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Mendelian randomization study on causal association of IL-6 signaling with pulmonary arterial hypertension

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ABSTRACT

Background: A recent Mendelian randomization (MR) did not support an effect of the lead interleukin-6 receptor (IL-6 R) variant on risk of pulmonary arterial hypertension (PAH). Thus, we used two sets of genetic instrumental variants (IVs) and publicly available PAH genome-wide association studies (GWAS) to reassess the genetic causal link between IL-6 signaling and PAH.

Methods: Six independent IL-6 signaling and 34 independent soluble IL-6 receptor (sIL-6 R) genetic IVs from recent MR reports and PAH GWAS including 162,962 European individuals were used to perform this two-sample MR study.

Results: We found that as IL-6 signaling genetically increased, the risk of PAH reduced using IVW (odds ratio [OR] = 0.023, 95% confidence interval [CI]: 0.0013–0.393; p = .0093) and weighted median (OR = 0.033, 95% CI: 0.0024–0.467; p = .0116). Otherwise, as sIL-6 R genetically increased, the risk of PAH increased using IVW (OR = 1.34, 95% CI: 1.16–1.56; p = .0001), weighted median (OR = 1.36, 95% CI: 1.10–1.68; p = .005), MR-Egger (OR = 1.43, 95% CI: 1.05–1.94; p = .03), and weighted mode (OR = 1.35, 95% CI for OR: 1.12–1.63; p = .0035).

Conclusion: Our analysis suggested the causal link between genetically increased sIL-6 R and increased risk of PAH and between genetically increased IL-6 signaling and reduced risk of PAH. Thus, higher sIL-6 R levels may be a risk factor for patients with PAH, whereas higher IL-6 signaling may be a protective factor for patients with PAH.

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IL-6; sIL-6R; PAH; Genome-wide association study; Mendelian randomization

Introduction

Pulmonary arterial hypertension (PAH) is a complex disease characterized by a progressive obliterative vasculopathy of the distal pulmonary arterial circulation (1). PAH leads to progressive increases in pulmonary vascular resistance, right heart failure, and death if untreated (2). Patients with PAH are associated with significant morbidity and mortality (3). Despite the availability over the past 15 years of multiple drugs interfering with the endothelin, nitric oxide and prostacyclin pathways, PAH remains a severe clinical condition (4).

Perivascular inflammation is a prominent pathologic feature in patients with PAH and animal models (5). Inflammation has been highlighted as a key factor in PAH development, particularly interleukin-6 (IL-6) (6). Thus, classical IL-6 signaling may be a promising therapeutic target for PAH (7). Isolated case reports have suggested regression of PAH with tocilizumab (a monoclonal antibody targeting IL-6 receptor) in associated systemic lupus erythematosus, mixed connective tissue diseases and Castleman's disease (8,9). However, one proof-of-concept open-label clinical trial demonstrated no significant effect of tocilizumab in prevalent group 1 PAH (10). The lack of efficacy in this trial would seem to contradict the extensive data in preclinical cell and animal models for an important potentially causal role for IL-6 (1,5,7,10,11).

Many factors including reverse causation and confounding bias observational studies and result in the absence of high-quality RCTs. Based on the principle that genetic variants are randomly allocated at meiosis, Mendelian randomization (MR) study is independent of many factors that bias observational studies and has been widely used to identify the causal link between an exposure and an outcome (12–18). However, a recent Mendelian randomization (MR) did not support an effect of IL-6 on PAH risk using a genetic variant in IL6R, rs7529229 and PAH genome-wide association studies (GWAS) including 11,744 European individuals (10). Thus, we used two sets of genetic instrumental variants (IVs) including six independent IL-6 signaling and 34 independent soluble IL-6 receptor (sIL-6 R) genetic IVs from recent MR reports and publicly available PAH GWAS including 162,962 European individuals to identify the genetic causal link between IL-6 signaling and PAH.

Materials and methods

Ethics approval and consent to participate

Our study was approved by the Ethics Committee of Beijing Institute of Brain Disorders in Capital Medical University. This article contains human participants collected by several

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previous studies to report the large-scale GWAS. All participants gave informed consent in all the corresponding original studies, as described in the Methods.

IL-6 signaling and sIL-6 R genetic instrumental variants (IVs)

Based on recent MR reports (19–22), IL-6 signaling and sIL-6 R genetic IVs were chosen. IL-6 signaling and sIL-6 R genetic IVs have been used to identify the causal link between IL-6 and autoimmune arthritis (19), ischemic stroke and other cardiovascular outcomes (22), depressive symptoms (21), cardiovascular diseases, immune-related disorders and longevity (20). Six IL-6 signaling and 34 sIL6R genetic IVs were generated from a meta-analysis of a large-scale chronic inflammation GWAS of 204,402 European individuals (23) and plasma proteome GWAS of 3,301 European individuals (24), respectively. "IL6 signaling" referred to IL-6 R genetic instruments and weighted by the level of CRP (21) and plasma sIL6R with sgp130 forms an inhibitory receptor to suppress IL-6 signaling (25). The Linkage disequilibrium (LD) matrix Tool was used to determine LD levels of SNPs (https://ldlink.nci.nih.gov/?tab=ldmatrix, CEU; $r^2 < 0.1$). Six potential IL-6 signaling genetic IVs and 34 potential sIL-6 R genetic IVs were shown in Table 1.

