Epstein-Barr virus-related antibody traits and idiopathic pulmonary fibrosis: a bidirectional Mendelian randomization study

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Abstract

Introduction: Previous studies have suggested an association between herpesvirus infections and idiopathic pulmonary fibrosis (IPF), but the causal relationship remains largely unclear. We used bidirectional Mendelian randomization (MR) to investigate the associations between genetically predicted antibody responses to herpesviruses and IPF risk.

Material and methods: The data for different antibodies against herpes simplex virus (HSV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV) were obtained from the IEU GWAS database (https://gwas.mrcieu.ac.uk/datasets/), and the data for IPF were obtained from the FinnGen GWAS database (https://r7.finngen.fi/). We selected eligible single nucleotide polymorphisms (SNPs) from summary-level data of GWAS as instrumental variables. The generalized summary data-based MR (GSMR) method was used as the main analysis method, complemented by inverse-variance weighted (IVW), MR-Egger, and weighted median analyses. Sensitivity analyses were conducted to check the robustness of the MR results, and reverse MR analyses were performed to assess potential reverse causation.

Results: Genetically predicted antibody responses to EBV viral capsid antigen (VCA) p18 were associated with IPF risk. However, GSMR and IVW results indicated that anti-EBV IgG levels were significantly negatively associated with IPF. Sensitivity analyses suggested limited influence of horizontal pleiotropy.

Conclusions: Genetically predicted EBV-related immune responses show heterogeneous associations with IPF risk. These findings suggest a potential role of EBV-related immune mechanisms in IPF, although causal interpretation should be made cautiously. Further studies are needed to clarify underlying biological mechanisms.

Key words: Mendelian randomization, idiopathic pulmonary fibrosis, herpesvirus-related antibody traits, antibody response.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease of unknown cause, characterized by gradual fibrosis of the lung tissue, leading to a progressive loss of lung function and, ultimately, death [1]. The disease predominantly affects older men, with a median

survival time of only 3 to 5 years after diagnosis [2]. The incidence of IPF has steadily increased over the years [3]. A recent study on Medicare beneficiaries in the United States reported a rise from 202 cases per 100,000 in 2001 to 495 cases in 2011 among those aged 65 and older [4]. Currently, lung transplantation remains the only definitive treatment for IPF. Nintedanib and pirfenidone are the main FDA-approved drugs used in clinical practice to slow the progression of IPF. The complexity of IPF and the unclear etiology contribute to the significant challenges in its treatment [5–7].

Repeated injury to the alveolar epithelium leads to fibrosis. Factors such as genetic predispositions, environmental influences, immune dysregulation, and microbial elements all act as triggers for alveolar damage in IPF [8]. Among microbial factors, viruses are the most extensively studied, with chronic viral infections – such as those caused by herpes simplex virus (HSV), Epstein-Barr virus (EBV), and cytomegalovirus (CMV) – being implicated in the onset or progression of IPF. A meta-analysis of studies from 10 countries, involving 1,287 participants, revealed that viral infections were significantly associated with the risk of developing IPF, but were not associated with exacerbation of the disease. All the analyzed viruses, including EBV, CMV, human herpesvirus 7 (HHV-7), and human herpesvirus 8 (HHV-8), were associated with an increased risk of developing IPF [9]. Herpesviruses, particularly, have been the subject of substantial research. They are categorized into three subfamilies based on their cellular targets and sites of latency: (i) α herpesviruses, such as HSV; (ii) β herpesviruses, such as CMV; and (iii) γ herpesviruses, such as EBV [10]. Once a herpesvirus infects a host, it establishes lifelong latency in the nuclei of host cells, persisting in an episomal form, and can periodically reactivate to produce infectious virions, leading to symptomatic recurrence [11]. The study by Lok *et al*. [12] in animal models demonstrated that herpesviruses do not directly induce pulmonary fibrosis, but viral replication can accelerate the fibrotic process in the presence of pre-existing lung injury.

Further evidence supporting the link between herpesvirus infections and IPF comes from a study showing elevated titers of HSV immunoglobulin G (IgG), CMV IgG, and EBV capsid antigen in IPF patients compared to healthy controls [13]. Additionally, high viral loads of EBV and CMV have been detected in the alveolar epithelial cells of IPF patients. Interestingly, EBV antigens have been associated with markers of endoplasmic reticulum (ER) stress, suggesting a mechanistic link between latent viral infections and the development of IPF [14]. However, the causal relationship between these chronic viral infections and IPF remains to be determined. In fact, current observational studies in this field have several limitations, such as small sample sizes, residual confounding, detection bias, and reverse causality [15, 16].

In this study, we aimed to analyze the potential causal relationship between genetically predicted antibody responses to herpesvirus antigens and IPF. Mendelian randomization (MR) is a commonly used epidemiological approach for causal inference that leverages summary-level data from genome-wide association studies (GWAS) to evaluate potential causal effects of exposures on outcomes [17]. Since alleles are randomly allocated during meiosis, genetic variants serve as instrumental variables for exposures, reducing – but not eliminating – the influence of confounding and reverse causation that affect observational studies [18, 19]. Routine two-sample MR methods employ multiple complementary approaches, including inverse variance weighting (IVW), MR-Egger, weighted median, and sensitivity analyses, to assess the robustness of results. In contrast to conventional MR, generalized summary-data-based MR (GSMR) offers several advantages: it corrects for biases using linkage disequilibrium (LD) matrices and employs the heterogeneity in dependent instruments (HEIDI) outlier test to identify and remove pleiotropic single nucleotide polymorphisms (SNPs), thereby improving the accuracy and reliability of causal inferences [20]. Given the lack of conclusive epidemiological evidence on the potential associations between genetically predicted antibody responses to herpesvirus antigens and IPF risk. We applied both bidirectional two-sample MR and GSMR methods to evaluate associations between genetically predicted antibody responses to three herpesvirus (HSV, CMV, EBV) and the risk of IPF.

