of differential biomarkers across studies revealed several pathways relevant to mitochondrial function (Fig. 2).

Results from the quantitative Functional Enrichment Analysis (FEA) are summarised in Fig. 2. In general, metabolic processes appeared to be more enriched in the downstream omic levels (i.e. RNA and protein levels in comparison to the genomic level)

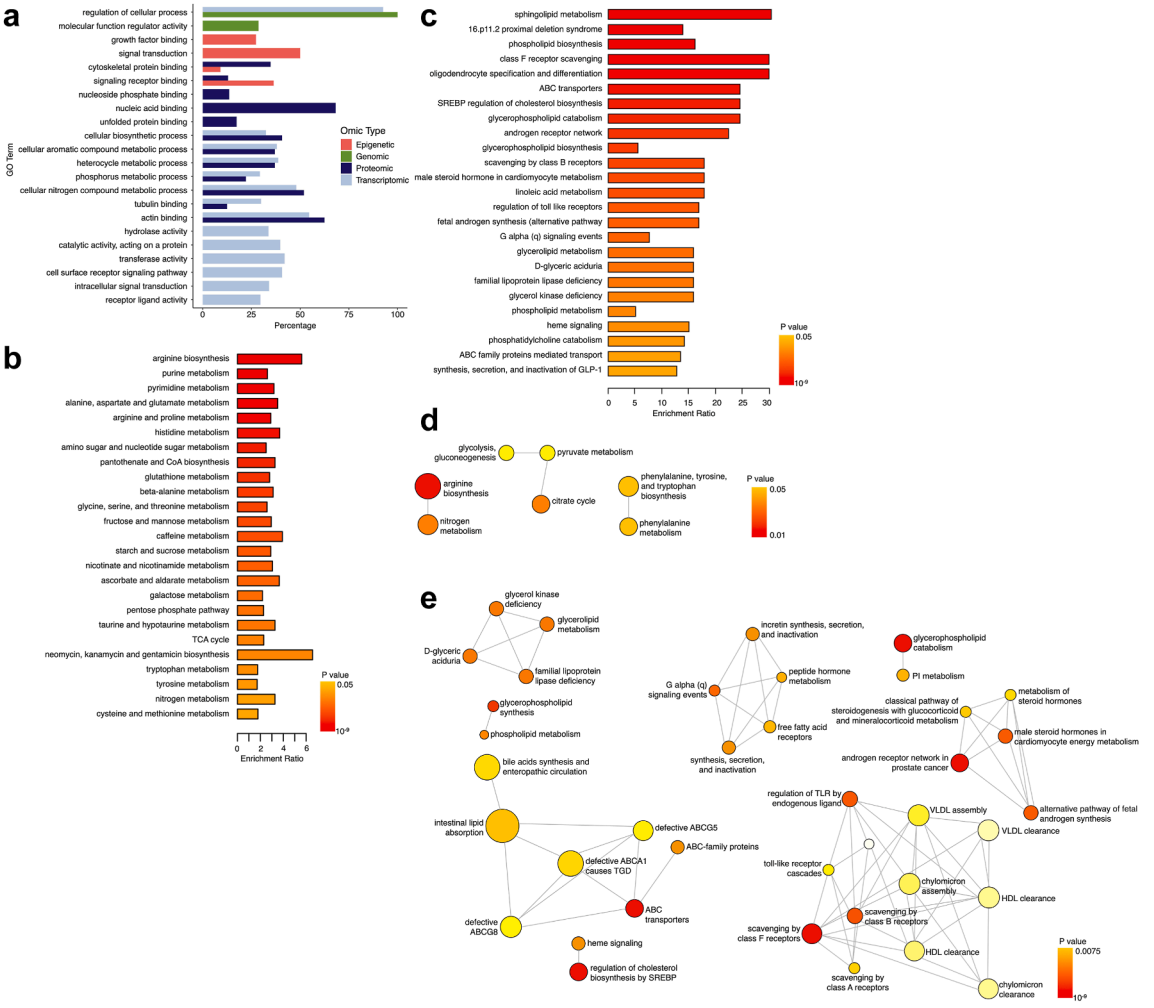

***Fig. 2:* Gene Ontology term analysis by study design.** From pooled gene list (total n = 6515; human DEGs n = 4931, mouse n = 576, rat n = 24, zebrafish n = 982, cell n = 1) (a) Gene Ontology (GO) Term analysis of differentially expressed genes and proteins. GO Terms for biological process and molecular function were included for graphical representation, with percentage of total genes in gene set annotated with each GO term displayed on x-axis. Transcriptomic analysis included 4170 differentially expressed genes, proteomic analysis included 173 differentially expressed proteins, epigenetic analysis included 73 differentially methylated genes, and genomic analysis included eight genes with SNPs determined to affect expression and phenotype. Top 25 (b) Metabolomic and (c) Lipidomic pathways displayed in descending order of significance. Colour represents p-value (Fisher's Exact Test, $p < 0.05$). (d) Metabolite and (e) lipid set analysis, size determined based on fold-enrichment and two metabolite sets are connected by an edge if the number of their shared metabolites was over 25% of combined metabolites. Colour represents p-value (Fisher's Exact test with Benjamini-Hochberg correction for FDR $p < 0.05$).

(Fig. 2a). Enriched molecular function terms represented at transcriptomic/proteomic levels included cellular biosynthetic processes, metabolic pathways in aromatic, heterocyclic, phosphoric, and nitrogenous processing, and enzymatic activities involving catalysis and transferases (Fisher's Exact Test, $p < 0.05$; Fig. 2a). The top enriched metabolomic pathways in CCHD were arginine biosynthesis, purine metabolism, and pyrimidine metabolism (Fig. 2b) and the top lipidomic pathways were sphingolipid metabolism, 16p11.2 proximal deletion syndrome, and phospholipid biosynthesis (Fig. 2c). Enrichment analysis of these metabolite and lipid datasets higlighted processes associated with mitochondrial function and energy production, such as glycolysis/gluconeogenesis, pyruvate metabolism, citrate cycle (Fig. 2d) and glycerolipid metabolism (Fig. 2e), which were enriched in CCHD.

