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Lipid-Lowering Drugs and Pulmonary Vascular Disease: A Mendelian Randomization Study

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ABSTRACT

The therapeutic value of lipid-lowering drugs in pulmonary vascular disease remains uncertain due to insufficient studies and evidence. This study aims to investigate the causal effects of lipid-lowering drugs (specifically, inhibitors of APOB, CETP, HMGCR, NPC1L1, and PCSK9) on pulmonary vascular diseases using a Mendelian randomization (MR) approach. We utilized summary-level statistics from genome-wide association studies (GWAS) to simulate the exposure to low-density lipoprotein cholesterol (LDL-C) and its outcomes on pulmonary arterial hypertension (PAH), pulmonary embolism (PE), and pulmonary heart disease (PHD). Single-nucleotide polymorphisms (SNPs) within or near drug target-associated LDL-C loci were selected as proxies for the lipid-lowering drugs. Data from the FinnGen cohort and UK Biobank (UKB) were incorporated to enhance the robustness and generalizability of the findings. The inverse variance weighted (IVW) and MR-Egger methods were employed to estimate MR effects. Our MR analysis indicated that LDL-C mediated by NPC1L1 (odds ratio [OR] = 104.76, 95% confidence interval [CI] = 2.01–5457.01, $p = 0.021$) and PCSK9 (OR = 10.20, 95% CI = 3.58–29.10, $p < 0.001$) was associated with an increased risk of PAH. In contrast, LDL-C mediated by APOB was associated with a decreased risk of PE (FinnGen: OR = 0.74, 95% CI = 0.60–0.91, $p = 0.005$; UKB: OR = 0.998, 95% CI = 0.996–1.000, $p = 0.031$) and PHD (FinnGen: OR = 0.73, 95% CI = 0.59–0.91, $p = 0.004$). However, LDL-C mediated by CETP and HMGCR did not show significant associations with the risks of PAH, PE, or PHD. This MR study revealed the causal effects of NPC1L1 and PCSK9 inhibitors on increased PAH risk, while APOB inhibitors appear to reduce the risks of PE and PHD. These findings enhance our understanding of the potential roles of lipid-lowering drugs in pulmonary vascular disease.

1 | Introduction

Pulmonary vascular diseases, including pulmonary arterial hypertension (PAH), pulmonary embolism (PE), and pulmonary heart disease (PHD), exhibit high morbidity, disability, and mortality rate [1–4]. PAH is characterized by increased pulmonary vascular resistance and elevated pulmonary arterial pressure due to various etiologies, which can lead to right heart failure and death [5]. The prevalence of PAH varies widely, ranging from 10.6 to 125 per million [6–8]. PE results from the obstruction of the pulmonary artery or its branches by an embolus, disrupting pulmonary circulation [9]. According to the 2016 Global Burden of Thrombosis Study, the annual incidence of PE is approximately 3.9 to 11.5 per million [10]. PHD involves increased pulmonary vascular resistance from various causes, resulting in PAH and increased right ventricular

afterload [11]. It has been reported that there are about 330 million cardiovascular disease patients in China, with 5 million suffering from PHD [12].

Currently, lipid-lowering drugs are widely used to lower blood lipids and prevent ischemic cardio-cerebral vascular diseases [13–15]. However, the value of lipid-lowering drugs in pulmonary vascular disease is not yet supported by sufficient studies and evidence. Some preclinical studies suggest that statins might improve pulmonary arterial pressure in PAH through various mechanisms [16–18]. but clinical studies have yielded inconsistent results [19–25]. Additionally, some studies have found that lipid-lowering drugs could decrease the risk of venous thromboembolism (VTE), which is the etiology of PE [26–28].

The purpose of this study was to investigate whether there is a relationship between lipid-lowering drugs and pulmonary vascular disease and its feasibility for clinical treatment by Mendelian randomization (MR) analysis. Most national and regional lipid management guidelines recommend low-density lipoprotein cholesterol (LDL-C) as the primary target of lipid-lowering therapy [29–31]. Genome-wide association studies (GWAS) have identified genome-wide genetic markers, such as single nucleotide polymorphisms (SNPs) in the classification of LDL-C-lowering drugs into five main targets: APOB, CETP, HMGCR, NPC1L1, and PCSK9 [32–35]. In this study, SNPs related to these targets were selected as instrumental variables (IVs) to assess the relationship between LDL-C and PAH, PE, and PHD, enabling causal inference.

