Computers in Biology and Medicine 175 (2024) 108507

Contents lists available at ScienceDirect

Computers in Biology and Medicine

journal homepage: www.elsevier.com/locate/compbiomed

Towards understanding post-COVID-19 condition: A systematic meta-analysis of transcriptomic alterations with sex-specific insights

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ARTICLE INFO

Keywords:
Long COVID
RNA-Seq
Meta-analysis
SARS-CoV-2
Histone expression
Sex-dependent gene expression
Disease enrichment
Hesperitin

ABSTRACT

Background: Post COVID-19 Condition (PCC), characterized by lingering symptoms post-acute COVID-19, poses clinical challenges, highlighting the need to understand its underlying molecular mechanisms. This meta-analysis aims to shed light on the transcriptomic landscapes and sex-specific molecular dynamics intrinsic to PCC.
Methods: A systematic review identified three studies suitable for comprehensive meta-analysis, encompassing 135 samples (57 PCC subjects and 78 recovered subjects). We performed meta-analysis on differential gene expression, a gene set enrichment analysis of Reactome pathways, and weighted gene co-expression network analysis (WGCNA). We performed a drug and disease enrichment analysis and also assessed sex-specific differences in expression patterns.
Key findings: A clear difference was observed in the transcriptomic profiles of PCC subjects, with 530 differentially expressed genes (DEGs) identified. Enrichment analysis revealed that the altered pathways were predominantly implicated in cell cycle processes, immune dysregulation and histone modifications. Antioxidant compounds such as hesperitin were predominantly linked to the hub genes of the DEGs. Sex-specific analyses highlighted disparities in DEGs and altered pathways in male and female PCC patients, revealing a difference in the expression of ribosomal proteins. PCC in men was mostly linked to neuro-cardiovascular disorders, while women exhibited more diverse disorders, with a high index of respiratory conditions.
Conclusion: Our study reveals the intricate molecular processes underlying PCC, highlighting that the differences in molecular dynamics between males and females could be key to understanding and effectively managing the varied symptomatology of this condition.

1. Introduction

Post COVID-19 Condition (PCC), also referred to as long COVID or post-acute sequelae of COVID-19, is a multisystem disease that includes different symptoms which persist for more than three months after an acute infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease (COVID-19) [1,2]. The World Health Organization (WHO) defines PCC by noting that these prolonged symptoms, not attributable to an alternative diagnosis, must persist for at least two months [3]. The most common manifestations of PCC are fatigue and dyspnea, yet individuals may also experience a spectrum of less frequent symptoms, such as headache, chest and

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https://doi.org/10.1016/j.compbiomed.2024.108507
Received 24 November 2023; Received in revised form 26 March 2024; Accepted 21 April 2024
Available online 22 April 2024

joint pain, cognitive and mental disorders, and an increased risk of developing conditions like cardiovascular diseases and type 2 diabetes [4]. Notably, various experts point out similarities between PCC and other post-viral diseases, such as Epstein-Barr [5], human herpes virus [6], influenza virus [7].

The global burden of PCC is substantial, with estimates indicating that at least 65 million individuals are afflicted worldwide, though actual numbers may be significantly higher due to the prevalence of undocumented cases [2]. The WHO estimates that approximately 10% people who have had COVID continue to experience symptoms beyond 12 weeks [8].

Regarding the pathogenesis of PCC, various overlapping factors are postulated to contribute to the disease's manifestation [9]. One prevailing hypothesis is the presence of active viral reservoirs in several tissues that persist after infection. In a recent study, circulating antigens of SARS-CoV-2 were observed for 2–12 months after infection in 65% of patients manifesting PCC [10]; other studies have also found SARS-CoV-2 RNA 7 months after being infected [11,12]. Furthermore, previous reports show that SARS-CoV-2 can deregulate the patient's immune response, weakening it, which could cause reactivation of the viral reservoir, or change in the genetic viral expression, triggering persistent symptoms [13]. On the other hand, it is speculated that the microbiome, which includes the virome, could also contribute to the persistence of PCC manifestations [14,15]. It was observed that the use of probiotics can mitigate or improve fatigue, mood, and overall quality of life in patients with post-infectious fatigue [16]. Autoimmunity, microvascular blood coagulation with endothelial dysfunction, and immune system priming via molecular mimicry, among other factors, have also been postulated as possible causes of this syndrome [13, 17–20].

In addition, studies on PCC consistently report an association between female sex and the likelihood of experiencing persistent symptoms [21,22]. In summary, the precise symptomatic manifestations of PCC are not fully understood since it is a disease that involves many phenotypes [23]. Moreover, PCC affects individuals who have been infected by SARS-CoV-2, regardless of the severity of the initial pathology and the age of the patient, though there are sex-specific trends. Consequently, predicting the onset of this syndrome continues to be an enigma [24,25]. Regarding treatment options, several drugs have yielded successful results in randomized controlled trials [26] and other approaches, such as hyperbaric oxygen therapy, also have demonstrated beneficial effects [27]. However, there are still no approved treatments for PCC [28].

In this context, we conducted a meta-analysis of transcriptomic studies pertaining to PCC, the first, according to our knowledge. By aggregating and synthesizing data from multiple sources, we are able to offer a comprehensive and robust overview that individual studies alone may not provide. Our focus resolved around studies that deposited in the Gene Expression Omnibus (GEO) database that contained data on subjects who had suffered COVID-19 a minimum of 4 months prior. This analysis aims to clarify which molecular mechanisms are altered in subjects suffering from PCC, as well as to identify potential drug candidates. Additionally, because PCC prevalence varies between genders, we also aimed to explore how these mechanisms are affected in a sex-dependent manner. Ultimately, we hope that understanding these molecular changes can help develop tailored management strategies for PCC, taking into account the diverse experiences of patients.

2. Material and methods

2.1. Literature review and study selection

The systematic search was performed between June and July 2023 following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [29]. We searched the GEO datasets database for the terms "long covid" OR "post-acute sequelae SARS-CoV-2" OR "PCC" OR "post-COVID-19" OR "long-haul covid" OR "chronic COVID". In GEO datasets, we filtered by "Expression profiling by array" and "Expression profiling by high throughput sequencing". Results were filtered by: 1) organism "Homo sapiens", 2) tissue "Blood" or Peripheral blood mononuclear cells ("PBMCs"), 3) study type "expression profiling by high throughput sequencing" or "expression profiling by array", 4) time between first COVID-19 diagnosis and sample extraction "more than 4 months". In addition, we searched PubMed with the same keywords in order to retrieve any publications that could contain transcriptome data associated with the study. The search period was from January 2020 (some months after the first outbreak in November 2019) to July 15, 2023. We discarded datasets according to the following criteria: (1) datasets without a clear separation of PCC and non- PCC patients; (2) no real bulk transcriptomic data (i.e., pseudo bulk RNA-Seq data derived from single cell RNA-Seq); (3) tissue other than blood; (4) less than 4 months between acute COVID-19 and sample extraction; (5) less than 4 months between acute COVID-19 and PCC diagnosis and (6) no information on patient sex. The PRISMA diagram related to this search is detailed in Fig. 1.