Following FEA, Kinase and Transcription Factor Enrichment analysis was conducted to infer upstream regulators (ie: kinases and TFs) using X2KWeb, which integrates data on TF-target protein relationships, protein interaction networks, and kinase-substrates

(Fig. 3).[15] Several transcription factors associated with mitochondrial pathways were enriched in CCHD (Fisher's Exact Test, $p < 0.05$), including SALL4, PPARG, and E2F1 (Fig. 3).

Cell component analysis

For this, 6515 DEGs compiled from literature were filtered for duplicates; 4931 unique human DEGs, along with 576, 24, 982, and 1 unique DEGs from mice, rat, zebrafish, and cell models, respectively, were entered into PANTHER GO for cell component analysis (Fig. 4A). Identified were 603 human DEGs with GO-CC annotations to the mitochondria (GO:0005739; herein referred to as 'mitoDEGs'), along with 115 mouse mitoDEGs, and 74 zebrafish mitoDEGs (Fig. 4B). From the rat DEGs list, none of the genes annotated for the mitochondrion did not meet the threshold for significance (Fisher's Exact Test, FDR <0.05). There were no human mitoDEGs reported from epigenomic studies. There was considerable overlap in the mitochondrial gene lists between the 603 human mitoDEGs and those identified in animal models—53/115 (46%) mouse mitoDEGs and 25/74 (34%) zebrafish mitoDEGs were also represented the human mitoDEG list. Multiple cell components associated with mitochondrial respiration (Fig. 4C) (e.g. respiratory chain complexes, tricarboxylic acid cycle enzymes, NADH dehydrogenase complex) and mitochondrial function (e.g. mitochondrial ribosomes, mitochondrial intermembrane transporter) were found in the top 25 enriched CC list from humans, mice, and zebrafish. Rat DEGs were enriched for nuclear components but not mitochondrial components.

Biological process analysis

Panther GO analysis for biological processes demonstrated enrichment of mitochondrial respiration processes from the pooled human, human mitoDEG, mouse, and zebrafish gene lists. 15 biological processes were found to be enriched between human, mouse, and zebrafish (Fig. 5A). Within the top 25 most enriched biological processes for human, mice, and zebrafish, there were multiple processes associated with mitochondrial aerobic respiration such as oxidative phosphorylation (GO:006119), and electron transport (GO:0022900) (Fig. 5). No biological processes were significantly enriched (Fisher's Exact Test, FDR < 0.05) from the rat gene list.

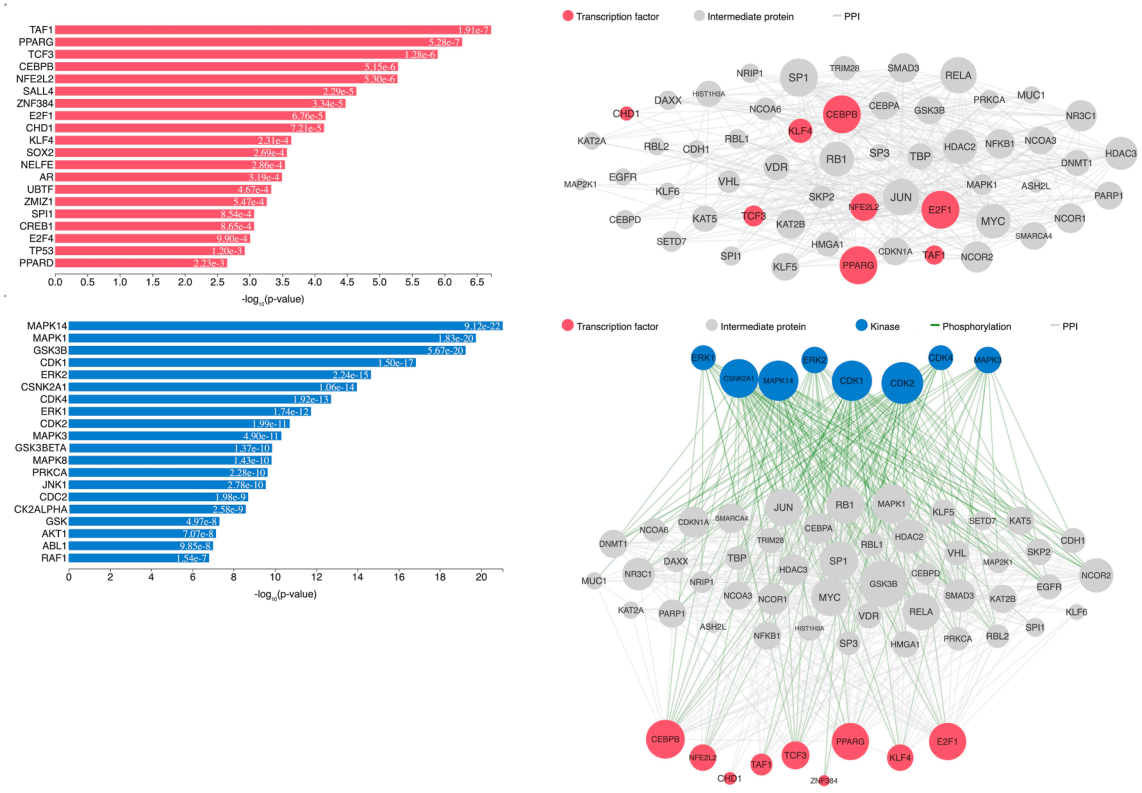

Fig. 3: **Kinase and Transcription Factor Enrichment Analysis.** Inferred enrichment of upstream kinase (blue) and transcription factors (red) from pooled gene list (n = 6515 DEGs) using X2KWeb (Fisher's Exact test, $p < 0.05$). Web diagrams demonstrate TF-intermediate protein relationships, protein–protein interaction (PPI) networks, kinase–substrate interactions, and phosphorylation events (green lines).

