Observational Study

Medicine

OPEN

Causal role of mitochondrial proteins in aortic aneurysms

Evidence from Mendelian randomization, transcriptomic analysis, and experimental validation

Adilai Abodulikemu, MD[a], Li Li, MD[b], Mukamengjiang Juaiti, MD[b,*]

Abstract

Mitochondrial dysfunction has been implicated in the pathogenesis of aortic aneurysms (AA); however, the causal role of mitochondrial-related proteins remains unclear. This study employs a Mendelian randomization (MR) approach to investigate the potential causal relationship between mitochondrial proteins and AA. Genetic instruments for mitochondrial proteins were obtained from the IEU Open genome-wide association study database, while AA-related genetic data were sourced from the FinnGen biobank. Inverse-variance weighting (IVW) served as the primary MR method, with MR-Egger and weighted median approaches utilized as complementary methods. Sensitivity analyses, including Cochran Q test, MR-Egger intercept, and MR-PRESSO, were performed to assess heterogeneity and pleiotropy. Reverse MR analysis was conducted to exclude the possibility of reverse causation. To enhance the robustness of the findings, replication was carried out using genome-wide association study Catalog data, and a meta-analysis was performed by integrating discovery and replication datasets. Gene expression validation was conducted using the Gene Expression Omnibus dataset, and gene set enrichment analysis (GSEA) was applied to explore relevant biological pathways. Additionally, in vitro experiments employing platelet-derived growth factor-BB-induced human aortic smooth muscle cells were performed to validate the expression patterns of mitochondrial-related proteins at both mRNA and protein levels. Through rigorous genetic variant selection, MR analysis using IVW, sensitivity analyses, replication, and meta-analysis, we identified iron–sulfur cluster assembly enzyme (ISCU), 39S ribosomal protein L14 (MRPL14), and mitochondrial peptide methionine sulfoxide reductase (MSRA) as mitochondrial proteins associated with AA. Sensitivity analyses confirmed the robustness of these findings, with no evidence of heterogeneity or pleiotropy. Reverse MR analysis ruled out reverse causation. Gene expression analysis demonstrated that ISCU was significantly upregulated, whereas MRPL14 and MSRA were downregulated in AA tissues. GSEA revealed that these proteins are involved in pathways related to inflammation, immune response, and vascular remodeling. In vitro experiments further corroborated these findings, demonstrating consistent expression patterns in platelet-derived growth factor-BB-induced human aortic smooth muscle cells. This study provides robust genetic and experimental evidence supporting the causal role of ISCU, MRPL14, and MSRA in AA pathogenesis. These mitochondrial proteins may serve as potential biomarkers and therapeutic targets for AA, warranting further investigation.

Abbreviations: AA = aortic aneurysms, GSEA = gene set enrichment analysis, ISCU = iron–sulfur cluster assembly enzyme, IVs = instrumental variables, IVW = inverse-variance weighting, MR = Mendelian randomization, MRPL14 = 39S ribosomal protein L14, MSRA = mitochondrial peptide methionine sulfoxide reductase, SNP = single nucleotide polymorphism, VSMC = vascular smooth muscle cell.

Keywords: aortic aneurysm, causal Inference, Mendelian randomization, mitochondrion, sensitivity analysis

The authors have no funding and conflicts of interest to disclose.

The datasets generated and analyzed in this study are available from publicly accessible databases, including the IEU OpenGWAS Project (https://gwas.mrcieu.ac.uk/), the FinnGen Project (https://www.finngen.fi/), and the GWAS Catalog (https://www.ebi.ac.uk/gwas/). Additionally, the GSE57691 microarray dataset was obtained from the NCBI Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). Any additional raw data supporting the conclusions of this study will be made available by the corresponding author upon reasonable request.

The datasets generated during and/or analyzed during the current study are publicly available.

Mendelian Randomization Section: This study utilized publicly available summary data from previously conducted GWAS. The original GWAS obtained informed consent from participants, and the data was de-identified and made openly accessible for research purposes. GEO Data Section: The gene expression dataset GSE57691 was obtained from a publicly available database and complied with all relevant ethical guidelines. Cell Experiment Section: All in vitro experiments were approved by the Ethics Committee of the Fourth Hospital of Changsha.

Supplemental Digital Content is available for this article.

[a] Department of Coronary Care Unit, Traditional Chinese Medical Hospital of Xinjiang Uygur Autonomous Region, The Affiliated Hospital of Traditional Chinese Medicine of Xinjiang Medical University, Urumqi, China, [b] Department of Cardiology, Changsha Institute of Cardiovascular Medicine, Changsha Fourth Hospital, Changsha, China.

** Correspondence: Mukamengjiang Juaiti, Department of Cardiology, Changsha Institute of Cardiovascular Medicine, Changsha Fourth Hospital, Changsha, China (e-mail: 632150871@qq.com).*

How to cite this article: Abodulikemu A, Li L, Juaiti M. Causal role of mitochondrial proteins in aortic aneurysms: Evidence from Mendelian randomization, transcriptomic analysis, and experimental validation. Medicine 2025;104:10(e41757).

Received: 5 November 2024 / Received in final form: 5 February 2025 / Accepted: 14 February 2025

http://dx.doi.org/10.1097/MD.0000000000041757

1. Introduction

Aortic aneurysm (AA) is a potentially life-threatening condition with severe consequences and extensive health risks.[1] Studies indicate that even in cases where the aneurysm has not ruptured, mortality rates remain significantly high.[2,3] In cases of rupture, the mortality rate can escalate to over 90%, making it a significant threat in the field of cardiovascular diseases.[4,5] Faced with this serious health challenge, we cannot ignore its underlying pathological mechanisms. Instead, it is essential to investigate its causes and potential contributing factors thoroughly. This study aims to explore the complex relationship between aortic aneurysms and the role of mitochondria, assessing how it significantly affects the functionality of aortic wall cells and overall vascular health, with the goal of identifying efficient preventive and treatment approaches.

In recent years, the relationship between mitochondria and AA has drawn increasing attention.[6] As the powerhouse of the cell, mitochondria provide adenosine triphosphate (ATP) through oxidative phosphorylation, supporting normal physiological functions. In the pathological process of AA, mitochondrial dysfunction is considered 1 of the key factors affecting the structural stability of the aortic wall.[7] When mitochondrial function is impaired, aortic smooth muscle cells fail to receive adequate energy support, leading to decreased cellular function and survival rates.[8] This, in turn, weakens the aortic wall, making it more prone to expansion and rupture, ultimately leading to the formation of an AA. Research has shown that mitochondrial dysfunction is a core mechanism in the development of aneurysms, particularly in vascular smooth muscle cells, where alterations in mitochondrial function profoundly affect the integrity of the aortic wall.[9] Targeting mitochondrial pathways is a possible approach for preventing and treating AA.[10] Dynamin-related protein 1 is a crucial regulatory protein in mitochondrial fission, and studies have found that Dynamin-related protein 1 expression is significantly elevated in aneurysmal tissue from AA patients, suggesting its role in driving mitochondrial fission and the progression of AA.[11] By inhibiting Dynamin-related protein 1 or other proteins involved in mitochondrial fission, the balance of mitochondrial dynamics can be regulated, protecting cellular function and delaying or preventing the onset and progression of AA. Furthermore, the mitochondrial quality control mechanism is central to maintaining mitochondrial health and functional stability.[12] The mitochondrial quality control mechanism ensures the dynamic balance of mitochondria within cells by processes such as mitophagy, fusion, and fission to remove damaged mitochondria. In AA patients, studies have indicated that mitophagy may be impaired, resulting in the accumulation of damaged mitochondria that are not effectively cleared. This imbalance exacerbates the decline in mitochondrial function. The increase in mitochondrial fission and the decrease in fusion lead to the accumulation of damage, increased cell apoptosis, and accelerated pathological progression of AA.[13] In summary, the crucial involvement of mitochondria in the development and advancement of AA indicates that managing mitochondrial function, especially the equilibrium between fission and fusion, presents novel strategies and potential targets for preventing and treating AA.

