Unveiling the Causal Link of herpes virus infection and Cutaneous Leukocytoclastic Angiitis: Insights from Mendelian Randomization

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Abstract

Cutaneous leukocytoclastic angiitis (CLA) is a condition of clinical interest, with previous studies suggesting an association with herpes virus infections. This study aimed to investigate the causal association relationship between the virus and CLA. Genetic variants linked to the virus were retrieved from the IEU open GWAS project and FinnGen database. Data on CLA were sourced from the FinnGen consortium R7. Mendelian randomization (MR) analysis, including the IVW, MR-Egger, and weighted median methods, was conducted. Sensitivity analyses were performed to ensure result accuracy. Among the six viruses investigated, only human herpesvirus 6 (HHV-6) demonstrated a causal association with CLA(odds ratio (OR) = 1.886, 95% confidence interval (CI) = 1.053–3.378, p = 0.033), indicating that HHV-6 infection significantly elevates the risk of CLA. Furthermore, both IVW and MR-Egger tests for heterogeneity confirmed homogeneous MR analysis results without evidence of horizontal pleiotropy (p>0.05). No significant causal relationship was observed for other viruses, such as herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Our MR analyses provide strong support for a causal relationship between HHV-6 and CLA, shedding light on the etiology of this condition and highlighting the potential therapeutic implications of targeting HHV-6 in CLA treatment.

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ABSTRACT

Cutaneous leukocytoclastic angiitis (CLA) is a condition of clinical interest, with previous studies suggesting an association with herpes virus infections. This study aimed to investigate the causal association relationship

between the virus and CLA. Genetic variants linked to the virus were retrieved from the IEU open GWAS project and FinnGen database. Data on CLA were sourced from the FinnGen consortium R7. Mendelian randomization (MR) analysis, including the IVW, MR-Egger, and weighted median methods, was conducted. Sensitivity analyses were performed to ensure result accuracy. Among the six viruses investigated, only human herpesvirus 6 (HHV-6) demonstrated a causal association with CLA(odds ratio (OR) = 1.886, 95% confidence interval (CI) = 1.053–3.378, p = 0.033), indicating that HHV-6 infection significantly elevates the risk of CLA. Furthermore, both IVW and MR-Egger tests for heterogeneity confirmed homogeneous MR analysis results without evidence of horizontal pleiotropy (p>0.05). No significant causal relationship was observed for other viruses, such as herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Our MR analyses provide strong support for a causal relationship between HHV-6 and CLA, shedding light on the etiology of this condition and highlighting the potential therapeutic implications of targeting HHV-6 in CLA treatment.

Keywords: Cutaneous Leukocytoclastic Angiitis, herpes virus, HHV-6, Mendelian Randomization, Causal Association

1 INTRODUCTION

Cutaneous leukocytoclastic angiitis (CLA) is characterized by inflammation of the superficial skin microvessels, typified by neutrophil inflammation and nuclear fragmentation [(1)]. Clinically, CLA predominantly presents as symmetric palpable purpura on the lower extremities and other dependent areas of the body[(2)]. Extensive research has identified various factors associated with CLA, including idiopathic causes, infection, inflammatory disease, drug intake, and malignancies[(3)]. Although many CLA cases resolve spontaneously within a few weeks, severe, intractable, chronic or recurrent cases may necessitate systemic treatment[(2)].

We studied some of the viruses implicated in CLA in the*herpesvirales, herpesviridae* : subfamily*Alphaherpesvirinae* , genus *Simplexvirus* , species*Simplexvirus humanalpha1* (HSV-1), subfamily*Alphaherpesvirinae* , genus *Simplexvirus* , species*Simplexvirus humanalpha2* (HSV-2), subfamily*Betaherpesvirinae* , genus *Roseolovirus* , species *Human herpesvirus 6* (HHV-6), subfamily *Alphaherpesvirinae* , genus*Varicellovirus* , species *Varicellovirus humanalpha3* (CMV), subfamily *Gammaherpesvirinae* , genus *Lymphocryptovirus* , species *Lymphocryptovirus humangamma4* (EBV) and subfamily*Alphaherpesvirinae* , genus *Varicellovirus* (VZV). Studies have indicated that HSV, including both HSV-1 and HSV-2, can incite angiitis through the reactivation of latent HSV infections, leading to perivascular inflammation, with a notable emphasis on HSV-2 infections[(4)]. VZV, a neurotropic alphaherpesvirus, can induce a sustained inflammatory response and pathological remodeling of blood vessels [(5)]. Instances have demonstrated cutaneous angiitis following CMV infection [(6)]. EBV, a widespread herpesvirus, has also been associated with angiitis[(7)]. HHV-6, encompassing HHV-6A and HHV-6B species, has shown links to angiitis based on biopsy specimens and molecular research [(8, 9)].

