Epstein-Barr virus infection is causally associated with idiopathic pulmonary fibrosis: a bidirectional Mendelian randomization study

Keywords
antibody, idiopathic pulmonary fibrosis, Mendelian randomization, herpes virus infection

Abstract

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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 the presence of reverse causality.

Results

We found that the levels of antibodies against the EBV viral capsid antigen (VCA) p18 were associated with an increased risk of IPF. GSMR and IVW results indicate that anti-EBV IgG levels were significantly negatively associated with IPF. Sensitivity analyses confirmed the robustness of our results.

Conclusions

Our results indicate that EBV infection increases the risk of IPF. Our findings enhance the understanding of the etiology of IPF. Targeting EBV infection may aid in the prevention and treatment of IPF.

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Keywords

Mendelian randomization, idiopathic pulmonary fibrosis, herpes virus infection, antibody

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 significantly increase the risk of developing IPF, but are not associated with the exacerbation of the disease. All the analyzed viruses, including EBV, CMV, human herpes virus 7 (HHV-7), and human herpes virus 8 (HHV-8), were associated with an increased risk of developing IPF(9). Herpes viruses, 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 herpes viruses, such as HSV; (ii) β herpes viruses, such as CMV; and (iii) γ herpes viruses, such as EBV(10). Once a herpes virus 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 herpes viruses 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 herpes virus infections and IPF comes from the 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 aim to analyze the causal relationship between herpes virus infections and IPF. Mendelian Randomization (MR) is a robust epidemiological tool for causal inference, utilizing summary-level data from genome-wide association studies (GWAS) to assess potential causal relationships between exposures and outcomes(17). Since alleles are randomly allocated during meiosis, genetic variants serve as unconfounded proxies for exposures, minimizing the risk of residual confounding and reverse causation inherent in observational studies(18, 19). Routine two-sample MR methods employ multiple complementary techniques to maintain the robustness of results, including inverse variance weighting (IVW), MR-Egger, weighted median, and sensitivity analyses. 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

causal relationship between herpes virus infections and IPF, we applied both bidirectional two-sample MR and GSMR methods to assess the causal relationship between three herpes virus (HSV, CMV, EBV) and the risk of IPF.

Methods

Data Sources

This study utilized 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 herpes virus 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 herpes virus infections (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. We provided detailed information on the selected datasets in Table 1 and Table S1.

This study utilized 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 herpes virus-related antibodies, except for the anti-EBV IgG levels, for which the GWAS significance threshold was set at $P < 5 \times 10^{-5}$ as there were too few SNPs (<3) for the procedure to work properly. 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 was presented in Tables S2 to S5.

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, including 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 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 Analyses

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 Infection on IPF

We first investigated the causal impact of HSV infection on IPF (Figure 1, Tables S6–

S8, and Figure 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 infection and IPF.

Effect of CMV Infection 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 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, Tables S6–S8, and Figures S4–S7).

Effect of EBV Infection on IPF

We then examined whether genetically elevated levels of five antibodies against EBV had causal effects on IPF (Figure 3 and 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 2). The calculated P-value of Egger intercept for EBV VCA p18 antibody levels was 0.061(Table 2), indicating no potential directional horizontal pleiotropy in the MR analysis.

Effect of IPF on Herpes virus Infection

In GSMR analyses, no significant causal relationship was observed between IPF and infection with any of the three tested herpes viruses (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 herpes virus infections and IPF has been unsettled for years. Clarifying the causal relationship between herpes virus infections and IPF 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 causally involved in IPF. 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%, with HSV

(77.7%) and EBV (72.0%) being the most common. The herpes virus 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 herpes viruses, 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 herpes virus infections, thereby elevating the risk of developing 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 herpes viruses, 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 not affected by confounding factors or reverse causality, making it similar to the random assignment method used in randomized controlled trials(32, 33). Therefore, we utilized MR analysis, which is less susceptible to confounding factors, to explore

the causal relationship between herpes virus infections and IPF. We demonstrated that genetically elevated anti-EBV VCA p18 levels are a causal risk factor for IPF and that genetically elevated anti-EBV IgG levels are a causal protective factor for IPF, though high-grade clinical evidence remains lacking. 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 utilized 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 depending on the prevalence and variability of each antibody in the population. Even if a biological link exists, some antibodies may lack sufficient power to exhibit a significant association. This conservative analytical method ensures that the observed associations are robust and not attributed to chance. 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). Thus, the presence of LMP1-specific antibodies may indicate a subset of IPF patients in whom EBV is actively influencing disease pathology(36, 41).