2 | Methods

2.1 | Data Sources

Summary-level statistics from genome-wide association studies (GWAS) were obtained from the Integrative Epidemiology Unit (IEU) (https://gwas.mrcieu.ac.uk). All data used in this study were sourced from the IEU. To enhance the robustness and external validity of our findings across European populations, we included data from the UK Biobank (UKB). The UKB provided summary-level data for PE and PHD. Together with the previously used FinnGen study data, this expanded dataset allowed for more comprehensive analyses.

2.2 | Drug Target Genetic Instrument Selection

The GWAS dataset from the Global Lipids Genetic Consortium (GLGC), which included 173,082 participants and was released in 2013, was used to identify single-nucleotide polymorphisms (SNPs) correlated with low-density lipoprotein cholesterol (LDL-C) as proxies for drug targets, including APOB, CETP, HMGCR, NPC1L1, and PCSK9 [33–35]. The selection of IVs followed three fundamental criteria: (1) Association with LDL-C: IVs should be significantly associated with LDL-C levels. (2) Independence from Confounding Factors: IVs should be independent of any confounding factors that might influence the outcomes. (3) Exclusive Effect on Pulmonary Vascular Disease through LDL-C: IVs should affect pulmonary vascular disease outcomes (PAH, PE, and PHD) exclusively through their impact on LDL-C. The SNPs within 100 kilobases of the drug target, with a genome-wide significance P less than 5×10^{-8} and a linkage disequilibrium R2 less than 0.3, were used as genetic instruments [34–36].

2.3 | Pulmonary Vascular Disease Outcomes

This study leveraged GWAS summary-level datasets from the FinnGen study and UKB to explore the genetic associations between lipid-lowering drug targets and pulmonary vascular diseases. The FinnGen data encompassed PAH, with 14,888 participants including 125 cases and 162,837 controls; PE, with 4185 cases and 214,228 controls; and PHD, with 4185 cases and 214,607 controls. To ensure broader applicability and enhance the robustness of the findings, complementary datasets from the UK Biobank were incorporated. The UKB dataset for PE (comprised 2118 cases and 359,076 controls, totaling 361,194 participants, while the dataset for PHD included 117 cases and 361,077 controls, also totaling 361,194 participants.

2.4 | Statistical Analyses

All outcome datasets were harmonized with the exposures-correlated genetic instruments. The inverse variance weighted (IVW) and MR-Egger methods were then used to derive MR effect estimates [37, 38]. In the meanwhile, pleiotropy and heterogeneity test statistics (Cochran Q-derived p) were calculated using the IVW, MR-Egger, and MR-Pleiotropy Residual Sum and Outlier methods (MR-PRESSO) [38–40]. Pleiotropy and heterogeneity were regarded as nonexistent when the *p*-value was higher than 0.05. IVW was used as the primary method in the absence of pleiotropy and heterogeneity; otherwise, MR-Egger was used. Finally, we utilized leave-one-out analysis to see if a single SNP was the source of the MR estimate bias [40]. The analyses were carried out using the R packages TwoSampleMR (version 0.5.6) and MendelR (version 2.1.2). All analyses were carried out in R. (version 4.0).

3 | Results

3.1 | Pulmonary Arterial Hypertension (PAH)

In the FinnGen dataset, NPC1L1-mediated LDL-C levels were significantly associated with an increased risk of PAH (Table 1: OR = 104.76, 95% CI = 2.01–5457.01, $p = 0.021$). Similarly, PCSK9-mediated LDL-C levels showed a strong positive association with PAH risk (Table 1: OR = 10.20, 95% CI = 3.58–29.10, $p < 0.001$). These results suggest that NPC1L1 and PCSK9 inhibitors may have protective effects against PAH (Figure 1). No significant associations were observed for LDL-C levels mediated by APOB, CETP, or HMGCR with PAH risk (Table 1; Figure 1).

