Exploring the causal association between mitochondrial dysfunction and idiopathic pulmonary fibrosis through two-sample Mendelian randomization

Keywords
idiopathic pulmonary fibrosis, mitochondrial dysfunction, causality, Mendelian randomization, transmembrane protein 70, sirtuin-5

Abstract

Introduction

Idiopathic pulmonary fibrosis (IPF) is a prevalent lung disease affecting mainly older adults, characterized by abnormal lung healing and an altered extracellular matrix. This study aims to explore the potential causal association between mitochondrial dysfunction and idiopathic pulmonary fibrosis.

Material and methods

This two-sample Mendelian randomization study analyzed GWAS data on mitochondrial dysfunction and IPF. The primary analysis employed the inverse variance weighted method, confirmed by the weighted median, weighted mode, and MR-Egger regression methods. Heterogeneity and pleiotropy were assessed using Cochran's Q-test and MR-Egger, with robustness evaluated through leave-one-out analysis.

Results

The genetic predictions indicated a potential inverse causal association of Transmembrane protein 70 with IPF in the IVW (OR = 0.83, 95% CI: 0.70-0.99, P = 0.03), without evidence of heterogeneity, horizontal pleiotropy, or outliers. The MR-PRESSO analysis showed one outlier for NAD-dependent protein deacylase sirtuin-5 and one outlier for Serine-tRNA ligase. After removing the outliers, NAD-dependent protein deacylase sirtuin-5 showed a suggestive positive association with IPF (OR = 1.25, 95% CI: 1.09-1.43, P = 0.007), without evidence of heterogeneity, horizontal pleiotropy, or outliers.

Conclusions

This MR analysis provides genetic evidence for potential causal associations of Transmembrane protein 70 and NAD-dependent protein deacylase sirtuin-5 with IPF. These proteins may represent therapeutic targets and enhance understanding of mitochondrial dysfunction in IPF. Further validation is needed before clinical application.

Exploring the causal association between mitochondrial dysfunction and idiopathic pulmonary fibrosis through two-sample Mendelian randomization

Running title: MR: mitochondrial dysfunction and IPF

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ABSTRACT

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Conclusions: This MR analysis provides genetic evidence for potential causal associations of Transmembrane protein 70 and NAD-dependent protein deacylase sirtuin-5 with IPF. These proteins may represent therapeutic targets and enhance understanding of mitochondrial dysfunction in IPF. Further validation is needed before clinical application.

Keywords: causality; idiopathic pulmonary fibrosis; Mendelian randomization; mitochondrial dysfunction; transmembrane protein 70; sirtuin-5.

Introduction

Idiopathic pulmonary fibrosis (IPF) results from an abnormal wound-healing process in the lung, resulting in the deposition of an altered extracellular matrix and the disruption of the alveolar architecture (1-3). IPF mainly affects adults aged 55-75 years and is more common in men than in women (1, 4). The estimated incidence of IPF is 3-9 per 100,000 person-years worldwide (1), and the prevalence is about 14-42.7 per 100,000 individuals (5, 6). The risk factors for IPF include cigarette smoking, genetic variants, exposure to metal and wood dust, gastroesophageal reflux, and certain viruses (1, 2, 7). Current management includes smoking cessation, pulmonary rehabilitation, and long-term oxygen therapy. Antifibrotic drugs such as pirfenidone and nintedanib may slow lung function decline, whereas corticosteroids, vasodilators, and interferon-based regimens are largely ineffective (2, 7). Nevertheless, despite best management, the median survival after diagnosis is about 3 years, and patients with acute exacerbations have ≥60% in-hospital mortality (2, 4). Due to its idiopathic nature, the etiology and pathogenesis remain poorly understood. Therefore, there is a need for a better understanding of IPF to improve its management.

Mitochondria play a central role in eukaryotic cells by generating ATP through oxidative phosphorylation, but they are also involved in fatty acid synthesis, calcium homeostasis, cell proliferation, and apoptosis(8-11)(12, 13). Dysfunctional mitochondria are involved in aging and several diseases (14, 15): cardiovascular diseases (16), diabetes (17), metabolic syndrome (18), autoimmune diseases (19), degenerative neurological and muscular diseases (20), psychiatric disorders (21), gastrointestinal diseases (22), fatiguing illnesses (23), and chronic infections (24). Growing evidence shows that mitochondrial dysfunction is involved

in IPF (25, 26) and fibrotic diseases in general (27, 28). Mechanistically, impaired electron transport reduces energy production while elevating ROS generation, driving oxidative stress—a hallmark of IPF. In addition, mitochondrial dysfunction alters cellular metabolism, promotes senescence, apoptosis, and stem cell exhaustion, and is linked to genomic instability, defective proteostasis, impaired autophagy, and telomere attrition (25, 26, 29-31). Research is focused on identifying potential therapeutic targets that could improve mitochondrial function and reduce oxidative stress in IPF. Strategies aimed at modulating pro-fibrotic pathways, such as TGF-β signaling, could also be beneficial. Promoting autophagy to remove dysfunctional mitochondria could be a potential therapeutic approach (8, 32, 33).