For CMV and HSV, we found no significant 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 demonstrated that there is 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 that elevated CMV and HSV IgG titers in IPF patients compared to controls. A meta-analysis showed that chronic infections with herpesviruses like EBV, CMV, HHV7, and HHV8 significantly increase the 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 this previous 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. It is worth noting that the exact mechanism by which HSV-1 contributes to fibrosis remains unclear, and further research is needed to determine whether it is a direct cause of fibrosis or merely a coexisting pathogen. Additionally, genetic and environmental factors may play important roles in determining the impact of the virus on IPF. Costa et al.(46) provides 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 causal relationship between herpes virus antibodies and IPF, the persistence of viral antigens, including those from EBV, could potentially influence 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) concerning Th17 cells and their involvement in such conditions, provides a comprehensive overview of how Th17 cells contribute to 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 could 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 EBV infection 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 GWAS for herpes virus infections was based on serological samples, and there is a distinction between herpes virus infections seropositivity and actual ongoing infection. Therefore, our results should be interpreted with caution, as false positives or negatives cannot be excluded. Lastly, 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 control group. At this stage, antiviral therapy cannot yet be considered an effective treatment for IPF, and further research is still needed in the future. Our study aims 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.

Conclusion

In conclusion, our integrative MR study, combining bidirectional and GSMR approaches, provides novel genetic evidence for a causal relationship between primary EBV infection and an increased risk of IPF. Specifically, elevated antibody levels against the EBV VCA p18 significantly increase the risk of developing IPF, whereas higher anti-EBV IgG levels appeared to exert a potential protective effect. These findings reveal the complex role of EBV-specific immune responses in the pathogenesis of IPF and suggest that chronic viral antigen stimulation may contribute to disease progression. By elucidating this causal relationship, our study offers new insights into early prevention and targeted antiviral or immunomodulatory therapeutic strategies for IPF.

Supplementary Information

This supplementary file includes: Figs. S1 to S12 and tables S1 to S8.

Abbreviations

CI, confidence interval; CMV, cytomegalovirus; EA-D, EBV early antigen-D; EBNA-1, EBV nuclear antigen-1; EBV, Epstein-Barr virus; EBV VCA p18, EBV viral capsid antigen p18; EBV ZEBRA, EBV Z EBV Replication Activator; GSMR, generalized summary data based MR; GWAS, genome-wide association studies; HEIDI, Heterogeneity in Dependent Instruments; HSV, herpes simplex virus; IgG, immunoglobulin G; IPF, idiopathic pulmonary fibrosis; IV, instrumental variable; IVW, inverse variance weighted; LD, linkage disequilibrium; MAF, minimum allele frequency; MR, Mendelian randomization; MR-PRESSO, MR pleiotropy residual sum and outliers; OR, odds ratio; SNPs, single nucleotide polymorphisms.

Declarations

Ethics approval statement

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

Patient consent statement

Not applicable.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Conflict of interest disclosure

The authors declare that they have no competing interests.

Funding statement

Not applicable.

Permission to reproduce material from other sources

Not applicable.

Clinical trial registration

Not applicable.

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Figure titles

Figure 1. The forest plot of the causal relationship between herpes simplex virus 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.

Figure 2. The forest plot of the causal relationship between cytomegalovirus 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. The forest plot of the causal relationship between Epstein-Barr virus 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.

Figure 4. The forest plot of the causal relationship between idiopathic pulmonary fibrosis and herpes virus infections. 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.

Table 1. Baseline characteristics for 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

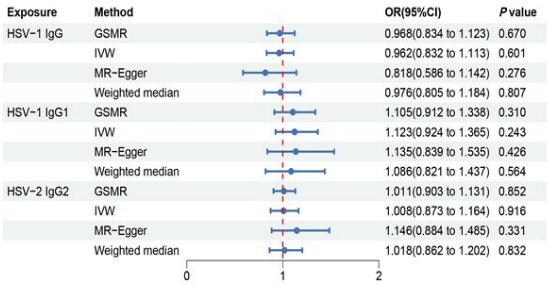
*Sample size shown as a total number for quantitative traits and cases/controls for binary traits.