Nevertheless, it remains uncertain whether these associations between herpes viruses and CLA are causal. This study seeks to explore the potential causal relationship between herpes viruses and CLA. Mendelian randomization(MR), an epidemiological approach, is employed to assess causality, utilizing genetic variation as instrumental variables (IVs) to mitigate potential confounding and reverse causality issues(10). This method proves particularly valuable when randomized controlled trials and observational studies are unfeasible[(10)]. In this investigation, we employ a two-sample MR (TSMR) analysis using summary-level data from comprehensive genome-wide association studies (GWASs) on herpes viruses and angiitis to examine these causal associations.

2 MATERIALS AND METHODS

2.1 Study design and assumption

The MR analysis aimed to assess the causal relationship between the human herpes virus family and leukocytoclastic angiitis. MR analysis relies on three fundamental assumptions:1)The genetic variants chosen as IVs must be significantly associated with the exposure (herpes virus). 2)The IVs should not be correlated

with any confounding factors. 3)The IVs should exclusively impact the outcome (leukocytoclastic angiitis) through the exposure, with no alternative pathways[11].

2.2 Exposure and Outcome

Genetic variants related to herpes virus were obtained from the IEU open GWAS project (*https://gwas.mrcieu.ac.uk/*) and the FinnGen database (*https://www.finngen.fi/*). Detailed information regarding the GWAS data can be found in*Supplemental Table 1.* For the outcome data, which comprised 262 cases of leukocytoclastic angiitis and 207,482 healthy controls, we accessed data from the FinnGen consortium R7 release in 2021 (*https://r7.finngen.fi/*). It's important to note that all the GWAS data used in this study originated from the European population.

2.3 Genetic instruments

Genetic IVs were chosen as independent single nucleotide polymorphisms (SNPs) with genome-wide significance (defined as $p < 1\times10\text{-}5$). To ensure independence among IVs for each exposure, we applied an LD threshold, $r2 < 0.001$, for SNPs within a 10,000 kb distance. We assessed the strength of the genetic instruments using F-statistics, with a strong instrument defined as an F-statistic > 10. Furthermore, we eliminated SNPs associated with potential confounding factors, such as height, white blood cell count, and body mass index (BMI), to meet the independence assumption of MR. We utilized the PhenoScannerV2 tool (*http://www.phenoscanner.medschl.cam.ac.uk/*) for this purpose [12, 13].

2.4 Statistical Methods

We conducted the primary MR analysis using the fixed effects inverse-variance weighted (IVW) method to assess the causal relationships between herpes viruses and leukocytoclastic angiitis. This method provides robust causal estimates, although it is somewhat sensitive to pleiotropy. In addition, we performed supplementary analyses using the weighted median method and MR-Egger regression[14, 15]. In the main analyses, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) for the IVs.

The heterogeneity was evaluated in the odds ratio using Cochran's Q test and visually inspected a funnel plot to assess symmetry, which indicates the absence of systematic bias [16]. We also conducted an MR-Egger intercept analysis to assess the presence of directional pleiotropy. Furthermore, we performed a leave-one-out sensitivity test to evaluate the impact of individual SNPs on the results, mitigating potential bias [14, 16, 17].

All statistical analyses were carried out using R Studio (v.4.3.1), with the MR analysis conducted using the 'TwoSampleMR' package[18]. (Figure. 1)

3 RESULT

3.1 Selection of Genetic Instruments

As previously mentioned, all SNPs used for genetic exposure prediction met the rigorous criteria of a genome-wide significance level ($p < 1\times10\text{-}5$) and the requirement of an F-statistic > 10, indicating that all instruments were strong. We further excluded SNPs associated with potential confounding factors using PhenoScannerV2. Ultimately, 4 to 21 SNPs per herpes virus satisfied harmonization criteria and were considered valid instruments.These SNPs data are visible in the*Supplemental Table 2.*

3.2 Causal Effects of HHV-6 on CLA

We explored the causal association between 14 SNPs for HHV-6 and CLA in individuals of European descent. The results from the IVW analysis indicated that HHV-6 significantly increased the risk of CLA (OR = 1.886, 95% CI = 1.053–3.378, p = 0.033). The MR-Egger method (OR = 1.503, 95% CI = 0.276–8.188; p = 0.646) and the weighted median (OR = 1.967, 95% CI = 0.894–4.325; p = 0.092) yielded consistent results, although they were not particularly significant. (Figure. 2). Moreover, we conducted a sensitivity analysis to verify the reliability of the IVW results. Sensitivity analyses confirmed the reliability of the IVW results, with tests for heterogeneity(IVW: Q = 12.508, p = 0.487; MR-Egger: Q = 12.426, p = 0.412) and horizontal pleiotropy(MR-Egger intercept = 0.032, p = 0.784) showing consistent results(Figure. 3 a).