2.2. Bioinformatic analyses

To enhance reproducibility, we downloaded and harmonized all the datasets in accordance with the pre-processing steps. With each dataset, we followed these steps: (1) data acquisition, (2) read quality assessment and preprocessing, (3) genome mapping, (4) summarization of mapped reads to genomic features, (5) exploratory analyses, (6) Differential gene expression (DGE) analyses. Finally, we (7) integrated single study DGE results using meta-analyses techniques and (8) performed a functional analysis using the meta-analysis results.

2.3. Data acquisition, preprocessing, genome mapping and summarization to genomic features

To ensure the consistency and reproducibility of our analysis, the standard established RNA-Seq pipeline was used. We downloaded the raw reads, assessed their quality pre and post preprocessing using FastQC [30] and MultiQC [31]. In between, we used trimmomatic [32] to trim the reads at the Illumina universal adapter and discard those shorter than 50 bases. We mapped the processed reads to the GRCh38 version of the human genome using STAR [33] and assigned the reads to their corresponding genes using the pair-end mode of featureCounts [34].

2.4. Differential gene expression

The gene counts matrices were imported into R 4.3.1 [35] and processed using DESeq2 [36]. For DEG analyses, we kept genes with more than 0.5 counts per million in at least 30% of the samples. We removed unwanted variation from the data using the residuals (RUVr function from the RUVSeq package [37]. Finally, we performed DEG analysis with DESeq2, adjusting the p-values using the Benjamini and Hochberg method and comparing PCC versus non-PCC patients. Our main goal was to evaluate the transcriptomic profile of PCC patients, including sex as a covariate, and to explore sex-specific trends in PCC by conducting separate analyses for male and female subjects. We annotated the log2 fold change confidence intervals and exported the results matrices. The connectivity between genes was assessed with Cytoscape [38] using the STRING database.

2.5. Weighted gene co-expression network analysis (WGCNA)

Gene counts matrices obtained after removal of unwanted variation and regularized transformation were used to build the networks for each dataset. Dataset GSE169687 as used to search the gene modules due to its larger sample size; the gene modules were subsequently assessed for

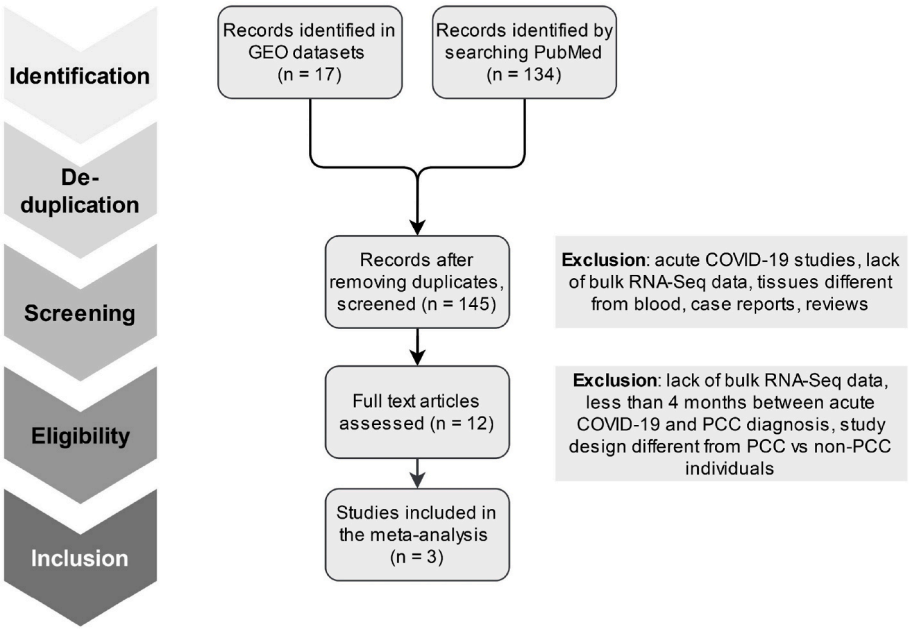


Fig. 1. PRISMA diagram.

conservation in the other datasets; we kept modules that had a Zsummary >10 and displayed the same tendency in the 3 datasets when comparing PCC and non-PCC patients. Additionally, to examine the consensus and conservation of the modules, we conducted Fisher's exact test between the modules from GSE169687 and those from the other datasets (Fig. S1). For these, we extracted the 50 genes with highest module membership (kME) and performed an over-representation analysis (ORA) of Reactome pathways. P-values were adjusted with the false discovery rate and values smaller than 0.1 were considered statistically significant.

2.6. Meta-analysis and functional enrichment analysis

The differentially expressed genes from three datasets were combined using the MetaVolcanoR method using the random effect model approach, which estimates a summary p-value. We used 0.01 as the threshold values for this summarized p-value.

Based on the combined differential expression results, we performed a gene set enrichment analysis (GSEA) from ReactomePA [39] and Reactome [40] pathways. Genes were sorted based on their combined log2 fold change. The pathways were assessed for statistical significance using adjusted p-values obtained through false discovery rate control. Pathways with adjusted p-values less than 0.1 were considered statistically significant.

Disease enrichment was performed with the DOSE R package [41]. DEGs were analysed using Network Analyzer [42] in Cytoscape [43], and interactions were loaded from the STRING database [44]. Hub genes were defined as those in the top 10% of interactions (10 or more direct connections). Drug enrichment analysis was performed with the list of hub genes using DSigDB [45] and the ClusterProfiler R package [46].

Availability of data and materials

In this study, transcriptomic data from the GEO database with accession numbers GSE169687, GSE194378 and GSE224615 were used. All the scripts corresponding to the read preprocessing, alignment and summarization, as well as DEG analysis, meta-analysis, WGCNA, GSEA and figure generation are available at zenodo: https://zenodo.org/doi/10.5281/zenodo.11032354. Results, corresponding to count matrices, Differentially Expressed Genes (DEGs) tables, combined DEGs table, cytoscape session and GSEA outputs are also available at the same link.

3. Results

3.1. Transcriptome datasets in PCC

Our systematic review meticulously examined 145 publications, ultimately identifying three studies eligible for inclusion in our analyses, as summarized in Table 1.

