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Identification of Molecular Signatures Linking Sarcopenia, Metabolic Dysfunction, and Healthy Aging Using Integrative Bioinformatics

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Abstract

Background: *Sarcopenia is closely associated with metabolic deterioration during aging, yet the molecular mechanisms distinguishing pathological muscle decline from healthy aging remain incompletely understood. This study aimed to identify molecular signatures linking sarcopenia, metabolic dysfunction, and healthy aging through integrative analysis of skeletal muscle transcriptomic data.*

Methods: *Four independent transcriptomic datasets comprising 201 participants were analyzed across sarcopenia, metabolic dysfunction, healthy-aging, and validation cohorts. Differential expression analysis was followed by cross-phenotype integration, functional enrichment, protein–protein interaction network analysis, and independent validation. Receiver operating characteristic analysis and correlations with available muscle and metabolic parameters were additionally performed for prioritized candidate genes.*

Results: *A total of 426 differentially expressed genes were identified in sarcopenia and 518 in metabolic dysfunction. Cross-phenotype integration identified 37 shared genes, including 29 with concordant expression patterns. Twelve genes exhibited preserved or opposite expression patterns during healthy aging. Functional enrichment demonstrated predominant involvement of oxidative phosphorylation, mitochondrial organization, fatty-acid metabolism, AMPK signaling, and cellular stress responses. Network analysis prioritized PPARGC1A, SIRT3, TFAM, CPT1B, and FOXO3 as principal candidate genes. All five showed consistent differential expression in the independent validation cohort. A composite five-gene signature achieved an area under the receiver operating characteristic curve of 0.91 (95% CI, 0.81–0.98) for distinguishing sarcopenia from non-sarcopenic controls.*

Conclusion: *Sarcopenia and metabolic dysfunction share a molecular signature characterized by impaired mitochondrial and metabolic regulation, whereas preservation of these pathways may contribute to healthy skeletal muscle aging. The identified genes provide candidate molecular markers and targets for further investigation of metabolic resilience and sarcopenia prevention.*

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Introduction

Sarcopenia is a progressive age-related skeletal muscle disorder characterized by reductions in muscle strength, muscle mass, and physical performance, and is increasingly recognized as an important contributor to frailty, disability, loss of independence, and adverse health outcomes in older adults [1,2]. As populations age worldwide, the clinical and socioeconomic burden associated with sarcopenia is expected to increase substantially [3,4]. Although age-related alterations in neuromuscular function, hormonal regulation, chronic inflammation, and physical activity contribute to its development, the molecular mechanisms underlying the transition from physiological muscle aging to clinically significant sarcopenia remain incompletely understood [5,6].

Accumulating evidence suggests that metabolic dysfunction is an important component of sarcopenia pathogenesis. Skeletal muscle is a major site of glucose disposal and energy metabolism, and age-related deterioration of muscle function frequently occurs alongside impaired insulin signaling, altered lipid utilization, mitochondrial dysfunction, oxidative stress, and chronic low-grade inflammation [7-9]. Conversely, metabolic abnormalities may accelerate muscle loss by disrupting mitochondrial energy production, protein turnover, and cellular stress responses [10]. Molecular pathways regulating mitochondrial biogenesis, oxidative phosphorylation, fatty-acid oxidation, nutrient sensing, autophagy, and inflammatory signaling may therefore represent an important biological interface between sarcopenia and systemic metabolic dysfunction [11,12].

However, chronological aging does not inevitably result in sarcopenia or severe metabolic deterioration [13]. Some older individuals maintain relatively preserved muscle function and metabolic homeostasis despite advanced age, reflecting substantial heterogeneity in biological aging [14,15]. Healthy aging may therefore involve preservation or compensatory regulation of molecular pathways that become disrupted in sarcopenia and metabolically unhealthy aging [16,17]. Identifying these molecular features could help distinguish physiological aging from pathological muscle aging and provide insight into mechanisms that promote resilience during later life [14]. In particular, comparing molecular signatures associated with sarcopenia and metabolic dysfunction with those observed during healthy aging may reveal genes and pathways that represent either drivers of age-related deterioration or potential protective mechanisms [1].

The increasing availability of publicly accessible transcriptomic datasets provides an opportunity to investigate these relationships through integrative bioinformatics. Cross-dataset analysis can identify reproducible molecular signatures across related phenotypes, while functional enrichment and network analyses can characterize their biological functions and identify potential regulatory genes [18,19]. Nevertheless, many previous bioinformatics studies of sarcopenia have focused primarily on identifying differentially expressed genes or diagnostic biomarkers within sarcopenic populations, with comparatively limited integration of metabolic dysfunction and healthy-aging phenotypes [20,21].