TABLE 1 | The Heterogeneity and pleiotropy analysis (FinnGen Data).

Outcome	Drug target	No. of SNPs	Heterogeneity (Q_*p* value)		Pleiotropy (*p* value)
			MR Egger	IVW	Egger intercept
Pulmonary arterial hypertension	APOB	20	0.8458	0.8335	0.3147
	CETP	3	0.4342	0.7280	0.9034
	HMGCR	7	0.9201	0.9504	0.6800
	NPC1L1	3	0.5606	0.8280	0.8760
	PCSK9	12	0.9962	0.9880	0.3039
Pulmonary embolism	APOB	20	0.1020	0.1137	0.4875
	CETP	3	0.9628	0.6962	0.5516
	HMGCR	7	0.8752	0.9231	0.7093
	NPC1L1	3	0.9849	0.8252	0.6469
	PCSK9	12	*0.0415*	0.0531	0.5935
Pulmonary heart disease	APOB	20	0.0947	0.1053	0.4834
	CETP	3	0.9539	0.6933	0.5500
	HMGCR	7	0.8782	0.9257	0.7141
	NPC1L1	3	0.9660	0.8227	0.6452
	PCSK9	12	*0.0420*	0.0532	0.5850

Abbreviations: IVW: inverse variance weighted; SNPs, single nucleotide polymorphisms.

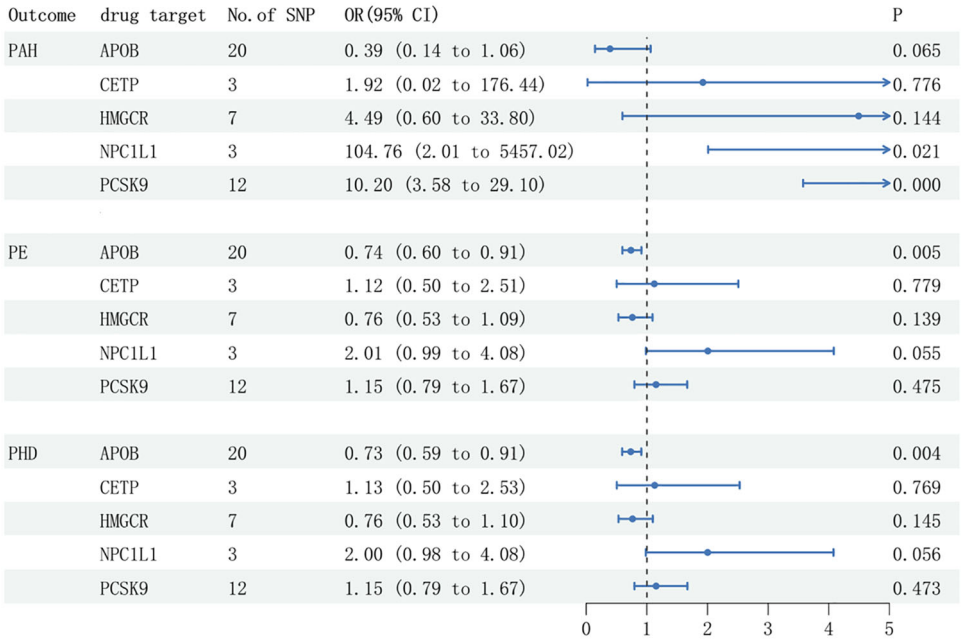

FIGURE 1 | Mendelian Randomization effect estimate for low-density lipoprotein cholesterol mediated by drug target and Pulmonary vascular disease outcomes (FinnGen Data). Abbreviations: PAH: pulmonary arterial hypertension, PE: pulmonary embolism, PHD: pulmonary heart disease.

3.2 | Pulmonary Embolism (PE)

APOB-mediated LDL-C levels were consistently associated with a reduced risk of PE in both datasets. In the FinnGen cohort, this association was statistically significant (Table 1: OR = 0.74, 95% CI = 0.60–0.91, $p = 0.005$). Similarly, in the UKB cohort, a borderline protective effect was observed (Table 2: OR = 0.998, 95% CI = 0.996–1.000, $p = 0.031$). No significant associations were identified for CETP-, HMGCR-, NPC1L1-, or PCSK9-mediated LDL-C levels with PE risk in either dataset (Tables 1 and 2; Figures 1 and 2).