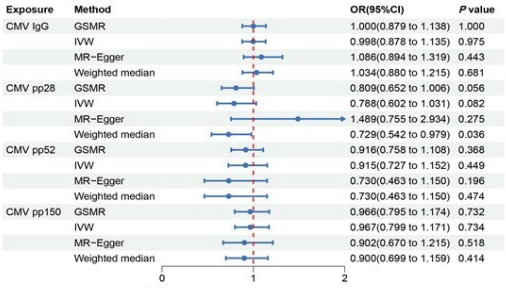
EBV, Epstein-Barr virus; CMV, cytomegalovirus; HSV, herpes simplex virus; EA, EBV early antigen; EBNA-1, EBV nuclear antigen-1; IgG, immunoglobulin G; EBV VCA p18, EBV viral capsid antigen p18; EBV ZEBRA, EBV Z EBV Replication Activator; IPF, idiopathic pulmonary fibrosis.

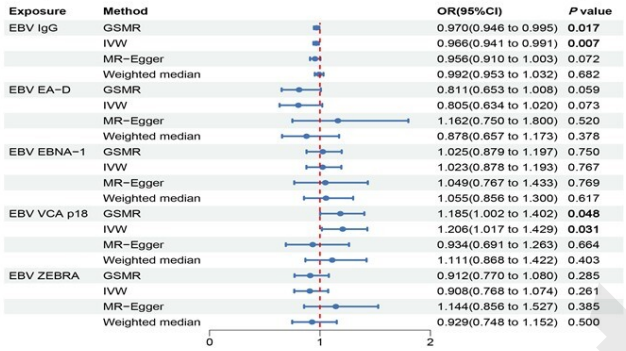
Table 2 The pleiotropic and heterogeneous results of five antibodies against EBV and IPF.

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

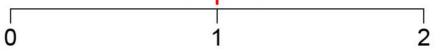
EBV, Epstein-Barr virus; EA, EBV early antigen; EBNA-1, EBV nuclear antigen-1; IgG, immunoglobulin G; EBV VCA p18, EBV viral capsid antigen p18; EBV ZEBRA, EBV Z EBV Replication Activator; IPF, idiopathic pulmonary fibrosis; IVW, inverse variance weighted.


Exposure
Method
OR(95%CI)
P value
HSV−1 IgG
GSMR
0.968(0.834 to 1.123)
0.670
IVW
0.962(0.832 to 1.113)
0.601
MR−Egger
0.818(0.586 to 1.142)
0.276
Weighted median
0.976(0.805 to 1.184)
0.807
HSV−1 IgG1
GSMR
1.105(0.912 to 1.338)
0.310
IVW
1.123(0.924 to 1.365)
0.243
MR−Egger
1.135(0.839 to 1.535)
0.426
Weighted median
1.086(0.821 to 1.437)
0.564
HSV−2 IgG2
GSMR
1.011(0.903 to 1.131)
0.852
IVW
1.008(0.873 to 1.164)
0.916
MR−Egger
1.146(0.884 to 1.485)
0.331
Weighted median
1.018(0.862 to 1.202)
0.832
0
1
2

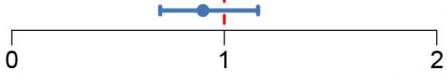

Exposure
Method
OR(95%CI)
P value
CMV IgG
GSMR
1.000(0.879 to 1.138)
1.000
IVW
0.998(0.878 to 1.135)
0.975
MR−Egger
1.086(0.894 to 1.319)
0.443
Weighted median
1.034(0.880 to 1.215)
0.681
CMV pp28
GSMR
0.809(0.652 to 1.006)
0.056
IVW
0.788(0.602 to 1.031)
0.082
MR−Egger
1.489(0.755 to 2.934)
0.275
Weighted median
0.729(0.542 to 0.979)
0.036
CMV pp52
GSMR
0.916(0.758 to 1.108)
0.368
IVW
0.915(0.727 to 1.152)
0.449
MR−Egger
0.730(0.463 to 1.150)
0.196
Weighted median
0.730(0.463 to 1.150)
0.474
CMV pp150
GSMR
0.966(0.795 to 1.174)
0.732
IVW
0.967(0.799 to 1.171)
0.734
MR−Egger
0.902(0.670 to 1.215)
0.518
Weighted median
0.900(0.699 to 1.159)
0.414
0
1
2