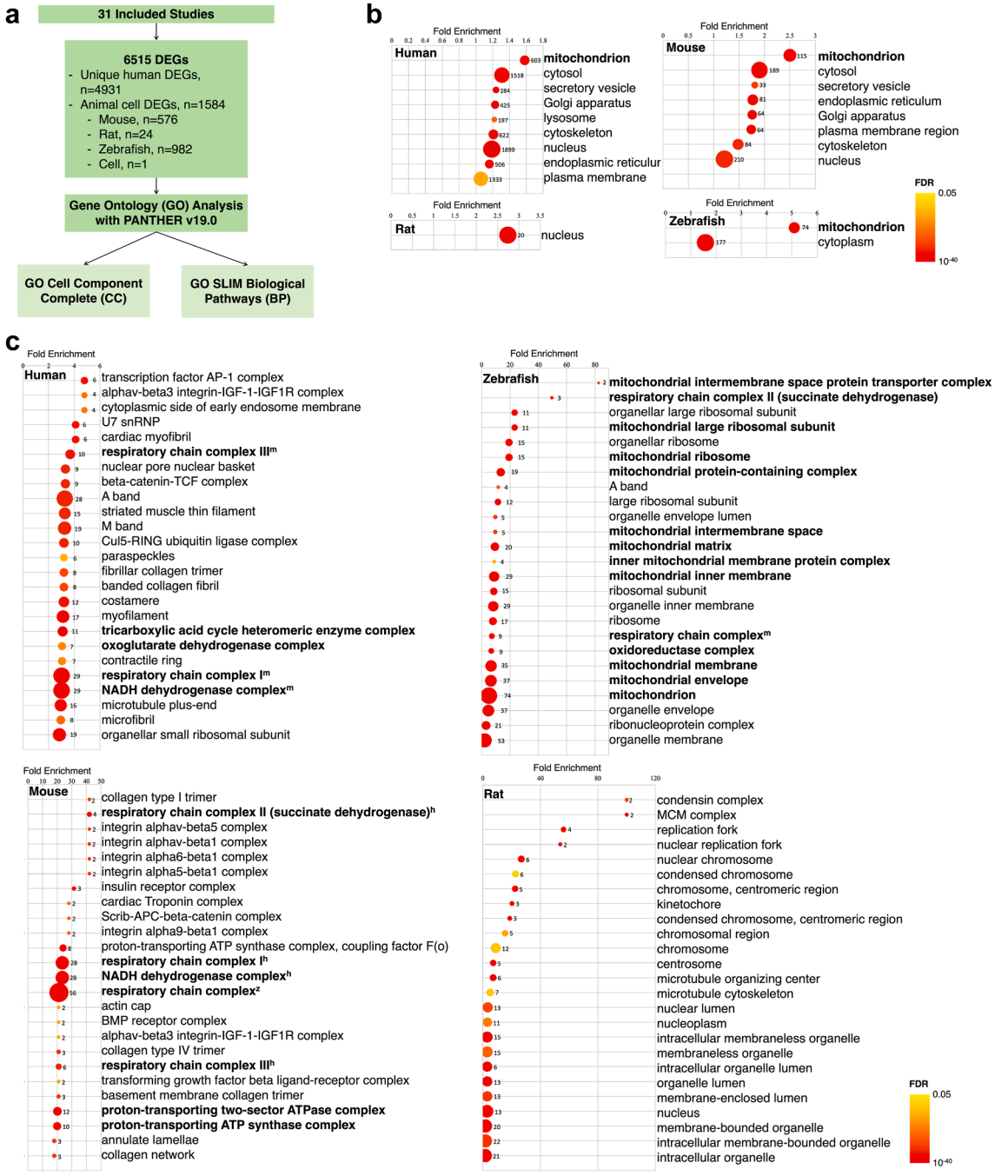

***Fig. 4:* Gene Ontology Analysis of Cellular Components.** (a) Workflow diagram of Gene Ontology (GO) analysis. (b) Selected cell compartments from GO Cellular Components (CC) from human n = 4931, mouse n = 576, rat, n = 24, and zebrafish n = 982 DEG lists. Number of differentially expressed genes (DEGs) within each GO-CC term are reported next to each bubble. Fisher's Exact Test and FDR correction $p < 0.05$. (c) Top 25 enriched GO-CC terms from human n = 4931, mouse n = 576, rat, n = 24, and zebrafish n = 982 DEG lists with Fisher's Exact Test and FDR $p < 0.05$ from human, mouse, rat, and zebrafish gene lists. Bolded CC terms are mitochondrial related. Superscript denotes convergent CC reported in other species (h = human, m = mouse, r = rat, z = zebrafish). NDUFV1 = NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial; NDUFV2 = NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial; NDUFA5 = NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5, NDUFS3 = NADH dehydrogenase [ubiquinone] iron-sulphur protein 3, mitochondrial; COX5A = Cytochrome *c* oxidase subunit 5A, mitochondrial; COQ7 = 5-demethoxyubiquinone hydroxylase, mitochondrial.