3.3 | Pulmonary Heart Disease (PHD)

In the FinnGen dataset, APOB-mediated LDL-C levels were significantly associated with a reduced risk of PHD (Table 1: OR = 0.73, 95% CI = 0.59–0.91, $p = 0.004$; Figure 1). However, in

TABLE 2 | The Heterogeneity and pleiotropy analysis (UK Biobank data).

Outcome	Drug target	No. of SNPs	Heterogeneity (Q_p value)		Pleiotropy (p value)
			MR Egger	IVW	Egger intercept
Pulmonary embolism	APOB	20	0.0435	0.0558	0.6843
	CETP	4	0.9350	0.9851	0.9100
	HMGCR	7	0.1881	0.1901	0.4022
	NPC1L1	2	NA	0.8044	NA
	PCSK9	14	0.2518	0.2663	0.4160
Pulmonary heart disease	APOB	20	0.8178	0.8510	0.6563
	CETP	4	0.5152	0.6799	0.7100
	HMGCR	7	0.9072	0.9526	0.8328
	NPC1L1	2	NA	0.7195	NA
	PCSK9	14	0.1863	0.1910	0.3881

Abbreviations: IVW: inverse variance weighted; SNPs, single nucleotide polymorphisms.

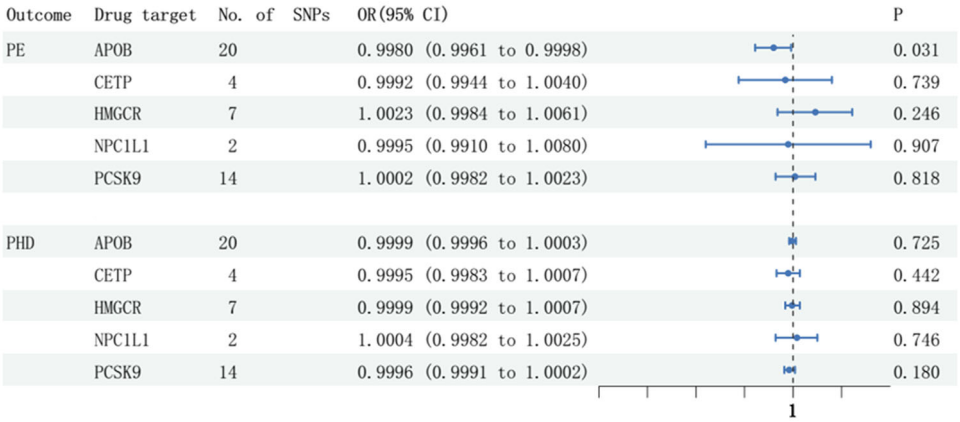

FIGURE 2 | Mendelian randomization effect estimate for low-density lipoprotein cholesterol mediated by drug target and pulmonary vascular disease outcomes (UK Biobank data). PAH: pulmonary arterial hypertension, PE: pulmonary embolism, PHD: pulmonary heart disease.

the UKB dataset, this association showed only a borderline protective effect that did not reach statistical significance (Table 2: OR = 0.998, 95% CI = 0.996–1.000, $p > 0.05$; Figure 2). CETP-, HMGCR-, NPC1L1-, and PCSK9-mediated LDL-C levels were not significantly associated with PHD risk in either dataset (Tables 1 and 2; Figures 1 and 2).

3.4 | Sensitivity Analyses

Figures 3 and 4 depict the results of leave-one-out sensitivity analyses for the FinnGen and UK Biobank datasets, respectively. These analyses confirm that no single SNP disproportionately influenced the MR effect estimates across all outcomes. This supports the robustness and consistency of the findings.