Pulmonary arterial hypertension (PAH) GWAS

To date, the largest PAH GWAS summary statistics was provided by ieu open GWAS project in 2021. This PAH GWAS consists of 125 PAH cases and 162,837 healthy controls from European ancestry. The summary statistics of pulmonary arterial hypertension (PAH) GWAS is available on ieu open GWAS project at https://gwas.mrcieu.ac.uk/datasets/finn-b-I9_HYPTENSPUL. The summary information about the largest PAH GWAS is shown in Table 2.

Association of IL-6 signaling and sIL-6 R genetic instrumental variants (IVs) in pulmonary arterial hypertension (PAH) GWAS

The LD proxy tool was used to identify potential proxy SNPs ($r^2 > 0.8$) when IL-6 signaling and sIL-6 R IVs could not be found in PAH summary statistics. All six independent IL-6 signaling genetic IVs were successively extracted from this PAH GWAS summary statistics. Of 34 independent sIL-6 R

Table 1. IL-6-signaling and sIL-6 R genetic instrumental variants (IVs).

Exposure	SNP	EA	NEA	Beta	SE	*p* val	Gene
IL-6-signaling	rs73026617	T	C	0.0474	0.0068	3.16E-12	IL6R
	rs12083537	A	G	0.0643	0.0053	7.14E-34	IL6R
	rs4556348	T	C	0.0541	0.0067	6.77E-16	IL6R
	rs2228145	A	C	0.0899	0.0042	1.21E-101	IL6R
	rs11264224	A	C	0.0465	0.0057	3.41E-16	ADAR
	rs12059682	T	C	-0.0441	0.0049	2.26E-19	ADAR
sIL-6 R	rs61806853	T	C	-0.4957	0.0573	5.01E-18	TPM3
	rs181862028	A	T	-0.4133	0.1042	7.24E-05	HAX1
	rs3103309	T	C	0.16	0.0261	8.51E-10	HAX1
	rs2297607	A	G	0.1756	0.0291	1.66E-09	ATP8B2
	rs56258967	T	C	0.4718	0.1151	4.17E-05	ATP8B2
	rs116568035	A	G	-0.3112	0.0696	7.76E-06	ATP8B2
	rs79438587	T	C	0.405	0.0338	4.07E-33	ATP8B2
	rs35717427	A	G	0.5238	0.036	5.62E-48	IL6R
	rs7525477	A	G	-0.3502	0.0261	4.79E-41	IL6R
	rs79778789	A	G	-0.7852	0.0887	8.32E-19	IL6R
	rs79219014	T	G	0.7582	0.0767	4.57E-23	IL6R
	rs139952834	T	C	-0.6506	0.1072	1.29E-09	IL6R
	rs113580743	A	G	-0.5141	0.0605	1.95E-17	IL6R
	rs4129267	T	C	1.1148	0.0157	1.00E-200	IL6R
	rs142712385	A	T	-0.2782	0.0534	1.91E-07	IL6R
	rs77741705	C	G	0.5205	0.0941	3.16E-08	IL6R
	rs79925547	T	C	0.752	0.1155	7.41E-11	IL6R
	rs147700711	T	G	-0.4972	0.119	2.95E-05	IL6R
	rs76518735	A	C	0.5646	0.0924	1.00E-09	SHE
	rs41269913	T	C	0.6983	0.0599	1.95E-31	SHE
	rs77994623	T	C	-0.6184	0.0307	2.75E-90	TDRD10
	rs4633282	T	C	0.6124	0.0286	6.31E-102	TDRD10
	rs116805289	A	C	0.6267	0.0822	2.51E-14	TDRD10
	rs76289529	T	C	0.6656	0.0678	9.77E-23	TDRD10
	rs115697580	A	G	-0.4684	0.0967	1.26E-06	TDRD10
	rs149551556	T	C	-0.6651	0.0947	2.19E-12	UBE2Q1
	rs67860750	C	G	0.3985	0.0353	1.41E-29	CHRNB2
	rs138398618	A	G	-0.5044	0.1027	9.12E-07	CHRNB2
	rs3766925	A	T	0.184	0.0294	3.89E-10	ADAR
	rs11264224	A	C	-0.4576	0.0336	3.89E-42	ADAR
	rs3766924	T	C	-0.3863	0.03	7.94E-38	ADAR
	rs115880387	A	G	-0.6209	0.1388	7.59E-06	ADAR
	rs147745605	T	C	0.5012	0.1034	1.26E-06	ADAR
	rs10752605	A	G	-0.3349	0.0374	3.63E-19	ADAR

IL-6: interleukin-6; sIL-6 R: soluble IL-6 receptor; IVs: instrumental variants; SNP: single-nucleotide polymorphism; EA: effect allele; NEA: non-effect allele; Beta: the regression coefficient based on the IL-6-signaling or sIL-6 R raising effect allele; SE: standard error.