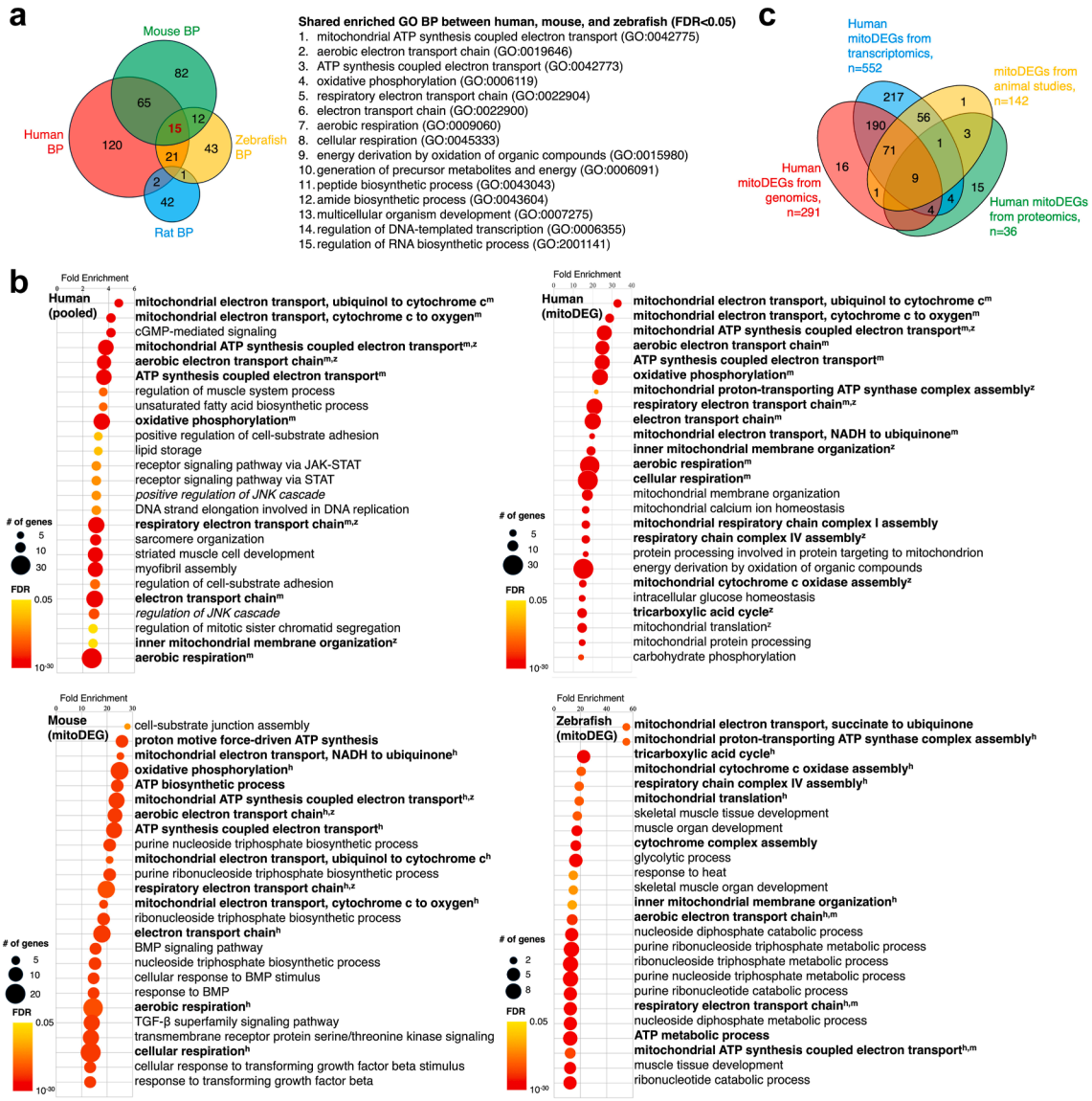

Fig. 5: **Gene Ontology Analysis of Biological Processes**. From human n = 4931, mouse n = 576, rat, n = 24, and zebrafish n = 982 DEGs, (a) Convergent biological processes between human, mouse, zebrafish, and rat. 15 Gene Ontology (GO) Biological Processes (BP) found to be convergent between human, mouse, and zebrafish with Fisher's Exact Test and FDR p < 0.05, depicted in list. (b) Venn diagram of GO-annotated mitochondrial localised differentially expressed genes (mitoDEGs) from human and animal studies. (c) Top 25 enriched biological processes with Fisher's Exact Test and FDR p < 0.05 from pooled human DEGs (n = 4931), GO-CC mitochondrion "mitoDEG" for human (n = 603), mouse (n = 576), and zebrafish (n = 982) gene lists. Bolded BP terms are related to mitochondrial respiration. Italicised BP terms are related to mitochondrial stress. Superscript denotes convergent CC reported in other species (h = human, m = mouse, r = rat, z = zebrafish).

Conservation of DEGs across species and omics methods

There was considerable overlap in enriched cell components and biological processes between species—15 enriched BPs, 8 of which are associated with aerobic respiration, were shared between human, mouse, and zebrafish (Fig. 5a). The 603 human DEGs were associated with mitochondrial cellular components (mitoDEGs); these were reported from genomic, transcriptomic, and proteomic studies (Fig. 5c). There was also conservation of DEGs across omics methods. Nine human mitoDEGs were reported by genomic, transcriptomic, proteomic studies, and animal models: NDUFV1,[19–21] NDUFV2,[19–21] NDUFA5,[6,19–23] NDUFS3,[6,7,19–21,24] COX5A,[7,19–21] COQ7,[12,19,21,25] VDAC1,[19,20,26,27] VDAC2,[19–21,27] VDAC3 [19–21,27] (Table 1). Further analysis of cell component and biological processes demonstrated 6/9 (NDUFV1, NDUFV2, NDUFA5, NDUFS3,