Mendelian randomization (MR), a technique used in epidemiology, utilizes genetic variants as instrumental variables (IVs) to evaluate the cause-and-effect relationships between risk factors and outcomes.[14] MR, a technique used in epidemiology, utilizes genetic variants as IVs to assess the cause-and-effect relationships between risk factors and outcomes. This approach is compelling in its capacity to reduce confounding by leveraging the random assortment of alleles at conception, which mimics the conditions of a randomized controlled trial. Unlike traditional observational studies, which are often susceptible to residual confounding and reverse causation, MR minimizes these biases and enhances the reliability of causal inferences. Furthermore, the use of large-scale genome-wide association study datasets strengthens statistical power and allows for robust assessments of causal relationships across diverse biological pathways.[15] Therefore, this study aims to employ MR to explore the possible cause-and-effect link between mitochondrial factors and AA, while also exploring the underlying biological mechanisms. This could provide valuable insights for the medical field and contribute to advancements in this area of research.

2. Materials and methods

2.1. Study design

This MR analysis relies on 3 core assumptions, as shown in Figure 1. First, the genetic instruments must have a strong association with the relevant exposure. Second, these genetic variants should not be related to any confounders. Third, the connection between the genetic instruments and the outcome must only arise through their effect on the exposure.[16]

2.2. Data source

Single nucleotide polymorphisms (SNP) linked to mitochondrial function were sourced from the IEU Open genome-wide association study (GWAS) project, a platform providing publicly available summary statistics for 66 mitochondrial-related proteins. These data were collected from a cohort of 3301 healthy blood donors of European ancestry enrolled in the INTERVAL study (Table S1, Supplemental Digital Content, http://links.lww.com/MD/O460). In compliance with national research ethics guidelines, all participants gave informed consent and provided comprehensive demographic information. To reduce potential biases arising from sample overlap, we used genetic association data related to aortic aneurysm from the FinnGen Biobank, which is publicly accessible at https://r11.finngen.fi/. This dataset includes 8923 cases of European ancestry and 420,324 controls. To assess the credibility of candidate mitochondrial proteins, we conducted replication and meta-analysis using data from the GWAS Catalog (GCST90044010), including 456,348 Europeans, of whom 258 were AA patients, and 456,090 were controls.[17]

2.3. Instrumental variables selection

The instrumental variables (IVs) were selected using a significance threshold of $P < 5 \times 10^{-6}$, with a clumping distance of 10,000 kb and an r^2 threshold of <.001 to minimize linkage disequilibrium. To evaluate the strength of the selected IVs, the *F*-statistic was calculated using the formula *F*-statistics ($F = beta^2/se^2$). This statistic measures how much variance in the exposure is accounted for by the selected IVs.[18] Generally, an *F*-statistic value below 10 suggests a weak instrument.[19] In this analysis, all SNPs had *F*-statistics >10, indicating that the IVs were strongly associated with the exposure (Table S2, Supplemental Digital Content, http://links.lww.com/MD/O460).

2.4. MR analysis

We examined the relationship between mitochondrial-related exposures and AA using 3 different MR methods: MR-Egger, inverse-variance weighting (IVW), and the weighted median approach. Among these, IVW is the most commonly used method in MR studies due to its ability to efficiently calculate a weighted average of effect estimates from each IV, assuming all IVs are valid.[20] Estimates with lower standard errors contribute more to the final result, improving precision and reliability.[21]

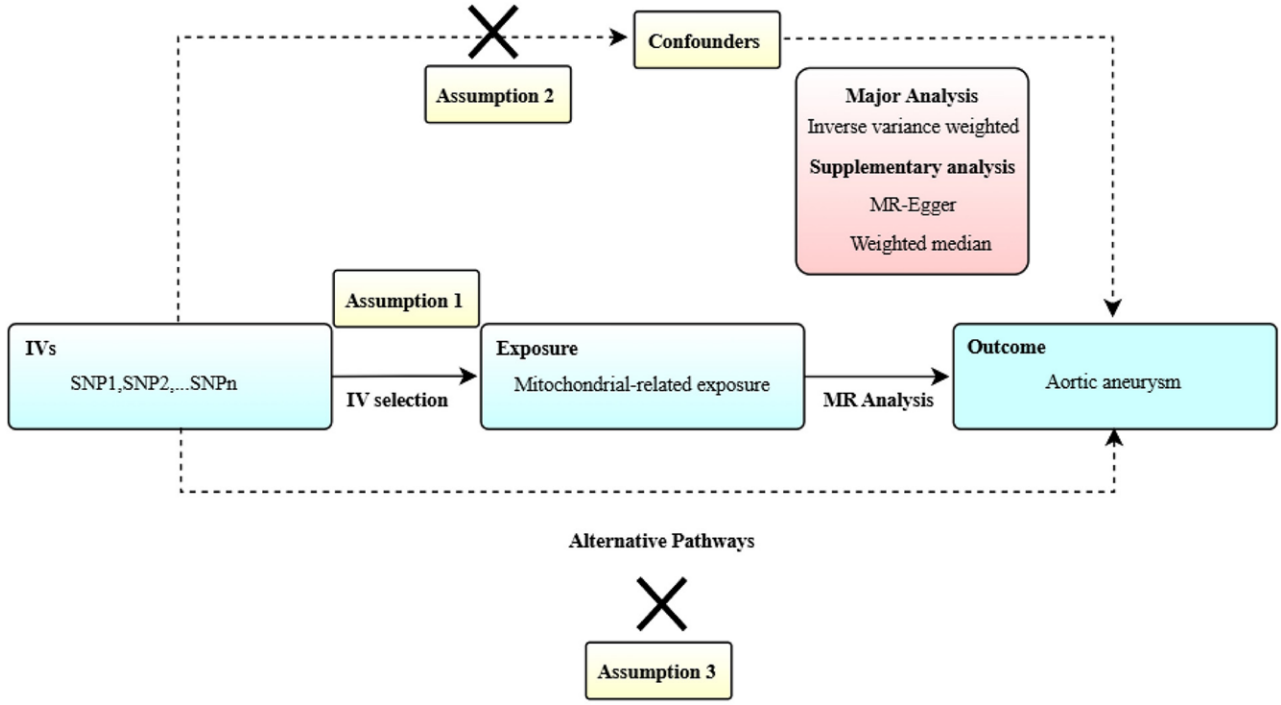

Figure 1. Study design.

Thus, IVW was reported as the primary analytical outcome. Additional methods were employed to ensure the robustness of our findings. MR-Egger regression is particularly advantageous for detecting and adjusting for horizontal pleiotropy, even when some IVs are invalid, under the InSIDE assumption.[22] The weighted mode approach estimates causal effects by focusing on the most frequently weighted values, reducing the influence of outliers and enhancing result stability.[23] Sensitivity analyses, including MR-Egger intercept and MR-PRESSO tests, were conducted to assess horizontal pleiotropy, with a *P*-value under .05 indicating its presence.[24] Cochran *Q*-test was applied to test for heterogeneity, where a *P*-value below .05 implied significant heterogeneity.[25] To further validate the robustness of our results, leave-one-out analysis was performed, confirming that excluding any individual SNP did not substantially alter the overall findings.