Table 2. Pulmonary arterial hypertension (PAH) genome-wide association study (GWAS).

GWAS ID	Year	Trait	ncase	ncontrol	nsnp	Population	Sex
finn-b-I9_HYPTENSPUL	2021	Hypertension, Pulmonary Arterial	125	162,837	16,380,163	European	Males and Females

PAH: pulmonary arterial hypertension; GWAS: genome-wide association study; GWAS ID: GWAS identity; ncase: the number of patients with PAH; ncontrol: the number of healthy controls; nsnp: the number of single-nucleotide polymorphism.

genetic IVs, 33 were successively extracted from this PAH GWAS summary statistics. The association of IL-6 signaling and sIL-6 R genetic IVs within PAH GWAS datasets was shown in Table 3.

Pleiotropy test

MR-Egger intercept and MR-pleiotropy residual sum and outlier (MR-PRESSO) tests have previously been described to test the pleiotropy (26). MR-Egger is based on the same regression model with inverse variance weighted (IVW), but allows and accounts for the potential pleiotropy using the MR-Egger intercept test (26–28). If the selected genetic variants are not pleiotropic, then the MR-Egger intercept term should tend to zero as the sample size increases (28). MR-PRESSO could detect and correct for the horizontal pleiotropy via outlier removal (the MR-PRESSO outlier test) (26). R Packages 'Mendelian Randomization' (29) and "MR-PRESSO" (26) was used to complete the statistical tests. Both MR-Egger intercept and MR-PRESSO methods were used to test the pleiotropy of independent IL-6 signaling or sIL-6 R genetic IVs in PAH GWAS dataset. The results about pleiotropy test were shown in Table 4. $p > .05$ represents no pleiotropy of independent IL-6 signaling or sIL-6 R genetic IVs in PAH GWAS.

Heterogeneity test

MR-Egger and inverse variance weighted (IVW) in Cochran's Q statistic have been broadly used to examine the heterogeneity (30,31). Cochran's Q statistic could provide evidence of heterogeneity due to pleiotropy or other causes (30).

Table 3. Association of IL-6-signaling and sIL-6 R genetic instrumental variants (IVs) with pulmonary arterial hypertension (PAH) GWAS.

		Exposure (IL-6 or sIL-6 R)			Outcome (PAH)		
Exposure	SNP	Beta	SE	*p* val	Beta	SE	*p* val
IL-6-signaling	rs11264224	0.047	0.006	3.41E-16	-0.565	0.192	0.003
	rs12059682	-0.044	0.005	2.26E-19	0.255	0.156	0.102
	rs12083537	0.064	0.005	7.14E-34	0.027	0.153	0.862
	rs2228145	0.090	0.004	1.21E-101	-0.340	0.139	0.015
	rs4556348	0.054	0.007	6.77E-16	-0.091	0.168	0.588
	rs73026617	0.047	0.007	3.16E-12	-0.413	0.186	0.026
sIL-6 R	rs10752605	-0.335	0.037	3.63E-19	-0.042	0.208	0.841
	rs11264224	-0.458	0.034	3.89E-42	-0.565	0.192	0.003
	rs113580743	-0.514	0.061	1.95E-17	-0.012	0.195	0.951
	rs115697580	-0.468	0.097	1.26E-06	-0.621	0.367	0.091
	rs115880387	-0.621	0.139	7.59E-06	-1.763	1.246	0.157
	rs116568035	-0.311	0.070	7.76E-06	-0.054	0.354	0.880
	rs116805289	0.627	0.082	2.51E-14	0.079	0.352	0.822
	rs138398618	-0.504	0.103	9.12E-07	-0.250	0.438	0.567
	rs139952834	-0.651	0.107	1.29E-09	0.037	0.370	0.920
	rs142712385	-0.278	0.053	1.91E-07	-0.008	0.234	0.971
	rs147700711	-0.497	0.119	2.95E-05	0.980	1.004	0.329
	rs147745605	0.501	0.103	1.26E-06	0.133	0.616	0.829
	rs149551556	-0.665	0.095	2.19E-12	0.610	0.756	0.420
	rs2297607	0.176	0.029	1.66E-09	0.063	0.135	0.642
	rs3103309	0.160	0.026	8.51E-10	-0.024	0.142	0.864
	rs35717427	0.524	0.036	5.62E-48	0.175	0.197	0.374
	rs3766924	-0.386	0.030	7.94E-38	-0.255	0.156	0.102
	rs3766925	0.184	0.029	3.89E-10	0.029	0.145	0.844
	rs41269913	0.698	0.060	1.95E-31	-0.620	0.435	0.154
	rs4129267	1.115	0.016	1.00E-200	0.342	0.139	0.014
	rs4633282	0.612	0.029	6.31E-102	0.189	0.135	0.162
	rs56258967	0.472	0.115	4.17E-05	0.193	1.135	0.865
	rs61806853	-0.496	0.057	5.01E-18	0.215	0.371	0.562
	rs67860750	0.399	0.035	1.41E-29	0.044	0.181	0.808
	rs7525477	-0.350	0.026	4.79E-41	0.089	0.129	0.489
	rs76289529	0.666	0.068	9.77E-23	1.377	0.691	0.046
	rs76518735	0.565	0.092	1.00E-09	0.083	0.348	0.811
	rs77741705	0.521	0.094	3.16E-08	0.515	0.719	0.474
	rs77994623	-0.618	0.031	2.75E-90	-0.283	0.192	0.139
	rs79219014	0.758	0.077	4.57E-23	-0.236	0.883	0.789
	rs79438587	0.405	0.034	4.07E-33	0.127	0.186	0.495
	rs79778789	-0.785	0.089	8.32E-19	-0.546	0.355	0.125
	rs79925547	0.752	0.116	7.41E-11	-0.881	1.338	0.510