The dataset encompassed a total of 135 samples, comprising 57 individuals with PCC and 78 subjects who exhibited a complete recovery from PCC within a normal time frame. Among the PCC patient group, 61.4% were female, while in the non-PCC group, only 46.2% of individuals were female. None of the patients received a COVID-19 vaccine at the time the samples were taken.

3.2. Differential gene expression and gene set enrichment analysis in PCC

As described in the Materials and Methods section, we performed individual differential gene expression analysis on each study and combined the results with MetaVolcanoR, using the random effect model. We obtained 530 DEGs with a combined p-value smaller than 0.01 (Fig. 2A).Afterwards, we wanted to evaluate whether there were some biological pathways consistently enriched in up- or down-regulated gene sets. To do this, we performed a gene set enrichment analysis of Reactome pathways using the fold change obtained from the meta-analysis. As detailed in the Materials and Methods section, and with the aim to gain further insight into the sex-specific molecular dynamics of PCC, we also performed this analysis contrasting only males and females separately (data shown in sections below). With the goal of obtaining the altered pathways that are most conserved within individuals, we intersected the pathways obtained from the sex-specific comparisons and whole data set and obtained a total of 28 altered pathways, as depicted in Fig. 2B and C. The clear majority of pathways are up-regulated in PCC subjects and mostly refer to cell cycle processes, histones modifications, senescence and human cytomegalovirus (HCMV) infection events, among others. Interestingly, it can be seen that most the pathways are up-regulated, and only "keratinization", "formation of the cornified envelope" and "interpheron alpha/beta signalling" appear to be down-regulated in the PCC sufferers.

Table 1
Characteristics of the selected studies.

Dataset	Submission date	Publication	Total samples (PCC samples)	Percentage of female subjects	Tissue	Time after SARS-CoV-2 infection	Platform	Region
GSE169687	2021-03-26	Ryan FJ, 2022 [47]	65 (21)	51%	Whole blood RNA	6 m	NovaSeq 6000	Australia
GSE194378	2022-01-25	Sparks R, 2023 [48]	34 (21)	53%	Whole blood RNA	6 m (average)	NovaSeq 6000/ NextSeq 500	USA
GSE224615	2023-02-06	Yin K, 2023 [49]	36 (23)	56%	Total PBMCs	8 m	HiSeq 4000	USA

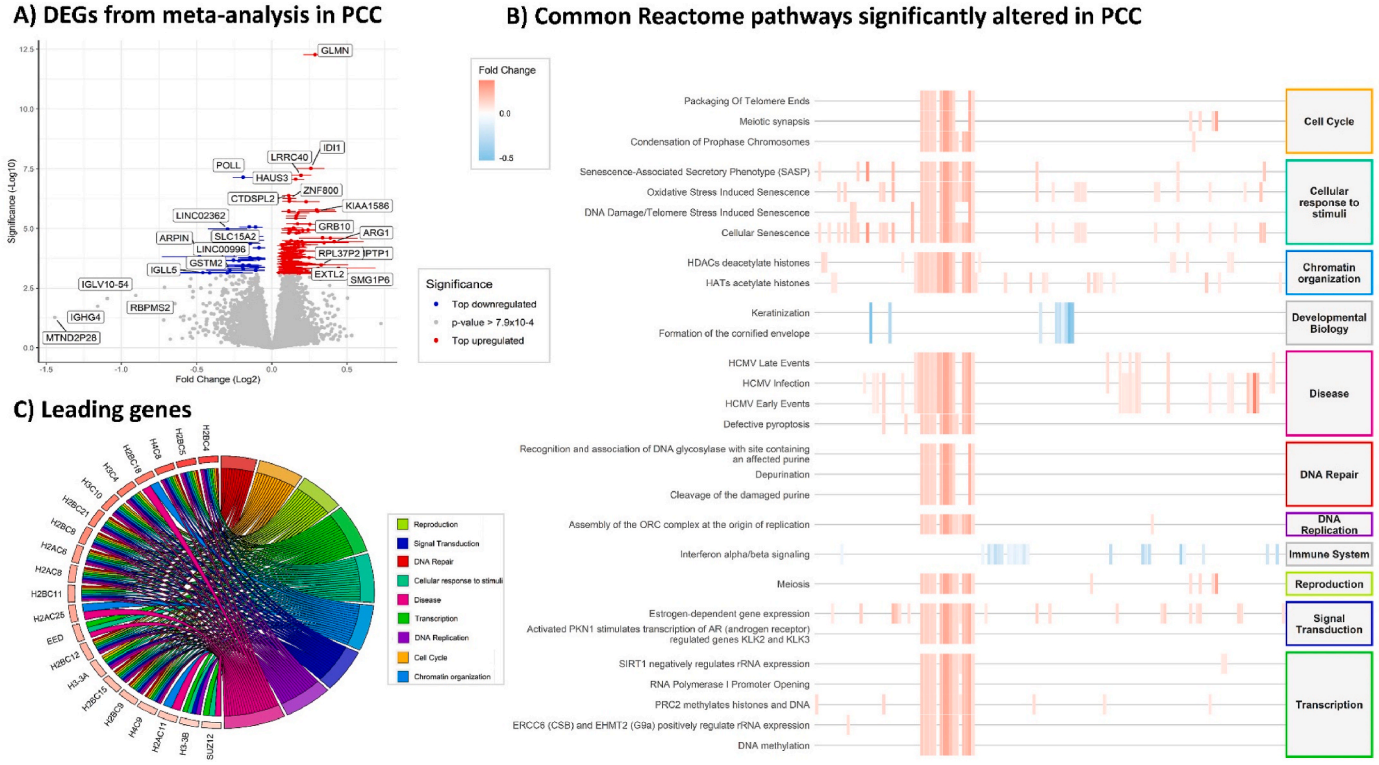


Fig. 2. DEGs and GSEA analysis. A) Volcano plot of DEGs in PCC versus non-PCC subjects. Errors bars correspond to the variation in the fold change values across the different datasets. The top 1% genes are highlighted, and interesting genes are labelled. Up-regulated genes are marked in red, down-regulated ones in blue and the bottom 99% of genes (according to p-value) are shown in grey. **B) Heatmap of the 28 most conserved altered Reactome pathways in PCC patients.** The y axis represents the pathways and x axis the genes that participate in these pathways. The figure is colour coded according to the mean fold change of each gene in PCC versus recovered subjects. **C) Circus plot of key gene-pathway relationships:** gene colours indicate log2 fold change, while pathway colours correspond to specific mechanisms.

Moreover, it can be noted that most up-regulated pathways share the same set of leading genes. Notably, these genes exclusively belong to the histone family, this is the reason because we have evaluated the most representative pathway of histones (HDACs deacetylate histones) to do the GSEA plot (Fig. 2C).