Material and methods

Data sources

This study used large publicly available GWAS databases. To minimize bias due to population stratification, we included study samples of European ancestry. We obtained the summary GWAS data for twelve antibodies against herpesvirus from the study by Butler-Laporte *et al.* [21]. Antibody titers were based on log-transformed units. This study comprehensively covers various phenotypes associated with antibody responses to herpesvirus antigens (HSV, CMV, EBV).

We accessed summary-level data for three HSV-related antibodies from the MRC-IEU UK Biobank and the IEU OpenGWAS project, including GWAS summary statistics for anti-HSV-1 IgG levels (GWAS ID: ieu-b-4906, n = 683), HSV-1 IgG1 antibody levels (GWAS ID: ebi-a-GCST90006918,

n = 6,199), and HSV-2 IgG2 antibody levels (GWAS ID: ebi-a-GCST90006920, *n* = 1,382). Similarly, we obtained GWAS summary data for four CMV-related antibodies, including anti-CMV IgG levels, CMV pp28 antibody levels, CMV pp52 antibody levels, and CMV pp150 antibody levels. These data came from GWAS analyses involving 5,010, 5,087, 5,681, and 5,136 participants, respectively, and were available in GWAS databases under the GWAS IDs ieu-b-4900, ebi-a-GCST90006894, ebi-a-GCST90006895, and ebi-a-GCST90006896. We also accessed five sets of GWAS summary statistics for EBV-related antibodies, including anti-EBV IgG levels (GWAS ID: ieu-b-4901, *n* = 5,010), EBV early antigen-D (EA-D) antibody levels (GWAS ID: ebi-a-GCST90006898, *n* = 7,763), EBV nuclear antigen-1 (EBNA-1) antibody levels (GWAS ID: ebi-a-GCST90006899, n = 7,972), EBV viral capsid antigen (VCA) p18 antibody levels (GWAS ID: ebi-a-GCST90006900, *n* = 8,518), and EBV Z EBV replication activator (ZEBRA) antibody levels (GWAS ID: ebi-a-GCST90006901, *n* = 8,191).

We sourced the GWAS summary data for IPF from the FinnGen cohort (https://r7.finngen.fi/), which comprised 1,514 IPF cases and 306,063 controls. All cases met the International Classification of Diseases (ICD-10) criteria. Detailed information on the selected datasets are provided in Table I and Supplementary Table SI.

This study used GWAS summary datasets for bidirectional MR analysis. We sourced all datasets from previously published publicly available GWAS studies that had obtained ethical approval and participant consent. The summary statistics were publicly available for download, fully de-identified, and could be accessed without any restriction.

Selection of instrumental variables

The following criteria were applied to select instrumental variables (IVs): (1) SNPs reaching GWAS significance threshold ($p < 5 \times 10^{-6}$) were included as IVs for herpesvirus-related antibodies, except for the anti-EBV IgG levels, for which the GWAS significance threshold was set at $p < 5 \times 10^{-5}$ to obtain a sufficient number of SNPs, as fewer than three genome-wide significant SNPs were available under the primary threshold. For reverse causal analyses, we used the same threshold ($p < 5 \times 10^{-6}$) to select SNPs associated with IPF. (2) To ensure independence and minimize the impact of LD, we clumped the GWAS significant SNPs from each split's GWAS with a clumping window of 10,000 kb and an r^2 threshold of 0.001. The reference panel used for LD estimation was the 1000 Genomes phase 3 European population. (3) We also excluded SNPs with a minimum allele frequency (MAF) less than 0.01 because the impact of these SNPs was not stable. Palindromic SNPs with intermediate allele frequencies were removed to avoid strand ambiguity. All non-matching alleles were aligned, and the signs of the beta estimates were flipped [22]. Then, the *F*-statistic was calculated for the selected SNPs, and strong instruments were defined as those with an F-statistic > 10 [23]. The summary of instrument counts, F-statistics, and related information for each antibody exposure is presented in Supplementary Tables SII–SV.