mitoDEG	GO cell components	GO biological process	Genomics	Transcriptomics	Proteomics	Animal model
NDUFV1	Respiratory chain complex I	Mitochondrial electron transport, NADH to ubiquinone	Bartoli-Leonard et al., 2024[19]	Ricci et al., 2012[12] Bartoli-Leonard et al., 2024[19]	Liu et al., 2023[20]	Huang et al., 2022[21]
NDUFV2			Bartoli-Leonard et al., 2024[19]	Bartoli-Leonard et al., 2024[19]	Liu et al., 2023[20]	Huang et al., 2022[21]
NDUFA5			Bartoli-Leonard et al., 2024[19]	Ghorbel et al. 2010[22]; Bartoli-Leonard et al., 2024[19]; Yu et al., 2022[23]	Liu et al., 2023[20]	Huang et al., 2022[21] Hwang et al., 2021[6]
NDUFS3			Bartoli-Leonard et al., 2024[19]	Bartoli-Leonard et al., 2024;[19] He et al., 2017[24]	Liu et al., 2023[20]	Hwang et al., 2021[6] Xu et al., 2021[7] Huang et al., 2022[21]
COX5A	Respiratory chain complex IV	Cytochrome-c oxidase activity	Bartoli-Leonard et al., 2024[19]	Bartoli-Leonard et al., 2024[19]	Liu et al., 2023[20]	Xu et al., 2021[7] Huang et al., 2022[21]
COQ7	Ubiquinone biosynthesis complex	Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen	Bartoli-Leonard et al., 2024[19]	Bartoli-Leonard et al., 2024 Ricci et al., 2012	Xia et al., 2013[25]	Huang et al., 2022[21]
VDAC1	Mitochondrial permeability transition pore complex	Voltage-gated monoatomic ion channel activity	Bartoli-Leonard et al., 2024[19]	You et al., 2020[27] Gu et al., 2013[26] Bartoli-Leonard et al., 2024[19]	Liu et al., 2023[20]	Huang et al., 2022[21]
VDAC2			Bartoli-Leonard et al., 2024[19]	Bartoli-Leonard et al., 2024[19] You et al., 2020[27]	Liu et al., 2023[20]	Huang et al., 2022[21]
VDAC3			Bartoli-Leonard et al., 2024[19]	Bartoli-Leonard et al., 2024[19]	Liu et al., 2023[20]	Huang et al., 2022[21]

Gene Ontology (GO) Cellular Components, GO Biological Processes, and corresponding study references for nine convergent mitochondrial differentially expressed genes (mitoDEGs) reported across multiple omics methods (genomics, transcriptomics, proteomics) and animal models, from Fig. 5c.

Table 1: **Summary of convergent mitochondrial findings from multiomic methods.**

COX5A, COQ7) are key components in the mitochondrial respiratory chain.

Discussion

This review examined mitochondrial dysfunction in CCHD from a systems biology perspective. We selected 31 studies based on our inclusion criteria, with studies using genomics, epigenomics, transcriptomics, proteomics, metabolomics, and lipidomics. Significant findings from studies were pooled and examined for GO, transcription factor, kinase, and metabolite/lipid enrichment.

Altered mitochondrial regulation in cyanotic heart disease

Among the DEGs reported in CCHD in humans, mice, and zebrafish, mitochondrial-located genes showed the highest fold enrichment (Fig. 4b). These mitochondrial-located genes were enriched for components involved in energy metabolism and aerobic respiration. Biological processes such as the tricarboxylic acid cycle, electron transport, and ATP synthesis were among the top ten enriched. Nine DEGs were conserved across multiple omics methods; these were found to all be associated with mitochondrial respiration, including subunits for ETC complex 1 (NDUFV1, NDUFV2, NDUFA5, NDUFS3), complex IV (COX5A), and outer mitochondrial proteins VDAC1, 2, and 3 (Fig. 5b–Table 1).

Several of these mitochondrial genes have been previously implicated in the pathogenesis and progression of cardiomyopathies. Variants in NDUFV1, NDUFV2, NDUFS3 have been associated with Leigh syndrome, a mitochondrial disease with dilated and hypertrophic cardiomyopathy phenotypes.[28–30] Mitochondrial complex 1 dysfunction has also been found to increase vulnerability to cardiomyocyte death and heart

failure during chronic increases in cardiac workload.[31] Furthermore, voltage-dependent anion channel (VDAC) proteins, which regulate mitochondrial membrane permeability and energy metabolism, were also found to be differentially expressed. VDAC2-knockout induced altered mitochondrial calcium homoeostasis with reduced contractility,[32] dilated cardiomyopathy, reduced ejection fraction, and early lethality in mice.[33] In chronic hypoxia states like those experienced in CCHD, accumulated electrons in ETC complexes can leak to form superoxide anions which overwhelm antioxidant clearance systems, leading to increases in reactive oxygen species and mitochondrial damage.[34,35]

These findings suggest that changes to mitochondrial genes and products may occur at multiple levels (genomic, transcriptomic, proteomic), and mitochondrial dysfunction may be bidirectional in CCHD, both affected by, and contributing to cardiac failure pathogenesis and progression.

Key mitochondrial processes in CCHD pathogenesis and/or progression involved the following main mitochondrial mechanisms: (1) *Dynamics*: fission/fusion, copy number; (2) *Metabolism*: oxidative phosphorylation, tricarboxylic acid cycle (TCA), fatty acid (FA), and amino acid (AA) metabolism; and finally (3) *ROS Management and Oxidative stress*.