3.3. Weighted gene co-expression network analysis

Next, we wanted to evaluate whether we could find specific gene modules composed of genes with similar expression patterns that could discern between PCC and non-PCC subjects. For this, we performed a Weighted Gene Network Correlation Analysis (WGCNA), using the dataset GSE169687 as reference (since this dataset holds nearly double the number of samples compared to the others). This yielded 20 gene modules, from which 12 were well conserved in the other two datasets (Fig. 3A) presenting a Zsummary higher than 10 in both cases. The representative metrics — eigenvalues, which reflect the collective expression pattern of each gene module — for modules 13, 14, and 20 were significantly different between PCC and non-PCC patients in at least one dataset, as displayed in Fig. 3B. Finally, we performed and over-representation analysis of Reactome pathways with the 50 genes most central to the module for those that displayed the same tendency when comparing PCC and non-PCC samples in the three datasets (Fig. 3C). Module 13 presented 49 significant pathways, while modules 14, 18 and 20 presented 3, 5 and 170 respectively.

3.4. Disease enrichment and relevant drugs

Then, we aimed to investigate the diseases in which the list of DEGs was enriched; we identified 198 diseases and conditions with an adjusted p-value <0.1. From the top 30 most significant conditions and diseases (Fig. 4A), all but optic nerve disease displayed a positive enrichment. DEGs were used to build a protein-protein interaction (PPI) network, and 52 genes were identified as hub noted (Fig. 4B); drug enrichment of these genes revealed 527 drugs with an adjusted p-value <0.01. The top 10 most significant ones are depicted in Fig. 4C, and we marked the targets in the PPI network for the 3 relevant compounds.

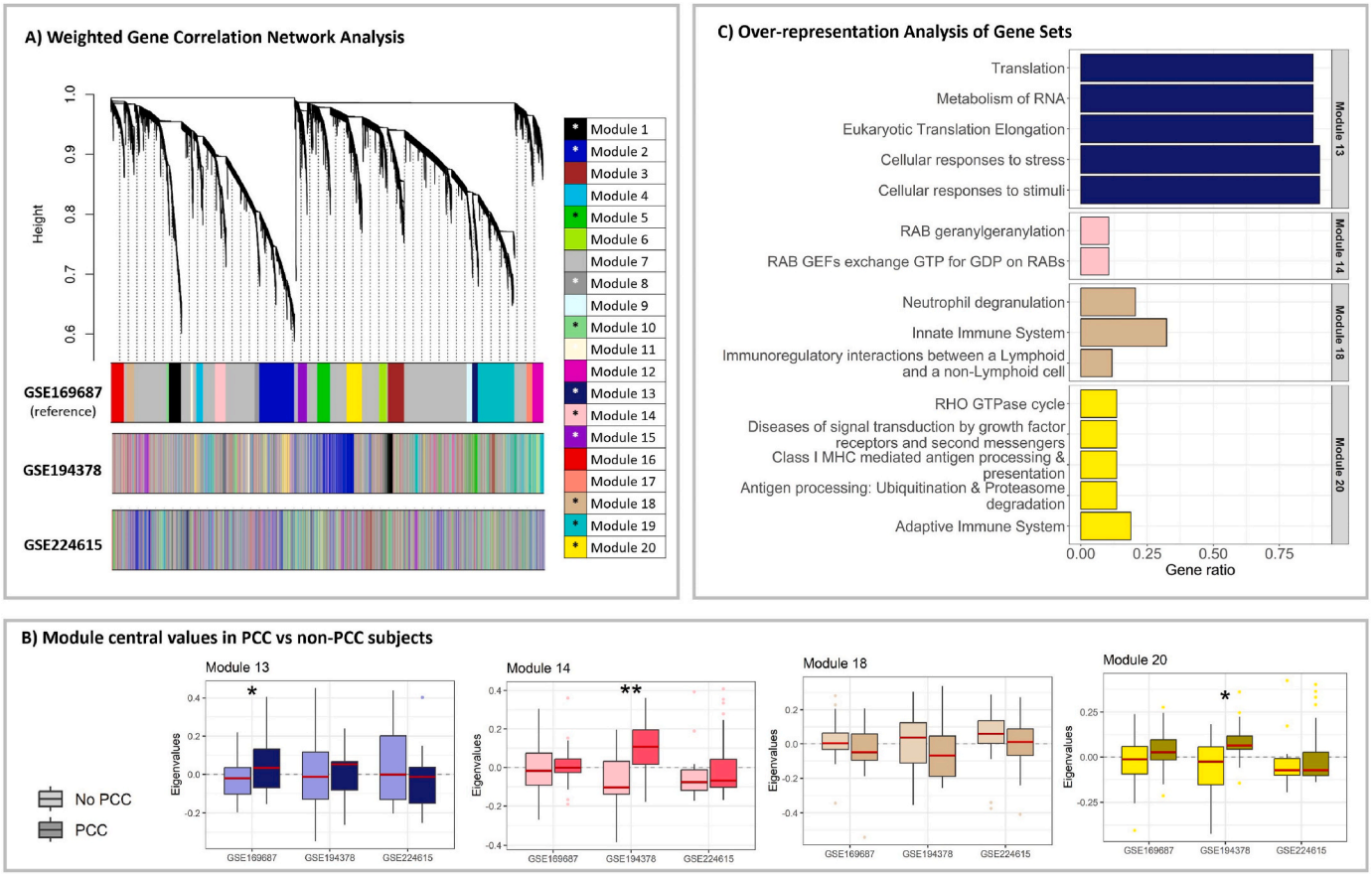


Fig. 3. Weighted Gene Co-Expression Network Analysis results. A) Cluster dendogram of the WGCNA; dataset GSE169687 was used as reference and generated 20 gene modules, that were subsequently searched in the other datasets. Stars in legend mark modules with a good conservation among the datasets. **B) Module central values depending on PCC status in each dataset**. Modules 13, 14, 18 and 20 are displayed; lighter colours correspond to PCC samples and darker colours to non-PCC individuals. T-tests were carried out to compute differences among PCC and non-PCC groups; p-values <0.05 are marked with stars. **C) Over-representation analysis of the 50 most central genes of the modules.** The five pathways with highest gene ratios are displayed (if more than 5 pathways were reported) for sets 13, 14, 18 and 20.

3.5. Sex-specific PCC transcriptomic dynamics

Next, we wanted to evaluate whether we could elucidate any sex-specific dynamics in PCC subjects. This is analysis is motivated by the different incidences and symptomatology reported depending on gender. Therefore, we performed a differential gene expression analysis and checked the contrast for female patients and for male patients independently. We obtained the DEGs depicted in Fig. 5, left for females and right for males. It can be noted that females presented more significatively down-regulated genes than men did, whereas the opposite is true for up-regulated genes.