Table I. Baseline characteristics of participants

Exposure/ outcome	Phenotype	GWAS ID	Data source	Sample size*	Population
Antibodies against HSV	Anti-HSV-1 IgG levels	ieu-b-4906	IEU OpenGWAS	683	European
	HSV-1 IgG1 antibody levels	ebi-a-GCST90006918	UK Biobank cohort	6,199	European
	HSV-2 IgG2 antibody levels	ebi-a-GCST90006920	UK Biobank cohort	1,382	European
Antibodies against CMV	Anti-CMV IgG levels	ieu-b-4900	IEU OpenGWAS	5,010	European
	CMV pp28 antibody levels	ebi-a-GCST90006894	UK Biobank cohort	5,087	European
	CMV pp52 antibody levels	ebi-a-GCST90006895	UK Biobank cohort	5,681	European
	CMV pp150 antibody levels	ebi-a-GCST90006896	UK Biobank cohort	5,136	European
Antibodies against EBV	Anti-EBV IgG levels	ieu-b-4901	IEU OpenGWAS	5,010	European
	EBV EA-D antibody levels	ebi-a-GCST90006898	UK Biobank cohort	7,763	European
	EBV EBNA-1 antibody levels	ebi-a-GCST90006899	UK Biobank cohort	7,972	European
	EBV VCA p18 antibody levels	ebi-a-GCST90006900	UK Biobank cohort	8,518	European
	EBV ZEBRA antibody levels	ebi-a-GCST90006901	UK Biobank cohort	8,191	European
IPF	–	Idiopathic pulmonary fibrosis	FinnGen cohort	1,514/ 306,063	European

**Sample size shown as a total number for quantitative traits and cases/controls for binary traits. EBV – Epstein-Barr virus, CMV – cytomegalovirus, HSV – herpes simplex virus, EA – EBV early antigen, EBNA-1 – EBV nuclear antigen-1, IgG – immunoglobulin G, EBV VCA p18 – EBV viral capsid antigen p18, EBV ZEBRA – EBV Z EBV replication activator, IPF – idiopathic pulmonary fibrosis.*

MR analyses

GSMR as the main analysis

We employed GSMR as the main analysis method. GSMR is a flexible approach that uses summary-level GWAS data from independent studies to conduct MR analysis. Multiple near-independent genetic instruments were selected to test causal relationships between risk factors (or phenotypes) and disease outcomes [24]. The HEIDI-outlier test was used to identify and exclude genetic variants exhibiting pleiotropy, thereby reducing bias in the causal effect estimates.

To select valid and independent IVs, we employed the clumping algorithm to identify genome-wide significant SNPs for each trait (r^2 threshold = 0.05, p-value threshold = 5×10^{-6}), using the 1000 Genomes phase 3 European samples as the reference for LD estimation. To address potential pleiotropy, the HEIDI-outlier test was implemented with a p-value threshold of 0.01 to filter SNPs exhibiting horizontal pleiotropy effects on both exposure and outcome traits [24].

Two-sample MR as supplemental analysis

We applied three additional MR methods as supplemental analyses for the antibody-disease associations examined in the main analysis-GSMR: the IVW, MR-Egger, and weighted median methods. Cochran's Q statistic was used to quantify heterogeneity among the IVs. A statistically significant Cochran's Q ($p < 0.05$) indicated heterogeneity among the IVs [25]. MR pleiotropy residual sum and outliers (MR-PRESSO) tests and the MR-Egger intercept were used to detect outliers and horizontal pleiotropy [26]. A significant intercept in the MR-Egger ($p < 0.05$) indicated the presence of pleiotropy. Leave-one-out sensitivity analyses were conducted to verify the robustness of the causal effect estimates.

Statistical analysis

MR analyses were conducted using the "TwoSampleMR" and "gsmr" packages in R version 4.3.0 (http://r-project.org/). Results were considered significant if the estimates from the four methods (GSMR, IVW, MR-Egger, and weighted median) were directionally consistent, GSMR result was significant ($p < 0.05$), and there was no significant heterogeneity or pleiotropy.

Results

Effect of HSV-related antibody traits on IPF

We first investigated the potential causal effects of genetically predicted HSV-related antibody traits on IPF (Figure 1, Supplementary Tables SVI–SVIII, and Supplementary Figures S1–S3). In GSMR analyses, the results did not support a causal effect of antibody levels targeting HSV-1 IgG1 (OR = 1.105, 95% CI: 0.912–1.338, p = 0.310), HSV-2 IgG2 (OR = 1.011, 95% CI: 0.903–1.131, p = 0.852), or anti-HSV-1 IgG (OR = 0.968, 95% CI: 0.834–1.123, p = 0.670) on IPF. Likewise, in analyses using three additional MR methods (IVW, MR-Egger, and weighted median), the results did not support a causal relationship between HSV-related antibody traits and IPF.

Effect of CMV-related antibody traits on IPF

In our main analyses, we assessed the relationships between four antibodies against CMV and IPF using GSMR. In GSMR, the results did not