Additionally, reverse causality between mitochondrial proteins and AA was examined by treating AA as the exposure and mitochondrial proteins as the outcome. SNPs strongly associated with AA ($P < 5 \times 10^{-6}$) were selected as IVs. This study followed the STROBE-R guidelines to ensure proper reporting, and dual-sample MR analyses were conducted using the "TwoSampleMR" package in R version 4.3.1.[26]

2.5. Gene expression data analysis and gene set enrichment analysis

The GSE57691 microarray dataset was obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/), utilizing the GPL10558 Illumina HumanHT-12 V4.0 platform. This dataset includes 10 control samples and 49 aortic tissue samples from AA patients. Differential gene expression analysis was performed using the limma package in R.[27,28] The expression levels of specific genes were visualized using boxplots to highlight their differential expression patterns. gene set enrichment analysis (GSEA) was conducted with the clusterProfiler package and the c2.cp.kegg.symbols.gmt gene set collection. The results were saved for further analysis.[29,30]

2.6. Cell culture and processing

Human aortic VSMCs (vascular smooth muscle cells), obtained from Guangzhou Gene Biotechnology Co., Ltd., were maintained in Dulbecco Modified Eagle Medium supplemented with 10% fetal bovine serum. Afterward, the cells were transferred to a serum-free medium for further culture. Platelet-derived growth factor-BB (PDGF-BB)-induced collagen deposition and disruption of extracellular matrix (ECM) homeostasis have been linked to increased arterial wall stiffness, potentially promoting the progression of aortic aneurysms.[31–33] In this study, we treated HASMCs with PDGF-BB to create an in vitro model of aortic aneurysms. Specifically, the cells were exposed to 20 ng/mL PDGF-BB (Sigma-Aldrich) for 12 hours.

2.7. Real-time quantitative PCR

The RNA from cells was extracted using Trizol (Beyotime, China) and reverse transcribed into cDNA through a synthesis kit containing specific primers and SYBR green reaction mix (Beyotime, China). Real-time qPCR was performed, followed by the calculation of relative gene expression levels using the 2–ΔΔCT approach. The primer sequences: ISCU forward: 5′-GTCTGGATGTGCTGGCTGAA-3′, reverse: 5′-TGCACTTCACGGGCTATCAA-3′; MRPL14 forward: 5′-GCGCTTGGCTGGATCTATCT-3′, reverse: 5′-GCCAGTAGTATCTGGTCGCC-3′; MSRA forward: 5′-TCCTCCTCCACAGCCTCTTT-3′, reverse: 5′-CGGATGTCGGTAGTGATGGG-3′.

2.8. Western blot

Proteins were extracted from cells using a protein extraction kit containing protease and phosphatase inhibitors (Qichun, China, YWB0501). Protein concentration was measured using the BCA protein assay kit (Beyotime, P0010). The proteins were denatured at 100 °C for 10 minutes, separated using a 10% SDS-PAGE gel, and transferred onto polyvinylidene fluoride membranes (Roche, Basel, Switzerland). The membranes were then blocked at room temperature for 10 minutes with a

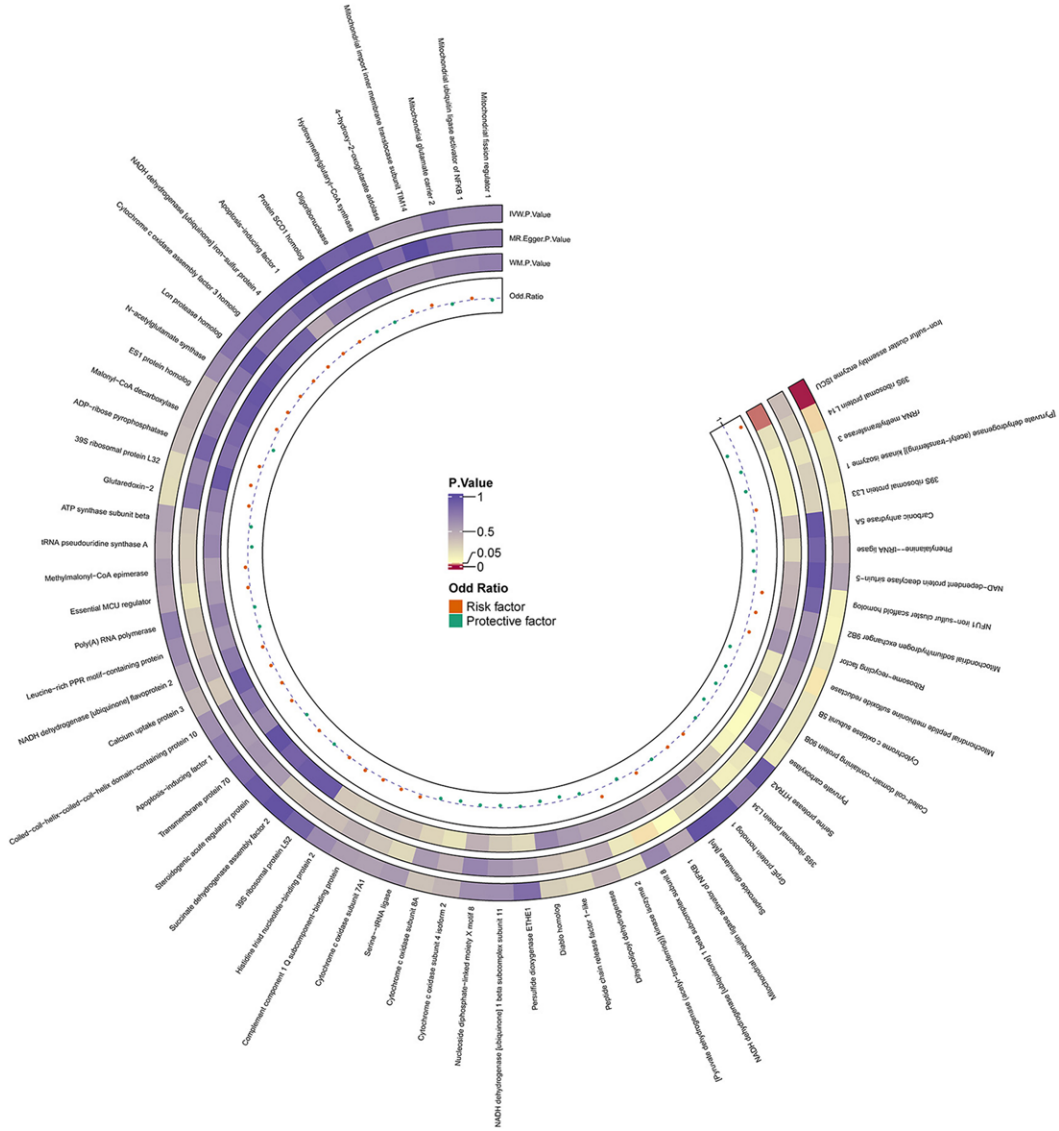

Figure 2. The causal relationship between mitochondrial proteins and aortic aneurysm of the discovery analysis.

blocking solution (Qichun, China, YWB0501). The membranes were then incubated overnight at 4 °C with primary antibodies. After washing the membranes 3 times, for 5 minutes each, they were probed with secondary antibodies at room temperature for 1 hour. The membranes were washed again 3 times, for 5 minutes each. Enhanced chemiluminescence (Qichun, China, PYT002-200) was used to detect the signal. Finally, signals were recorded using an imaging system. All primary antibodies were obtained from Proteintech (Wuhan, China): ISCU (Cat No. 14812-1-AP), MRPL14 (Cat No. 15040-1-AP), and MSRA (Cat No. 14547-1-AP).

2.9. Statistical analysis

Statistical analyses were conducted using SPSS version 18.0. Data are presented as means ± SD or means ± SEM. A 1-tailed Student *t* test was used to compare differences between 2 groups, each with 3 replicates. A *P* value of <.05 was considered statistically significant. Experimental data were derived from 3 independent replicates for each group unless otherwise noted. Statistical significance is indicated as follows: **P* < .05, ***P* < .01, ****P* < .001, *****P* < .0001, and ns (not significant).

3. Result

3.1. The causal effect between mitochondrial-associated proteins and AA

To explore the causal effect between mitochondrial-associated proteins and AA, we employed 3 MR analysis methods – MR-Egger, IVW, and weighted median – with IVW serving as the primary approach (Fig. 2). The IVW results demonstrated that several mitochondrial-related proteins are genetically linked to AA, including ISCU (iron–sulfur cluster assembly enzyme; OR = 1.162, 95% CI = 1.054 to 1.280, *P* = .002), MRPL14 (39S ribosomal protein L14; OR = 0.896, 95% CI = 0.806 to 0.995, *P* = .041), and MSRA (Mitochondrial peptide methionine sulfoxide reductase; OR = 0.910, 95% CI = 0.829 to 0.998, *P* = .045; Fig. 3). These findings imply that these protein variations may affect the risk or progression of aortic aneurysm. Scatter plots from the discovery analysis are shown in Figure 4A to C.