Afterwards, we wanted to examine the differentially regulated pathways in men and women, depicted in Fig. 6. To achieve this, we performed a GSEA for Reactome pathways in each sex independently. We found 257 significantly up-regulated pathways in males and 70 in females, while only 23 pathways were significantly down-regulated in males and 122 in females (Fig. 6A). The 28 pathways observed in both sexes are illustrated in the previous Fig. 3. Conversely, 104 pathways are significantly up-regulated in male PCC subjects when comparing to non-PCC males, while down-regulated in female PCC individuals in contrast to non-PCC females (Fig. 6B).

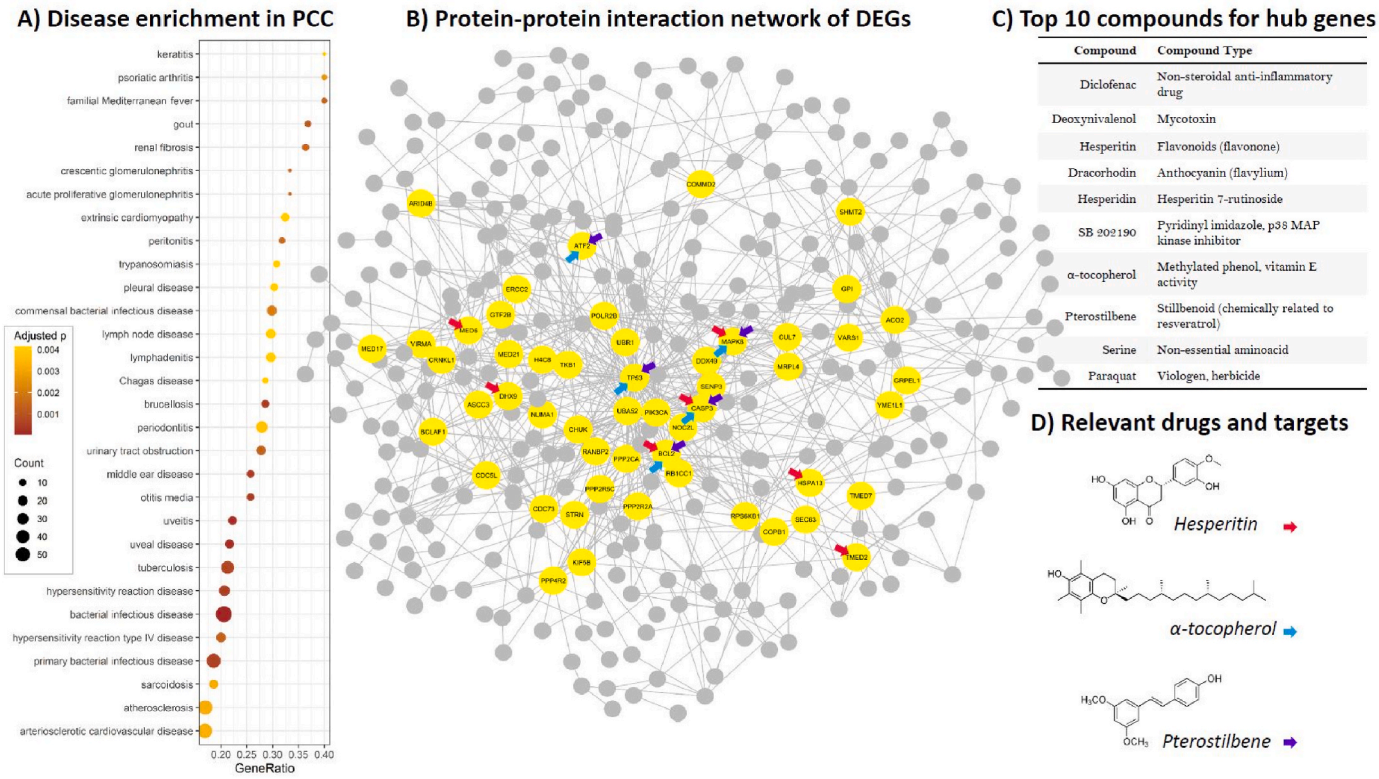
Focusing on the top 10 pathways with the highest normalized enrichment score (NES) for each sex (Fig. 6C), 8 were common to both but were regulated inversely: down-regulation was observed in females with PCC and up-regulation in males with PCC. These pathways primarily fell into the "Metabolism of proteins" category, while common pathways were found also in the "Disease", "Metabolism", and "Metabolism of RNA" categories. Overall, these largely related to translation processes (eukaryotic translation initiation and elongation, peptide chain elongation). Viral mRNA translation, selenocysteine synthesis and nonsense mediated decay were also noted. The analogous distributions of the fold changes for each pathway suggest that these are led by the same set of genes (Fig. 6D), which mainly belong to different large and small ribosomal proteins. Several translation initiation and elongation factors were also identified, in addition to some signal recognition particles, albeit in fewer numbers.

Finally, and given that different symptoms of PCC depend on sex, we wanted to investigate whether altered genes related to different diseases in men and women (Fig. 7). DEGs found in women led to 86 enriched diseases/conditions, while only 8 diseases were enriched for men.

4. Discussion

At present, given the novelty of the syndrome, PCC is a disease whose pathogenesis is complex and still elusive [23]. In this meta-analysis, by integrating information from three different datasets, we have been able to improve the understanding of the molecular mechanisms involved in the pathogenesis of PCC.

After performing pathway enrichment with the 530 DEGs, we found significant alterations in immune dysregulation, also reported by other authors [50]. Specifically, we found a down-regulations of the interferon α/β signaling pathway, a key strategy in the infection of SARS-Co-V-2



Compound	Compound Type
Diclofenac	Non-steroidal anti-inflammatory drug
Deoxynivalenol	Mycotoxin
Hesperitin	Flavonoids (flavonone)
Dracorhodin	Anthocyanin (flavylium)
Hesperidin	Hesperitin 7-rutinoside
SB 202190	Pyridinyl imidazole, p38 MAP kinase inhibitor
α-tocopherol	Methylated phenol, vitamin E activity
Pterostilbene	Stillbenoid (chemically related to resveratrol)
Serine	Non-essential aminoacid
Paraquat	Viologen, herbicide

Fig. 4. Disease enrichment and relevant drug candidates for PCC. A) GSEA analysis of disease ontology. B) Protein-protein interaction network of DEGs. Yellow nodes display 10 or more direct connections. **C) Top 10 compounds for the hub genes**, sorted by adjusted p-value. **D) Relevant drugs**, with targets marked in the PPI network for hesperitin (pink), α-tocopherol (blue) and pterostilbene (purple).