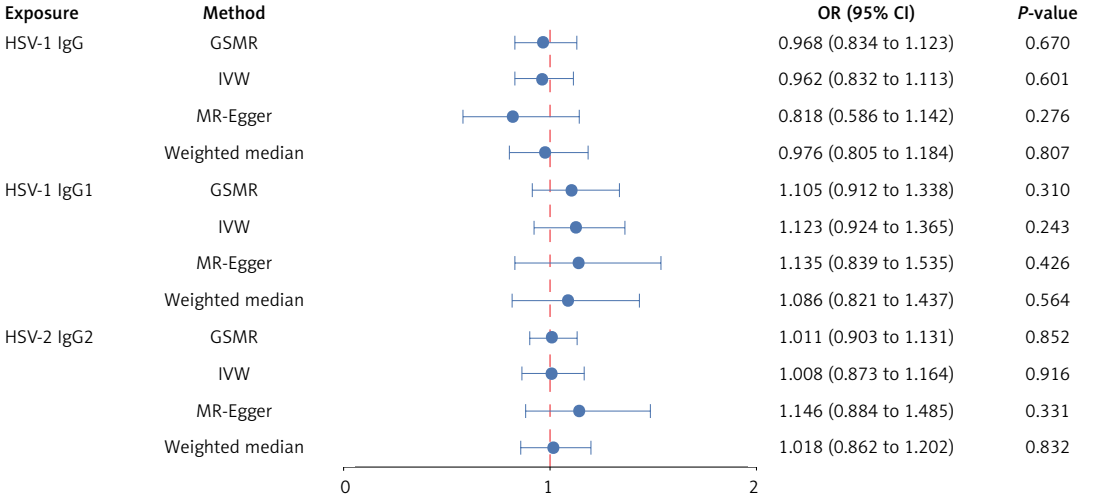

Figure 1. Forest plot of the causal association between herpes simplex virus-related antibody traits and idiopathic pulmonary fibrosis

HSV – herpes simplex virus, OR – odds ratio, CI – confidence interval, GSMR – generalized summary data based Mendelian randomization, IVW – inverse variance weighted.

support causal effects of antibody levels against CMV pp28 (OR = 0.809, 95% CI: 0.652–1.006, p = 0.056), CMV pp52 (OR = 0.916, 95% CI: 0.758–1.108, p = 0.368), CMV pp150 (OR = 0.966, 95% CI: 0.795–1.174, p = 0.732), or anti-CMV IgG (OR = 1.000, 95% CI: 0.879–1.138, p = 1.000) on IPF (Figure 2, Supplementary Tables SVI–SVIII, and Supplementary Figures S4–S7).

Effect of EBV-related antibody traits on IPF

We then examined whether genetically elevated levels of five antibodies against EBV showed potential causal associations with IPF (Figure 3, Supplementary Figures S8–S12). In GSMR, genetically increased antibody levels against EBV VCA p18 were positively associated with IPF (OR = 1.185, 95% CI: 1.002–1.402, p = 0.048). In IVW, the association remained significant (OR = 1.206, 95% CI: 1.017–1.429, p = 0.031). However, in GSMR, anti-EBV IgG levels were negatively associated with IPF (OR = 0.970, 95% CI: 0.946–0.995, p = 0.007). In IVW, the association remained significant, confirming the protective effect of anti-EBV IgG levels on IPF (OR = 0.966, 95% CI: 0.941–0.991, p = 0.007). There was no other evidence to support a causal relationship between the other EBV antibody levels and IPF.

No heterogeneity was found with the Cochran's Q for EBV VCA p18 antibody levels (p = 0.549) (Table II). The calculated p-value of Egger intercept for EBV VCA p18 antibody levels was 0.061 (Table II), indicating no potential directional horizontal pleiotropy in the MR analysis.

Effect of IPF on herpesvirus-related antibody traits

In GSMR analyses, no significant causal relationship was observed between IPF and genetically predicted herpesvirus-related antibody traits (Figure 4). Likewise, in IVW, MR-Egger, and weighted median analyses, the results did not support a causal relationship between IPF and HSV, CMV, or EBV.

Discussion

The relationship between herpesvirus-related immune responses and IPF has been unsettled for years. Clarifying these associations could provide a theoretical basis for future large-scale antiviral therapeutic trials in IPF. In the MR analyses, we found that genetically predicted anti-EBV VCA p18 antibodies and anti-EBV IgG levels were associated with IPF risk, consistent with a potential causal relationship under MR assumptions. Early detection and appropriate treatment of EBV infection may help alleviate the progression or worsening of IPF symptoms.

Viruses have long been thought to play an important role in the development and progression of IPF. In a systematic review and meta-analysis by Mostafaei *et al*. [27], the overall prevalence of viruses in IPF patients was found to be 53.72%,