We also performed Cochran *Q* test, which indicated no significant heterogeneity among the mitochondrial-related protein variables. Further validation through MR-PRESSO detected no outliers in the data. The MR-Egger intercept test revealed no evidence of pleiotropy, as the intercept results were not statistically

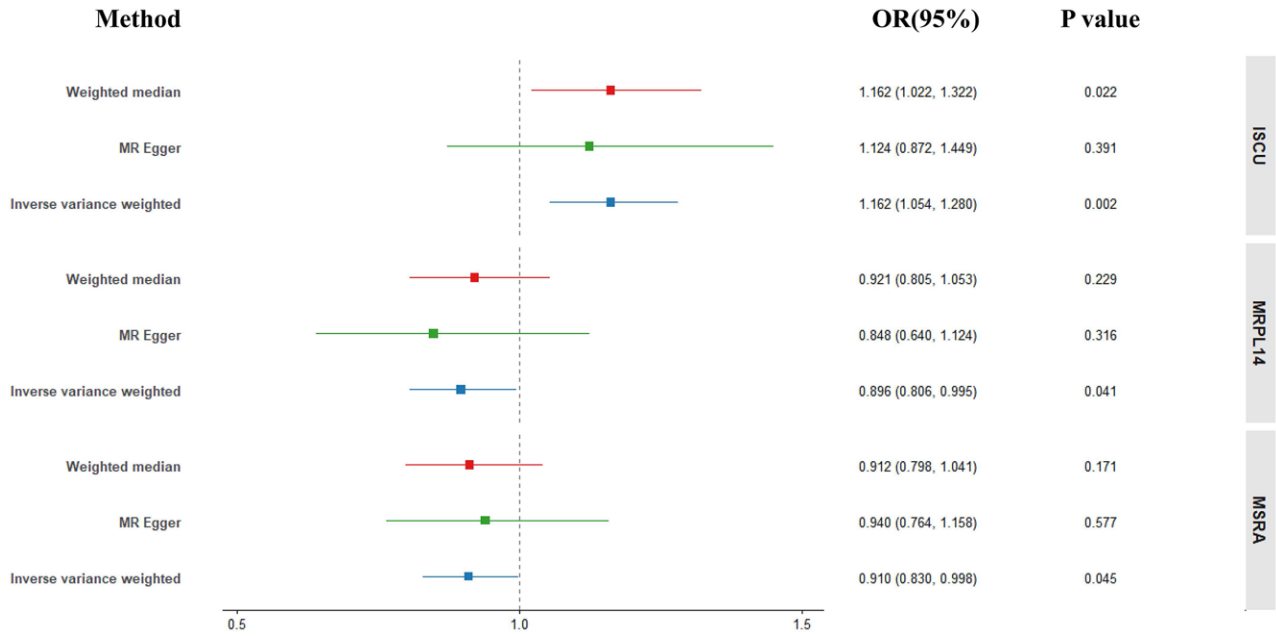

Figure 3. Forest map of the causal relationship between mitochondrial proteins and aortic aneurysm of the discovery analysis.

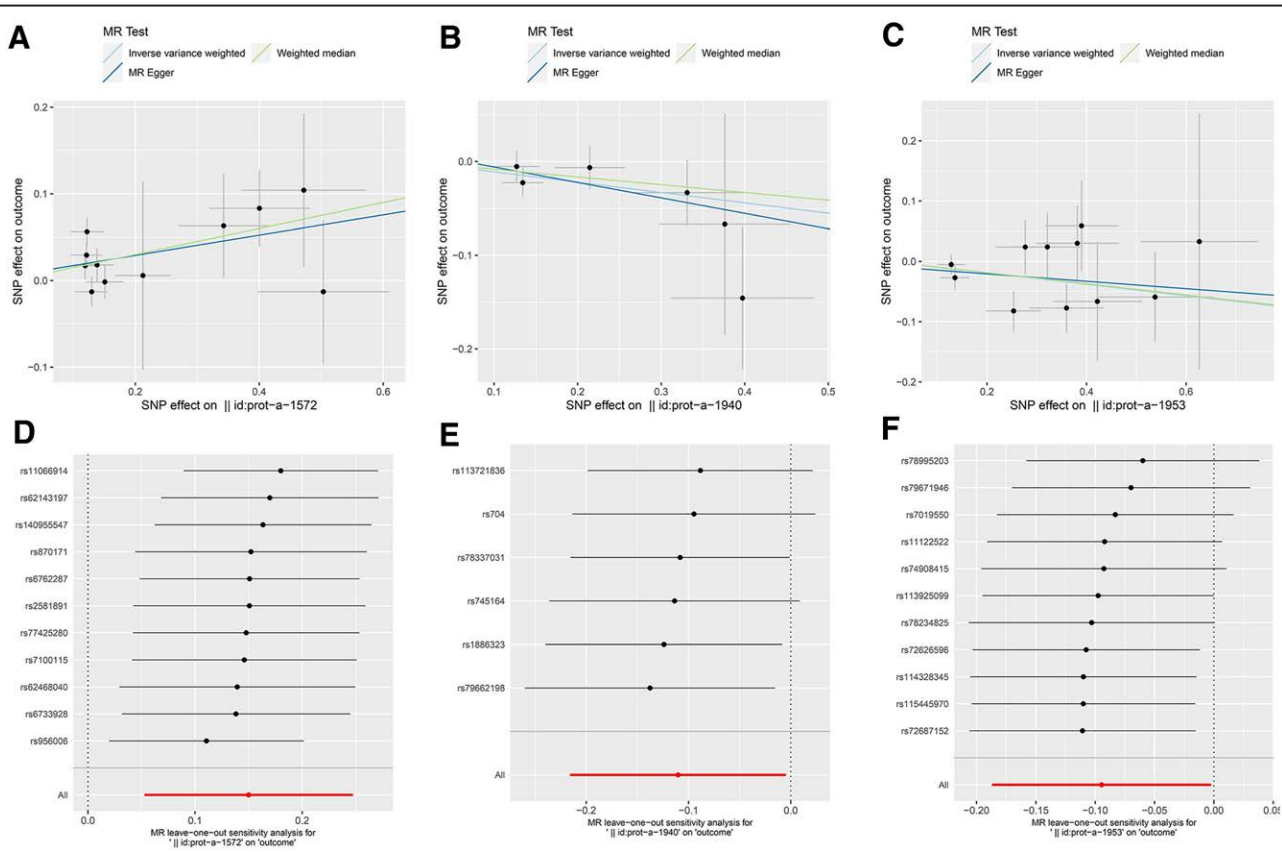

Figure 4. Scatter plots and Leave-one-out analysis of the main results of the discovery analysis. (A) Scatter plots of iron–sulfur cluster assembly enzyme ISCU, mitochondrial. (B) Scatter plots of 39S ribosomal protein L14, mitochondrial. (C) Scatter plots of mitochondrial peptide methionine sulfoxide reductase. (D) Leave-one-out analysis of iron–sulfur cluster assembly enzyme ISCU, mitochondrial. (E) Leave-one-out analysis of 9S ribosomal protein L14, mitochondrial. (F) Leave-one-out analysis of mitochondrial peptide methionine sulfoxide reductase. ISCU = iron–sulfur cluster assembly enzyme.

significant, suggesting that horizontal pleiotropy does not bias our results. While MR-Egger is a widely used method for detecting horizontal pleiotropy, it has theoretical limitations, particularly its reduced statistical power when the number of IVs is small. However, given that our results across multiple sensitivity analyses are consistent and robust, it is unlikely that undetected pleiotropy has materially influenced our findings. Therefore, these sensitivity checks strongly support the robustness and