The recent decades have seen the completion of many genome-wide association studies (GWASs) that provided data for millions of genetic variations and their association with phenotypes, including diseases (34, 35). Mendelian randomization (MR) relies on the common genetic variations for different environmental exposures and enables the exploration of possible causal associations between exposures and diseases (36-38). Two-sample MR uses the associations between single-nucleotide polymorphism (SNPs) and exposure and between SNPs and outcomes from different GWASs to combine them into an estimation of the causal associations. Recent MR-based studies have successfully applied this approach to explore causal links between various exposures, such as environmental factors or organ function, and the risk of chronic diseases (39, 40). These examples highlight the robustness and versatility of the MR framework, providing a strong methodological precedent for its application in investigating the role of mitochondrial dysfunction in IPF.

The available data about the association between mitochondrial dysfunction and IPF mainly come from epidemiological studies that can suffer from confounding and reverse causation.

Under key assumptions, MR reduces the reverse causation and confounding that impede or mislead the interpretation of results from epidemiological studies (41). Although there is evidence for causality between mitochondrial dysfunction and fibrotic diseases, including IPF (26, 42), the involvement of specific mitochondrial proteins remains to be examined. Determining causality between specific mitochondrial proteins could hint toward the mechanisms involved and potential therapeutic targets.

Hence, this study used the MR methodology to examine the causal association between mitochondrial dysfunction and IPF. The results could help improve our understanding of IPF pathogenesis.

Material and methods

Study design

This study used publicly available data from GWASs to investigate the causal association between mitochondrial dysfunction and IPF (Figure 1). Since the data were from studies that already adhered to the Declaration of Helsinki, no additional ethical approval was necessary. This study assumed that the SNPs used as instrumental variables (IVs) for the exposure are associated with mitochondrial dysfunction, that there are no common causes to the SNPs and the outcome (IPF), and that there are no independent pathways between the SNPs and IPF other than through mitochondrial dysfunction (41).

Data sources

The GWAS data for the outcome (IPF) was from FinnGen, which is a large project in genomics and personalized medicine that collected and analyzed the genetic and health outcomes of 500,000 biobank donors in Finland. The IPF dataset

(https://risteys.finregistry.fi/endpoints/IPF) was used in the present study. It contains 2189 patients with IPF and 407,609 controls.

The GWAS for mitochondrial 2,4-dienoyl-CoA reductase 1 is from a published study (43) and includes 1296 individuals and 18,162,745 SNPs. The other mitochondrial protein data are from 3301 individuals of European origin (10,534,735 SNPs) (44) (https://gwas.mrcieu.ac.uk/), including 2,4-Dienoyl-CoA Reductase, Diablo homolog, Persulfide dioxygenase ETHE1, Ribosome-recycling factor, Serine-tRNA ligase, Mitochondrial import inner membrane translocase subunit, NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 8, NADH dehydrogenase (ubiquinone) iron-sulfur protein 4, NAD-dependent protein deacylase sirtuin-5, NADH dehydrogenase (ubiquinone) flavoprotein 2, and Transmembrane protein 70. GWAS information for all outcomes and exposures is shown in Supplementary Table S1.

Instrument variable selection

The IVs included in this study had to meet the following criteria. First, the SNPs significantly associated with mitochondrial proteins in the entire genome were screened out based on $P < 5\times10^{-8}$. However, because of the limited sample size of the available GWASs, very few SNPs reached this stringent threshold for some exposures. Therefore, in line with common practice in MR studies, we relaxed the threshold to $P < 5\times10^{-6}$ or $P < 5\times10^{-5}$ to ensure adequate instrument numbers while maintaining analytical power. To minimize potential weak instrument bias, the strength of each SNP was evaluated, and only those with F-statistics >10 were retained(45). Second, the SNPs with a minimum minor allele frequency (MAF) of >0.01 were removed (46). Finally, linkage disequilibrium (LD) among SNPs was removed based on $R^2<0.001$ and a window size of 10,000 kb (47). If a SNP identified for exposure was not

found in the outcome data, then proxy SNPs were identified based on higher LD (R^2=0.8) (48). The strength of the IVs was evaluated using the F-value for each SNP to assess the potential weak instrument bias using the formula $F=R^2\times(N-2)/(1-R^2)$, where R^2 represents the proportion of exposure variance explained by the SNP in the IV. F- statistics >10 indicated sufficient instrument strength and reduced the likelihood of weak instrument bias.