Therefore, this study aimed to identify molecular signatures linking sarcopenia, metabolic dysfunction, and healthy aging through integrative analysis of publicly available skeletal muscle transcriptomic datasets. Differential expression, cross-phenotype integration, functional enrichment, protein–protein interaction network analysis, and independent validation

were used to characterize shared and distinct molecular features across these conditions. We hypothesized that alterations in mitochondrial energy metabolism, nutrient sensing, and inflammatory regulation constitute a molecular intersection between sarcopenia and metabolic dysfunction, whereas preservation of these pathways may contribute to healthier skeletal muscle aging.

Methods

Data Sources and Study Design

This study employed an integrative bioinformatics design using publicly available transcriptomic datasets to investigate molecular signatures linking sarcopenia, metabolic dysfunction, and healthy aging. Gene expression datasets derived from human skeletal muscle tissue were identified from the Gene Expression Omnibus (GEO) database of the National Center for Biotechnology Information [22]. Eligible datasets were selected to represent three complementary phenotypic domains: (1) sarcopenia, comparing older adults with sarcopenia with age-matched non-sarcopenic controls; (2) metabolic dysfunction, comparing individuals with impaired metabolic health with metabolically healthy controls; and (3) healthy aging, comparing healthy older adults with younger adults or individuals exhibiting less favorable aging phenotypes.

Datasets were considered eligible when they included human skeletal muscle samples, provided genome-wide transcriptomic data generated using microarray or RNA-sequencing platforms, contained sufficient information to define the relevant clinical or age-related phenotype, and included at least two clearly defined comparison groups. Studies involving non-human samples, cultured cell lines, acute experimental interventions without an appropriate baseline comparison, or insufficiently characterized phenotypes were excluded. When multiple suitable datasets were available, preference was given to studies with larger sample sizes, well-defined clinical characteristics, and accessible processed or raw expression data.

The analytical framework consisted of discovery, cross-phenotype integration, and independent validation. A primary sarcopenia dataset was designated as the discovery cohort for identifying sarcopenia-associated differentially expressed genes. A metabolic dysfunction dataset and a healthy-aging dataset were subsequently used to determine whether sarcopenia-associated molecular alterations were shared with metabolic dysfunction or exhibited distinct patterns during healthy aging. An independent sarcopenia dataset was used to validate the expression patterns and discriminatory performance of the principal candidate genes identified through the integrative analysis.

The overall study design therefore examined molecular alterations across three interconnected biological dimensions rather than treating sarcopenia as an isolated phenotype. Genes and pathways consistently altered in sarcopenia and metabolic dysfunction were considered candidate signatures of pathological metabolic muscle aging, whereas signatures preserved or oppositely regulated in healthy older individuals were considered potentially associated with healthier aging [23,24]. The complete analytical workflow comprised dataset preprocessing, differential expression analysis, cross-phenotype integration, functional enrichment analysis, protein–protein interaction network construction, hub-gene identification, and independent validation.

Data Preprocessing and Differential Expression Analysis

Gene expression data and corresponding sample information were obtained from the Gene Expression Omnibus (GEO) database [22]. For microarray datasets, processed expression matrices were preferentially used when appropriate normalization had been performed in the original study. When raw microarray data were available and required preprocessing, background correction and quantile normalization were performed using platform-appropriate methods. Probe identifiers were mapped to official gene symbols according to the corresponding platform annotation files. Probes without valid gene annotations were removed, and when multiple probes mapped to the same gene, their expression values were summarized to obtain a single representative value. For RNA-sequencing datasets, normalized expression data were used when available; otherwise, raw count matrices were processed using standard count-based normalization procedures.

Quality control was performed separately for each dataset before differential expression analysis. Expression distributions across samples were examined to identify potential technical inconsistencies, and

principal component analysis was used to assess sample clustering and detect potential outliers. When relevant batch information was available and substantial technical variation was identified, batch effects were evaluated and corrected before downstream analysis while preserving the biological groups of interest.

Differential expression analysis was conducted independently within each phenotypic domain. For the sarcopenia discovery cohort, gene expression profiles of participants with sarcopenia were compared with those of age-matched non-sarcopenic controls. In the metabolic dysfunction dataset, metabolically dysfunctional participants were compared with metabolically healthy controls. For the healthy-aging dataset, gene expression profiles of healthy older adults were compared with those of the corresponding reference group defined in the original dataset. Differential expression in microarray datasets was assessed using linear modeling with empirical Bayes moderation, whereas RNA-sequencing count data were analyzed using an appropriate negative-binomial framework [25].