3.3 Causal Effects of HSV on CLA

For HSV, we selected 4 SNPs for HSV-1,10 SNPs for HSV-2 and 19 SNPs for HSV infection and examined their effects on CLA. The results showed no significant association between HSV-1 (OR = 0.896, 95% CI = 0.141-5.691, p = 0.908), HSV-2 (OR = 1.075, 95% CI = 0.737-1.568, p = 0.706), and HSV infection (OR = 1.119, 95% CI = 0.826-1.515, p = 0.467) with CLA. The MR-Egger and weighted median also yielded similar results(all $p > 0.05$) (Figure. 2). Horizontal pleiotropy did not affect the MR analyses based on the MR-Egger regression intercept test, with intercept p-values of 0.998 for HSV-1, 0.954 for HSV-2, and 0.160 for HSV infection. The Cochran's Q test results showed no heterogeneity for HSV-1 (p = 0.148) and HSV-2 (p = 0.250), but heterogeneity was detected for HSV infection (p = 0.040) (Figure. 3 b-d).

3.4 Causal Effects of VZV on CLA

Regarding VZV, we separately screened 8 SNPs for varicella and 16 SNPs for zoster virus. The IVW results did not support a causal effect of varicella or zoster virus (OR = 1.013, 95% CI = 0.803-1.277, p = 0.914; OR = 1.163, 95% CI = 0.762-1.777, p = 0.484) on CLA. The MR-Egger and weighted median methods also yielded p-values above 0.05(Figure. 2). Likewise, there was no horizontal pleiotropy for varicella (MR-Egger intercept = -0.047, p = 0.717) and the zoster virus (MR-Egger intercept = 0.053, p = 0.594). Additionally, Cochrane's Q statistics results showed no heterogeneity for varicella (p = 0.906), but heterogeneity existed for zoster virus (p = 0.006) (Figure. 3 e-f).

3.5 Causal Effects of CMV on CLA

Similarly, we assessed 18 SNPs for CMV but found no evidence of a causal relationship with CLA in the IVW analysis (OR = 0.863, 95% CI = 0.538-1.384, p = 0.540). MR-Egger (OR = 5.694, 95% CI = 0.694-46.740, p = 0.125) and weighted median analyses (OR = 0.800, 95% CI = 0.429-1.493, p = 0.483) also did not yield statistically significant results ($p > 0.05$) (Figure. 2). Tests for horizontal pleiotropy (MR-Egger intercept = -0.336, p = 0.091) and heterogeneity(p = 0.314) were consistent with these findings. (Figure. 3 g).

3.6 Causal Effects of EBV on CLA

For EBV, we explored the relationship between eight EBV-related antibodies and CLA. The MR-Egger analysis indicated a potential reduction in the risk of CLA for the EBV EA-D antibody levels (OR = 0.145, 95% CI = 0.038–0.551, p = 0.016). However, IVW and weighted median analyses did not find significant associations for any of the eight antibodies, with p-values exceeding 0.05(Figure. 2).

For the eight EBV-related antibodies, no unbalanced horizontal pleiotropy was found in any of the instruments ($p > 0.05$). Two antibodies (EBV VCA p18 antibody levels and Anti-EBV EA IgG levels) showed heterogeneity (p = 0.043; p = 0.031), while there was no evidence of heterogeneity among the remaining six antibodies (Figure. 3h-o).

Furthermore, all funnel plots indicated a slight presence of pleiotropy, suggesting a potential single instrumental variable affecting the estimated causal effect (Figure. 4). The leave-one-out analysis showed that no single SNP strongly influenced the overall effect of exposure on the outcome (Figure. 5).

In summary, our results suggest a causal relationship between HHV-6 and CLA, while no such relationships were observed for other herpes viruses. Sensitivity analyses and tests for pleiotropy and heterogeneity support the robustness of these findings.

4 DISCUSSION

Infection, particularly viral infection, is a significant factor in the development of angiitis, which is directly related to the occurrence and development of angiitis. Our study using MR analysis revealed a clear link between CLA and HHV-6, a member of the herpesvirus family. HHV-6 infection directly contributes to the development of CLA, while other viruses such as EBV, HSV-1, HSV-2, varicella virus, zoster virus, and CMV are not causally related to CLA.

Previous studies have indicated the association between HHV-6 and leukocytoclastic angiitis, specifically CLA. Serology tests showed positive HHV-6 IgG and IgM antibodies in patients with CLA, while tests for other viruses were negative [19]. Research shows that over 90% of individuals are infected with HHV-6B during early childhood, and the virus remains dormant in the body throughout their lifetime. Thus, we believe that the occurrence of CLA should be related to the primary infection or reactivation of HHV-6. However, subsequent observational studies did not include HHV-6 infection as part of their examinations. Additionally, around half of CLA cases do not have an underlying cause [20, 21].