IL-6: interleukin-6; sIL-6 R: soluble IL-6 receptor; IVs: instrumental variants; PAH: pulmonary arterial hypertension; GWAS: genome wide association study; SNP: single-nucleotide polymorphism; Beta: the regression coefficient based on IL-6-signaling or sIL-6 R raising effect allele; SE: standard error.

Table 4. Pleiotropy and heterogeneity test of IL-6-signaling and sIL-6 R genetic instrumental variants (IVs) in pulmonary arterial hypertension (PAH) GWAS.

	Pleiotropy test				Heterogeneity test					
	MR_Egger			PRESSO	MR Egger			IVW		
Exposure	Intercept	SE	*p* val	*p* val	Q	Q_df	*Q_p* val	Q	Q_df	*Q_p* val
IL-6-signaling	-0.304	0.342	0.425	0.177	8.011	4	0.091	9.589	5	0.088
sIL-6 R	-0.037	0.081	0.646	0.827	25.098	31	0.763	25.313	32	0.793

IL-6: interleukin-6; sIL-6 R: soluble IL-6 receptor; IVs: instrumental variants; PAH: pulmonary arterial hypertension; GWAS: genome wide association study; IVW: inverse variance weighted; SE: standard error. *p* val > 0.05 represent no significant pleiotropy. *Q_p* val > 0.05 represents no significant heterogeneity.

R Packages 'Mendelian Randomization' (29) was used to complete the statistical tests. Both MR-Egger and Inverse variance weighted (IVW) in Cochran's Q statistic were used to test the heterogeneity of independent IL-6 signaling or sIL-6 R genetic IVs in PAH GWAS dataset. The results about heterogeneity test were shown in Table 4. *Q_p* val > 0.05 represents no heterogeneity of independent IL-6 signaling or sIL-6 R genetic IVs in PAH GWAS.

MR analysis

The IVW was selected as the main MR analysis method to combine the variant-specific Wald estimators by taking the inverse of their approximate variances as the corresponding weights (28). In addition, we also selected the weighted median that could produce consistent estimates even up to 50% of selected genetic variants are not valid (26–28). R Packages 'Mendelian Randomization' (29) was used to complete all the statistical tests in MR analysis. Four MR analysis methods including MR-Egger, weighted median, IVW, and weighted mode were used to analyze the causal association of IL-6 signaling or sIL-6 R levels with PAH. The results about MR analysis were shown in Table 5. $p < .05$ represents the causal association of IL-6 signaling or sIL-6 R levels with PAH. To analyze the single SNP effect, individual causal effect of and single effect size of IL-6 signaling or sIL-6 R-associated SNPs on PAH were analyzed and shown in Figure 1 and Figure 2, respectively.

Table 5. The causal association of IL-6-signaling and sIL-6 R with pulmonary arterial hypertension (PAH).

Exposure	Method	nsnp	Beta	SE	*p* val	Beta_lci95	Beta_uci95	OR	OR_lci95	OR_uci95
IL-6-signaling	IVW	6	-3.79	1.46	0.0093	-6.653	-0.935	0.023	0.0013	0.393
	Weighted median	6	-3.41	1.35	0.0116	-6.051	-0.762	0.033	0.0024	0.467
sIL-6 R	IVW	33	0.29	0.08	0.0001	0.146	0.444	1.343	1.1569	1.5588
	Weighted median	33	0.31	0.11	0.0049	0.093	0.521	1.360	1.0975	1.6844
	MR Egger	33	0.36	0.16	0.0286	0.052	0.664	1.430	1.0538	1.9416
	Weighted mode	33	0.30	0.10	0.0035	0.114	0.488	1.351	1.1203	1.6293

IL-6: interleukin-6; sIL-6 R: soluble IL-6 receptor; PAH: pulmonary arterial hypertension. IVW: inverse variance weighted; nsnp: the number of single-nucleotide polymorphism; Beta: the regression coefficient based on IL-6-signaling or sIL-6 R raising effect allele; SE: standard error; $p < 0.05$ represents the causal association of the increased levels of IL-6-signaling or sIL-6 R with PAH; Beta_lci95: Lower limit of 95% confidence interval for beta; Beta_uci95: Upper limit of 95% confidence interval for beta; OR: Odds ratio; OR_lci95: Lower limit of 95% confidence interval for OR; OR_uci95: Upper limit of 95% confidence interval for OR.