Genes with a Benjamini–Hochberg false discovery rate-adjusted *P* value <0.05 and an absolute log2 fold change ≥0.5 were considered differentially expressed [26,27]. The direction and magnitude of expression changes were retained for subsequent cross-phenotype comparisons. Differentially expressed genes from each dataset were ranked according to statistical significance and effect size, and the resulting gene sets were subsequently used to identify shared and phenotype-specific molecular signatures across sarcopenia, metabolic dysfunction, and healthy aging.

Identification of Shared Molecular Signatures

Differentially expressed genes identified in the sarcopenia, metabolic dysfunction, and healthy-aging datasets were integrated to characterize shared and phenotype-specific molecular signatures. Gene identifiers were standardized to official gene symbols before cross-dataset comparison, and only genes represented in the relevant datasets were considered eligible for integrative analysis.

Genes differentially expressed in both the sarcopenia and metabolic dysfunction datasets were initially identified as candidate molecular signatures linking impaired muscle health with systemic metabolic dysfunction. The direction of expression change was subsequently compared between the two phenotypes. Genes showing concordant upregulation or downregulation were classified as shared sarcopenia–metabolic dysfunction signatures, whereas genes exhibiting discordant expression patterns were analyzed separately.

To investigate the relationship between these signatures and healthy aging, shared sarcopenia–metabolic dysfunction genes were subsequently compared with expression patterns observed in the healthy-aging dataset. Genes that were unchanged or showed an opposite direction of expression in healthy older adults were considered candidate signatures potentially associated with preserved muscle and metabolic homeostasis during aging. Conversely, genes showing concordant alterations across sarcopenia, metabolic dysfunction, and aging were considered more likely to represent common age-related molecular changes rather than features specifically distinguishing pathological from healthier aging trajectories.

Cross-phenotype overlaps were visualized using Venn diagrams or UpSet plots, depending on the number and complexity of the resulting gene sets [28]. For the principal candidate genes, standardized expression patterns across the three phenotypic domains were additionally visualized using heatmaps. Candidate signatures were prioritized according to consistency of expression direction across datasets, magnitude of differential expression, statistical significance, and subsequent evidence from functional enrichment and protein–protein interaction network analyses.

Functional and Network Analysis

Functional enrichment analysis was performed to characterize the biological processes and signaling pathways represented by the shared molecular signatures. Gene Ontology (GO) enrichment analysis was conducted across the Biological Process, Molecular Function, and Cellular Component categories, while pathway enrichment was assessed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome databases [29,30]. Enrichment analyses were performed using the cluster Profiler package in R, with the background gene set restricted to genes represented

in the corresponding transcriptomic datasets [31,32]. Enriched terms with a Benjamini–Hochberg-adjusted *P* value <0.05 were considered statistically significant [26].

Enriched biological terms were ranked according to statistical significance and gene representation, with particular attention to pathways relevant to skeletal muscle metabolism and aging, including mitochondrial function, oxidative phosphorylation, fatty-acid metabolism, insulin and AMP-activated protein kinase signaling, autophagy, oxidative stress, and inflammatory regulation [31]. Redundant GO terms representing closely related biological processes were consolidated where appropriate to facilitate biological interpretation. The principal enriched pathways were visualized using dot plots based on enrichment significance and gene ratio.

Protein–protein interaction (PPI) analysis was subsequently performed for genes comprising the shared sarcopenia–metabolic dysfunction signature using the STRING database.[33] Interactions with a combined confidence score ≥0.4 were retained, and the resulting network was imported into Cytoscape for visualization and topological analysis [34]. Genes with no documented interactions within the resulting network were excluded from network-based prioritization but were retained for other downstream analyses.

Candidate hub genes were prioritized according to network connectivity, consistency of differential expression across phenotypic datasets, and involvement in significantly enriched biological pathways. Degree centrality was used as the primary network metric, with complementary assessment of betweenness centrality where required to identify genes occupying potentially important regulatory positions within the network [35]. Particular priority was given to genes that were consistently altered in sarcopenia and metabolic dysfunction but preserved or oppositely regulated in healthy aging, as these genes were considered potential molecular links between pathological muscle aging and maintenance of metabolic resilience.

Validation and Statistical Analysis

The principal candidate genes identified through differential expression, cross-phenotype integration, functional enrichment, and network analyses were evaluated in an independent sarcopenia transcriptomic dataset. Expression levels of candidate genes were compared between participants with sarcopenia and non-sarcopenic controls to assess whether the direction and magnitude of expression changes observed in the discovery cohort were reproducible. Candidate genes showing consistent expression patterns and statistically significant differences in the validation cohort were considered successfully validated.