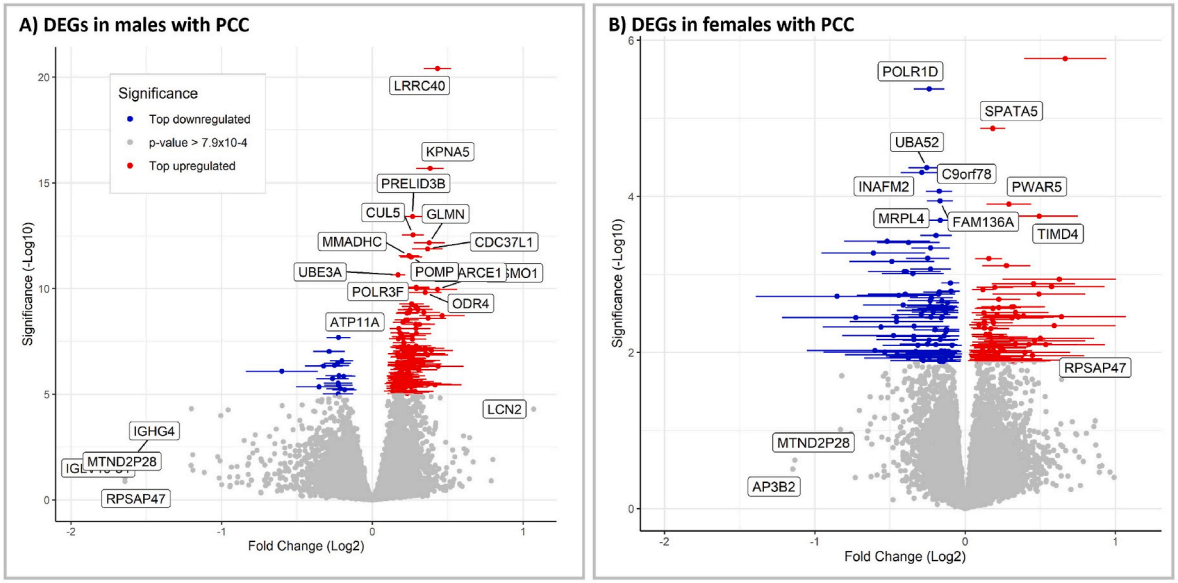


Fig. 5. Volcano plot of differentially expressed genes in PCC versus non-PCC subjects depending on sex. A) Females; B) Males. Errors bars correspond to the variation in the fold change values across the different datasets. The top 1% genes are highlighted, and interesting genes are labelled. Up-regulated genes are marked in red, down-regulated ones in blue and the bottom 99% of genes (according to p-value) are shown in grey. Note that the y-axis has different scales.

[51], and the WGNCA analysis yielded an enrichment in MHC class I-mediated antigen presentation, antigen processing and adaptive immune system. Additionally, we report an impairment in DNA damage repair mechanisms that has been linked to PCC [52–55], represented here by an increased activity of DNA glycosylases and an induction of depurination. These processes have the potential to lead to senescence [56], a process that is also up-regulated in PCC as demonstrated by our data and the one from Xu et al. and Badaras et al. [57,58]. Other pathways related to translation and RNA metabolism are also positively enriched, including RNA polymerase I promoter opening, and assembly of the ORC complex at the origin of replication, which can be traced to viral persistence in some individuals with PCC [59,60]. Our findings also