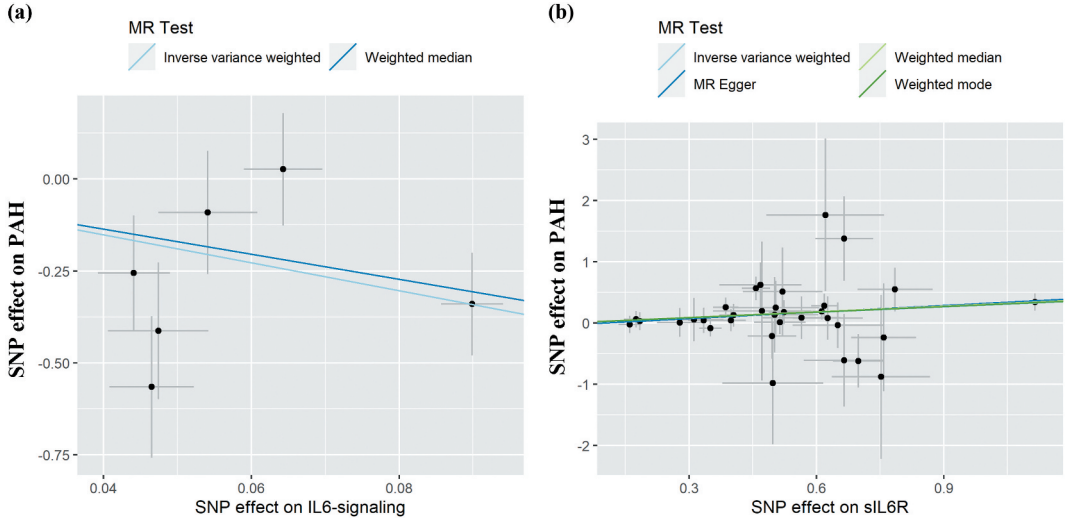

Figure 1. Individual estimates about the causal effect of IL-6 signaling and its negative regulator sIL-6 R on pulmonary arterial hypertension (PAH). The x-axis shows the single SNP (single nucleotide polymorphism) effect (beta value: the regression coefficient based on IL-6 signaling or sIL-6 R raising effect allele, dark dots) and standard error (SE, horizontal cross lines) of six IL-6 signaling (A) or 33 sIL-6 R (B) associated SNPs on IL-6 signaling (A) or sIL-6 R (B) levels, respectively. The y-axis shows the single SNP effect (beta value, dark dots) and standard error (SE, vertical cross lines) of six IL-6 signaling (A) or 33 sIL-6 R (B) associated SNPs on PAH risk. The regression lines for inverse variance weighted (IVW) and weighted median (A) or IVW, weighted median, MR-Egger, and weighted mode (B) are shown.

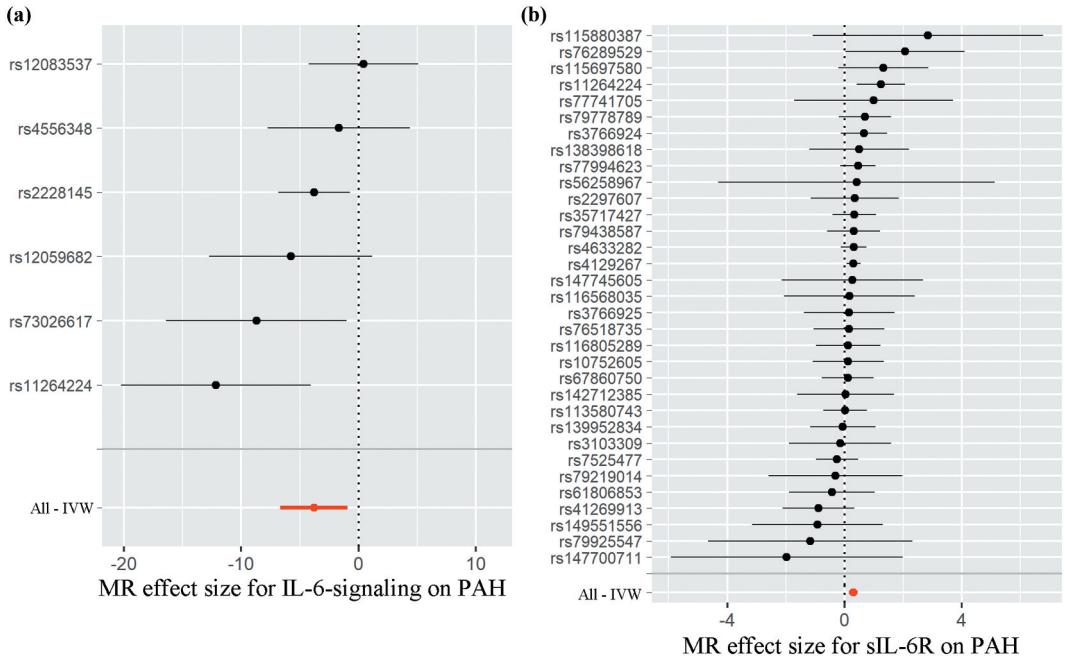

Figure 2. Forest plot of IL-6 signaling and its negative regulator sIL-6 R-associated SNPs with the risk of pulmonary arterial hypertension (PAH). The x-axis shows the MR effect size for six IL-6 signaling (A) or 33 sIL-6 R (B) associated SNPs on PAH. The y-axis shows the analysis for each and the total of six IL-6 signaling (A) or 33 sIL-6 R (B) associated SNPs using IVW methods. Dark dots: the single SNP effect (beta value). Red dot: the total SNP effect (beta value). Horizontal cross lines: standard error (SE). Vertical dotted line denotes the beta of 0.