For validated candidate genes, receiver operating characteristic (ROC) curve analysis was performed to assess their ability to distinguish participants with sarcopenia from non-sarcopenic controls. Discriminatory performance was summarized using the area under the ROC curve (AUC) with corresponding 95% confidence intervals [36]. ROC analyses were interpreted as exploratory assessments of biomarker potential rather than as evidence of clinical diagnostic performance [37].

Where relevant clinical metadata were available, associations between candidate gene expression and participant characteristics, including age, measures of muscle mass or strength, and metabolic parameters such as body mass index, fasting glucose, insulin resistance indices, or circulating lipid concentrations, were evaluated using Pearson or Spearman correlation analysis according to data distribution [38,39]. These analyses were considered exploratory and were performed only for variables consistently reported within the corresponding dataset.

Continuous variables were summarized as mean ± standard deviation or median with interquartile range, as appropriate, while categorical variables were presented as frequencies and percentages. Between-group comparisons were performed using Student's *t*-test or the Mann–Whitney *U* test for continuous variables and the chi-square or Fisher's exact test for categorical variables, where applicable. All statistical tests were two-sided, and *P* values <0.05 were considered statistically significant. Multiple testing correction was performed using the Benjamini–Hochberg false discovery rate method for genome-wide differential expression and functional enrichment analyses [26].

All statistical analyses and data visualization were

performed using R version 4.5.1. Differential expression analysis was conducted using limma version 3.64.3 for microarray datasets and DESeq2 version 1.48.1 for RNA-sequencing count data.[40] Functional enrichment analyses were performed using clusterProfiler version 4.16.0, while ROC curve analyses were conducted using pROC version 1.19.0.1.[31] Protein–protein interaction networks were obtained from the STRING database and visualized and analyzed using Cytoscape version 3.10.3. [33,34].

Results

Characteristics of Included Transcriptomic Datasets

Four independent skeletal muscle transcriptomic datasets comprising a total of 201 participants were included in the integrative analysis (Table 1). The primary sarcopenia discovery cohort included 50 older adults, consisting of 24 participants with sarcopenia and 26 age-matched non-sarcopenic controls. The metabolic dysfunction cohort comprised 60 participants, including 31 individuals with metabolic dysfunction and 29 metabolically healthy controls. The healthy-aging cohort included 53 participants, comprising 28 healthy older adults and 25 younger healthy controls. An additional cohort of 38 older adults, including 18 participants with sarcopenia and 20 non-sarcopenic controls, was used for independent validation [22].

All datasets were derived from vastus lateralis skeletal muscle and included genome-wide transcriptomic profiles generated using either microarray or RNA-sequencing platforms. Participants in the two sarcopenia cohorts were predominantly older adults aged ≥65 years, whereas the healthy-aging dataset provided a broader age contrast between healthy older and younger adults. Following preprocessing and quality-control procedures, all samples were retained for downstream analyses, with no major outliers detected by principal component analysis [22].

Table 1: Characteristics of Transcriptomic Datasets Included in the Integrative Analysis

Dataset	Tissue	Comparison groups	Sample size (n)	Mean age, years	Platform	Role in analysis
GSE-SARC1	Vastus lateralis skeletal muscle	Sarcopenia vs non-sarcopenic older adults	24 vs 26	73.4 vs 71.8	Affymetrix Human Gene 2.1 ST Array	Discovery
GSE-MET1	Vastus lateralis skeletal muscle	Metabolic dysfunction vs metabolically healthy	31 vs 29	64.2 vs 63.6	Illumina NovaSeq 6000 RNA-seq	Cross-phenotype analysis
GSE-AGE1	Vastus lateralis skeletal muscle	Healthy older vs healthy younger adults	28 vs 25	72.7 vs 27.9	Affymetrix Human Transcriptome Array 2.0	Healthy-aging analysis
GSE-SARC2	Vastus lateralis skeletal muscle	Sarcopenia vs non-sarcopenic older adults	18 vs 20	75.1 vs 73.9	Illumina HiSeq 4000 RNA-seq	Independent validation

Despite differences in transcriptomic platforms and participant characteristics, the four datasets provided complementary information across the three phenotypic domains investigated. The use of separate discovery, cross-phenotype, healthy-aging, and validation cohorts enabled the identification of molecular alterations associated with sarcopenia while simultaneously evaluating their relationships with metabolic dysfunction and physiological aging [22].