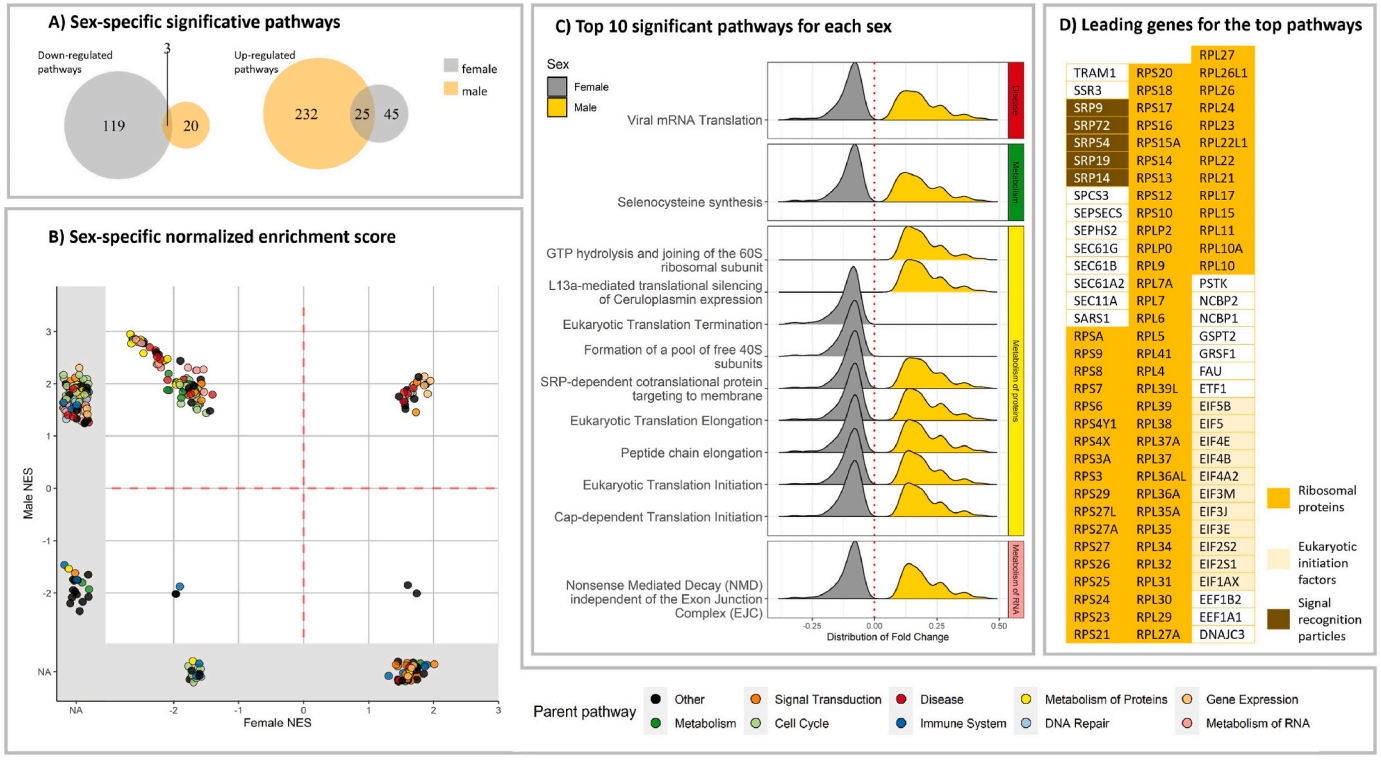


Fig. 6. Comparison of sex-specific pathway analysis. A) Intersection of pathways depending on sex. Common significant down-regulated and up-regulated pathways in males and females. **B) Comparison of the normalized enrichment scores (NES) of significant pathways** found in the sex-specific analyses, grouped by category. Pathways plotted on the grey areas present non-significant values in one of the sexes. **C) Top 10 pathways depending on sex.** Ridgeplot of the top 10 pathways with absolute larger NES for female PCC subjects (grey) and male PCC subjects (yellow), grouped according to pathway category. **D) Genes leading the sex-dependent pathways.** Prominent genes from major pathways with cell colouring based on pathway type: yellow for ribosomal proteins, cream for eukaryotic initiation factors, and brown for signal recognition particles.

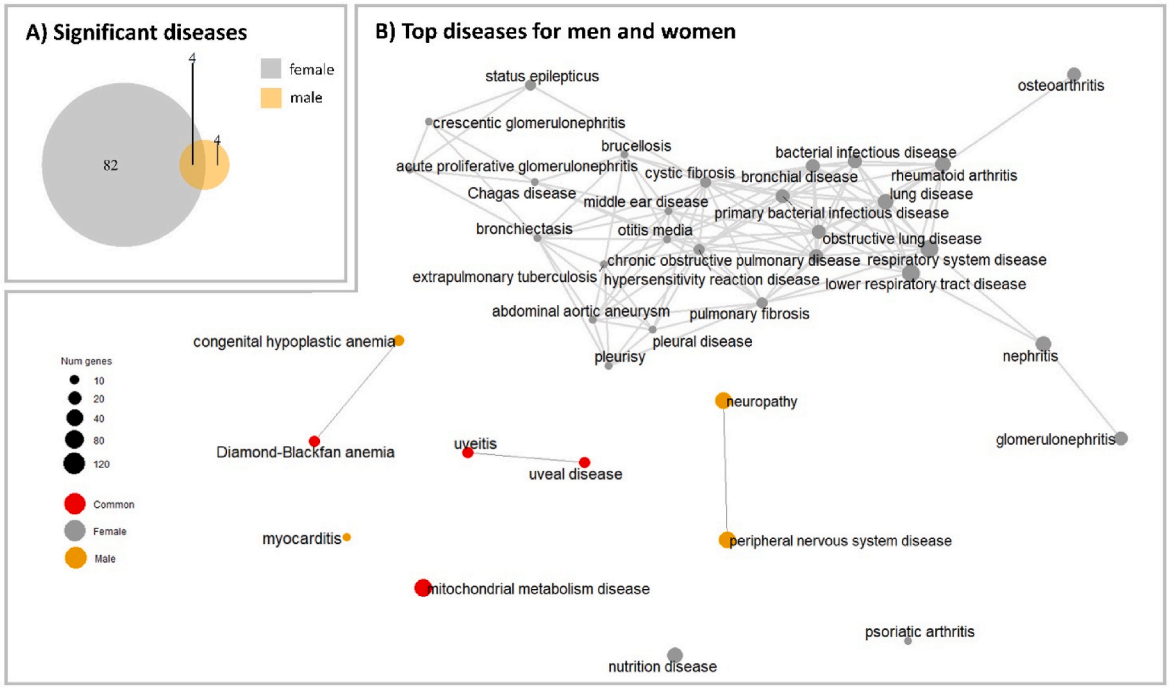


Fig. 7. Sex-dependant disease enrichment result. A) Intersection of diseases depending on sex. B) Top 30 most significant diseases in women and all significant diseases in men compared. Node sizes depend on the number of core enrichment genes. Common diseases are shown in red, while diseases and conditions unique to female in grey, and in gold for the ones in men.

point to the reactivation of dormant viruses, such as cytomegalovirus (CMV), also reported elsewhere [61]. Interestingly, several of the significant pathways directly involve or are led by histones, overexpressed in PCC. SARS-CoV-2 infection has been reported to promote persistent DNA methylation elsewhere [62–64].

The disease enrichment analysis revealed a link to several cardiovascular diseases, including atherosclerosis. The risk of cardiovascular diseases has been shown to increase after COVID-19 in several large-cohort studies [65–67] and, recently, Eberhardt and colleagues demonstrated SARS-CoV-2 presence and replication in macrophages from coronary vasculature [68]. Interestingly, several vision-related conditions also appear enriched, including uveitis, uveal disease and keratitis. Then, we built the PPI of DEGs and extracted the hub genes to perform a drug enrichment analysis. The drug with the most targets among the list of hub genes is the non-steroidal anti-inflammatory diclofenac. Among the top 10 most significant drugs, we found several phytochemicals with antioxidant properties, such as the chemically-related flavonoids hesperitin and its aglycone hesperidin, dracorhodin, an anthocyanin, and α-tocopherol and pterostilbene. In recent publications, a pivotal aspect of PCC has been identified, involving decreased antioxidant defences and increased oxidative stress toxicity [69], and antioxidant supplementation has been proposed as a viable adjuvant therapy for COVID-19 [70,71] as well as for PCC [72]. More specifically, flavonoids have been postulated to possess potent ACE1 inhibitory and ACE2 stimulatory effects, therefore being able to disrupt the interaction between ACE2 and SARS-CoV-2 [73]. The identification of drugs with antioxidant properties, such as diclofenac and hesperitin, highlights the function of oxidative stress in the pathology of PCC, echoing the growing consensus on the utility of antioxidants in mitigating the aftermath of post-viral syndromes highlighted by Mahmud et al. [74].Concurrently, our findings, reinforced by prior research, suggest the therapeutic potential of anti-inflammatory agents [75] in addressing PCC.

Another aspect to consider is that women are more prone to develop PCC [21] and PCC symptomatology is dependent on sex [22]. Our analysis revealed opposite expression patterns between male and female in response to PCC. When analysing the 10 top pathways most implicated in PCC in both women and men separately, we discovered that 8 of them were common but displayed divergent regulation—being down-regulated in women and up-regulated in men. Most of these pathways are related to protein synthesis (transcription and translation processes). For men, the up-regulation of these pathways might be associated with the observed higher instances of weight loss [76], suggesting a metabolic adaptation or stress response to the condition. In contrast, the down-regulation seen in women could reflect a distinct pathophysiological response, potentially contributing to the varied symptomatology reported between genders. Interestingly, the majority of the leading genes encode ribosomal proteins, critical for cellular protein synthesis. In this sense, SARS-CoV-2 infection was shown to affect ribosome biogenesis and lead to immune evasion and viral replication [77,78]. Additionally, since in another study that evaluated sex differences in the ribosomal profile of the immune response after a stroke was found a similar pattern [79], it can be suggested that the expression of sex-specific differential ribosome genes in PCC may be a key mechanism underlying the sexually dysmorphic difference in the immune response.