One of the possible mechanisms of CLA is the formation and deposition of immune complexes in and around vessel walls [22]. Studies have shown a higher presence of IgA, IgM, and IgG in the affected skin of CLA patients compared to unaffected skin[23]. A comparative study also proposed that Helper T cells and granzyme B may also play a role in the inflammatory process of CLA [24]. HHV-6 primarily infects CD4+T cells and upregulates the expression of granzyme B in these cells, leading to disruption of the body's antiviral immunity and potentially contributing to inflammation. It plays an important role in various inflammations. In addition, HHV-6 infection induces the release of cytokines,like tumor necrosis factor-alpha (TNF-α) and interleukin 1-β, which may further contribute to inflammation and autoimmune reactions[25]. TNF- α can induce the expression of protease-3 (PR3) and myeloperoxidase (MPO) on the cell membrane, leading to angiitis [26]. Further research is needed to determine whether similar mechanisms exist in CLA.

Various studies have explored the connection between the herpesvirus family and different types of angiitis. For example, EBV may be associated with anti-neutrophil cytoplasmic antibody-associated angiitis (AAV), Henoch-Schönlein purpura (HSP) and systemic vasculitis, varicella zoster virus causes giant cell arteritis, Acute Retinal Necrosis (ARN) and lgA angiitis, HSV infection is linked to cerebrovascular disease, and CMV infection can cause skin necrotizing angiitis[4, 6, 27-31]. However, our MR analysis did not find a causal relationship between these viruses and CLA. It is important to investigate the relationship between the herpesvirus family and angiitis, including other forms of angiitis, through further research.

This study had several strengths, including the use of two-sample MR analysis, which helps overcome limitations in conventional epidemiological studies. Sensitivity analyses were conducted, and consistent estimates from different models increased confidence in the associations identified. However, there were also limitations to consider. More genetic variations as IVs were for sensitivity analysis and pleiotropy detection. The SNPs used in the analysis did not meet the traditional GWAS significance threshold, but this was deemed acceptable[32]. Additionally, the GWAS databases used were limited to individuals of European ancestry, reducing the universality of our results. Further studies should investigate whether our results hold for other ancestries. Finally, the limited number of SNPs associated with other viruses (HSV-1, HSV-2, CMV, Anti-EBV VCA IgG levels, Anti-EBV EA IgG levels and Anti-EBV IgG seropositivity could introduce weak instrument bias.

5 CONCLUSION

In conclusion, our study provides evidence of a causal relationship between HHV-6 infection and CLA. This finding helps us understand the etiology of CLA and emphasizes the role of HHV6 in CLA in future studies. Further research is required to understand the impact of HHV-6 primary infection versus reactivation on the risk of CLA. We recommend testing for HHV-6 infection in patients with unexplainable Cutaneous leukocytoclastic angiitis. The relationship between herpesviruses and other forms of angiitis warrants further investigation.

STATEMENTS AND DECLARATIONS

Author contributions

S.-X. Z. and Y.-H. H. designed the study. H.-Y. Z., J.-L. X., and Y.-F. W. collected and analyzed data. S.-X. Z. H.-Y. Z., and T. C. contributed to the analysis and interpretation of the data. H.-Y. Z, J.-L. X., and Y.-F. W. wrote the manuscript. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. S.-X. Z. and

Y.-H. H. had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest disclosure

The authors declare no conflict of interest.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval statement

The original GWAS data have obtained prior approval from relevant ethics review boards and the summary-level statistics don't contain any personal information, thus no additional ethics approval was required.

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We thank the IEU open GWAS project for providing summary statistics data of CLA and viral infections for our analyses.

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Figure. Legends

Figure. 1 Workflow and Key Assumptions in the Current Mendelian Randomization (MR) Study. IVs, instrumental variables; MR, Mendelian randomization; GWAS, genome-wide association study; SNPs, single-nucleotide polymorphisms