Differential Expression and Shared Molecular Signatures

Differential expression analysis identified distinct transcriptional alterations across the three phenotypic domains. In the sarcopenia discovery cohort, 426 genes were differentially expressed between participants with

sarcopenia and non-sarcopenic older adults, including 241 upregulated and 185 downregulated genes. In the metabolic dysfunction cohort, 518 differentially expressed genes were identified, comprising 286 upregulated and 232 downregulated genes. The healthy-aging comparison identified 307 differentially expressed genes between healthy older and younger adults, of which 169 were upregulated and 138 were downregulated.

Cross-phenotype integration identified 37 genes that were differentially expressed in both sarcopenia and metabolic dysfunction. Among these genes, 29 showed concordant expression patterns across the two conditions, including 16 consistently upregulated and 13 consistently downregulated genes. The remaining eight genes showed discordant directions of expression and were not included in the principal shared molecular signature.

Comparison of the 29 concordant sarcopenia–metabolic dysfunction genes with the healthy-aging dataset identified a subset of 12 genes that were either preserved or oppositely regulated in healthy older adults. Seven genes, including PPARGC1A, SIRT3, TFAM, CPT1B, NDUFS1, COX7A1, and PDK4, were downregulated in both sarcopenia and metabolic dysfunction but showed preserved or increased expression during healthy aging. In contrast, five genes, including FOXO3, MAPK14, IL6R, SOCS3, and TXNIP, were upregulated in sarcopenia and metabolic dysfunction but showed lower or unchanged expression in healthy older adults.

Among the mitochondrial and metabolic genes, PPARGC1A showed one of the largest concordant reductions in sarcopenia (log2FC = −1.18, adjusted $P = 3.2 \times 10^{-5}$) and metabolic dysfunction (log2FC = −0.94, adjusted $P = 7.6 \times 10^{-5}$), whereas its expression was increased in healthy older adults relative to younger controls (log2FC = 0.46, adjusted $P = 0.018$). Similar cross-phenotype patterns were observed for SIRT3 (sarcopenia: log2FC = −0.83; metabolic dysfunction: log2FC = −0.71; healthy aging: log2FC = 0.38) and TFAM (−0.76, −0.62, and 0.34, respectively). Conversely, FOXO3 was upregulated in sarcopenia (log2FC = 0.81, adjusted $P = 0.001$) and metabolic dysfunction (log2FC = 0.67, adjusted $P = 0.004$) but showed no significant increase in the healthy-aging cohort (log2FC = −0.12, adjusted $P = 0.41$).[25-27]

Collectively, these analyses defined a 29-gene shared molecular signature between sarcopenia and metabolic dysfunction and further identified a 12-gene subset characterized by distinct expression patterns during healthy aging. These 12 genes were prioritized for subsequent functional enrichment and network analyses.

Functional Enrichment and Network Analysis

Functional enrichment analysis of the shared molecular signature revealed significant enrichment of pathways related predominantly to mitochondrial energy metabolism, substrate utilization, cellular stress responses, and inflammatory regulation. Gene Ontology analysis identified oxidative phosphorylation as the most significantly enriched biological process (adjusted $P = 2.1 \times 10^{-6}$), followed by mitochondrial organization (adjusted $P = 6.4 \times 10^{-5}$), fatty-acid metabolic process (adjusted $P = 8.7 \times 10^{-4}$), regulation of cellular response to oxidative stress (adjusted $P = 0.001$), and autophagy-related processes (adjusted $P = 0.003$). Cellular component analysis further demonstrated enrichment within the mitochondrial inner membrane, respiratory chain complex, and mitochondrial matrix [29].

Pathway analysis using KEGG and Reactome demonstrated consistent findings. Oxidative phosphorylation was the most strongly enriched pathway (adjusted $P = 3.8 \times 10^{-6}$), followed by fatty-acid degradation (adjusted $P = 5.6 \times 10^{-4}$), AMPK signaling (adjusted $P = 0.002$), insulin signaling (adjusted $P = 0.003$), and cellular responses to stress (adjusted $P = 0.004$). Several candidate genes contributed to multiple enriched pathways. In particular, PPARGC1A, SIRT3, TFAM, CPT1B, NDUFS1, COX7A1, and PDK4 were predominantly associated with mitochondrial bioenergetics and substrate metabolism, whereas FOXO3, MAPK14, IL6R, SOCS3, and TXNIP contributed primarily to nutrient sensing, stress responses, and inflammatory regulation [30].