Mendelian randomization analysis

The primary analysis used in MR studies is the inverse variance weighted (IVW) method (49). In MR, the IVW method is a popular approach for estimating causal effects, where genetic variants are used as IVs to infer causality between an exposure and an outcome, even in the presence of unmeasured confounders (50). If the IVW analysis reveals a significant causal association ($P < 0.05$), then the strength of the association can be tested using the MR-Egger (51), weighted median (52), and weighted mode (53) methods. Those methods are used to support the robustness of the IVW results because their use without the IVW method could lead to bias and unsteady results (50). The MR analysis was performed using the "TwoSampleMR" package in R 4.3.0 (The R Project for Statistical Computing, www.r-project.org). The results were presented as odds ratios (ORs) and 95% confidence intervals (CIs)

Sensitivity analysis

Heterogeneity can bias the observed causal associations between exposure and outcomes. Heterogeneity among IVs was detected using Cochran's Q test; P-values >0.05 indicated low heterogeneity (54). In addition, according to the third MR assumption, MR analyses are only valid in the absence of horizontal pleiotropy, which was detected using the MR-Egger regression method to explore horizontal pleiotropy based on an intercept term approaching 0

or $P > 0.05$ (54). Outliers can also bias causal associations and be detected using the MR-PRESSO method based on $P < 0.05$ (54, 55). The outliers were removed, and the ORs were recalculated to correct for horizontal pleiotropy. Finally, a leave-one-out analysis was used to assess the robustness and consistency of the results (56).

Results

Instrumental variable selection

This study screened out 143 IVs related to mitochondrial function. The mean F-value was 36.21 (range, 20.89-1787.59). The IVs, F-values, and non-matching SNPs and their proxies are summarized in Table 1. All F-values were >10, indicating the absence of weak instrumental bias (Supplementary Table S2).

Mendelian randomization analysis results

The genetic predictions indicated that there were no statistically significant associations between mitochondrial proteins and IPF, except for a possible inverse causal association of genetically predicted transmembrane protein 70 with genetically predicted IPF in the IVW (OR=0.83, 95%CI: 0.70-0.99, $P = 0.03$) (Table 2 and Figure 2). The other factors showed no genetically predicted causal associations with IPF (Table 2 and Figures S1-10). Furthermore, the MR-PRESSO analysis showed one outlier for NAD-dependent protein deacylase sirtuin-5 (rs10733789) and one outlier for Serine-tRNA ligase (rs1294404). After removing the outlier, NAD-dependent protein deacylase sirtuin-5 showed a positive causal association with IPF (OR = 1.25, 95% CI: 1.09-1.43, $P = 0.007$) (Table 4). Conversely, the exclusion did not alter the outcomes for Serine-tRNA ligase (Table 4), maintaining the initial observations.

Sensitivity analyses

Sensitivity analyses attest to the robustness of the findings. While Cochrane's Q-test revealed significant heterogeneity for 2,4-Dienoyl-CoA Reductase (P=0.028), Serine-tRNA ligase (*P* = 0.008), NAD-dependent protein deacylase sirtuin-5 (P=0.033), and Diablo homolog (*P* = 0.043) (Table 3), but IVW is a random-effect statistical method that can tolerate some degree of heterogeneity (57). The MR-Egger regression results suggested no evidence of horizontal pleiotropy (all $P > 0.05$) (Table 3). The leave-one-out analyses revealed that no SNPs drove the results (Figure 2 and Figures S1-10). These collective findings underscore the resilience of the conclusions to sensitivity analyses and support the validity of our causal inference.

Discussion

This two-sample MR study explored the potential causal association between mitochondrial dysfunction and IPF. The results suggest that genetically predicted Transmembrane protein 70 may have a protective association with IPF. NAD-dependent protein deacylase sirtuin-5 might also be associated, but the association was observed after removing one outlier, and the result should be taken cautiously. The other mitochondrial proteins were not associated with IPF, and more in-depth research is necessary.

Transmembrane protein 70 is a mitochondrial membrane protein that possibly plays a role in the biogenesis of mitochondrial ATP synthase (58, 59). Indeed, mutations in the *TMEM70* gene are associated with neonatal mitochondrial encephalocardiomyopathy due to ATP synthase deficiency (60, 61). A study showed that knocking down *TMEM70* did not affect p16 and p21 (two markers of cellular senescence) in human bronchial epithelial cells (62). A deficiency in Transmembrane protein 70 has been reported to be associated with pulmonary hypertension in newborns (63). For now, no other evidence is available to link

Transmembrane protein 70 with IPF, and additional research is necessary. Nevertheless, mitochondrial dysfunction is involved in IPF, and the aging lung is characterized by decreased mitochondrial respiration, mitophagy, and mitochondrial biogenesis (25). Since IPF is observed in adults aged 55-75 years (1, 4), the mitochondrial pathogenesis of IPF is highly likely. As Transmembrane protein 70 participates in forming ATP synthase (58, 59), lower Transmembrane protein 70 could contribute to decreased mitochondrial respiration and mitochondrial biogenesis. Nevertheless, whether a lower formation of ATP synthase, decreased mitochondrial respiration, and impaired mitochondrial biogenesis, all due to a lower Transmembrane protein 70 expression, participate in IPF remains uncertain and requires confirmation.