Dynamics

Mitochondria respond to environmental cues with fission and fusion, both to generate new mitochondria and mitigate stress-induced damage by re-distributing damaged contents.[36] We identified substantial evidence of altered dynamics in CCHD, driven primarily by epigenetic and transcriptomic data.[6,18,37,38] A SNP encoding microRNA-196 (miR-196) was associated with a 32–84% increased susceptibility to CHD depending on the base pair change.[18] miR-196 inhibits mitofusin-1 (MFN1), an essential fusion protein.[39] In the 2009 study by Xu et al., although only 33% of study participants had CCHD or complex heart lesions,[18] the results are comparable to another epigenetic study consisting entirely of cases with TOF versus controls, which identified significantly increased DNA methylation of the EGFR gene (Mann–Whitney test; $p = 0.0042$).[38] EGFR encodes the EGF receptor, an integral component inducing mitochondrial fission.[40] These epigenetic data indicating the role of mitochondrial dynamics in cyanotic heart disease in human patients are further supported by in vivo transcriptomic evidence.[6] In a mouse model of TOF, three RNAs encoding fission proteins (DRP1, MFF, and OPA1) were significantly downregulated versus controls.[6] Subsequent immunoblot validation demonstrated that the encoded proteins initially increased in abundance (with RV hypertrophy) then decreased (with progressive RV failure). Although the relationship between mitochondrial mass, dynamics, and mtDNA copy number has not completely been elucidated, patients with TOF seem to have increased mitochondrial volume and mtDNA versus controls.[3] This increase may be PGC-1α-mediated in response to TOF-related hypoxia.[3] Furthermore, our TF enrichment analysis identified E2F1 which is upregulated in hypertrophic cardiomyopathy,[41] activates miR-421 expression, and produces cardiomyocyte mitochondrial fragmentation.[42] E2F1 has also been linked to suppressed cardiac neovascularisation following ischaemic injury by vascular-endothelial growth factor and placental growth factor regulation.[43] Combined, these findings highlight that mitochondrial dynamics may be involved not only in the early stages of CCHD, but also during disease progression.

Metabolism

Most current evidence regarding CCHD mitochondrial alterations stems from metabolic and energetic processes (epigenetic, genetic, transcriptomic, metabolomic/lipidomic, and multi-level integrative studies). In one epigenetic TOF study examining DNA methylation, NK2 homoeobox 5 gene (NKX2-5) was hypermethylated (Mann–Whitney test; $p = 0.0013$) versus controls, suggesting relative deactivation of NKX2-5 in the disease state.[44] NKX2-5 has many functions with respect to cardiac differentiation and development, including mitochondrial dysfunction: neonatal cardiomyocytes with mutant NKX2-5 have decreased mitochondrial respiration[45] and NKX2-5 knockout zebrafish have altered mitochondrial translation, and tricarboxylic acid (TCA) cycle defects.[46]

The most robust genomic evidence for mitochondrial energetics in CCHD stems from whole exome analysis of 100 individuals with RV outflow obstructive defects.[47] Several predicted damaging Phosphoenolpyruvate carboxykinase (PCK2) variants were detected. PCK2 encodes a rate-limiting TCA enzyme (oxaloacetate to phosphoenolpyruvate). Damaging PCK2 variants could thus shunt oxaloacetate away from TCA, impacting energy production.[48]

Robust transcriptomic evidence also links dysregulated mitochondrial metabolism with SV disease pathogenesis and progression.[4] An RNA study of over 50 SV tissues (failing and non-failing) and controls[4] identified profiles of decreased energy generation, CPTI/CPTII enzyme activity, oxidative phosphorylation, amino acid metabolism, TCA, and lipid metabolism. Metabolomic analysis affirmed that both failing and non-failing SV have altered AA metabolism, TCA function, oxidative phosphorylation, pyruvate, and FA metabolism.[4] Non-failing SVs appeared as an intermediate phenotype with potential susceptibility towards future failure. Differential RNAs included PCK2 which aligns with aforementioned genetic evidence.[47] Energetic changes in chronic hypoxia are further supported by metabolomics; triglyceride, fructose, aspartic acid, sucrose, pyruvate, organic acid, and energy storage metabolic

processes are altered in TOF and ASD myocardium.[20] Furthermore, previously-repaired TOF adults have altered oxidative reduction processes which may be related to downstream pathogenic RV remodelling.[49] RV remodelling in TOF and HLHS is also associated with increased glycolysis and reduced FA metabolism.[12,25]

Another multiomic human study reported differences in cardiac metabolism during puberty in patients with CCHD, potentially mediated by Hypoxia-inducible factor 1-alpha (HIF-1α) regulation. Compared to prepubertal patients with CCHD, post-pubertal patients demonstrated decreased myocardial uptake of glucose, but an increase in fatty acids. [50] Furthermore, HIF-1α knockout and pharmacological inhibition with Pioglitazone in a rodent model abrogated hypoxia-related metabolic changes and improved cardiac function in pubertal CCHD animals.[50] Such exciting preclinical evidence suggests existing medical therapies may have potential applications in CCHD and is consistent with transcriptomic evidence of compensatory HIF-1α downregulation in CCHD.[51,52]

Additionally, as patients with SV grow and progress in Fontan stage, significant differences in mitochondrial respiratory reserve and maximal respiratory capacity increase in magnitude compared to controls.[7] Differences are most pronounced among failing, and to lesser extent, non-failing SVs, suggesting that CCHD-related mitochondrial dysregulation occurs progressively. Thus, this pathway may have applications to identify those at highest risk of adverse outcomes from disease progression. Xu et al. corroborated these findings using an established murine model of hypoplastic left heart syndrome using *Ohia* mice affirmed transcriptomic alterations in metabolic pathways, thermogenesis, FA degradation, AA metabolism, oxidative phosphorylation, and PPAR signalling.[7] In fact, both RVs and LVs showed significant differences in Seahorse assay mitochondrial respiration rate. Zhang et al. have also reported increased expression of carnitines butyrylcarnitine, hexanoylcarnitine, and tetradecanoylcarnitine in CHD, which play crucial roles in transporting long-chain fatty acids into the mitochondrial for lipid metabolism and energy production.[53]