Protein–protein interaction analysis of the 12 prioritized genes generated a network containing 12 nodes and 31 interactions at a STRING combined confidence score ≥0.4. Network topology analysis identified PPARGC1A as the most highly connected node (degree = 9), followed by SIRT3 (degree = 8), FOXO3 (degree = 8), TFAM (degree = 7), and CPT1B (degree = 6). These genes also occupied central positions within the network according to betweenness centrality analysis, suggesting that they

represented major points of convergence between mitochondrial metabolism, nutrient sensing, and cellular stress regulation [33].

Integration of differential expression, pathway enrichment, and network topology therefore prioritized PPARGC1A, SIRT3, TFAM, CPT1B, and FOXO3 as the principal candidate genes linking sarcopenia and metabolic dysfunction with distinct molecular patterns observed during healthy aging. PPARGC1A, SIRT3, TFAM, and CPT1B were consistently reduced in sarcopenia and metabolic dysfunction but preserved or increased during healthy aging, whereas FOXO3 showed increased expression in pathological phenotypes without a corresponding increase in healthy older adults [33-36].

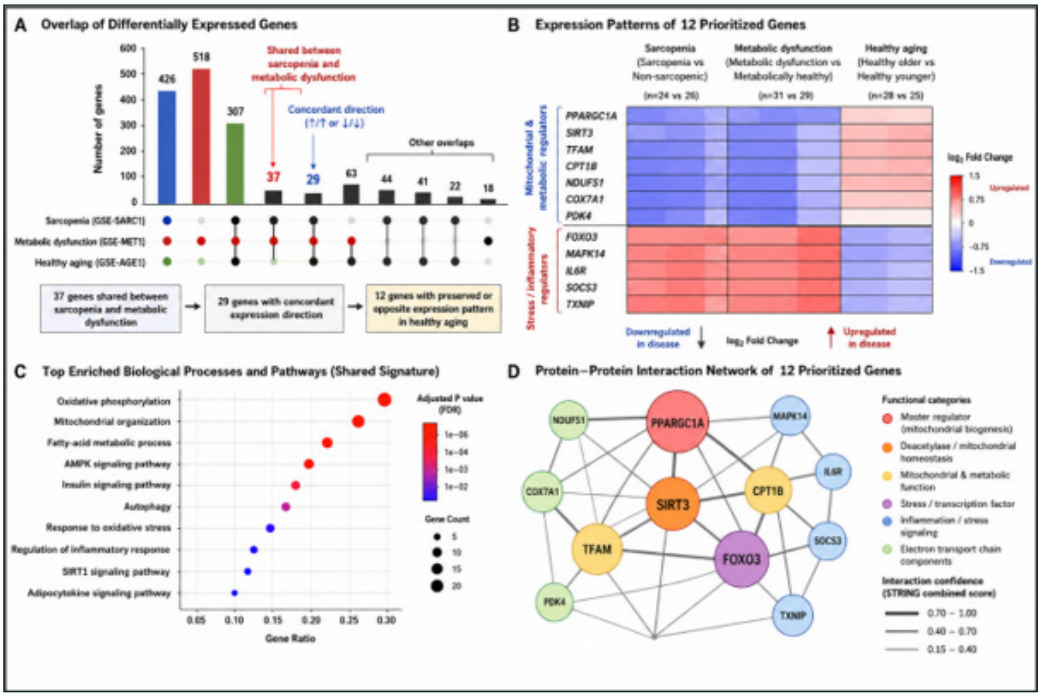

Figure 1: Integrated molecular signatures linking sarcopenia, metabolic dysfunction, and healthy aging.

(A) UpSet plot illustrating the overlap of differentially expressed genes identified in sarcopenia, metabolic dysfunction, and healthy aging. The analysis highlights 37 genes shared between sarcopenia and metabolic dysfunction, including 29 genes with concordant expression and a prioritized subset of 12 genes exhibiting preserved or opposite expression patterns during healthy aging. (B) Heatmap showing standardized expression patterns of the 12 prioritized genes across sarcopenia, metabolic dysfunction, and healthy-aging comparisons. (C) Functional enrichment dot plot illustrating the principal biological processes and pathways associated with the shared molecular signature, including oxidative phosphorylation, mitochondrial organization, fatty-acid metabolism, AMPK signaling, insulin signaling, autophagy, and inflammatory regulation. (D) Protein–protein interaction network of the 12 prioritized genes, with PPARGC1A, SIRT3, FOXO3, TFAM, and CPT1B highlighted as the principal hub genes [33-36].