Leave-one-out effect analysis

We examined the potential impact of outlying and pleiotropic SNPs on causal estimates adopting a leave-one-out strategy, under the IVW (random effects) model (32). This method as the sensitivity analysis methods performs the MR analysis but leaves out each SNP in turn to identify whether a single SNP is driving the association (32). Leave-one-out effect of IL-6 signaling or sIL-6 R-associated SNPs on PAH was shown in Figure 3.

Results

IL-6 signaling and sIL-6 R genetic instrumental variants (IVs) have no significant pleiotropy or heterogeneity in pulmonary arterial hypertension (PAH) GWAS

Six independent IL-6 signaling genetic IVs and 34 independent sIL-6 R genetic variants were used as potential genetic IVs (Table 1). Six independent IL-6 signaling genetic IVs and 33 independent sIL-6 R genetic IVs were successfully extracted from PAH GWAS (Table 2). The association of six

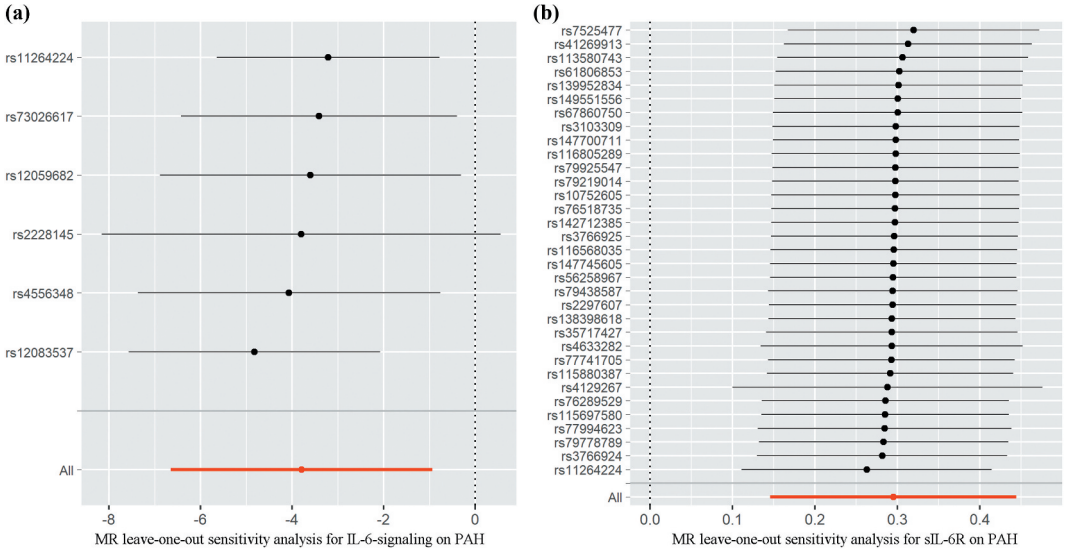

Figure 3. MR leave-one-out sensitivity analysis for the effect of IL-6 signaling and its negative regulator sIL-6 R SNPs on pulmonary arterial hypertension (PAH). The x-axis shows the MR leave-one-out sensitivity analysis for six IL-6 signaling (A) or 33 sIL-6 R (B) associated SNPs on PAH. The y-axis shows the analysis for the effect of leave-one-out and the total of six IL-6 signaling (A) or 33 sIL-6 R (B) associated SNPs on PAH using IVW methods. Dark dots: the single SNP effect (beta value). Red dot: the total SNP effect (beta value). Horizontal cross lines: standard error (SE). Vertical dotted line denotes the beta of 0.

independent IL-6 signaling genetic IVs and 33 independent sIL-6 R genetic IVs in PAH GWAS is shown in Table 3. We found no pleiotropy or heterogeneity of six independent IL-6 signaling genetic IVs and 33 independent sIL-6 R genetic IVs in PAH GWAS dataset (Table 4). Thus, all selected IL-6 signaling and sIL-6 R genetic variants can be taken as the effective IVs in our MR study.