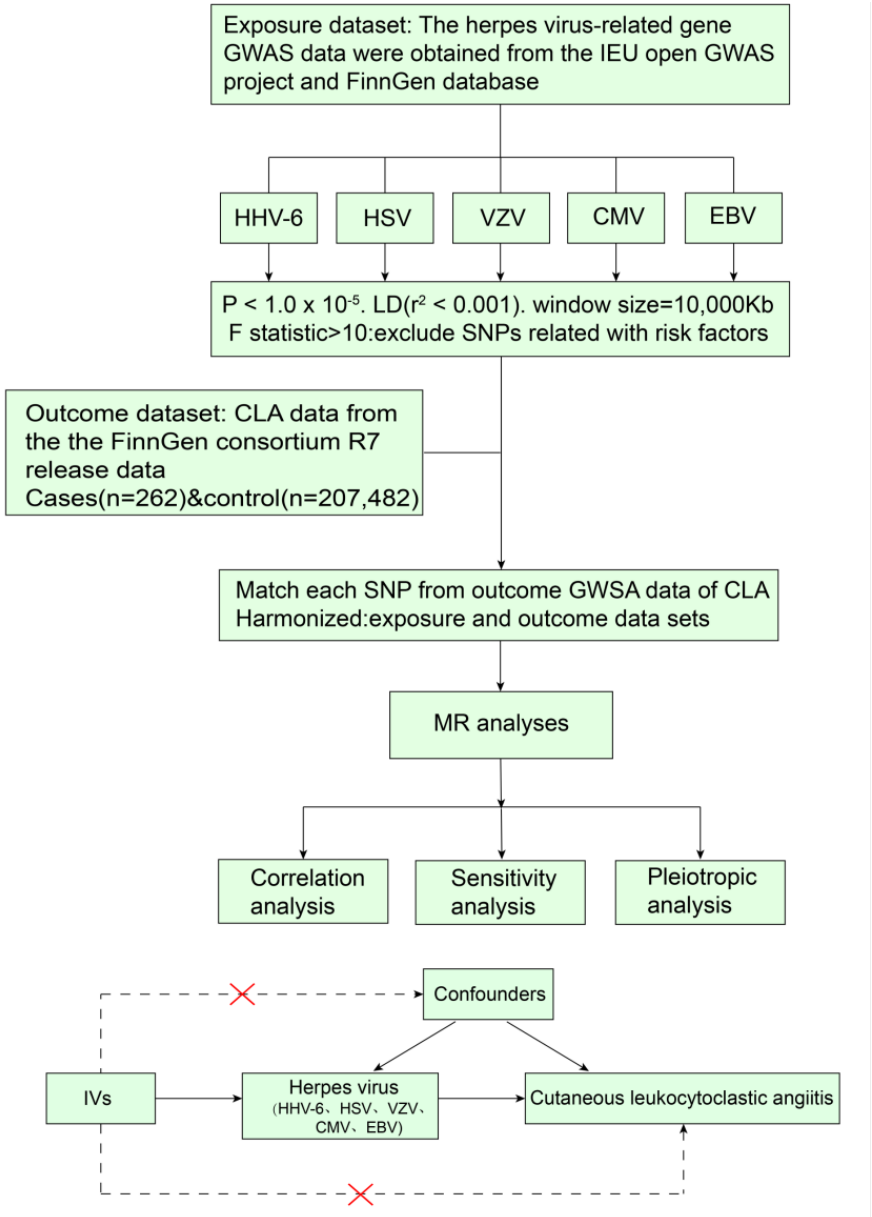


Figure. 2 Causal relationship between herpes virus and Cutaneous leukocytoclastic angiitis(CLA) evaluated using two-sample Mendelian Randomization (TSMR) methods and sensitivity analysis