Exposure
Method
OR(95%CI)
P value
EBV IgG
GSMR
0.970(0.946 to 0.995)
0.017
IVW
0.966(0.941 to 0.991)
0.007
MR−Egger
0.956(0.910 to 1.003)
0.072
Weighted median
0.992(0.953 to 1.032)
0.682
EBV EA−D
GSMR
0.811(0.653 to 1.008)
0.059
IVW
0.805(0.634 to 1.020)
0.073
MR−Egger
1.162(0.750 to 1.800)
0.520
Weighted median
0.878(0.657 to 1.173)
0.378
EBV EBNA−1
GSMR
1.025(0.879 to 1.197)
0.750
IVW
1.023(0.878 to 1.193)
0.767
MR−Egger
1.049(0.767 to 1.433)
0.769
Weighted median
1.055(0.856 to 1.300)
0.617
EBV VCA p18
GSMR
1.185(1.002 to 1.402)
0.048
IVW
1.206(1.017 to 1.429)
0.031
MR−Egger
0.934(0.691 to 1.263)
0.664
Weighted median
1.111(0.868 to 1.422)
0.403
EBV ZEBRA
GSMR
0.912(0.770 to 1.080)
0.285
IVW
0.908(0.768 to 1.074)
0.261
MR−Egger
1.144(0.856 to 1.527)
0.385
Weighted median
0.929(0.748 to 1.152)
0.500
0
1
2

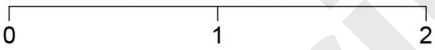
Exposure	Method	OR(95%CI)	*P* value
HSV−1 IgG	GSMR	0.968(0.834 to 1.123)	0.670
	IVW	0.962(0.832 to 1.113)	0.601
	MR−Egger	0.818(0.586 to 1.142)	0.276
	Weighted median	0.976(0.805 to 1.184)	0.807
HSV−1 IgG1	GSMR	1.105(0.912 to 1.338)	0.310
	IVW	1.123(0.924 to 1.365)	0.243
	MR−Egger	1.135(0.839 to 1.535)	0.426
	Weighted median	1.086(0.821 to 1.437)	0.564
HSV−2 IgG2	GSMR	1.011(0.903 to 1.131)	0.852
	IVW	1.008(0.873 to 1.164)	0.916
	MR−Egger	1.146(0.884 to 1.485)	0.331
	Weighted median	1.018(0.862 to 1.202)	0.832



Exposure	Method	OR(95%CI)	*P* value
CMV IgG	GSMR	1.000(0.879 to 1.138)	1.000
	IVW	0.998(0.878 to 1.135)	0.975
	MR−Egger	1.086(0.894 to 1.319)	0.443
	Weighted median	1.034(0.880 to 1.215)	0.681
CMV pp28	GSMR	0.809(0.652 to 1.006)	0.056
	IVW	0.788(0.602 to 1.031)	0.082
	MR−Egger	1.489(0.755 to 2.934)	0.275
	Weighted median	0.729(0.542 to 0.979)	0.036
CMV pp52	GSMR	0.916(0.758 to 1.108)	0.368
	IVW	0.915(0.727 to 1.152)	0.449
	MR−Egger	0.730(0.463 to 1.150)	0.196
	Weighted median	0.730(0.463 to 1.150)	0.474
CMV pp150	GSMR	0.966(0.795 to 1.174)	0.732
	IVW	0.967(0.799 to 1.171)	0.734
	MR−Egger	0.902(0.670 to 1.215)	0.518
	Weighted median	0.900(0.699 to 1.159)	0.414