4 | Discussion

The present drug target MR found suggestive evidence for a positive association between NPC1L1-mediated and PCSK9-mediated LDL-C levels and the risk of pulmonary arterial hypertension (PAH). This indicates a protective effect of NPC1L1 and PCSK9 inhibitors against PAH. Conversely, APOB-mediated LDL-C levels were negatively associated with the risk of PE and PHD, suggesting a harmful effect of APOB inhibitors on these conditions. No associations were found between CETP- or HMGCR-mediated LDL-C levels and the risks of PAH, PE, or PHD.

According to Mendelian principles, alleles are randomly assigned to offspring, similar to the randomization process in controlled trials [41]. This reduces confounding from environmental and behavioral factors, making effect estimates more accurate [42, 43]. In this study, we found that NPC1L1-mediated and PCSK9-mediated lower LDL-C levels on PAH risk with a protective effect. Cholesterol homeostasis is regulated by a combination of endogenous cholesterol synthesis and intestinal uptake, absorption, transport, metabolism and secretion of cholesterol [44]. Among them, Niemann-Pick C1-like protein 1 (NPC1L1) is abundantly expressed in intestinal epithelial cells and is a cholesterol absorption transporter protein in the small intestine, central to cholesterol uptake by intestinal epithelial cells [45, 46]. The inhibitors of NPC1L1 inhibit cholesterol absorption in the small intestine by inhibiting the activity of NPC1L1, thereby reducing serum cholesterol levels [47].