Another mitochondrial protein, NAD-dependent protein deacylase sirtuin-5, may also be involved in IPF pathogenesis, although the association was only observed after removing one outlier, and thus should be regarded as hypothesis-generating. NAD-dependent protein deacylase sirtuin-5 has been implicated in various diseases, including cancer, neurodegenerative disorders, and potentially IPF (31, 64). Nevertheless, sirtuins, including NAD-dependent protein deacylase sirtuin-5, are involved in lung fibrosis (65) and pulmonary cell senescence (31, 66), underscoring their relevance in the context of IPF and warranting further rigorous examination. NAD-dependent protein deacylase sirtuin-5 is a member of the sirtuin family, primarily expressed in the mitochondrial matrix, and is crucial for maintaining mitochondrial function and cellular homeostasis (64, 67). NAD-dependent protein deacylase sirtuin-5 regulates proteins involved in glycolysis, the tricarboxylic acid (TCA) cycle, fatty acid oxidation, the electron transport chain, ketone body formation, nitrogenous waste management, and ROS detoxification (64, 68). Some studies suggest that NAD-dependent

protein deacylase sirtuin-5 deficiency or dysfunction may contribute to the development of IPF (31, 69). Specifically, NAD-dependent protein deacylase sirtuin-5-mediated desuccinylation may prevent mitochondrial dysfunction in alveolar epithelial cells, a key event in IPF (69).

The lack of associations for most other mitochondrial proteins in this study requires careful interpretation. First, some proteins may truly have no causal role in IPF. Second, statistical power might be insufficient to detect weak effects given the limited sample size of current GWASs. Third, tissue specificity could play a role, as the pQTL data used for mitochondrial proteins were derived mainly from blood, which may not reflect protein regulation in lung tissue. Fourth, mitochondrial pathways are highly interconnected, and the effects of individual proteins may be compensated by others, obscuring their independent associations. From a clinical perspective, these findings highlight TMEM70 and SIRT5 as potential therapeutic targets, as both proteins are involved in mitochondrial function and cellular metabolism. Specifically, TMEM70 plays an essential role in ATP synthase biogenesis, and its deficiency has been linked to mitochondrial diseases such as ATP synthase deficiency (60). SIRT5, by regulating diverse metabolic and cellular processes, has also emerged as a potential therapeutic target in fibrotic diseases including IPF (64, 68, 70, 71). The present results therefore not only deepen our understanding of the contribution of mitochondrial dysfunction to IPF pathogenesis, but also suggest that, after further validation, genetic variants in these proteins may eventually contribute to risk stratification in susceptible populations. Nevertheless, the current evidence remains limited, and more studies are needed before these proteins can be translated into clinical management.

The main strength of MR studies is the use of large-scale GWAS data from thousands of individuals and millions of SNPs. On the other hand, the study also had limitations. First, the GWAS data were derived exclusively from individuals of European ancestry, which may limit the generalizability of our findings to other populations with different genetic backgrounds and environmental exposures. Future studies including more diverse populations are needed to validate these associations. Second, there may be sample overlap between the exposure and outcome GWAS datasets, which could potentially introduce bias. Although the exact degree of overlap could not be determined, we sought to mitigate this risk. Sample overlap does not invalidate the MR estimates, but it can amplify the bias from weak instruments (72). Therefore, by ensuring that all our instruments had an F-statistic greater than 10, we minimized the potential for weak instrument bias and, consequently, reduced the impact of any potential sample overlap. Finally, several SNPs could be selected as IVs, affecting the causal associations.

Conclusions

The MR analysis results suggest a potential protective association of genetically predicted Transmembrane protein 70 with IPF and a possible positive association of SIRT5, although the latter finding requires cautious interpretation. These proteins may represent potential therapeutic targets that improve understanding of mitochondrial dysfunction in IPF, but the current evidence is preliminary. Further biological and clinical validation is required before these findings can be translated into clinical practice. The other mitochondrial proteins were not associated with IPF, and more in-depth research is necessary.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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None.

Authors contributions

Ming Yu and Linlin Ye carried out the studies, participated in collecting data, and drafted the manuscript. Ming Yu and Linlin Ye performed the statistical analysis and participated in its design. Ming Yu and Linlin Ye participated in the acquisition, analysis, or interpretation of data and drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors report there are no competing interests to declare.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Figure legends

Figure 1. Schematic representation of the Mendelian randomization study assumptions.

Figure 2. Mendelian randomization analysis of the possible causal association between transmembrane protein 70 and idiopathic pulmonary fibrosis: scatter plot (top left), forest plot (top right), funnel plot (bottom left), and leave-one-out forest plot (bottom right).