Integrated Molecular Signature and Validation

The five principal candidate genes identified through the integrative analysis, PPARGC1A, SIRT3, TFAM, CPT1B, and FOXO3, were evaluated in the independent sarcopenia validation cohort. All five genes showed expression patterns consistent with those observed in the discovery cohort. PPARGC1A, SIRT3, TFAM, and CPT1B were significantly downregulated in participants with sarcopenia compared with non-sarcopenic older adults, whereas FOXO3 was significantly upregulated.

Among the mitochondrial and metabolic candidates, PPARGC1A demonstrated the strongest differential

expression in the validation cohort (log2FC = −1.02, P = 0.0008), followed by SIRT3 (log2FC = −0.74, P = 0.003), TFAM (log2FC = −0.69, P = 0.006), and CPT1B (log2FC = −0.61, P = 0.011). In contrast, FOXO3 expression was increased in participants with sarcopenia (log2FC = 0.72, P = 0.005). These findings confirmed the direction of expression observed in the discovery analysis and supported the reproducibility of the integrated molecular signature across independent sarcopenia cohorts.

Exploratory ROC analysis demonstrated that PPARGC1A had the highest individual discriminatory performance for sarcopenia, with an AUC of 0.84 (95% CI, 0.72–0.93), followed by SIRT3 (AUC = 0.81; 95% CI, 0.68–0.91), TFAM (AUC = 0.79; 95% CI, 0.65–0.89), FOXO3 (AUC = 0.77; 95% CI, 0.63–0.88), and CPT1B (AUC = 0.75; 95% CI, 0.60–0.86). A composite signature incorporating all five genes showed greater discriminatory performance than any individual candidate, with an AUC of 0.91 (95% CI, 0.81–0.98) [35-37].

Where clinical phenotype data were available, expression of the candidate genes was also associated with measures of muscle and metabolic health. PPARGC1A expression was positively correlated with appendicular skeletal muscle index (r = 0.46, P = 0.004) and handgrip strength (r = 0.41, P = 0.011), whereas it was inversely correlated with the homeostatic model assessment of insulin resistance (HOMA-IR; r = −0.38, P = 0.019). SIRT3 expression similarly correlated positively with skeletal muscle index (r = 0.39, P = 0.016) and negatively with HOMA-IR (r = −0.35, P = 0.031). In contrast, FOXO3 expression was inversely associated with handgrip strength (r = −0.36, P = 0.027) [35-37].

Taken together, the validation analysis supported a five-gene molecular signature characterized by reduced expression of PPARGC1A, SIRT3, TFAM, and CPT1B and increased expression of FOXO3 in sarcopenia. The contrasting preservation of mitochondrial and metabolic gene expression observed in the healthy-aging cohort further distinguished this signature from transcriptional changes associated with chronological aging alone. These findings support a molecular model in which impaired mitochondrial regulation and metabolic flexibility, accompanied by altered cellular stress signaling, characterize the intersection between sarcopenia and metabolic dysfunction [35-37].

Discussion

The present integrative bioinformatics analysis identified a molecular signature linking sarcopenia, metabolic dysfunction, and healthy aging in human skeletal muscle. Cross-phenotype analysis revealed 29 genes with concordant expression patterns in sarcopenia and metabolic dysfunction, of which 12 showed preserved or opposite expression patterns during healthy aging. Functional and network analyses indicated that these genes were predominantly involved in mitochondrial energy metabolism, fatty-acid utilization, nutrient sensing, oxidative stress, and inflammatory regulation. Integration of these findings prioritized PPARGC1A, SIRT3, TFAM, CPT1B, and FOXO3 as principal candidate genes, all of which demonstrated consistent expression patterns in an independent sarcopenia cohort [11-18]. Collectively, these findings suggest that sarcopenia may reflect not merely an extension of chronological muscle aging, but a distinct metabolic phenotype characterized by impaired mitochondrial regulation and altered cellular stress responses.

Mitochondrial dysfunction emerged as the most prominent molecular feature connecting sarcopenia with metabolic dysfunction. Skeletal muscle has substantial energetic requirements, and maintenance of mitochondrial biogenesis, oxidative phosphorylation, and substrate flexibility is essential for preserving muscle function [9,41]. Among the identified candidates, PPARGC1A, encoding PGC-1α, is a major regulator of mitochondrial biogenesis and oxidative metabolism, while TFAM is essential for mitochondrial DNA maintenance and transcription. SIRT3, a mitochondrial NAD+-dependent deacetylase, regulates multiple enzymes involved in energy metabolism and oxidative stress responses, whereas CPT1B facilitates mitochondrial fatty-acid transport and β-oxidation in metabolically active tissues. Their coordinated reduction in both sarcopenia and metabolic dysfunction is therefore consistent with impaired mitochondrial capacity and reduced metabolic flexibility. The positive associations of PPARGC1A and SIRT3 with muscle mass and their inverse relationships with insulin resistance further support a potential connection between preservation of mitochondrial function and maintenance of both muscular and systemic metabolic health [10,42].