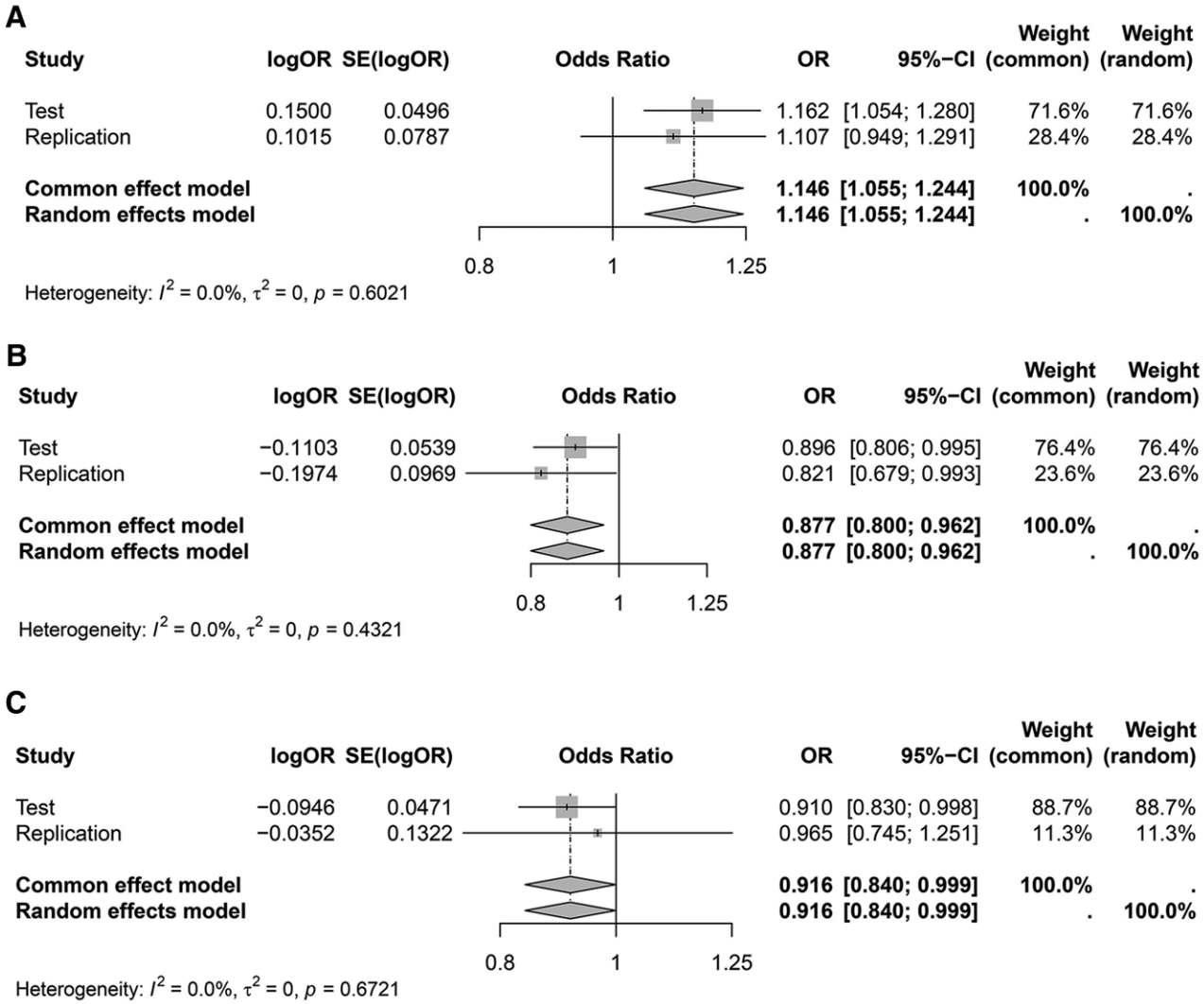

Figure 5. Meta-analysis of suggestively significant associations between mitochondrial proteins and aortic aneurysm (A) Iron–sulfur cluster assembly enzyme ISCU, mitochondrial; (B) 39S ribosomal protein L14, mitochondrial; (C) mitochondrial peptide methionine sulfoxide reductase. ISCU = iron–sulfur cluster assembly enzyme.

reliability of the identified causal link between mitochondrial-related proteins and AA (Table S3, Supplemental Digital Content, http://links.lww.com/MD/O460). The leave-one-out analysis indicated that no single SNP had a significant impact on the overall causal estimate (Fig. 4D–F).

3.2. Reverse causal effect between mitochondrial-associated proteins and AA

A reverse MR analysis was performed on the 3 mitochondrial-related proteins that were previously found to be causally linked to aortic aneurysm in the forward MR analysis to assess whether a reverse causal relationship exists. The results indicated that there was no reverse causal association between these proteins and AA. Sensitivity analysis further confirmed this, as Cochran Q test, MR-Egger intercept test, and MR-PRESSO global test all revealed no significant heterogeneity or horizontal pleiotropy (Table S4, Supplemental Digital Content, http://links.lww.com/MD/O460).

3.3. Replication and meta-analysis of data

As expected, similar trends for certain mitochondrial proteins were observed in the replication GWAS dataset (Table S5, Supplemental Digital Content, http://links.lww.com/MD/O460). Meta-analysis of the discovery and replication datasets further demonstrated that ISCU was associated with an increased risk of AA (OR = 1.146, 95% CI = 1.055 to 1.244, $P < .05$), MRPL14 was associated with a reduced risk of AA (OR = 0.877, 95% CI = 0.800 to 0.962, $P < .05$), and MSRA was associated with a reduced risk of AA (OR = 0.916, 95% CI = 0.840 to 0.999, $P < .05$). These results further enhance the robustness of our findings (Fig. 5).

3.4. Expression profiles of ISCU, MRPL14, and MSRA in AA tissues and gene set enrichment analysis

We further validated the expression levels of ISCU, MRPL14, and MSRA using the GEO dataset GSE57691. The results showed that ISCU expression was significantly upregulated in AA tissue samples, whereas MRPL14 and MSRA were relatively downregulated (Fig. 6A). Additionally, we performed GSEA by dividing the samples into high-expression and low-expression groups based on the expression levels of ISCU, MRPL14, and MSRA, respectively, to analyze the pathways potentially influenced by these genes (Fig. 7). ISCU was enriched in Gene Ontology (GO) terms related to bacterial