Table 1. Selection of the IVs

Mitochontrial proteins	Selection threshold	IVs, n	Mean F	F range	Non-matching SNPs and proxy
2,4-Dienoyl-CoA Reductase	$P < 5\times10^{-6}$	10	23.09	21.53-27.18	rs1284877 -> rs1284878 (R^2=0.0171)
Ribosome-recycling factor	$P < 5\times10^{-5}$	15	23.25	21.05-30.06	rs552107732, no proxy rs9674789 -> rs12936590 (R^2=0.0067)
NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 8	$P < 5\times10^{-5}$	13	22.43	21.04-24.98	rs58906847, no proxy
NADH dehydrogenase (ubiquinone) iron-sulfur protein 4	$P < 5\times10^{-6}$	19	115.00	20.89-1787.59	rs143366101, no proxy rs115713928 -> rs72834339 (R^2=0.0066) rs34140990 -> rs7128448 (R^2=0.0068)
NADH dehydrogenase (ubiquinone) flavoprotein 2	$P < 5\times10^{-5}$	7	23.05	21.24-28.89	-
Serine-tRNA ligase	$P < 5\times10^{-6}$	11	23.03	21.03-26.26	rs12292833, no proxy rs850388 -> rs917177 (R^2=0.0065)

NAD-dependent protein deacylase sirtuin-5	$P < 5\times10^{-6}$	16	25.97	20.93-60.55	rs545964696, no proxy rs983355 -> rs1480318 (R^2=0.0064) rs8139620 was eliminated later
Transmembrane protein 70	$P < 5\times10^{-6}$	10	22.51	20.84-25.32	rs569797207, no proxy rs4310923, no proxy
Diablo homolog	$P < 5\times10^{-6}$	18	23.81	21.01-43.04	rs111261668 -> rs62015875 (R^2=0.0069) rs1718860 -> rs7685467 (R^2=0.0129)
Mitochondrial import inner membrane translocase subunit	$P < 5\times10^{-5}$	11	22.82	21.15-30.68	-
Persulfide dioxygenase ETHE1	$P < 5\times10^{-6}$	14	26.74	21.00-51.10	rs8055509 -> rs13337159 (R^2=0.0066)

IV: instrumental variable; SNP: single-nucleotide polymorphism.

Table 2. The association between genetically predicted mitochondrial function and the risk of idiopathic pulmonary fibrosis

Exposures	Outcome	Methods	SNPs, n	P	OR (95% CI)
2,4-Dienoyl-CoA Reductase	IPF	IVW	9	0.65	1.02 (0.92-1.14)
2,4-Dienoyl-CoA Reductase		MR Egger	9	0.54	0.95 (0.81-1.11)
2,4-Dienoyl-CoA Reductase		Weighted median	9	0.87	0.99 (0.90-1.09)
2,4-Dienoyl-CoA Reductase		Weighted mode	9	0.82	0.99 (0.89-1.10)
Ribosome-recycling factor		IVW	12	0.59	0.96 (0.83-1.11)
Ribosome-recycling factor		MR Egger	12	0.52	0.91 (0.67-1.21)
Ribosome-recycling factor		Weighted median	12	0.53	0.94 (0.77-1.14)
Ribosome-recycling factor		Weighted mode	12	0.59	0.94 (0.76-1.17)
NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 8		IVW	12	0.96	1.00 (0.85-1.18)
NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 8		MR Egger	12	0.87	0.97 (0.64-1.45)
NADH dehydrogenase (ubiquinone) 1 beta		Weighted median	12	0.64	0.95 (0.78-1.16)

subcomplex subunit 8				
NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 8	Weighted mode	12	0.68	0.92 (0.64-1.33)
NADH dehydrogenase (ubiquinone) iron-sulfur protein 4	IVW	18	0.32	0.97 (0.91-1.03)
NADH dehydrogenase (ubiquinone) iron-sulfur protein 4	MR Egger	18	0.33	0.96 (0.88-1.04)
NADH dehydrogenase (ubiquinone) iron-sulfur protein 4	Weighted median	18	0.46	0.98 (0.91-1.04)
NADH dehydrogenase (ubiquinone) iron-sulfur protein 4	Weighted mode	18	0.50	0.98 (0.91-1.05)
NADH dehydrogenase (ubiquinone) flavoprotein 2	IVW	6	0.49	1.07 (0.89-1.28)
NADH dehydrogenase (ubiquinone) flavoprotein 2	MR Egger	6	0.79	0.95 (0.66-1.36)
NADH dehydrogenase (ubiquinone) flavoprotein 2	Weighted median	6	0.88	1.02 (0.8-1.29)
NADH dehydrogenase (ubiquinone) flavoprotein 2	Weighted mode	6	0.98	1.00 (0.75-1.34)