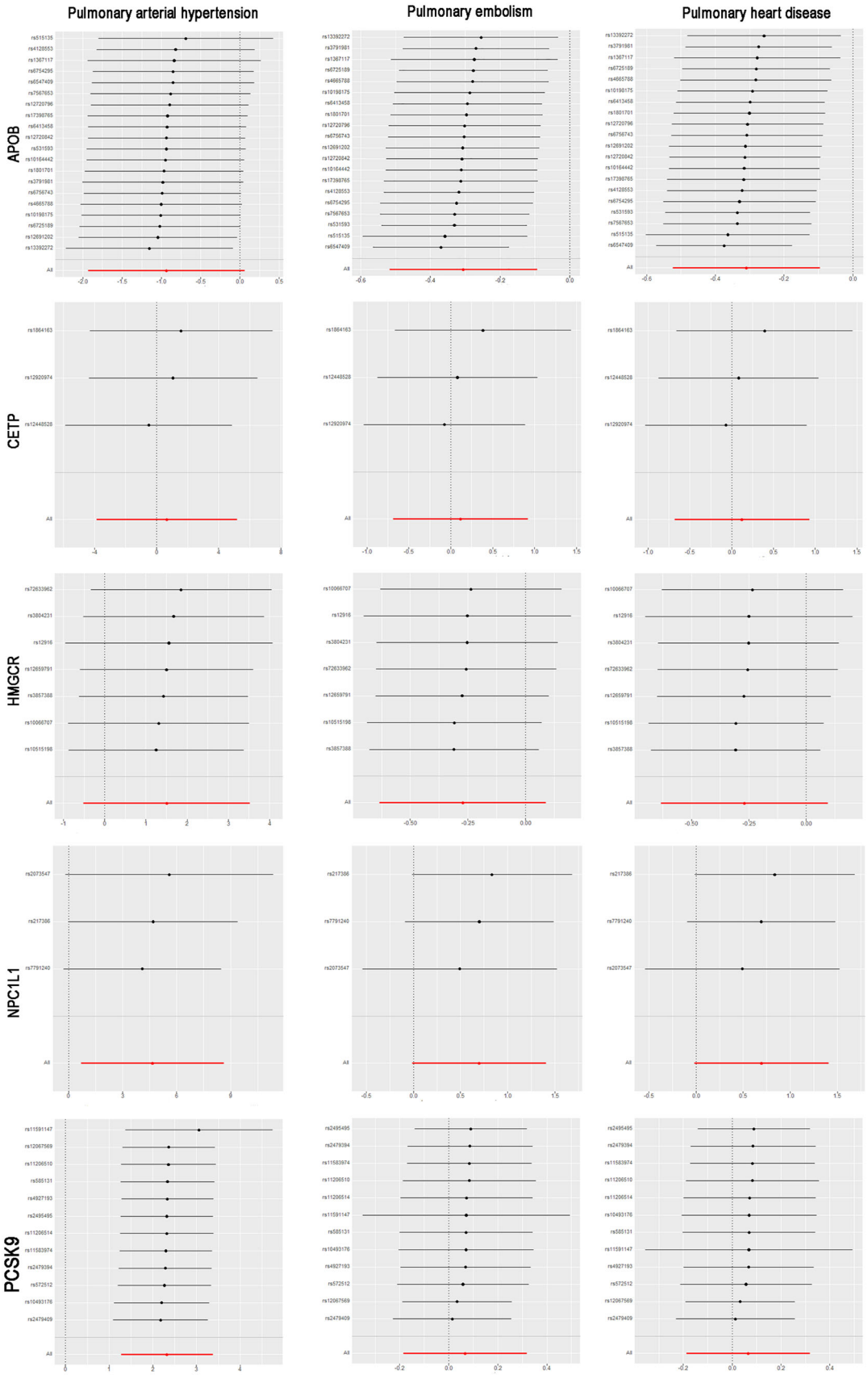

FIGURE 3 | Leave-One-Out Analysis (FinnGen Data). The figure showed the leave-one-out analysis of genetic instruments. The first column displayed the pulmonary arterial hypertension risk, the second column displayed the pulmonary embolism risk, and the third column displayed the pulmonary heart disease risk. The first row displayed the APOB, the second row displayed the CETP, the third row displayed the HMGCR, the fourth row displayed the NPC1L1 and the fifth row displayed the PCSK9.