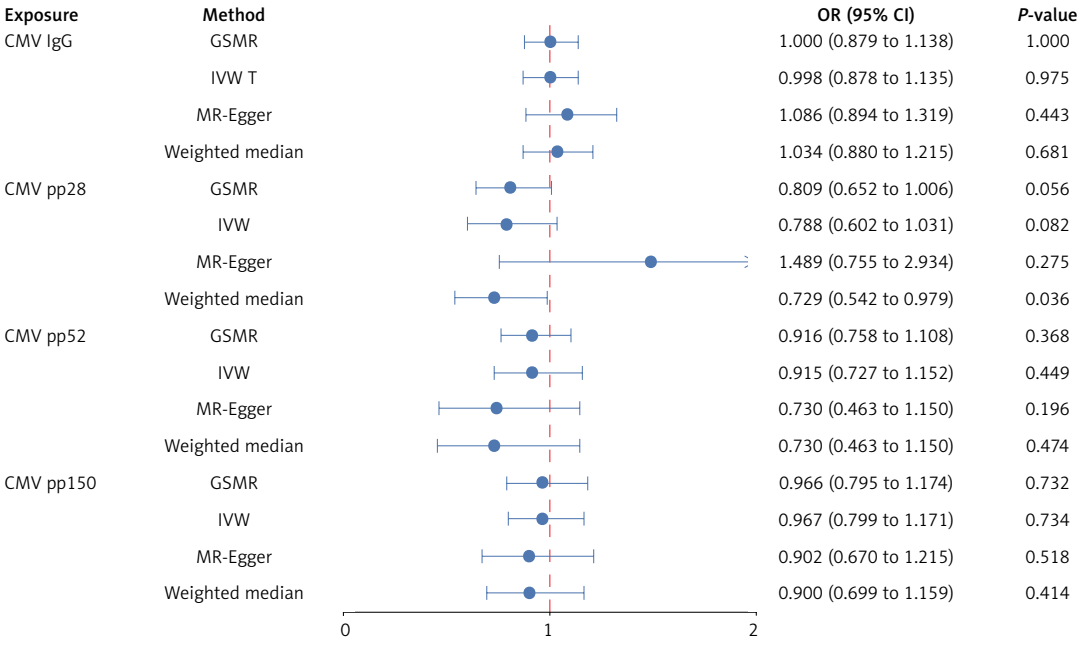

Figure 2. Forest plot of the causal association between cytomegalovirus-related antibody traits and idiopathic pulmonary fibrosis

CMV – cytomegalovirus, OR – odds ratio, CI – confidence interval, GSMR – generalized summary data based Mendelian randomization, IVW – inverse variance weighted.

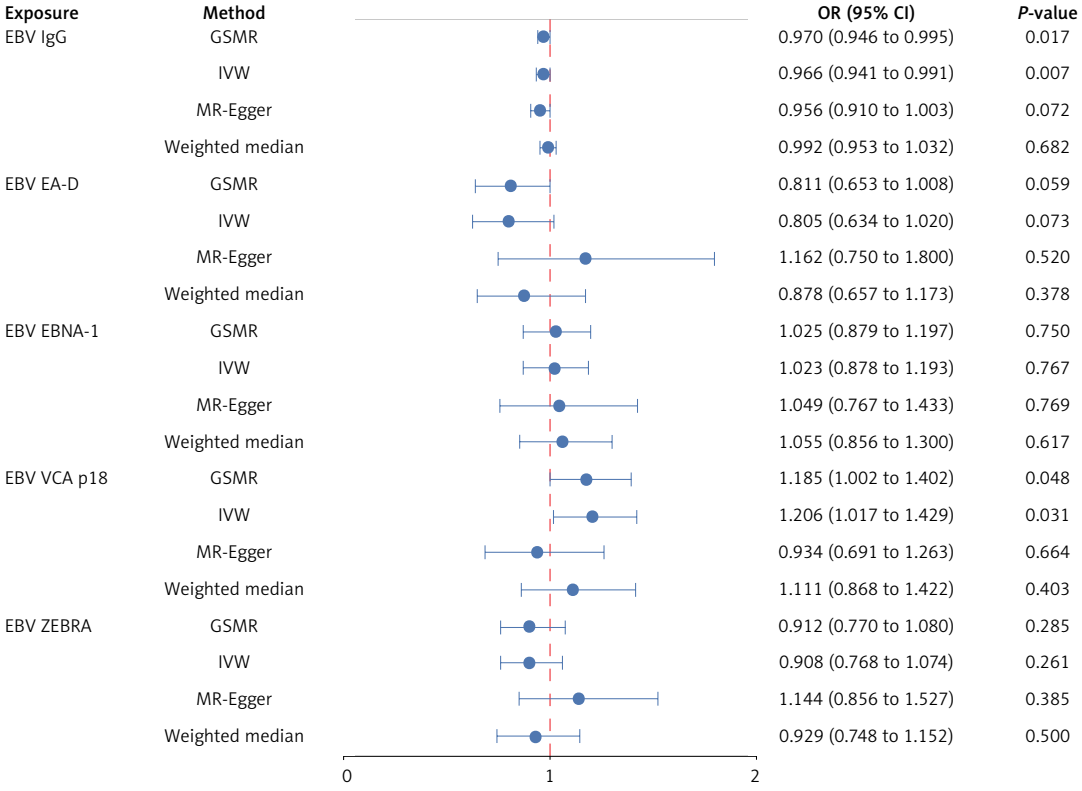

Figure 3. Forest plot of the causal association between Epstein-Barr virus-related antibody traits and idiopathic pulmonary fibrosis

EBV – Epstein-Barr virus, OR – odds ratio, CI – confidence interval, GSMR – generalized summary data based Mendelian randomization, IVW – inverse variance weighted.

Table II. Pleiotropic and heterogeneous results of five antibodies against EBV and IPF

Exposure	Pleiotropy test						Heterogeneity test			
	MR-Egger			MR-PRESSO test			MR-Egger		IVW	
	Intercept	SE	*P*-value	Beta (raw)	Beta (outlier)	*P*-value (global test)	Q	*P*-value	Q	*P*-value
Anti-EBV IgG levels	0.006	0.013	0.627	–0.035	NA	0.173	87.877	0.256	88.138	0.275
EBV EA-D antibody levels	–0.064	0.034	0.096	–0.217	NA	0.107	7.600	0.473	11.155	0.265
EBV EBNA-1 antibody levels	–0.004	0.025	0.862	0.023	NA	0.722	11.361	0.787	11.392	0.835
EBV VCA p18 antibody levels	0.045	0.023	0.061	0.187	NA	0.142	19.567	0.549	23.479	0.375
EBV ZEBRA antibody levels	–0.056	0.029	0.084	–0.096	NA	0.240	5.316	0.869	8.994	0.622