Serine-tRNA ligase	IVW	11	0.88	1.02 (0.80-1.31)
Serine-tRNA ligase	MR Egger	11	0.93	0.96 (0.39-2.37)
Serine-tRNA ligase	Weighted median	11	0.75	0.96 (0.76-1.21)
Serine-tRNA ligase	Weighted mode	11	0.67	0.94 (0.70-1.25)
NAD-dependent protein deacylase sirtuin-5	IVW	14	0.13	1.15 (0.96-1.39)
NAD-dependent protein deacylase sirtuin-5	MR Egger	14	0.49	0.73 (0.31-1.73)
NAD-dependent protein deacylase sirtuin-5	Weighted median	14	0.06	1.22 (0.99-1.50)
NAD-dependent protein deacylase sirtuin-5	Weighted mode	14	0.08	1.5 (0.99-2.27)
Transmembrane protein 70	IVW	9	0.03	0.83 (0.70-0.99)
Transmembrane protein 70	MR Egger	9	0.11	0.65 (0.41-1.04)
Transmembrane protein 70	Weighted median	9	0.14	0.84 (0.66-1.06)
Transmembrane protein 70	Weighted mode	9	0.60	0.91 (0.66-1.26)
Diablo homolog	IVW	18	0.79	1.02 (0.88-1.19)
Diablo homolog	MR Egger	18	0.62	1.12 (0.72-1.74)
Diablo homolog	Weighted median	18	0.16	1.13 (0.95-1.33)

Diablo homolog	Weighted mode	18	0.19	1.18 (0.93-1.51)
Mitochondrial import inner membrane translocase subunit	IVW	8	0.89	1.01 (0.83-1.23)
Mitochondrial import inner membrane translocase subunit	MR Egger	8	0.82	1.08 (0.58-2.01)
Mitochondrial import inner membrane translocase subunit	Weighted median	8	0.59	1.07 (0.84-1.35)
Mitochondrial import inner membrane translocase subunit	Weighted mode	8	0.61	1.08 (0.81-1.45)
Persulfide dioxygenase ETHE1	IVW	14	0.55	1.05 (0.90-1.21)
Persulfide dioxygenase ETHE1	MR Egger	14	0.40	0.84 (0.56-1.25)
Persulfide dioxygenase ETHE1	Weighted median	14	0.27	1.12 (0.92-1.35)
Persulfide dioxygenase ETHE1	Weighted mode	14	0.34	1.16 (0.87-1.56)

IVW: inverse variance weighted; OR: odds ratio; CI: confidence interval; IPF: idiopathic pulmonary fibrosis.

Table 3. Heterogeneity and pleiotropy testing of the instrumental variables for the exposures and IPF as the outcome

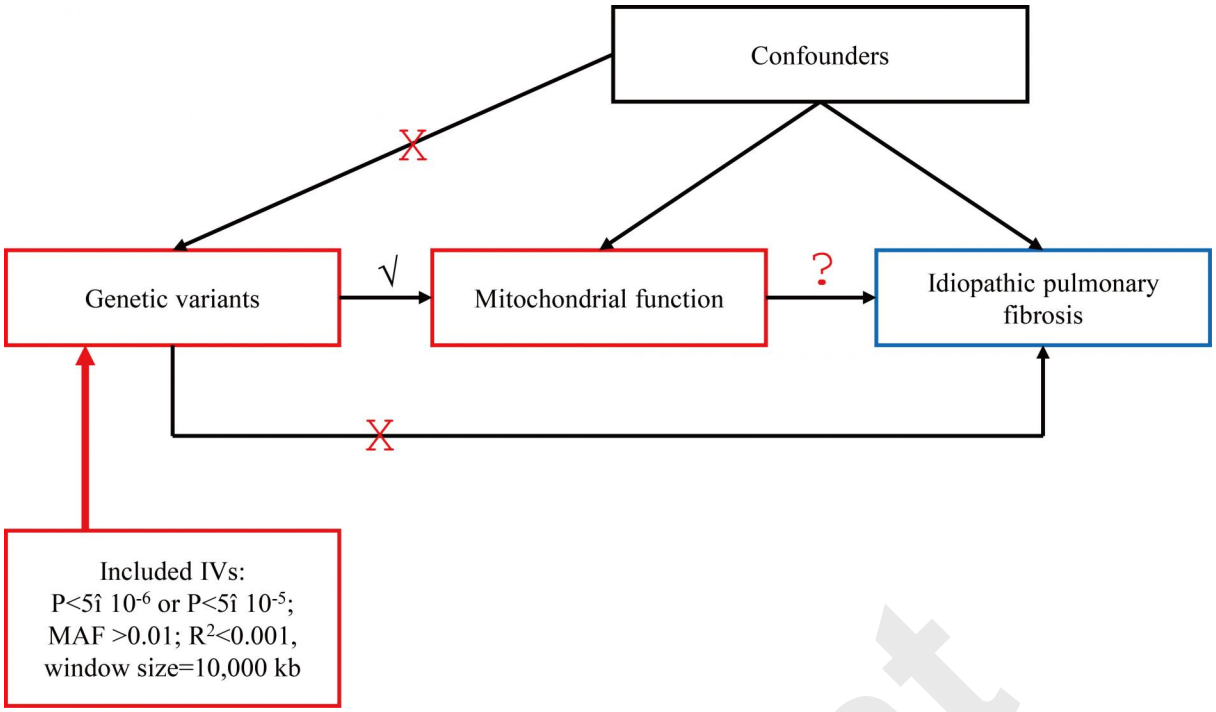
Exposures	Heterogeneity		Pleiotropy	
	Q	P	Egger intercept	P
2,4-Dienoyl-CoA Reductase	17.23	0.028	77.00	0.250
Ribosome-recycling factor	8.35	0.682	0.01	0.656
NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 8	17.79	0.087	0.01	0.844
NADH dehydrogenase (ubiquinone) iron-sulfur protein 4	7.89	0.969	0.01	0.674
NADH dehydrogenase (ubiquinone) flavoprotein 2	4.62	0.464	0.03	0.510
Serine-tRNA ligase	23.88	0.008	0.01	0.890
NAD-dependent protein deacylase sirtuin-5	23.85	0.033	0.07	0.311
Transmembrane protein 70	6.28	0.616	0.04	0.310
Diablo homolog	28.14	0.043	-0.02	0.661