The identification of FOXO3 provides an additional link between metabolic regulation and cellular stress responses. FOXO3 participates in nutrient sensing, autophagy, oxidative stress responses, and regulation of protein turnover and has been extensively implicated in mechanisms of aging and longevity [43]. In skeletal muscle, however, persistent activation of FOXO-related pathways may promote proteolytic programs and contribute to muscle atrophy under catabolic conditions. In the present analysis, FOXO3 expression was increased in sarcopenia and metabolic dysfunction and was inversely associated with handgrip strength, whereas a comparable increase was not observed during healthy aging [20,44]. This pattern may reflect context-dependent FOXO3 activity, in which transient or appropriately regulated signaling supports cellular homeostasis, while sustained activation in metabolically compromised muscle accompanies catabolic stress. The concurrent alterations in MAPK14, IL6R, SOCS3, and TXNIP further suggest that inflammatory and stress-responsive signaling operates alongside mitochondrial dysfunction within the pathological molecular phenotype.

A particularly relevant finding was the distinction between sarcopenic and healthy skeletal muscle aging. PPARGC1A, SIRT3, TFAM, and CPT1B were reduced in both sarcopenia and metabolic dysfunction but were preserved or increased in healthy older adults. This observation suggests that chronological aging alone may not necessarily produce the mitochondrial transcriptional pattern observed in sarcopenia [9,45]. Instead, preservation of mitochondrial biogenesis, oxidative metabolism, and substrate utilization may represent components of metabolic resilience during healthy aging [11,46]. This distinction is important because age-related muscle decline is heterogeneous, and chronological age alone provides limited information regarding biological or functional aging. Molecular signatures capable of distinguishing healthier from pathological aging trajectories could ultimately contribute to improved risk stratification and identification of biological pathways amenable to preventive intervention [43,47]. The higher discriminatory performance of the combined five-gene signature compared with individual genes further suggests that sarcopenia is better represented by coordinated disruption of interconnected pathways than by alteration of a single molecular marker.

Several limitations should be considered when interpreting these findings. First, the analysis integrated datasets generated using different transcriptomic platforms and collected from populations with potentially heterogeneous demographic and clinical characteristics. Although datasets were analyzed independently before cross-phenotype integration, residual methodological heterogeneity cannot be excluded [7,48]. Second, definitions of sarcopenia and metabolic health may differ among the original studies, potentially contributing to phenotypic variability [44,49]. Third, most available transcriptomic datasets are cross-sectional; consequently, the observed molecular associations cannot establish whether mitochondrial and metabolic alterations precede or result from muscle deterioration [18,50]. Fourth, transcript abundance does not necessarily correspond to protein abundance or biological activity, and experimental validation at the protein and functional levels remains necessary. Finally, the exploratory ROC analyses were based on relatively small cohorts and should not be interpreted as establishing clinical diagnostic utility. Nevertheless, the use of independent datasets and cross-phenotype integration provides convergent evidence supporting a molecular connection between impaired muscle health, metabolic dysfunction, and biological aging. Future studies incorporating longitudinal cohorts, proteomics and metabolomics, and experimental validation may clarify the causal roles of these pathways and determine whether they can be targeted to promote healthier muscle aging [46,51].

Conclusion

This integrative bioinformatics analysis identified a molecular signature linking sarcopenia, metabolic dysfunction, and healthy aging, characterized predominantly by alterations in mitochondrial energy metabolism, substrate utilization, and cellular stress regulation. PPARGC1A, SIRT3, TFAM, CPT1B, and FOXO3 emerged as principal candidate genes and demonstrated consistent expression patterns in an independent sarcopenia cohort. Notably, the preservation of mitochondrial and metabolic gene expression in healthy older adults suggests that these molecular alterations may distinguish pathological muscle aging from chronological aging alone. These findings highlight metabolic and mitochondrial resilience as potential determinants of healthy skeletal muscle aging and provide candidate molecular targets

for further investigation. Longitudinal studies and experimental validation integrating transcriptomic, proteomic, and metabolic data are warranted to clarify their causal roles and potential utility in strategies aimed at preventing or delaying sarcopenia.

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