EBV – Epstein-Barr virus, EA – EBV early antigen, EBNA-1 – EBV nuclear antigen-1, IgG – immunoglobulin G, EBV VCA p18 – EBV viral capsid antigen p18, EBV ZEBRA – EBV Z EBV replication activator, IPF – idiopathic pulmonary fibrosis, IVW – inverse variance weighted.

with HSV (77.7%) and EBV (72.0%) being the most common. The herpesvirus family has received the most attention as a causative and exacerbating factor in IPF. Significant associations with IPF risk have been found for HSV-1, EBV, CMV, HHV-7, and HHV-8 [9, 28]. Furthermore, Tang *et al*. [29] detected herpesvirus DNA in 97% of IPF patients' lung tissue samples, with EBV being the predominant virus. Their study also found that sporadic IPF patients often harbor two or more herpesviruses, suggesting that genetic factors may render familial IPF patients more susceptible to fewer viral infections, while sporadic IPF may be linked to multiple viral infections. Genetic predispositions could potentially increase susceptibility to herpesvirus infections, thereby elevating the risk of develop-

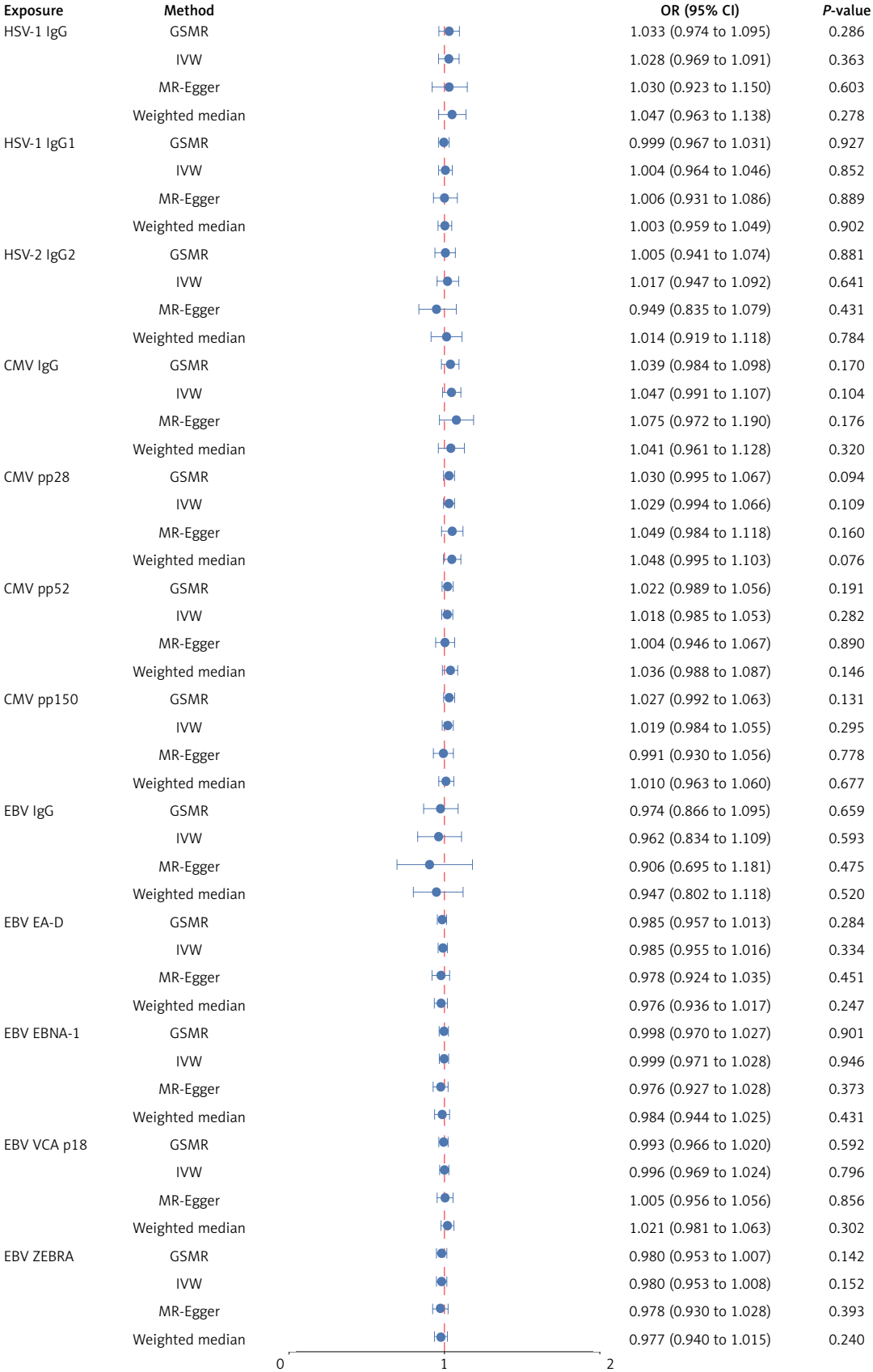

Figure 4. Forest plot of the causal association between idiopathic pulmonary fibrosis and herpesvirus-related antibody traits

HSV – herpes simplex virus, CMV – cytomegalovirus, EBV – Epstein-Barr virus, OR – odds ratio, CI – confidence interval, GSMR – generalized summary data based Mendelian randomization, IVW – inverse variance weighted.

ing IPF. Seibold *et al.* [30] reported that a specific polymorphism in the MUC5B gene, strongly associated with IPF risk, might enhance susceptibility to viral infections, including herpesviruses, by modulating lung immune responses.