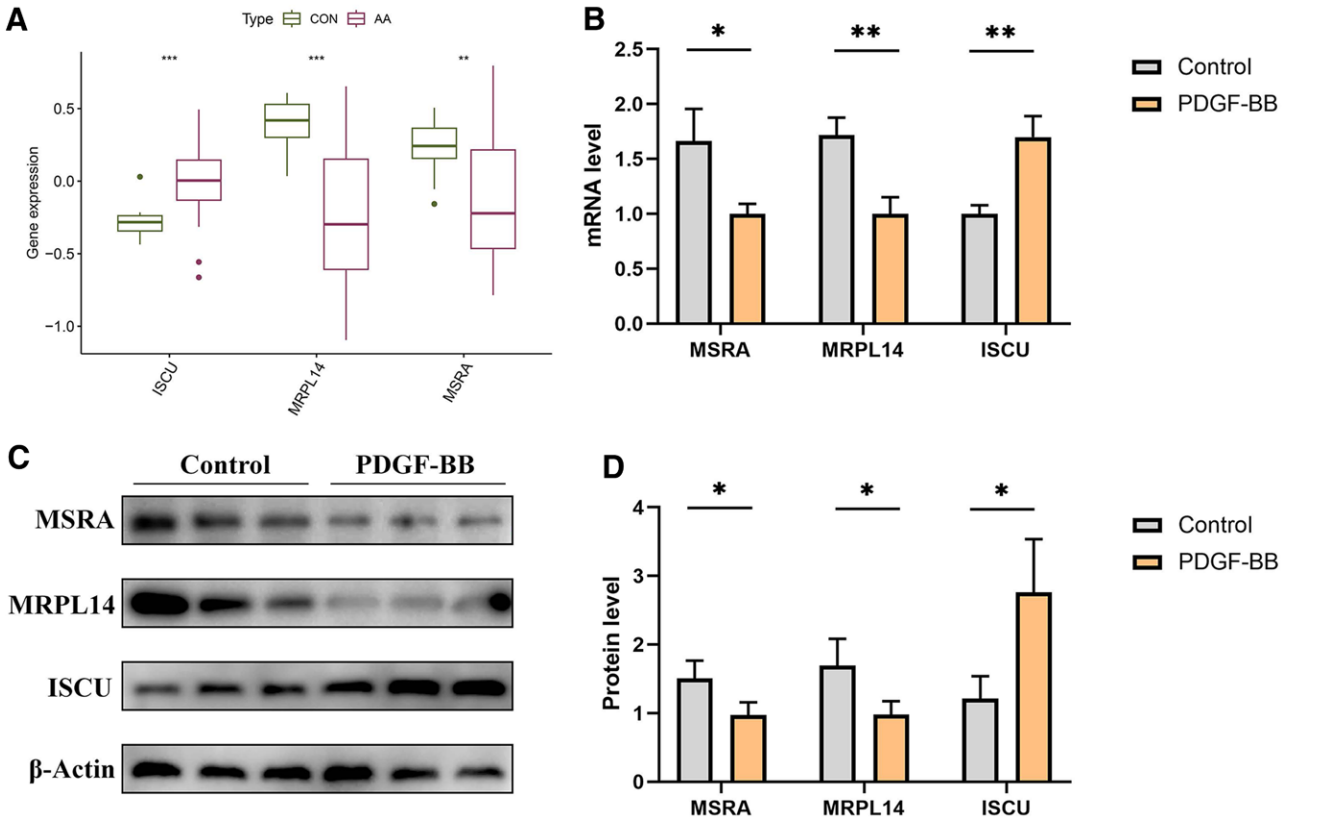

Figure 6. Validation of ISCU, MRPL14, and MSRA Expression in Aortic Aneurysm: Transcriptomic and Experimental Evidence: (A) Differential expression analysis of ISCU, MRPL14, and MSRA in aortic aneurysm tissues using the GEO dataset (GSE57691); (B) qPCR validation of ISCU, MRPL14, and MSRA expression levels in PDGF-BB-induced human aortic smooth muscle cells. (C) Western blot analysis of ISCU, MRPL14, and MSRA protein expression in PDGF-BB-induced human aortic smooth muscle cells; (D) Quantification of Western blot results for ISCU, MRPL14, and MSRA protein expression. GEO = Gene Expression Omnibus, ISCU = iron–sulfur cluster assembly enzyme, MRPL14 = 39S ribosomal protein L14, MSRA = mitochondrial peptide methionine sulfoxide reductase, PDGF-BB = platelet-derived growth factor-BB.

response, protein localization, wound healing, and cell junctions, while Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed enrichment in adipocytokine signaling, complement and coagulation cascades, mitogen-activated protein kinase (MAPK) signaling, primary immunodeficiency, ribosome, and vascular endothelial growth factor (VEGF) signaling. MRPL14 was associated with protein localization, wound healing, bacterial response, and cell junctions in GO analysis, and enriched in complement and coagulation cascades, MAPK signaling, primary immunodeficiency, ribosome, Toll-like receptor signaling, and VEGF signaling in KEGG analysis. MSRA was linked to B cell proliferation, protein localization, wound healing, bacterial response, and cell junctions in GO analysis, while KEGG analysis showed enrichment in complement and coagulation cascades, MAPK signaling, primary immunodeficiency, ribosome, Toll-like receptor signaling, and VEGF signaling.

3.5. In vitro validation of mitochondrial protein and gene expression in PDGF-BB-induced aortic smooth muscle cells

To validate the expression levels of the 3 mitochondrial-related proteins and their corresponding mRNA levels in vitro, we employed a PDGF-BB-induced aortic smooth muscle cell model. qPCR analysis revealed significant changes in the mRNA expression of the 3 mitochondrial-related proteins, with ISCU being upregulated and MRPL14 and MSRA downregulated in PDGF-BB-induced aortic smooth muscle cells (Fig. 6B). Consistently, Western blot results corroborated these findings at the protein level, showing elevated ISCU protein levels and reduced MRPL14 and MSRA protein levels in the same model (Fig. 6C–D). These findings are consistent with our previous observations.

4. Discussion

Using a 2-sample MR approach, we analyzed data from the IEU OpenGWAS database, which included 66 mitochondrial function-related exposures, as well as aortic aneurysm data from 2 independent datasets. Through IVW as the primary MR method, complemented by sensitivity analyses, replication, and meta-analysis, we identified ISCU, MRPL14, and MSRA as mitochondrial proteins associated with AA. Specifically, ISCU was linked to an increased risk of AA, while MRPL14 and MSRA were associated with a reduced risk. These findings align with recent studies emphasizing mitochondrial dysfunction as a key factor in vascular pathologies, yet our study is the first to establish a causal relationship using MR analysis.[34] Furthermore, these associations were validated using gene expression data from the GEO database, along with functional validation in TGF-β1-induced aortic smooth muscle cells, reinforcing the involvement of mitochondrial proteins in aneurysm-related cellular dysfunction.

The investigation of mitochondrial proteins in relation to AA holds substantial clinical significance. This study reveals significant alterations in the expression of 3 key mitochondrial proteins in AA patients, suggesting that mitochondrial dysfunction plays a crucial role in aneurysm pathogenesis. Mitochondrial impairment has been increasingly recognized as

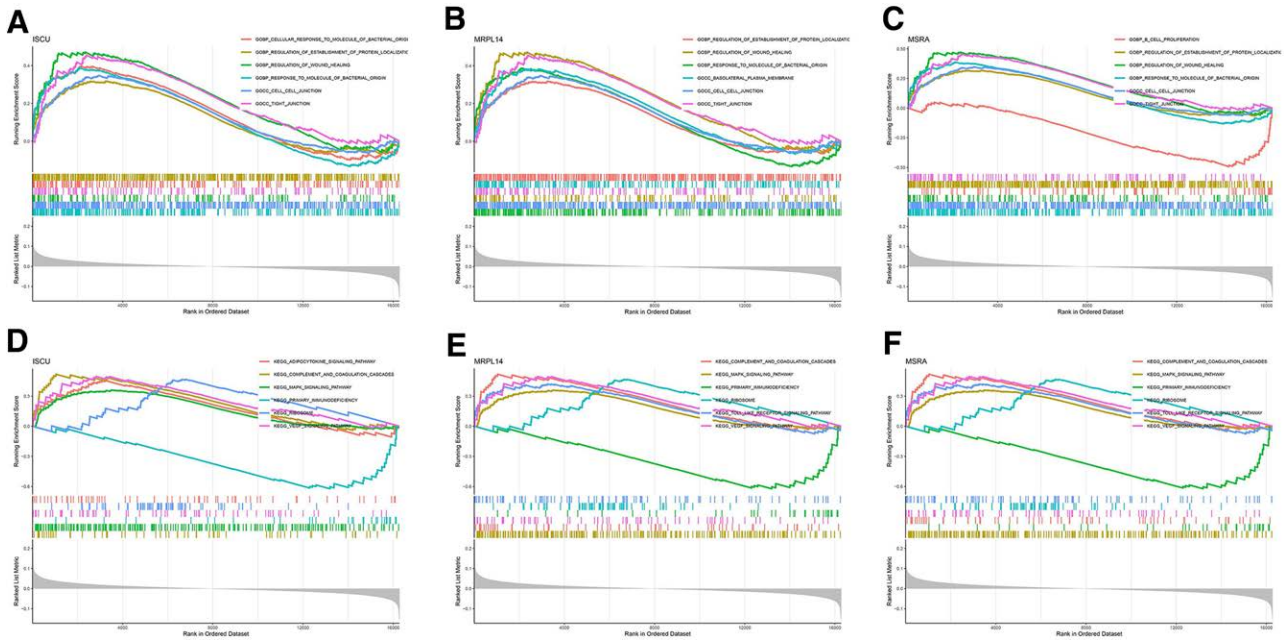

Figure 7. The gene set enrichment analysis result. (A–C): GO enrichment analysis of ISCU, MRPL14, and MSRA;(D-E) KEGG pathway analysis of ISCU, MRPL14, and MSRA. GO = Gene Ontology, ISCU = iron–sulfur cluster assembly enzyme, KEGG = Kyoto Encyclopedia of Genes and Genomes, MRPL14 = 39S ribosomal protein L14, MSRA = mitochondrial peptide methionine sulfoxide reductase,

a central contributor to vascular remodeling and arterial wall instability.[35] However, previous studies have primarily focused on mitochondrial DNA mutations and respiratory chain defects rather than specific mitochondrial proteins involved in AA.[6,36] Our findings highlight ISCU, MRPL14, and MSRA as potential contributors to these processes, bridging a critical gap between mitochondrial dysfunction and AA development.