Mitochondrial import inner membrane translocase subunit	5.20	0.636	-0.01	0.845
Persulfide dioxygenase ETHE1	17.01	0.199	0.04	0.261

Table 4. MR-PRESSO results

Outcome	Exposure	Raw		Outlier corrected		Global P	Number of outliers	Distortion P
		OR (CI%)	*P*	OR (CI%)	*P*			
Idiopathic pulmonary fibrosis	2,4-Dienoyl-CoA Reductase, mitochondrial levels	1.02 (0.92-1.14)	0.665	/	/	/	/	/
	Diablo homolog, mitochondrial	1.02 (0.88-1.19)	0.790	/	/	/	/	/
	Mitochondrial import inner membrane translocase subunit	1.01 (0.86-1.2)	0.881	/	/	/	/	/
	NAD-dependent protein deacylase sirtuin-5, mitochondrial	1.16 (0.97-1.37)	0.120	1.25 (1.09-1.43)	0.007	0.031	1 (rs10733789)	0.424
	NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 8, mitochondrial	1 (0.85-1.18)	0.964	/	/	/	/	/
	NADH dehydrogenase	1.11 (0.93-	0.276	/	/	/	/	/

(ubiquinone) flavoprotein 2, mitochondrial	1.32)									
NADH dehydrogenase (ubiquinone) iron-sulfur protein 4, mitochondrial	0.97	1.01)	(0.93-	0.165	/	/	/	/	/	
Persulfide dioxygenase ETHE1, mitochondrial	1.05	1.21)	(0.9-	0.561	/	/	/	/	/	
Ribosome-recycling factor, mitochondrial	0.96	1.09)	(0.85-	0.550	/	/	/	/	/	
Serine-tRNA ligase, mitochondrial	1.02	1.31)	(0.8-	0.878	0.96	1.16)	(0.76-	0.563	0.006	1 (rs1294404)	0.281
Transmembrane protein 70, mitochondrial	0.83	0.97)	(0.71-	0.044	/	/	/	/	/	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Genetic variants
√
Mitochondrial function
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Idiopathic pulmonary fibrosis
X
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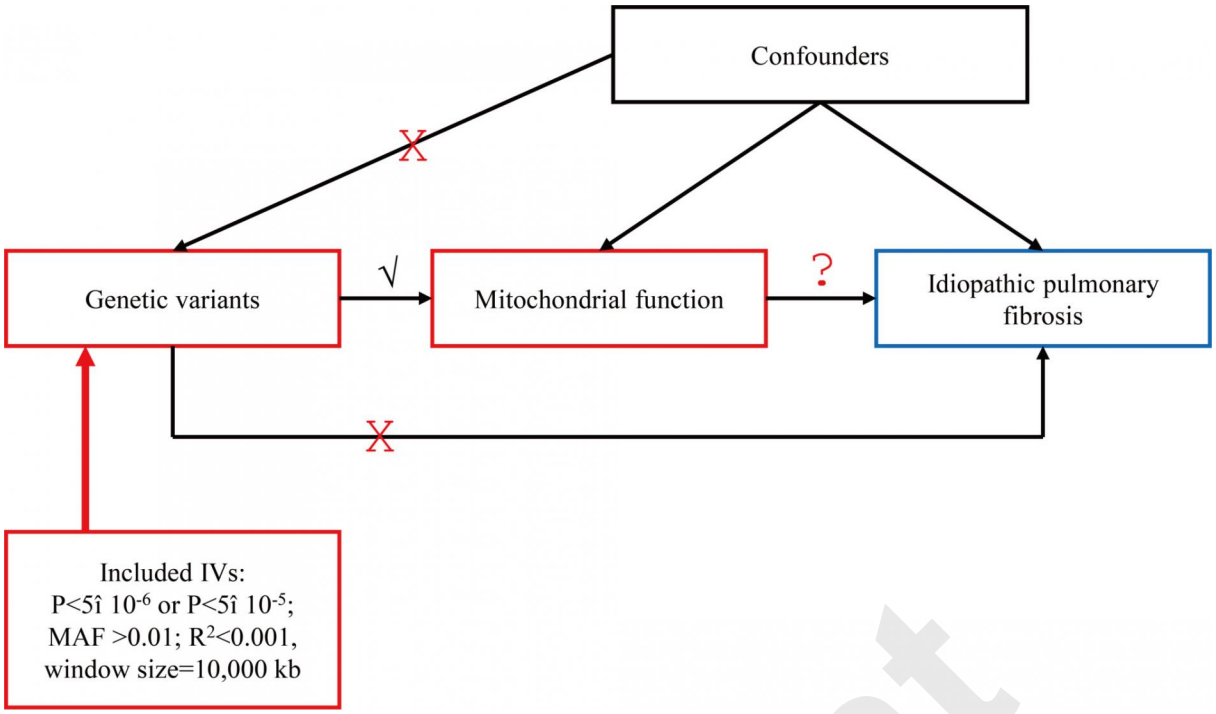