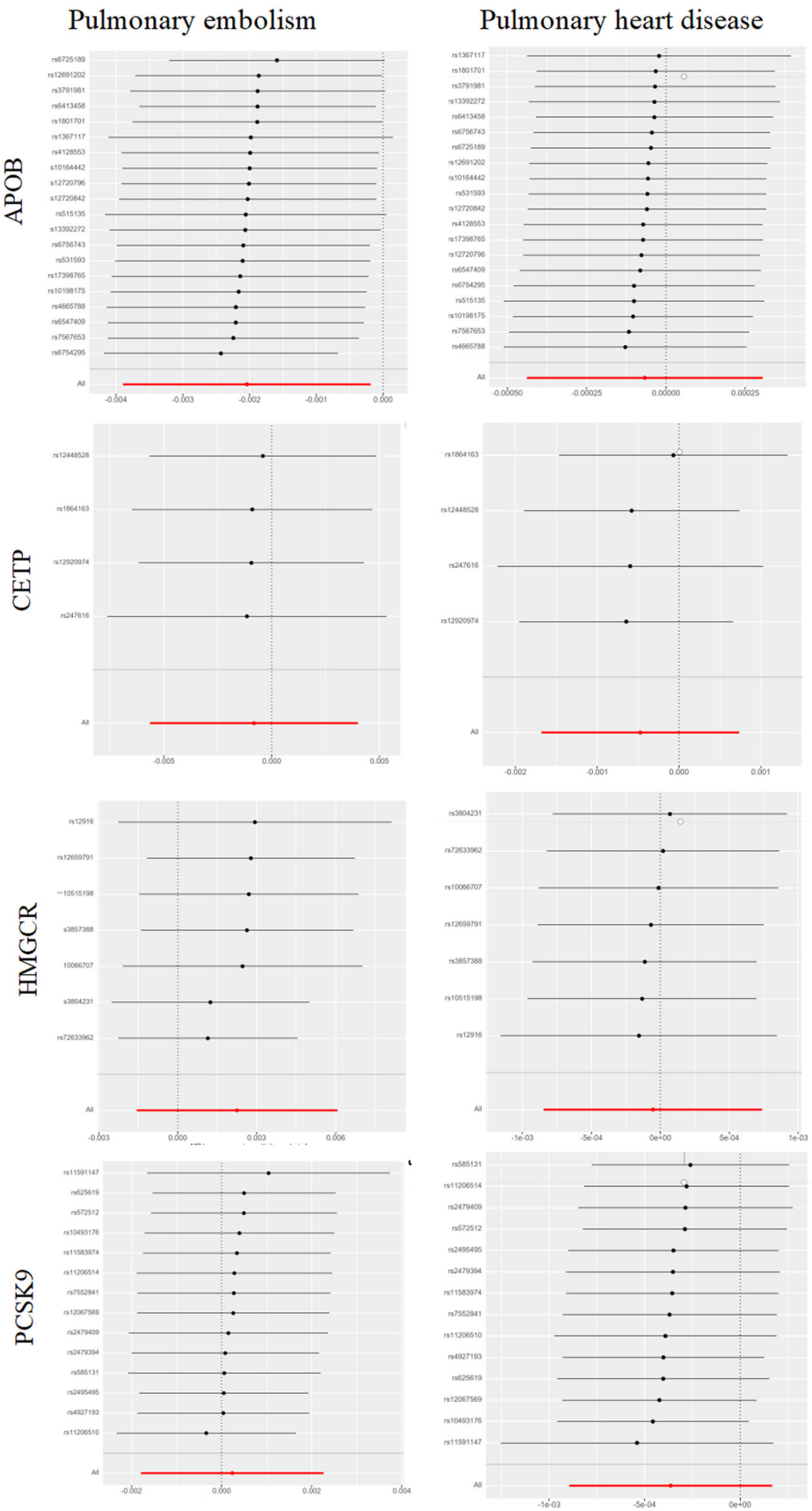

FIGURE 4 | Leave-One-Out Analysis (UK Biobank Data). The figure showed the leave-one-out analysis of genetic instruments. The first column displayed the pulmonary arterial hypertension risk, the second column displayed the pulmonary embolism risk, and the third column displayed the pulmonary heart disease risk. The first row displayed the APOB, the second row displayed the CETP, the third row displayed the HMGCR, and the fifth row displayed the PCSK9.

In addition, the proprotein convertase subtilisin/kexin type 9 (PCSK9) has the effect of increasing plasma LDL-C levels in vivo by binding to the low-density lipoprotein receptor (LDLR) and reducing serum cholesterol levels [48]. Inhibitors of PCSK9 can neutralize or inhibit PCSK9 protein, blocking its mediated LDLR degradation process and upregulating cell surface LDLR levels thereby enhancing the body's ability to metabolize LDL-C [48]. In addition, recent studies have shown that PCSK9 inhibitors are pleiotropic and have potential anti-inflammatory [49], anti-arterial and venous thrombosis [50], and enhanced efficacy of tumor immunosuppressants [51]. Meanwhile, a previous study found that patients with hypercholesterolemia treated with PCSK9 monoclonal antibody (mAb), could observe a significant decrease in platelet activity, which was significantly correlated with LDL-C and PCSK9 levels [52]. Furthermore, the main pathogenesis of PAH is pulmonary vasodilatory dysfunction, progressive pulmonary vascular remodeling, and persistently elevated pulmonary artery pressure [53], which results in intimal fibrosis, intimal thickening, and in situ thrombosis of small pulmonary arteries [54]. Among them, thrombotic lesions are a common pathological alteration in PAH, and platelets play an important role in the thrombosis process [55, 56]. It had reported that activated platelets can secrete pro-vasoconstrictive factors, growth factors, inflammatory factors and other active substances that act on pulmonary vascular cells, which also promote the development of PAH [55–57]. Moreover, previous studies have shown that the lung is an important site of platelet production, which also suggests that platelets play an important role in the pathogenesis of PAH [57]. These results suggested the ability of PCSK9 mAb to lower lipids, neutralize PCSK9 level, and correlate with platelet function. Therefore, inhibitors of NPC1L1 and PCSK9 reduced PAH risk, possibly by lowering LDL-C levels or modulating platelet function.