The disease enrichment analysis illustrates the sex-specific impact of PCC. For men, the disease was associated with neuro-cardiovascular disorders, while women show a broad spectrum of diseases and conditions, mainly focused on respiratory disorders, though renal and immunological conditions also appear associated. The enriched diseases identified in our study are consistent with clinical observations that women are more likely to report symptoms such as dyspnea, fatigue, chest pain, and palpitations [80]. This suggests a predisposition to developing more severe respiratory and autoimmune conditions post-COVID-19, potentially exacerbated by a female-predominant immune dysregulation, which could be influenced by the persistent immune dysregulation observed in women [76]. The exacerbated autoimmune response in women, potentially linked to the reduced oestrogen production during COVID-19 infection, may contribute to the heightened susceptibility to developing more severe conditions post-COVID-19 [81].

This is the first meta-analysis that integrates data from three independent RNA-seq datasets to delve into the pathogenesis of PCC at the gene expression level. While prior bioinformatics studies, such as those conducted by Mahmud et al. [74] and Lv et al. [75], have identified shared genes between COVID-19 and conditions like idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, or myalgic encephalomyelitis/chronic fatigue syndrome. Also, Zhang et al. [82] have investigated microRNA-gene interactions during COVID-19 infection, our analysis not only confirms these discoveries but also sheds light on the differential pathomechanisms of PCC by gender. In relation to the studies whose datasets we used, Feargal et al. reported an enrichment in pathway related to transcription, translation and ribosome synthesis in their dataset, but they did not report the sex-specific dynamic we observed [47]. One the other hand, Sparks et al. did report a sex dimorphic effect of mild cases of COVID-19 (independently of PCC status) [48]. The pre-print work of Yin et al. reports systemic inflammation and immune dysregulation in PCC subjects [49]. However, none of these studies finds significantly altered histone expression, which we could detect likely due to an increased statistical power; nor do they report a sex divergent expression of ribosomal proteins or link PCC to other conditions and propose therapeutic strategies.

The implemented bioinformatic workflow allowed us to characterize PCC, underscored by a complex interplay of immune dysregulation, metabolic shifts, and neuropsychiatric disturbances. By integrating data from multiple independent RNA-seq datasets and analyzing robust changes in gene expression across these datasets to identify highly connected genes, we have linked these gene expression changes with key pathways, diseases, and drugs. The implications of this study extend beyond the analysis of PCC. The methodologies applied—combining data integration, pathway analysis, and disease association mapping—offer a powerful approach to elucidate the pathophysiological mechanisms of various syndromes [74,75,83–85], enhancing our capacity to identify potential biomarkers and therapeutic targets. Importantly, our study's pathway analysis reveals key molecular pathways in PCC, while disease association mapping provides insights into related conditions. Identifying drugs like antioxidants and anti-inflammatory agents highlights promising interventions.

However, the study is not without limitations; the assessment of PCC symptoms and patients classification is beyond our control in this analysis and varies from study to study. Data on important covariates such as patient age or COVID-19 severity is not available for all of the datasets. Two of the three datasets extracted RNA from whole blood, while the latter used PBMCs samples, which do not include neutrophils, eosinophils, or reticulocytes. Due to the lack of sufficient datasets reporting transcriptomic profiles in other tissues, we have not been able to investigate gene expression in tissues directly affected by PCC, such as the central nervous system, nor in tissues primarily infected by SARS-CoV-2. Finally, the data presented here is on unvaccinated individuals while, as of today, 70.5% of the world population has received at least one dose of a COVID-19 vaccine [86]. Thus, these results may not generalize as effectively in light of the widespread vaccination.

5. Conclusion

In conclusion, this meta-analysis sheds light on the intricate molecular landscape and pathogenesis underlying PCC. We report a persistent immune dysregulation in patients with PCC, as well as alterations in DNA methylation and histone expression. Remarkably, our findings highlight an up-regulation of the histone family of proteins in PCC patients. We identify of drugs with antioxidant properties, such as

hesperitin, which can potentially mitigate the oxidative stress characteristic of PCC. In addition, our analysis reveals distinct sex-specific patterns, with ribosomal proteins emerging as central players in driving differential gene expression responses between males and females. These sex-specific differences extend to the associated diseases, with men exhibiting a predisposition to neuro-cardiovascular disorders, while women manifest a broader spectrum of mostly respiratory, but also renal and immunological conditions. Our study not only enhances our understanding of PCC pathophysiology but also underscores the importance of considering sex-specific dynamics in disease manifestation.

Funding

This study has been funded by funds from Agència de Gestió d'Ajuts Universitaris de Recerca (AGAUR 2017 SGR 00030–2021 Teresa Auguet), the Investigador actiu Program from the URV (2021-10-837-AUGUET to Teresa Auguet) and own financing.

CRediT authorship contribution statement

Elena Cristina Rusu: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Pablo Monfort-Lanzas:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Laia Bertran:** Writing – review & editing, Writing – original draft, Conceptualization. **Andrea Barrientos-Riosalido:** Writing – review & editing, Writing – original draft, Conceptualization. **Emilia Solé:** Writing – review & editing, Data curation. **Razieh Mahmoudian:** Writing – original draft, Data curation. **Carmen Aguilar:** Writing – original draft, Data curation. **Silvia Briansó:** Writing – review & editing, Data curation. **Fadel Mohamed:** Writing – review & editing, Data curation. **Susana Garcia:** Writing – review & editing, Data curation. **Javier Camaron:** Writing – review & editing, Data curation. **Teresa Auguet:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We extend our deepest gratitude to Dr. Johanna M. Gostner and Dr. Hubert Hackl from the Medical University of Innsbruck for their invaluable guidance and insightful critiques during the development of this study. Their expertise significantly enhanced the quality and depth of our research. We would also like to thank Dr David Lynn and Feargal Ryan from the South Australian Health and Medical Research Institute for promptly resolving our questions regarding their metadata. Part of the analysis described in this work were performed on the HPC cluster Garnatxa at Institute for Integrative Systems Biology (I2SysBio), I2SysBio is a mixed research centre formed by University of Valencia (UV) and Spanish National Research Council (CSIC).

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.compbiomed.2024.108507.

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