IL-6 signaling genetically reduces pulmonary arterial hypertension (PAH) risk

As IL-6 signaling levels genetically increased, the risk of PAH reduced using IVW (Beta = -3.79, 95% CI for beta: -6.653–-0.935; OR = 0.023, 95% CI for OR: 0.0013–0.393; p = .0093) and weighted median (Beta = -3.41, 95% CI for beta: -6.051–-0.762; OR = 0.033, 95% CI for OR: 0.0024–0.467; p = .0116) (Table 5). The individual MR estimates of the causal effect demonstrated that as the effect of single SNP on IL-6 signaling increased, the suppressive effect of single SNP increased on PAH using IVW and weighted median (Figure 1A). Each effect size analysis suggested that each effect of six IL-6 signaling-associated SNPs on PAH was similar and there was no lead IVs (Figure 2A). MR leave-one-out sensitivity analysis showed that removing a specific SNP of six IL-6 signaling-associated SNPs did not change the results (Figure 3A). Collectively, our data suggested the causal association of genetically increased IL-6 signaling levels with reduced risk of PAH.

sIL-6 R genetically promotes pulmonary arterial hypertension (PAH) risk

As sIL-6 R levels genetically increased, the risk of PAH increased using IVW (Beta = 0.29, 95% CI for beta: 0.146–0.444; OR = 1.343, 95% CI for OR: 1.1569–1.5588; p = .0001), weighted median (Beta = 0.31, 95% CI for beta: 0.093–0.521; OR = 1.360, 95% CI for OR: 1.0975–1.6844; p = .0049), MR-Egger (Beta = 0.36, 95% CI for beta: 0.052–0.664; OR = 1.430, 95% CI for OR: 1.0538–1.9416; p = .0286), and weighted mode (Beta = 0.30, 95% CI for beta: 0.114–0.488; OR = 1.351, 95% CI for OR: 1.1203–1.6293; p = .0035) (Table 5). The individual MR estimates of the causal effect demonstrated that as the effect of single SNP on sIL-6 R levels increased, the promoting effect of single SNP increased on PAH using IVW, weighted median, MR-Egger, and weighted mode (Figure 1B). Each effect size analysis suggested that each effect of 33 sIL-6 R-associated SNPs on PAH were similar and there was not lead IVs (Figure 2B). MR leave-one-out sensitivity analysis showed that removing a specific SNP of 33 sIL-6 R-signaling-associated SNPs did not change the results (Figure 3B). Collectively, our data suggested the causal association of genetically increased sIL-6 R levels with increased risk of PAH.

Discussion

The present MR study demonstrated the causal link between genetically increased sIL-6 R levels and increased risk of PAH and between genetically increased IL-6 signaling and reduced risk of PAH. Thus, genetic predisposition to a higher sIL-6 R or lower IL-6 signaling level may be genetically associated with higher risk of PAH, whereas higher IL-6 signaling may be genetically associated with lower risk of PAH. Our findings showed corroborating evidence that the overactive IL-6 signal pathway led to the reduced risk of PAH, whereas its negative regulator sIL-6 R levels genetically increased the risk of PAH.

Toshner et al. recently found that the lead IL-6 R variant had no effect on risk of PAH using MR study (10). They used a genetic variant in IL6R, rs7529229 and total sample size (11,744). Low statistical power is a major limitation in MR study (33). To improve the statistical power, we used two sets of genetic instrumental variants (IVs) including six independent IL-6 signaling and 34 independent sIL-6 R genetic IVs and, to date, the largest PAH genome-wide association studies (GWAS) with total sample size (162,962 European individuals). Because of a higher statistical power, we found this causal association of genetically increased sIL-6 R levels with increased risk of PAH and genetically increased IL-6 signaling with reduced risk of PAH.

sIL-6 R also binds with IL-6 ("trans-signaling") to suppress the effect of IL-6 such as IL-6-induced inflammation (34). sIL-6 R concentrations [69.7 (IQR 60.4–84.4) vs 45.7 (IQR 34.6–70.3) ng/ml, p = .0036] increased in patients with PAH compared to control subjects (35). Thus, IL-6 trans-signaling is enhanced in PAH (35). Elevated concentration of sIL-6 R suggests its potential unfavorable role in systemic amplification of IL-6 signaling in PAH (35). IL-6 trans-signaling contributes to chronic hypoxia-induced pulmonary hypertension (36). Consistent with these studies, our study suggests a causal association of genetically increased sIL-6 R levels with increased risk of PAH. Thus, sIL-6 R may be a risk factor for PAH.

In PAH, with the exception of targeting proliferation with imatinib (37), no new hypothesis has survived through to phase 3 of drug development in the last 20 years (38). Increases in lung and serum IL-6 have been associated with PAH (39). Many studies have shown a rapid rise in lung IL-6 mRNA levels following exposure of mice to hypoxia, peaking at 24 h and remaining elevated for one week (40,41). These studies emphasize the importance of IL-6 as therapeutic target for treatment of PAH. Isolated case reports have suggested that tocilizumab has an effect in PAH associated systemic lupus erythematosus, mixed connective tissue diseases and Castleman's disease (8,9). However, a phase 2 open-label clinical trial demonstrated no significant effect of tocilizumab in idiopathic or heritable PAH and PAH associated with CTD excluding SLE, RA and mixed CTD (10). Consistent with this phase 2 open-label clinical trial, our analytical study also does not support IL-6 signaling may be a promising therapeutic target for PAH.