Our study also found evidence of a deleterious effect of APOB-mediated lower LDL-C level on the risk of PE and PHD. Apolipoproteins are the protein fraction of plasma lipoproteins, binding and transporting blood lipids to various tissues of the body for metabolism and utilization [58, 59]. Apolipoprotein B (ApoB) particles can increase the risk of thrombosis by inhibiting the fibrinolytic system and stimulating the production of cytokines [60]. Moreover, there is a correlation between ApoB and the degree of stenosis. PE is not an independent disease, which is mainly caused by VTE [3, 61]. Chronic thromboembolic pulmonary hypertension (CTEPH) is a form of pulmonary hypertension that occurs as a complication in patients who have experienced acute thrombotic events [62]. CTEPH is associated with proximal pulmonary artery obstruction and vascular remodeling as one of the etiologies of pulmonary hypertension [63]. Furthermore, PAH is a key link in the pathological process of PHD [64]. Meanwhile, some studies have shown that immune inflammation and coagulation disorders are involved in the development of PAH, which also become an important influencing factor in PHD [65, 66]. Hence, our results are contradictory to previous studies. We assume that the increased risk of PE and PHD due to APOB inhibitors may be due to off-target effects rather than to lipid-lowering or antithrombotic effects.

We also confirmed that there is no evidence of an association between CETP-mediated or HMGCR-mediated LDL-C level and the risk of PAH, PE or PHD. CETP can encode a lipoprotein that mediates the transfer of cholesteryl esters from HDL-C to ApoB-containing lipoproteins in exchange for TG and is directly involved in the cholesterol reversal transport process [67]. Some studies have found that inhibition of CETP could increase HDL-C levels, but could not show a cardiovascular benefit [67–72]. Hence, our MR study found no evidence of an association between CETP and the risk of pulmonary vascular disease, which might be a similar mechanism for the lack of clear benefit or therapeutic value for cardiovascular disease. In addition, hydroxymethylglutaryl coenzyme A reductase, an enzyme encoded by HMGCR, has an important regulatory role in the synthesis and metabolism of cholesterol and is inhibited by statins [73]. Some studies have found that statins couldn't improve pulmonary arterial pressure, exercise capacity and cardiac index in patients with PAH [21, 22, 25]. But it can improve pulmonary arterial pressure in patients with pulmonary hypertension due to chronic obstructive pulmonary disease [25]. Meanwhile, rosuvastatin could decrease the risk of VTE. We assumed that the negative results of HMGCR-mediated LDL-C on PAH and PE risk might be due to the complexity of the disease etiology.

This study has several limitations that should be considered. The lack of replication for PHD associations in the UK Biobank cohort, likely due to the small number of cases, and the absence of PAH data in the UK Biobank highlight the need for validation in larger cohorts. Additionally, the limited number of PAH cases in the FinnGen dataset may reduce the robustness of the findings. The study did not perform subgroup analyses to differentiate the etiologies of PAH and PE, which could provide more detailed insights. Furthermore, as the datasets were predominantly derived from individuals of European ancestry, the generalizability of these findings to other populations remains uncertain. Lastly, the observed effects of lipid-lowering drugs may reflect off-target mechanisms rather than solely LDL-C reduction. Future studies, including larger and more diverse cohorts as well as clinical trials, are necessary to confirm these results and explore underlying mechanisms.

In conclusion, this MR analysis demonstrated a causal relationship between NPC1L1 and PCSK9 inhibitors and a reduced risk of PAH. Meanwhile, it revealed a causal association between APOB inhibitors and an increased risk of PE and PHD. These findings suggest the potential therapeutic benefits of targeting NPC1L1 and PCSK9 in PAH and indicate the need for further investigation into the effects of APOB inhibitors on PE and PHD risks.

Author Contributions

Hong P proposed the idea; Hong P and Yuan X acquired the data. Hong P and Yuan X analyzed the data. Hong P wrote the first draft; Yuan X revised the draft. All authors have approved the final article.

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Ethics Statement

This study makes use of publically accessible GWAS summary data. The FinnGen study was approved by the Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (number HUS/990/2017). The UK Biobank data were collected under approval from the UK Biobank Research Ethics Committee as part of a larger initiative (application ID 17618). The data from the GLGC was a meta-analysis, which the ethics approval was not applicable. All participants signed written informed consent forms in their original studies. Because there were no direct participants in this study, informed consent for this study was not applicable.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data analyzed in this study are available on the IEU public availability (https://gwas.mrcieu.ac.uk/).

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