Mitochondrial dysfunction contributes to AA through mechanisms such as oxidative stress, apoptosis, extracellular matrix remodeling, and vascular inflammation. ISCU plays a central role in iron–sulfur cluster assembly, which is essential for mitochondrial respiration, DNA repair, and redox homeostasis. Aberrant regulation of ISCU has been implicated in mitochondrial dysfunction, leading to excessive reactive oxygen species production and oxidative stress.[37] In our study, ISCU was significantly upregulated in AA tissues and TGF-β1-treated vascular smooth muscle cells, suggesting that dysregulated ISC assembly may contribute to aneurysm formation. Mechanistically, increased ISCU expression may lead to mitochondrial iron overload, causing oxidative stress via the Fenton reaction, which generates highly reactive hydroxyl radicals.[38] This process has been linked to lipid peroxidation, protein oxidation, and mitochondrial DNA damage, all of which are known contributors to vascular remodeling and ECM degradation in AA. Additionally, excess reactive oxygen species (ROS) can activate Nuclear factor kappa-light-chain-enhancer of activated B cells and MAPK signaling pathways, leading to increased expression of pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor-alpha, further exacerbating vascular inflammation and aortic wall weakening.[39]

MRPL14 is a mitochondrial ribosomal protein required for mitochondrial translation and oxidative phosphorylation. Its downregulation has been associated with reduced mitochondrial biogenesis, impaired ATP production, and increased susceptibility to apoptosis.[40] In our study, MRPL14 expression was significantly reduced in AA tissues, which may contribute to mitochondrial dysfunction and vascular degeneration. Loss of MRPL14 could lead to impaired mitochondrial electron transport chain function, resulting in increased ROS production and mitochondrial depolarization, both of which have been shown to promote vascular smooth muscle cell apoptosis and ECM degradation.[41] Additionally, defective mitochondrial translation may impair the synthesis of respiratory chain components, reducing ATP availability and triggering AMPK activation, a known modulator of VSMC contractility and survival.[42] These processes collectively contribute to aortic wall weakening and aneurysm formation.

MSRA is an enzyme responsible for repairing oxidized methionine residues in proteins, thereby protecting cells from oxidative stress. Reduced MSRA expression has been linked to increased oxidative damage and impaired cellular homeostasis.[43] Our study found that MSRA expression was significantly lower in AA tissues, which may indicate a reduced capacity for oxidative stress repair. MSRA deficiency may exacerbate ROS-induced protein oxidation, leading to structural instability of key mitochondrial and ECM proteins.[44] Furthermore, oxidized proteins can activate autophagic and apoptotic pathways, leading to increased smooth muscle cell death and progressive ECM remodeling, both of which are hallmarks of aneurysm pathology.[45] This suggests that MSRA downregulation may contribute to AA progression by impairing the mitochondrial stress response and increasing oxidative damage to the vascular wall.

Mitochondrial dysfunction has been increasingly linked to vascular diseases, yet its role as a potential therapeutic target in AA remains largely unexplored. This study emphasizes the importance of ISCU, MRPL14, and MSRA in mitochondrial homeostasis and their possible contributions to aneurysm formation. Notably, our findings suggest that targeting mitochondrial oxidative stress pathways could be a viable strategy for AA intervention. While existing AA treatments primarily focus on surgical repair and blood pressure control, emerging evidence supports the potential of mitochondrial-targeted therapies, such as antioxidants and mitochondrial biogenesis enhancers, in vascular diseases.[46] However, whether modulating ISCU, MRPL14, or MSRA expression could mitigate aneurysm progression requires further investigation.

Despite the strengths of this study, several limitations should be acknowledged. First, the dataset primarily consists of

individuals of European ancestry, limiting the generalizability of our findings across diverse populations. Genetic variation and environmental influences may lead to population-specific differences in AA susceptibility and mitochondrial function.[47] Future studies should include multi-ethnic cohorts to enhance external validity. Additionally, the dataset lacks critical demographic details such as age and sex, which may influence mitochondrial protein expression and AA risk. Moreover, our study establishes a causal relationship between mitochondrial proteins and AA but does not fully elucidate the underlying molecular mechanisms. Although GSEA identified key pathways involving inflammation, immune regulation, and oxidative stress, further experimental validation is required to confirm their direct roles in AA pathogenesis. Future research should incorporate high-resolution techniques such as single-cell RNA sequencing or proteomics to provide cell-type-specific insights into mitochondrial dysfunction in AA. Lastly, a multivariable MR analysis was not performed, mainly due to sample size limitations.[48] Expanding datasets and improving statistical power will be essential for a more comprehensive analysis of mitochondrial proteins in AA.

5. Conclusion

This study provides novel insights into the mitochondrial mechanisms underlying AA pathogenesis, identifying ISCU, MRPL14, and MSRA as key contributors. By integrating MR analysis, gene expression validation, and functional enrichment analysis, our findings offer a comprehensive perspective on mitochondrial dysfunction in AA. The identification of these mitochondrial proteins as potential therapeutic targets underscores the need for further research into mitochondrial-directed interventions for AA prevention and treatment.

Author contributions

Conceptualization: Li Li, Mukamengjiang Juaiti.
Data curation: Adilai Abodulikemu, Li Li, Mukamengjiang Juaiti.
Formal analysis: Adilai Abodulikemu, Li Li, Mukamengjiang Juaiti.
Funding acquisition: Mukamengjiang Juaiti.
Investigation: Mukamengjiang Juaiti.
Methodology: Adilai Abodulikemu, Li Li, Mukamengjiang Juaiti.
Project administration: Mukamengjiang Juaiti.
Resources: Adilai Abodulikemu, Li Li, Mukamengjiang Juaiti.
Software: Adilai Abodulikemu, Li Li.
Supervision: Adilai Abodulikemu, Li Li.
Validation: Adilai Abodulikemu.
Visualization: Adilai Abodulikemu.
Writing – review & editing: Adilai Abodulikemu, Mukamengjiang Juaiti.
Writing – original draft: Mukamengjiang Juaiti.