Most existing studies are retrospective case-control studies or meta-analyses, or conducted on relatively small cohorts. Due to the low incidence of IPF, conducting large-scale prospective studies to investigate actual causal relationships is challenging. MR is a method that leverages genetic variants associated with specific exposures as IVs to infer causal effects while minimizing confounding [31]. MR methods use SNPs as IVs to infer causal relationships between exposures and outcomes. Variants are assigned randomly to offspring by their parents at conception. Therefore, the MR method is generally less susceptible to confounding and reverse causality, making it conceptually analogous to the random assignment method used in randomized controlled trials [32, 33]. Therefore, we used MR analysis to explore the potential association between genetically predicted herpesvirus-related antibody traits and IPF risk. We found that genetically predicted elevated anti-EBV VCA p18 levels were positively associated with IPF, whereas genetically predicted anti-EBV IgG levels were inversely associated with IPF. These findings suggest heterogeneous associations between EBV-related immune response traits and IPF risk under Mendelian randomization assumptions. However, further prospective and mechanistic studies are required to clarify these relationships. EBV is known to establish latent infections in host epithelial and B cells, leading to chronic antigen stimulation and persistent inflammatory responses. This chronic stimulation can activate various signaling pathways, including NF-κB and PI3K/AKT, which are crucial for cell survival, proliferation, and cytokine production [34]. Such sustained activation can contribute to tissue fibrosis and immune dysregulation, aligning with our observations of EBV's strong effect. Furthermore, EBV's ability to evade host immune responses through mechanisms such as downregulation of MHC class I molecules and expression of viral proteins that inhibit apoptosis can exacerbate epithelial injury and immune dysregulation [35]. The involvement of EBV in autoimmune diseases, as suggested by its association with systemic lupus erythematosus and rheumatoid arthritis, further underscores its role in immune dysregulation [35].

It is crucial to note that EBV infection triggers a multifaceted immune response, leading to the production of distinct antibodies targeting various viral antigens. These antibodies can reflect different stages and characteristics of the infection, ranging from acute to latent phases [36, 37]. For example, antibodies against EBV VCA and EA-D are often associated with active or recent infection, while antibodies against nuclear antigens are indicative of a more chronic or latent infection state [13]. EBV proteins, such as EBNA-1, ZEBRA, EA-D, and VCA-p18, are key targets in serology assays. Different serological profiles may be associated with the incubation and clearance phases of EBV infection [38, 39]. These phases could contribute to IPF progression through distinct mechanisms. During latency, chronic antigen stimulation may trigger persistent inflammatory responses, leading to fibrosis, whereas acute inflammation and tissue damage during viral clearance could further accelerate pulmonary fibrosis [29]. One of the key factors contributing to progressive pulmonary fibrosis is the excessive accumulation of extracellular matrix (ECM), primarily derived from myofibroblasts [1]. Prolonged EBV infection may lead to excessive deposition of ECM [40]. Additionally, Sides *et al.* [41] found that EBV can promote epithelial-mesenchymal transition (EMT), which subsequently generates myofibroblasts. All of these mechanisms of chronic immune stimulation and the initiation of fibrosis further support our findings.

The differential association of EBV antibodies with disease risk can be explained by statistical explanation and their distinct biological roles. In this study, we adopted a relatively conservative statistical approach to enhance the reliability of the results. Specifically, we used a comprehensive set of genetic instruments for each antibody, which allowed us to discern these subtle differences in associations. The statistical power to detect associations varies across antibody traits and is influenced by differences in sample size and the proportion of variance explained by the genetic instruments. Consequently, some antibody phenotypes may have limited power to detect modest causal effects. The use of stringent instrument selection criteria and multiple sensitivity analyses helps to improve the robustness of the findings and reduce the likelihood of spurious associations, although it does not eliminate the possibility of bias. From a biological perspective, antibodies such as IgA against VCA, which signify active viral replication, may correlate with ongoing tissue damage and inflammation in the lungs, processes that are known to contribute to the pathogenesis of IPF [40]. This is supported by studies showing a higher prevalence of EBV DNA and active replication markers in the lung tissues of IPF patients compared to controls [36, 37]. Antibodies like IgG against EBNA, which reflect a latent infection, may not directly contribute to the acute inflammatory processes but could indicate a chronic state of viral persistence. This persistent infection might

contribute to a low-grade, chronic inflammatory milieu that, over time, could facilitate fibrotic changes in the lung tissue [13]. Furthermore, the presence of EBV latent membrane protein 1 (LMP1) antibodies, specifically associated with more severe disease progression in IPF, suggests a role for this viral protein in modulating host cellular responses. LMP1 is known to mimic a constitutively active TNF receptor, activating signaling pathways that can lead to EMT, a process implicated in the development of pulmonary fibrosis [41]. These biological findings provide a potential mechanistic context for observed associations between EBV-related immune response traits and idiopathic pulmonary fibrosis [36, 41].