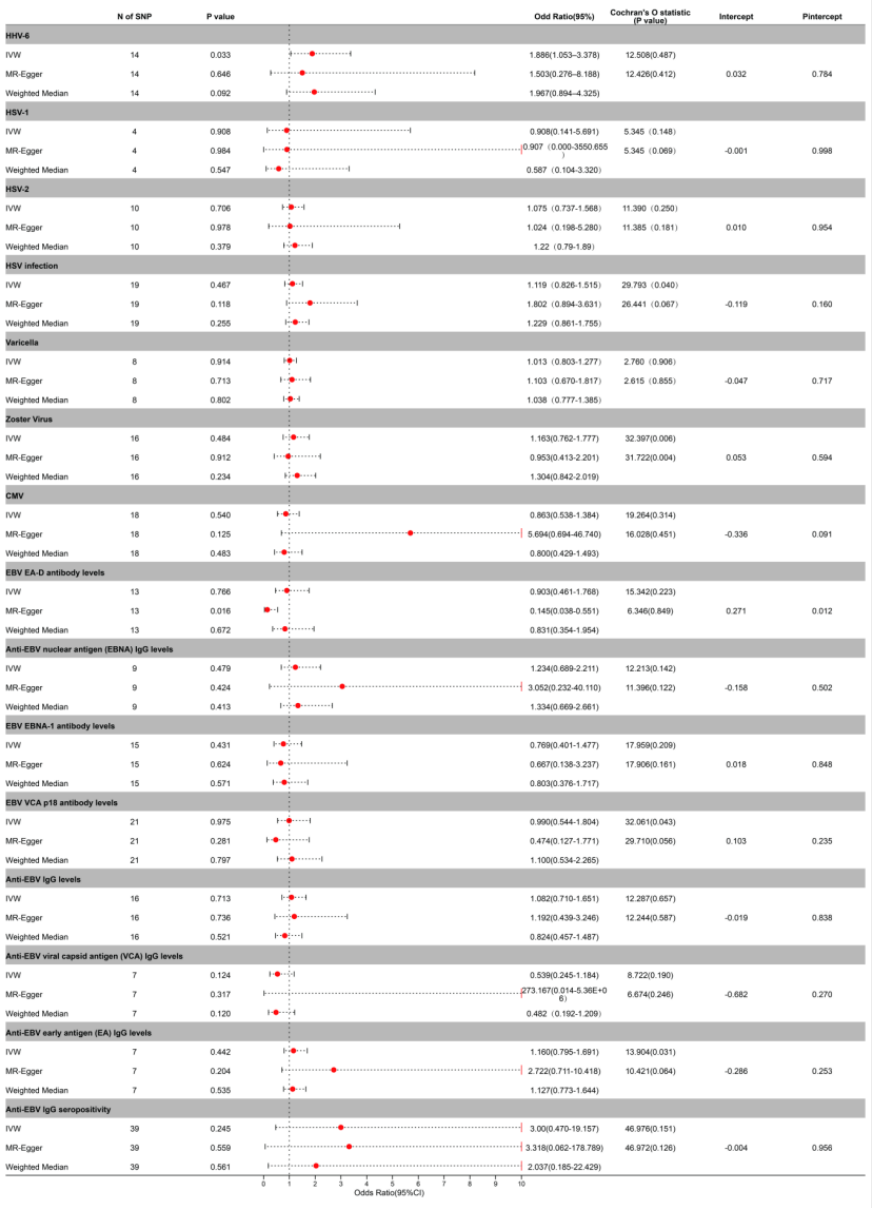


Figure. 3 Scatter plots for IVW, MR-Egger, and WM analysis methods highlighting the impact of various herpes viruses and infections on CLA. (a)HHV-6, (b)HSV-1, (c)HSV-2, (d)HSV infection, (e-f)VZV, (g)CMV, (h-o)EBV, respectively

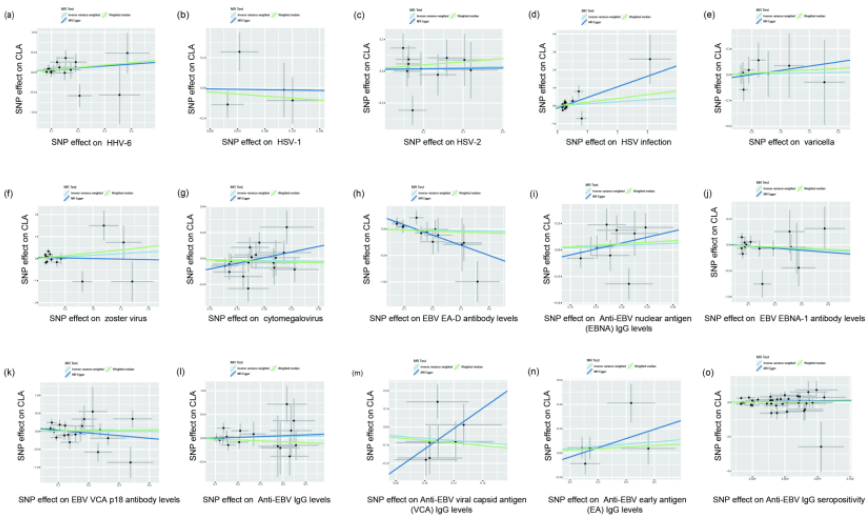


Figure. 4 Funnel Plots Showing Genetic Liability Effects of Various Herpes Viruses and Infections on CLA. (a)HHV-6, (b)HSV-1, (c)HSV-2, (d)HSV infection, (e-f)VZV, (g)CMV, (h-o)EBV, respectively