Another pre-clinical multiomic SV study (genomic, transcriptomic, proteomic) by Garcia et al. examined primary cardiomyocytes treated with serum from patients with SV disease. Although mitochondrial DNA (mtDNA) copy number was not significantly altered, treatment of cardiomyocytes with serum from patients with failing SV decreased mitochondrial cardiolipin levels (membrane phospholipid involved in mitochondrial dynamics) compared to those treated with serum from biventricular non-failing controls.[4,54] There were also transcriptomic changes as lipid and metabolic remodelling enzymes were also differentially expressed.[4,54] Treated cells also had evidence of increased ROS and reduced mitochondrial respiration versus controls. Interestingly, pharmacological treatment (Sildenafil) improved mitochondrial coupling efficiency and ATP production and reduced oxidative stress. Complementing this finding, hyperacetylation of mitochondrial proteins in failing versus non-failing SV hearts has been observed and sildenafil regulates Sirtuin 3 (SIRT3) which is associated with mitochondrial protein deacetylation.[55] These findings bolster additional evidence that Sildenafil may be beneficial in SV during the failure stage, but not in non-failing counterparts.[56]

Interestingly, in patients with SV undergoing surgery, serum FBXL3, ACTR2, PRKAR1A, and QKI RNAs were significantly upregulated, whereas SF3A2, SPATC1L, AZU1, and ELANE were significantly downregulated in those who experienced postoperative low cardiac output syndrome (LCOS) versus those with no LCOS.[13] These transcriptomic signatures reflect TCA and electron transport, mitochondrial protein import, AA metabolism, and FA oxidation. These findings highlight the potential application of mitochondrial targets as predictive biomarkers for postoperative complications, which would be particularly valuable for complex lesions.

Lastly, peroxisome proliferator-activated receptor gamma (PPAR-γ) and Sal-like protein 4 (SALL4) were transcription factors highlighted by our enrichment analysis (Fig. 2) that have been previously linked to mitochondrial metabolism. Cardiac PPAR-gamma modulates energy metabolism through its effects on extra-cardiac tissues,[57] increases FA oxidation and lipoprotein triglyceride uptake, leading to increased lipid and glycogen stores, altered mitochondrial inner matrix architecture, and altered cristae.[58] Similarly, although not specific to cardiomyocytes, SALL4 increases oxidative phosphorylation, oxygen consumption, mitochondrial membrane potential, and ATP generation.[59] Additional investigations are warranted to elucidate the role of SALL4 within cardiac tissue.

ROS management and oxidative stress

Oxidative phosphorylation in cardiac mitochondria is a major source of ROS production, and to lesser extent, cytokines and growth factors such as angiotensin II, PDGF, and TNF-a.[2] ROS generation has been linked to heart failure and CHD, though current evidence remains conflicting.[2] Chronic hypoxia in CCHD increases susceptibility to oxidative stress due to reduced antioxidant reserve through significantly increased myocardial COX11.[22] COX11 may have a role in counteracting hypoxia-mediated ROS through haem A biosynthesis.[22]

Impact of primary diagnosis and disease state on type of mitochondrial dysregulation

From this systematic review, we identified that altered dynamics were more implicated in TOF and obstructive

lesions producing a hypertrophic phenotype.[6,38,39,44] On the other hand, respiration defects appeared to play a greater role in SV conditions[4,7,13] where failure was more predominant. There was also direct evidence that mitochondrial changes occur as disease state progresses from a hypertrophy to a failure stage.[6] Additionally, respiration defects seem to begin in compensated patients with SV and progress with time as patients age, progress in Fontan stage, and/or develop progressive heart failure. Further analysis performing detailed comparisons between different CCHD subtypes are warranted to elucidate specific biomarkers that could be used to predict failure, postoperative complications, or other adverse outcomes, by linking such biomarkers to hypertrophy, cyanosis, or failure.

Hwang et al. observed an increase of transcripts of mitochondrial fission proteins DRP1 and MFF in a mouse model of TOF with RV hypertrophy, but a subsequent decrease of these transcripts in RV failure,[6] raising questions around whether these changes are compensatory or maladaptive. Mitochondrial fission in the early stages may be a compensatory mechanism to boost energy production, followed by decline in mitochondrial fission as hearts progress towards failure. This would be essential information for potential therapeutics to effectively leverage the compensatory and suppress the maladaptive stages. Still, the finding that mtDNA copy number is unchanged in RV hypertrophy compared to control suggests that overall mitochondrial number and density may only play a limited role in comparison to overall metabolism and mitochondrial bioenergetics.[6] This is supported by Hwang et al.'s findings in compensated RV hypertrophy, demonstrating reduced mitochondrial energy generation even when mitochondrial biogenesis through fission is maintained.[6] The dilemma of elucidating adaptive versus maladaptive mitochondrial alterations may also be less pronounced in the context of mitochondrial respiratory targets as it would be counterintuitive that any reduction in mitochondrial energetics could be anything other than maladaptive.

Clinical translation

Overall, systems biology suggests that mitochondrial clinical targets should be further explored in CCHD. Both Sildenafil and Pioglitazone are approved drugs which are currently in clinical practice and could potentially be applied to CCHD. The former may improve mitochondrial function in SV heart failure through SIRT3-mediated deacetylation of mitochondrial proteins.[55] Importantly, it is not immediately evident what clinical effect may be expected from such treatments: perhaps reduction in risk of progressive heart failure, improvement in ejection fraction, or enhanced exercise tolerance could be investigated. Additionally, several adult mitochondrial drugs in preclinical or clinical trial phases for heart failure could be explored such as: Edaravone (free radical scavenger), Idebenone (free radical scavenger), Elamipretide (cardiolipin protection), Vericiguat (soluble guanylate cyclase stimulator), and others.[2]

Another interesting and unusual emerging therapy is autologous mitochondrial transplant.[60] This technique involves delivering skeletal muscle-derived isolated mitochondria to cardiac muscle tissue. The therapy has preliminary evidence of safety in a small clinical trial of CCHD with refractory heart failure on ECMO. The proposed mechanism of action involves additional ATP production from the delivered mitochondria to support cardiac muscle contraction, though questions remain. Transplanted mitochondria have never been shown to remain viable; it is unclear how they might translocate into cells or remain viable extracellularly. Extracellular ATP would not necessarily impact cellular contraction. Overall, the mechanism should be fully elucidated before embarking on detailed clinical trials.