For CMV and HSV, we found no evidence of a causal relationship between HSV and CMV antibodies and the risk of IPF. This finding is consistent with the results reported by Yan *et al.* [42], who also observed no causal association between herpesvirus infections – including HSV and CMV – as well as herpesvirus-related IgG levels, and IPF. This suggests that HSV and CMV infections may not play a direct causal role in the development and progression of IPF, and also reflects the complexity of interactions between CMV and the host, which may vary depending on factors such as age, immune status, and genetic predisposition [43]. However, Yonemaru *et al.* [13] found elevated CMV and HSV IgG titers in IPF patients compared to controls. A meta-analysis showed that chronic infections with herpesviruses such as EBV, CMV, HHV7, and HHV8 were significantly associated with increased risk of IPF [9]. Lasithiotaki *et al.* [44] attempted to infect primary macrophages from IPF patients and healthy controls with wild-type HSV-1, and found that latent HSV-1 infection may have regulatory effects on inflammation, fibrosis, angiogenesis, and wound healing. Zhang *et al.* [45] used HSV infection-related GWAS data from the FinnGen study and conducted an analysis via the IVW method. They reported a potential causal effect of HSV infection on the risk of developing IPF. Our study findings differ from those of the abovementioned research, and this discrepancy may arise from differences in study design, sample size, or the specific IVs used. To enhance the robustness of our results, our study analyzed antibody-related data for HSV, CMV, and EBV using the GSMR method. The biological mechanisms underlying the potential relationship between HSV-1-related immune response traits and fibrosis remains unclear, and further research is needed to determine whether the findings reflect causal effects, shared immune pathways, or non-specific host–virus interactions. Additionally, genetic and environmental factors may play important roles in determining the impact of the virus on IPF. Costa *et al.* [46] provide valuable insights into how iNK cell receptors can modulate the immune response during chronic viral infections such as HIV. In the context of our study, it is plausible that similar mechanisms involving iNK cells could contribute to the chronic inflammation observed in IPF. While our research focused primarily on the potential causal relationship between herpesvirus antibodies and IPF, existing evidence suggests that the persistence of viral antigens, including those from EBV, may influence immune regulation, including the expression and function of iNK cells. This, in turn, might affect the balance between pro-inflammatory and anti-inflammatory responses, contributing to the fibrotic process. Murdaca *et al.* [47] provide a comprehensive overview of the involvement of Th17 cells in the pathogenesis of various autoimmune and inflammatory diseases. Th17 cells are known to produce interleukin-17 (IL-17), a pro-inflammatory cytokine that can drive tissue inflammation and damage. Given the chronic inflammatory nature of IPF, it is conceivable that Th17 cells and IL-17 may play a role in the progression of the disease.

There are some limitations to our study. First, our sample was composed of individuals of European ancestry, which may limit the generalizability of our findings to other ethnic groups. Second, we found a significant association between genetically predicted Epstein–Barr virus (EBV)-related antibody traits and IPF only in the GSMR and IVW analyses, and further research is needed to confirm and expand on these findings, especially in larger clinical cohorts. Third, the exposure variables were derived from GWAS of serologically measured antibody titers, which reflect host immune response to viral antigens rather than direct measures of active or persistent infection. Therefore, these traits may not fully capture ongoing viral activity, and interpretation in terms of infection status should be made with caution. Finally, we lacked additional details related to IPF, such as family history, genetic factors, age, sex, lifestyle, and comorbidities, preventing us from performing further stratified analyses. Future studies should focus on collecting data from independent populations and obtaining more SNPs or increasing the sample size.

Some studies have shown that antiviral therapy may have a positive impact on delaying disease progression in IPF patients. Tang *et al.* [29] observed improvement in two EBV-positive IPF patients after short-term valganciclovir treatment and found that EBV was no longer detectable in their sputum. Egan *et al.* [48] treated 14 EBV-positive patients with advanced IPF using ganciclovir, and after 8 weeks of treatment, 64.3% (9/14) of the patients showed symptom improvement.

However, these studies have limitations such as small sample size, short treatment time, and lack of a control group. At this stage, antiviral therapy cannot yet be considered an effective treatment for IPF, and further research is still needed. Our study aimed to contribute to future therapeutic strategies and elucidate the underlying mechanisms of IPF. Nevertheless, the role of EBV antibody levels in IPF progression and the influence of individual genetic susceptibility remain unclear. Future research using longitudinal multi-omics analyses, integrating various types of data, may help clarify these uncertainties.

In conclusion, our integrative MR study, combining bidirectional and GSMR approaches, provides novel evidence suggesting an association between EBV-related antibody response traits and the risk of IPF. Specifically, elevated antibody levels against EBV VCA p18 were significantly associated with risk of developing IPF, whereas higher anti-EBV IgG levels showed an inverse association. These findings suggest heterogenous effects of EBV-specific immune response traits on IPF susceptibility and highlight the need for further studies to clarify underlying mechanisms. By suggesting a potential causal relationship between EBV-related antibody response traits and IPF, our study offers new insights into the potential involvement of host–virus immune interactions in disease pathogenesis and may inform future mechanistic and translational research.

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Ethical approval

The study used the large publicly available GWAS databases, which have received approval from their relevant ethical review board and participants.

Conflict of interest

The authors declare no conflict of interest.

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