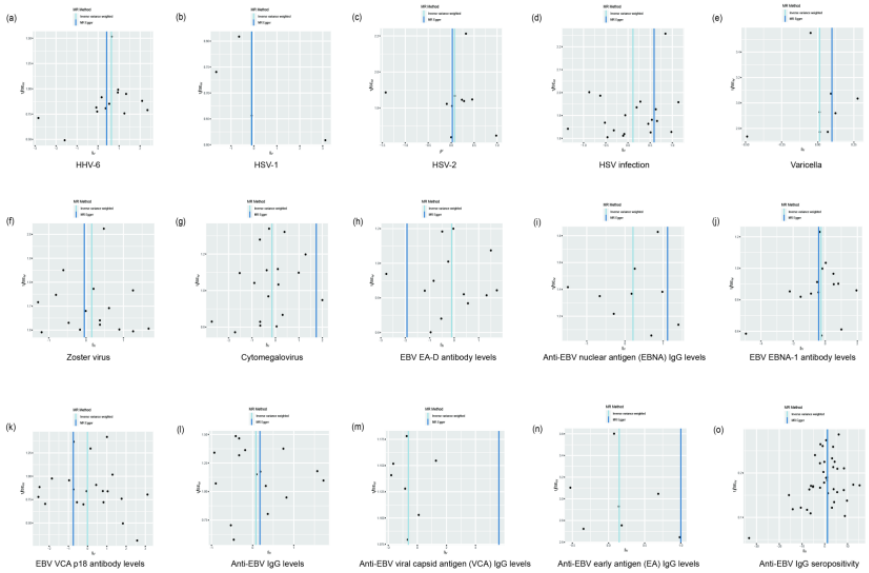
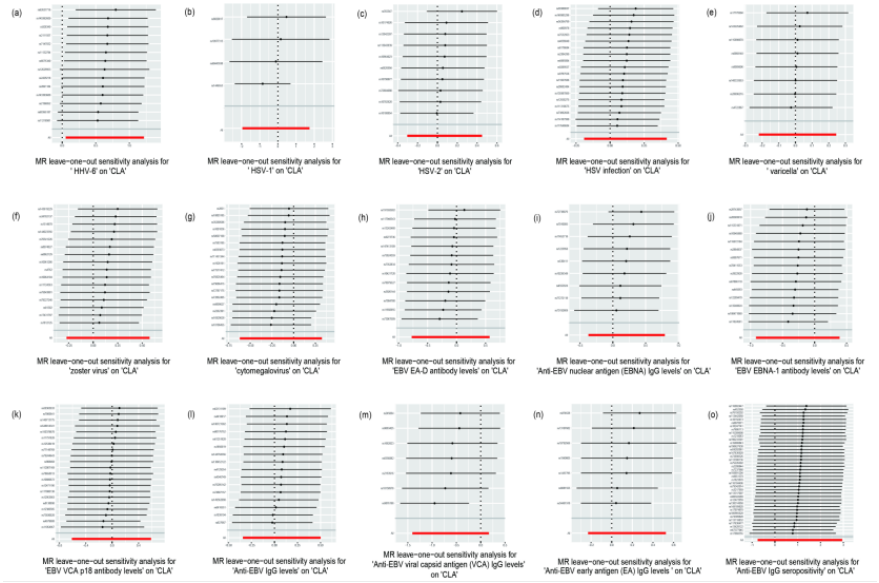