Exposure	Method	OR(95%CI)	*P* value
EBV IgG	GSMR	0.970(0.946 to 0.995)	**0.017**
	IVW	0.966(0.941 to 0.991)	**0.007**
	MR−Egger	0.956(0.910 to 1.003)	0.072
	Weighted median	0.992(0.953 to 1.032)	0.682
EBV EA−D	GSMR	0.811(0.653 to 1.008)	0.059
	IVW	0.805(0.634 to 1.020)	0.073
	MR−Egger	1.162(0.750 to 1.800)	0.520
	Weighted median	0.878(0.657 to 1.173)	0.378
EBV EBNA−1	GSMR	1.025(0.879 to 1.197)	0.750
	IVW	1.023(0.878 to 1.193)	0.767
	MR−Egger	1.049(0.767 to 1.433)	0.769
	Weighted median	1.055(0.856 to 1.300)	0.617
EBV VCA p18	GSMR	1.185(1.002 to 1.402)	**0.048**
	IVW	1.206(1.017 to 1.429)	**0.031**
	MR−Egger	0.934(0.691 to 1.263)	0.664
	Weighted median	1.111(0.868 to 1.422)	0.403
EBV ZEBRA	GSMR	0.912(0.770 to 1.080)	0.285
	IVW	0.908(0.768 to 1.074)	0.261
	MR−Egger	1.144(0.856 to 1.527)	0.385
	Weighted median	0.929(0.748 to 1.152)	0.500



Exposure	Method	OR(95%CI)	*P* value
HSV−1 IgG	GSMR	1.033(0.974 to 1.095)	0.286
	IVW	1.028(0.969 to 1.091)	0.363
	MR−Egger	1.030(0.923 to 1.150)	0.603
	Weighted median	1.047(0.963 to 1.138)	0.278
HSV−1 IgG1	GSMR	0.999(0.967 to 1.031)	0.927
	IVW	1.004(0.964 to 1.046)	0.852
	MR−Egger	1.006(0.931 to 1.086)	0.889
	Weighted median	1.003(0.959 to 1.049)	0.902
HSV−2 IgG2	GSMR	1.005(0.941 to 1.074)	0.881
	IVW	1.017(0.947 to 1.092)	0.641
	MR−Egger	0.949(0.835 to 1.079)	0.431
	Weighted median	1.014(0.919 to 1.118)	0.784
CMV IgG	GSMR	1.039(0.984 to 1.098)	0.170
	IVW	1.047(0.991 to 1.107)	0.104
	MR−Egger	1.075(0.972 to 1.190)	0.176
	Weighted median	1.041(0.961 to 1.128)	0.320
CMV pp28	GSMR	1.030(0.995 to 1.067)	0.094
	IVW	1.029(0.994 to 1.066)	0.109
	MR−Egger	1.049(0.984 to 1.118)	0.160
	Weighted median	1.048(0.995 to 1.103)	0.076
CMV pp52	GSMR	1.022(0.989 to 1.056)	0.191
	IVW	1.018(0.985 to 1.053)	0.282
	MR−Egger	1.004(0.946 to 1.067)	0.890
	Weighted median	1.036(0.988 to 1.087)	0.146
CMV pp150	GSMR	1.027(0.992 to 1.063)	0.131
	IVW	1.019(0.984 to 1.055)	0.295
	MR−Egger	0.991(0.930 to 1.056)	0.778
	Weighted median	1.010(0.963 to 1.060)	0.677
EBV IgG	GSMR	0.974(0.866 to 1.095)	0.659
	IVW	0.962(0.834 to 1.109)	0.593
	MR−Egger	0.906(0.695 to 1.181)	0.475
	Weighted median	0.947(0.802 to 1.118)	0.520
EBV EA−D	GSMR	0.985(0.957 to 1.013)	0.284
	IVW	0.985(0.955 to 1.016)	0.334
	MR−Egger	0.978(0.924 to 1.035)	0.451
	Weighted median	0.976(0.936 to 1.017)	0.247
EBV EBNA−1	GSMR	0.998(0.970 to 1.027)	0.901
	IVW	0.999(0.971 to 1.028)	0.946
	MR−Egger	0.976(0.927 to 1.028)	0.373
	Weighted median	0.984(0.944 to 1.025)	0.431
EBV VCA p18	GSMR	0.993(0.966 to 1.020)	0.592
	IVW	0.996(0.969 to 1.024)	0.796
	MR−Egger	1.005(0.956 to 1.056)	0.856
	Weighted median	1.021(0.981 to 1.063)	0.302
EBV ZEBRA	GSMR	0.980(0.953 to 1.007)	0.142
	IVW	0.980(0.953 to 1.008)	0.152
	MR−Egger	0.978(0.930 to 1.028)	0.393
	Weighted median	0.977(0.940 to 1.015)	0.240