References

[1] Bossone E, Eagle KA. Epidemiology and management of aortic disease: aortic aneurysms and acute aortic syndromes. Nat Rev Cardiol. 2021;18:331–48.
[2] Raaymakers TW, Rinkel GJ, Limburg M, Algra A. Mortality and morbidity of surgery for unruptured intracranial aneurysms: a meta-analysis. Stroke. 1998;29:1531–8.
[3] Schermerhorn ML, Bensley RP, Giles KA, et al. Changes in abdominal aortic aneurysm rupture and short-term mortality, 1995-2008: a retrospective observational study. Ann Surg. 2012;256:651–8.
[4] Flora GD, Nayak MK. A brief review of cardiovascular diseases, associated risk factors and current treatment regimes. Curr Pharm Des. 2019;25:4063–84.
[5] Altobelli E, Rapacchietta L, Profeta VF, Fagnano R. Risk factors for abdominal aortic aneurysm in population-based studies: a systematic review and meta-analysis. Int J Environ Res Public Health. 2018;15:2805.
[6] Summerhill VI, Sukhorukov VN, Eid AH, Nedosugova LV, Sobenin IA, Orekhov AN. Pathophysiological aspects of the development of abdominal aortic aneurysm with a special focus on mitochondrial dysfunction and genetic associations. Biomol Concepts. 2021;12:55–67.
[7] Liu Y, Huang Y, Xu C, et al. Mitochondrial dysfunction and therapeutic perspectives in cardiovascular diseases. Int J Mol Sci. 2022;23:16053.
[8] Chistiakov DA, Shkurat TP, Melnichenko AA, Grechko AV, Orekhov AN. The role of mitochondrial dysfunction in cardiovascular disease: a brief review. Ann Med. 2018;50:121–7.
[9] Rega S, Farina F, Bouhuis S, et al. Multi-omics in thoracic aortic aneurysm: the complex road to the simplification. Cell Biosci. 2023;13:131.
[10] Oller J, Gabandé-Rodríguez E, Ruiz-Rodríguez MJ, et al. Extracellular tuning of mitochondrial respiration leads to aortic aneurysm. Circulation. 2021;143:2091–109.
[11] Cooper HA, Cicalese S, Preston KJ, et al. Targeting mitochondrial fission as a potential therapeutic for abdominal aortic aneurysm. Cardiovasc Res. 2021;117:971–82.
[12] Tian H, Chen X, Liao J, et al. Mitochondrial quality control in stroke: from the mechanisms to therapeutic potentials. J Cell Mol Med. 2022;26:1000–12.
[13] Ouyang M, Wang M, Yu B. Aberrant mitochondrial dynamics: an emerging pathogenic driver of abdominal aortic aneurysm. Cardiovasc Ther. 2021;2021:6615400.
[14] Sekula P, Del Greco M F, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. J Am Soc Nephrol. 2016;27:3253–65.
[15] Sanderson E, Glymour MM, Holmes MV, et al. Mendelian randomization. Nat Rev Methods Primers. 2022;2:6.
[16] Yang Q, Sanderson E, Tilling K, Borges MC, Lawlor DA. Exploring and mitigating potential bias when genetic instrumental variables are associated with multiple non-exposure traits in Mendelian randomization. Eur J Epidemiol. 2022;37:683–700.
[17] Jiang L, Zheng Z, Fang H, Yang J. A generalized linear mixed model association tool for biobank-scale data. Nat Genet. 2021;53:1616–21.
[18] Palmer TM, Lawlor DA, Harbord RM, et al. Using multiple genetic variants as instrumental variables for modifiable risk factors. Stat Methods Med Res. 2012;21:223–42.
[19] Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. Stat Methods Med Res. 2017;26:2333–55.
[20] Lin Z, Deng Y, Pan W. Combining the strengths of inverse-variance weighting and Egger regression in Mendelian randomization using a mixture of regressions model. PLoS Genet. 2021;17:e1009922.
[21] Dudbridge F. Polygenic Mendelian randomization. Cold Spring Harb Perspect Med. 2021;11:a039586.
[22] Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol. 2017;32:377–89.
[23] Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol. 2016;40:304–14.
[24] Bowden J, Del Greco M F, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. Int J Epidemiol. 2016;45:1961–74.
[25] Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity analyses for robust causal inference from Mendelian randomization analyses with multiple genetic variants. Epidemiology. 2017;28:30–42.
[26] Skrivankova VW, Richmond RC, Woolf BAR, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian Randomization: the STROBE-MR Statement. JAMA. 2021;326:1614–21.
[27] Schnell PM. Controlling the false-discovery rate when identifying the subgroup benefiting from treatment. Clin Trials. 2023;20:394–404.
[28] Juaiti M, Feng Y, Tang Y, Liang B, Zha L, Yu Z. Integrated bioinformatics analysis and experimental animal models identify a robust biomarker and its correlation with the immune microenvironment in pulmonary arterial hypertension. Heliyon. 2024;10:e29587.
[29] Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16:284–7.

[30] Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA. 2005;102:15545–50.
[31] Holycross BJ, Blank RS, Thompson MM, Peach MJ, Owens GK. Platelet-derived growth factor-BB-induced suppression of smooth muscle cell differentiation. Circ Res. 1992;71:1525–32.
[32] Su M, Yue Z, Wang H, et al. Ufmylation is activated in vascular remodeling and lipopolysaccharide-induced endothelial cell injury. DNA Cell Biol. 2018;37:426–31.
[33] Meng W, Liu S, Li D, et al. Expression of platelet-derived growth factor B is upregulated in patients with thoracic aortic dissection. J Vasc Surg. 2018;68:3S–13S.
[34] Yu E, Foote K, Bennett M. Mitochondrial function in thoracic aortic aneurysms. Cardiovasc Res. 2018;114:1696–8.
[35] Xia Y, Zhang X, An P, Luo J, Luo Y. Mitochondrial homeostasis in VSMCs as a central hub in vascular remodeling. Int J Mol Sci. 2023;24:3483.
[36] Wang D, Jia L, Zhao C, et al. Mitochondrial quality control in abdominal aortic aneurysm: From molecular mechanisms to therapeutic strategies. FASEB J. 2023;37:e22969.
[37] Crooks DR, Jeong SY, Tong WH, et al. Tissue specificity of a human mitochondrial disease: differentiation-enhanced mis-splicing of the Fe-S scaffold gene ISCU renders patient cells more sensitive to oxidative stress in ISCU myopathy. J Biol Chem. 2012;287:40119–30.
[38] Wu H, Wei H, Zhang D, et al. Defective mitochondrial ISCs biogenesis switches on IRP1 to fine tune selective mitophagy. Redox Biol. 2020;36:101661.
[39] Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. Biochem Pharmacol. 2009;78:539–52.
[40] Lin X, Guo L, Lin X, Wang Y, Zhang G. Expression and prognosis analysis of mitochondrial ribosomal protein family in breast cancer. Sci Rep. 2022;12:10658.
[41] Kim HJ, Nguyen QK, Jung SN, et al. Mitochondrial ribosomal protein L14 promotes cell growth and invasion by modulating reactive oxygen species in thyroid cancer. Clin Exp Otorhinolaryngol. 2023;16:184–97.
[42] Chen X, Yang Y, Zhou Z, et al. Unraveling the complex interplay between Mitochondria-Associated Membranes (MAMs) and cardiovascular inflammation: molecular mechanisms and therapeutic implications. Int Immunopharmacol. 2024;141:112930.
[43] Cabreiro F, Picot CR, Friguet B, Petropoulos I. Methionine sulfoxide reductases: relevance to aging and protection against oxidative stress. Ann N Y Acad Sci. 2006;1067:37–44.
[44] Lourenço Dos Santos S, Petropoulos I, Friguet B. The oxidized protein repair enzymes methionine sulfoxide reductases and their roles in protecting against oxidative stress, in ageing and in regulating protein function. Antioxidants (Basel). 2018;7:191.
[45] Chandran S, Binninger D. Role of oxidative stress, methionine oxidation and Methionine Sulfoxide Reductases (MSR) in Alzheimer's disease. Antioxidants (Basel). 2023;13:21.
[46] Tavris BS, Peters AS, Böckler D, Dihlmann S. Mitochondrial dysfunction and increased DNA damage in vascular smooth muscle cells of abdominal aortic aneurysm (AAA-SMC). Oxid Med Cell Longevity. 2023;2023:6237960.
[47] Wolff JN, Pichaud N, Camus MF, Côté G, Blier PU, Dowling DK. Evolutionary implications of mitochondrial genetic variation: mitochondrial genetic effects on OXPHOS respiration and mitochondrial quantity change with age and sex in fruit flies. J Evol Biol. 2016;29:736–47.
[48] Ko DS, Kim YH. Mendelian randomization studies in atherosclerotic cardiovascular diseases. J Lipid Atheroscler. 2024;13:280–91.