Figure 1. Schematic representation of the Mendelian randomization study assumptions.

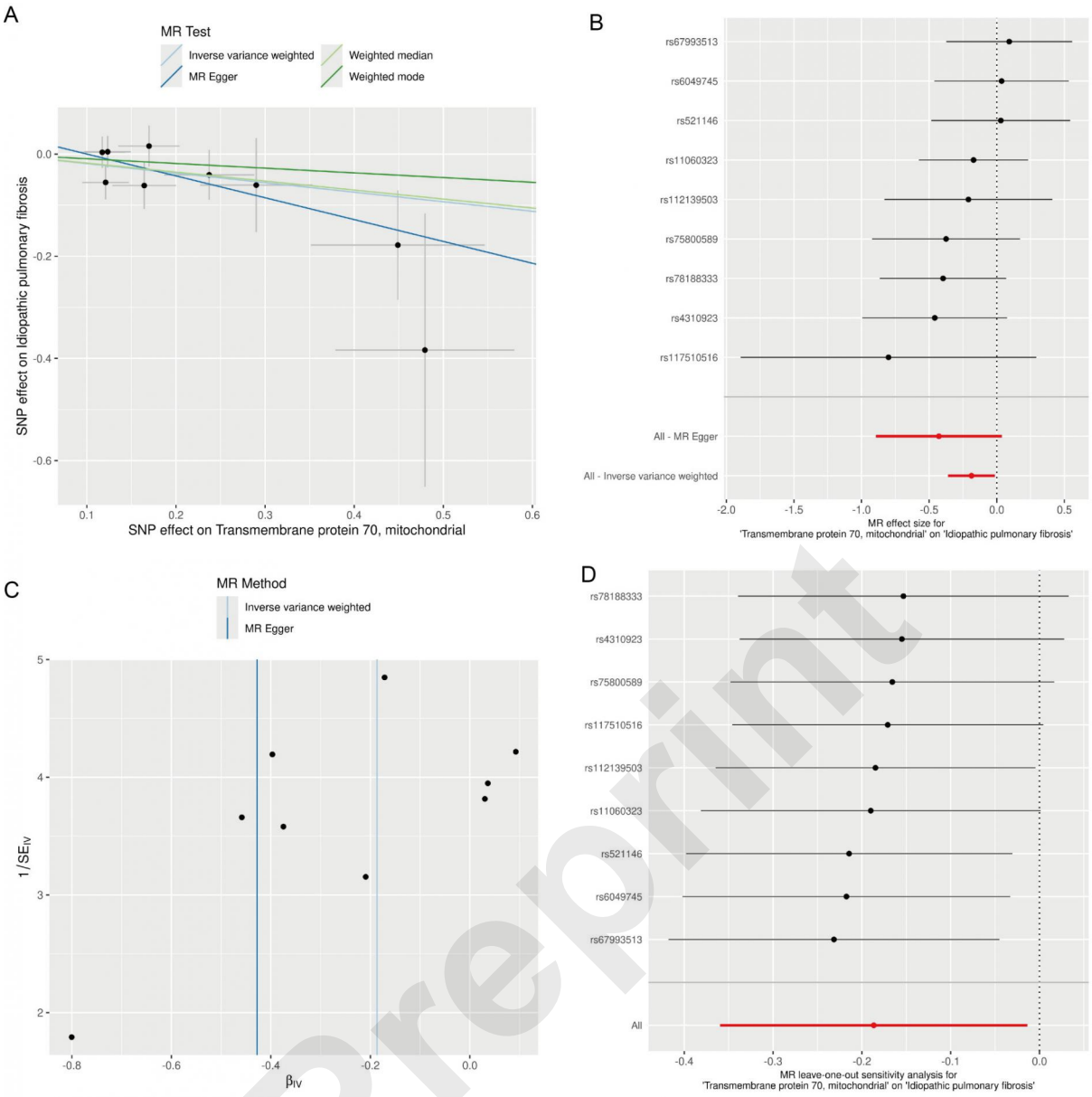

Figure 2. Mendelian randomization analysis of the possible causal association between transmembrane protein 70 and idiopathic pulmonary fibrosis: scatter plot (top left), forest plot (top right), funnel plot (bottom left), and leave-one-out forest plot (bottom right).