Another promising potential application of mitochondrial biomarkers is to prognosticate surgical outcomes, responsiveness to treatment, and to follow disease progression over the long term when considering the risk of heart failure development, particularly in SV heart failure conditions[4,7,61] however further investigation of other CCHDs that are currently underrepresented in literature (e.g. mixing lesions) may follow similar trends. Such biomarker-based risk stratification could guide timing of surgery to mitigate postoperative complications and/or predict future transplant.

Study strengths and limitations

By integrating multiple omics approaches, including transcriptomics, proteomics, epigenomics, and metabolomics, this study provides a comprehensive overview of differentially expressed genes and metabolites in CCHD. Additionally, the inclusion of human and animal model data provides additional strength to the study by enabling cross-species comparisons, which corroborate identified differential biological processes.

Although it is very difficult to apply quality control metrics to systems biology datasets, unlike in patient cohort metanalyses, we made an effort to examine and assess the clinical reporting features. For instance, although included studies were required to have CCHD, no single study directly confirmed included study subjects were actively cyanotic at the time of analysis. Thus, it is challenging to ascertain which decompensatory processes are driving these mitochondrial changes: cyanosis, hypertrophy, or progressive heart failure. Although it was not the objective of the present study to specifically elucidate which mitochondrial changes are resulting from cyanosis specifically, these considerations represent an important potential area for future research. The significant

heterogeneity between analytical techniques and study populations precluded possibility of formal meta-analysis but can be explored as more data emerge. Additionally, overall sample sizes in most studies were limited (<100) and a few studies only had a subset of samples with cyanotic lesions. More extensive analyses to confirm the generalisability of these findings are required.

Furthermore, biventricular mixing lesions (which can be challenging to accumulate in large numbers) were under-represented including transposition of great arteries, truncus arteriosus, and total anomalous pulmonary venous return. There were also limitations from animal models used in the non-human studies include which may not fully replicate the pathophysiology of CCHD. For example, there is lack of evidence that serum-treated cardiomyocytes fully recapitulate the physiologic environment seen in CHD. Further, the Tetralogy of Fallot mouse model primarily reflects RV pressure overload with pulmonary arterial constriction,[6] but does not recapitulate the subpulmonary hypertrophy, valvular abnormalities, or potential intrinsic myocardial differences in the "true" TOF RV. This model may, in fact, be better suited as a model of RV failure more broadly. Limitations from the review process include the focus on untargeted systems biology methods only, and missing data in some studies meant it was not possible to obtain complete gene lists. Finally, while most included studies reported sex of subjects, no studies presented sex-disaggregated data, and thus, it was not possible to conduct sex-specific subanalyses in this review.

Outstanding questions

Currently, there remains a far greater number of transcriptomic studies compared to proteomic analyses on cyanotic heart disease. RNA and protein profile correlation is generally poor, due to greater susceptibility of RNA to degradation. So, the ongoing application of proteomic approaches in disease should provide an excellent opportunity to profiling the phenotypes associated during disesea development. Another area which remains unexplored are post-translational modifications of these proteins. Phosphorylation, acetylation, and ubiquitination for instance, drastically impact protein activity and are strongly influenced by environmental cues. Therefore, robust post-translational modification proteomic profiling remains valuable—an untapped area for both therapeutic and prognosticative targets in CCHD. Unlike nucleotide sequencing, these techniques are labour-intensive, costly, and require access to mass spectrometry, but have been used to perform high-throughput analyses in other cardiovascular diseases. Finally, given the focus of this review on systems biology techniques, morphological alterations in mitochondria which may be involved in cyanotic disease processes, such as those evidenced by electron microscopy, are outside the scope.[62] Still, such analyses offer complementary insights into pathophysiologic pathways which may be leveraged for clinical translation.

Conclusion

Systems biology data do highlight mitochondrial dysregulation through metabolism, and fission/fusion, are key to CCHD pathogenesis, and progression to decompensation. Preliminary evidence associates mitochondrial biomarkers with risk of perioperative complications, secondary heart failure, and substrates for existing and potential pharmacologic agents. More robust proteomics, larger scale epigenomics, and multiomic integrative analyses are required to prioritise targets for detailed validation.

Contributors

M.E. conceived and designed the study, A.R.W carried out the literature search. M.E., Y.T.L., collected data, performed GO and X2KWeb analysis, and wrote the original manuscript. Y.T.L updated the search, collected data, performed GO and X2KWeb analysis, and created Figs. 1 and 2, Supplementary Tables S1–S8. M.H. updated the search, collected data, performed GO analysis of biological pathways and cell components, created Figs. 3–5 and Table 1, and contributed to Supplementary Tables S1–S8. M.E., and Y.T.L wrote the original manuscript. M.H. revised the manuscript text with input from Y.T.L, J.C., S.M., O.H., D.J.B., and A.O.G. All authors accessed all included data and approved the final draft of the manuscript.

Data sharing statement

All data used for this manuscript, namely the pooled gene lists and all PANTHER GO output data, are shared as Supplementary files.

Declaration of interests

S.M. holds the Heart and Stroke Foundation of Canada/Robert M Freedom Chair of Cardiovascular Science and serves on the advisory board for Bristol-Myers-Squibb, Tenaya Therapeutics, Rocket Pharmaceuticals, and an NIH RO1 grant. D.J.B. previously served as an expert witness for a case of congenitally corrected transposition in the United Kingdom. All other authors declare no conflicts of interest.

Acknowledgements

Malak Elbatarny was supported by a Vanier CIHR Scholarship. Yu Tong Linda Lu was supported by a University Health Network (UHN) Research Studentship. Mostin Hu was supported by an Ontario Graduate Scholarship. Anthony Gramolini was supported by Translational Biology and Engineering Program seed operating funds and research funding from the Heart and Stroke Foundation of Canada.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105839.

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