IL-6 concentrations were increased in pulmonary artery smooth muscle cells (PASMCs), but not endothelial cells, suggesting PASMCs may be a source of increased IL-6 (42). Whether local vascular and autocrine production of IL-6 is more important than inflammatory cells is an unanswered question but the repeated demonstration of PASMC does at the very least suggest a mechanism whereby IL-6 signaling locally could be uncoupled from classical pro-inflammatory cytokines (38). The classical pathway of IL-6 signaling mediated T cell-driven immune responses via membrane-

bound IL-6 Rα (mIL-6 Rα), whereas its trans-signaling contributes only at the local site, that is, in the affected tissues via sIL-6 Rα (43). Thus, local sIL-6 Rα induced perivascular inflammation that is a prominent pathologic feature in most animal models of pulmonary hypertension (PH) as well as in pulmonary arterial hypertension (PAH) patients (5). Consistent with these studies, a previous study pointed out that the role of IL-6 in homeostasis is mediated through the classic-signaling pathway, whereas pathology-associated responses are mediated by IL-6 trans-signaling (36,44). It may have clinical implications when specific inhibition of IL-6 trans-signaling should only block the pro-inflammatory effects of IL-6 without inhibiting its important homeostatic properties (36).

IL-6 has context-dependent anti-inflammatory properties (44). IL-6 protects against fatal lung pathology (45). IL-6 also induces expression of the IL-1 receptor antagonist and the soluble p55 receptor for tumor-necrosis factor (44,46). These findings are consistent with the ability of IL-6 to promote an alternatively activated macrophage phenotype associated with wound healing and its ability to inhibit the microbicidal activities of macrophages and the production of pro-inflammatory cytokines (44,47,48). In addition, IL-6 promotes the production of IL-10 by T cells, which would restrict many inflammatory processes (49,50). These studies suggest anti-inflammatory effects of IL-6. Consistent with anti-inflammatory role of IL-6, our study suggests a causal association of genetically increased IL-6 signaling with reduced risk of PAH. IL-6 signals through mIL-6 Rα to induce anti-inflammatory responses (36). Thus, future studies need to consider the complex role of IL-6 classical pathway via mIL-6 Rα and its trans-signaling via IL-6 Rα in the PAH disease process might be worthwhile.

This study has several strengths. First, six independent IL-6 signaling and 34 independent sIL-6 R genetic IVs were chosen from a previously reported large-scale IL-6 signaling GWAS of 204,402 European individuals (23) and plasma proteome (sIL-6 R) GWAS of 3,301 European individuals (24) and have broadly used in recent MR reports (19–22). Second, all six independent IL-6 signaling and 33 of 34 independent sIL-6 R genetic IVs were successively extracted from PAH GWAS. Third, we used four different MR analysis methods demonstrated no significant pleiotropy or heterogeneity of IL-6 signaling and sIL-6 R genetic IVs as the effective IVs. Fourth, two MR analysis methods including IVW and weighted median proved the causal link between genetically increased IL-6 signaling and reduced risk of PAH and four MR analysis including IVW, weighted median, MR-Egger, and weighted mode proved the causal link between genetically increased sIL-6 R levels and increased risk of PAH. Fifth, all three methods demonstrated that each effect of IL-6 signaling and sIL-6 R SNPs on PAH was no obvious bias. Finally, we used IL-6 signaling levels and its negative regulator sIL-6 R and critically, they showed the opposite result.

This study has several limitations. First, our IL-6 signaling and sIL-6 R IVs and PAH GWAS datasets are from European ancestry. Therefore, our results need be proven in other ancestries. Second, randomized controlled trials are required to clarify whether blockade of IL-6 signaling or sIL-6 R could is effective in some subsets of PAH. Third, the underlying mechanism by which IL-6 signaling or sIL-6 R genetically affects the risk of PAH is still unclear and worth to explore in the future.

In summary, our results suggested the causal association of genetically increased sIL-6 R levels with increased risk of PAH and genetically increased IL-6 signaling with reduced risk of PAH. Thus, a higher sIL-6 R levels may be a risk factor for patients with PAH, whereas a higher IL-6 signaling may be a protective factor for patients with PAH.

Abbreviations

PAH: pulmonary arterial hypertension; IL-6: interleukin-6; sIL-6R: soluble IL-6 receptor; GWAS: Genome-wide association study; MR: Mendelian randomization; SNP: Single nucleotide polymorphism; IVW: Inverse variance weighted.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Authors' contributions

RW conceived and initiated the project. SZ and GZ analyzed the data and wrote the manuscript. All authors contributed to the interpretation of the results and critical revision of the manuscript, and approved the final version of the manuscript. All authors read and approved the final manuscript.

Data availablity statement

The summary statistics of pulmonary arterial hypertension (PAH) GWAS (GWAS ID: finn-b-I9_HYPTENSPUL) is available on ieu open gwas project at https://gwas.mrcieu.ac.uk/datasets/. The MR analysis code can be found at https://mrcieu.github.io/TwoSampleMR/articles/index.html.

Ethics statement

The study was reviewed and approved by the Ethics Committee of Beijing Institute of Brain Disorders in Capital Medical University.

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