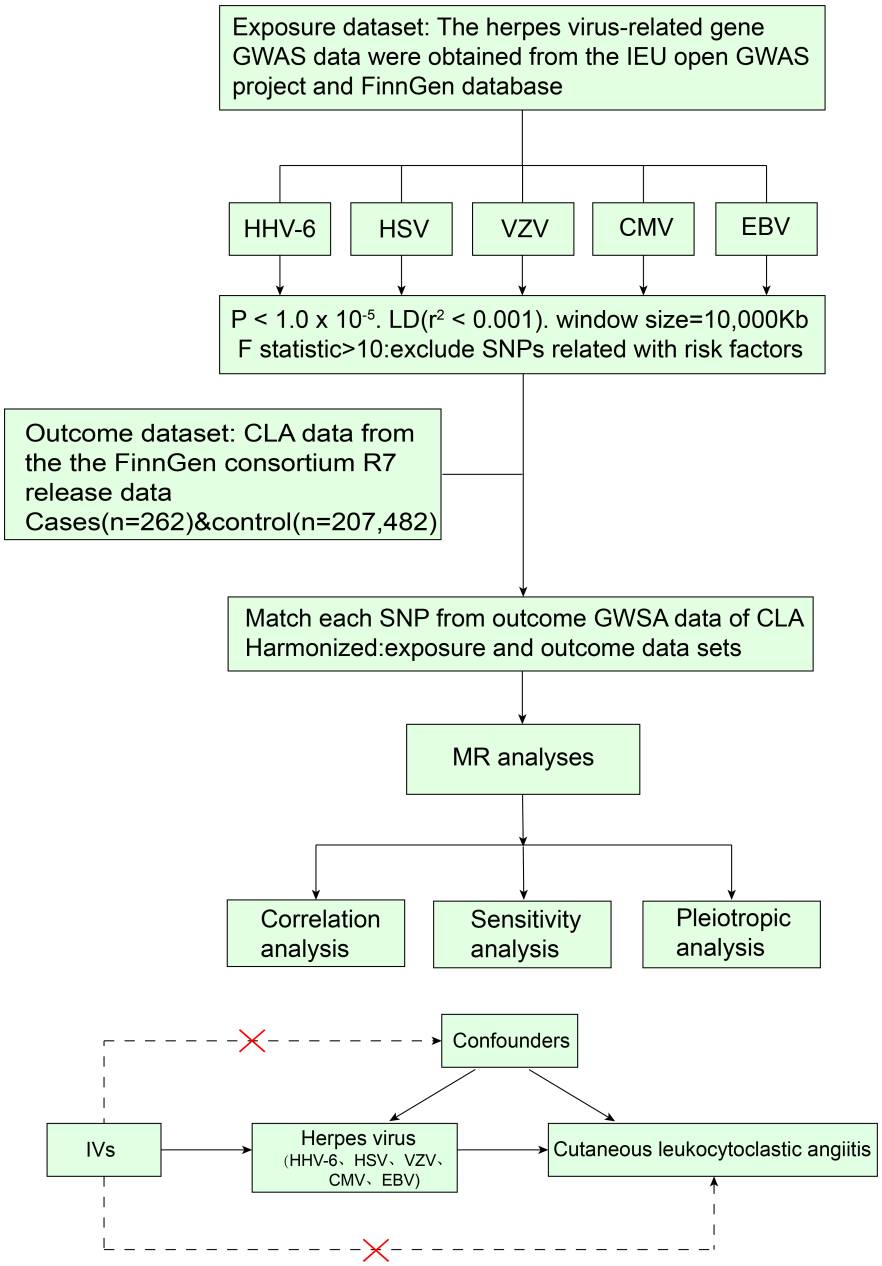
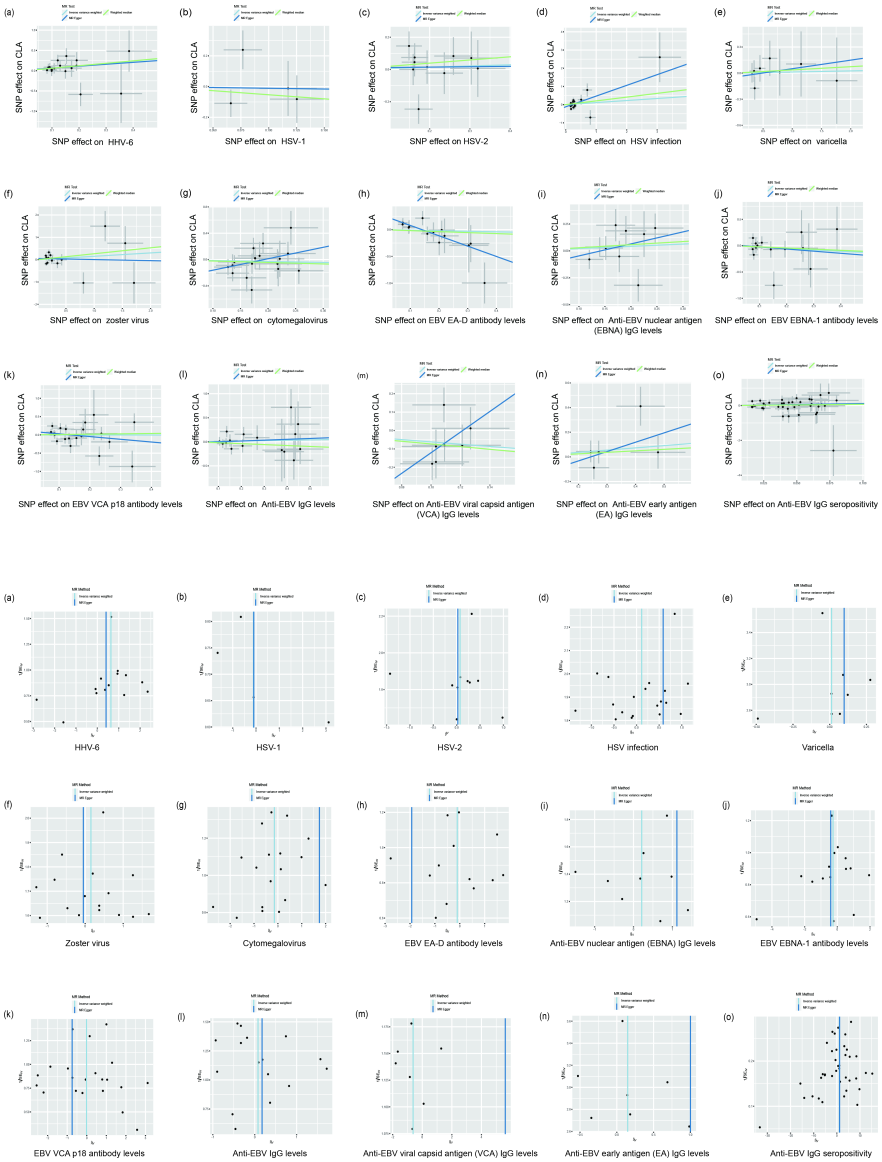
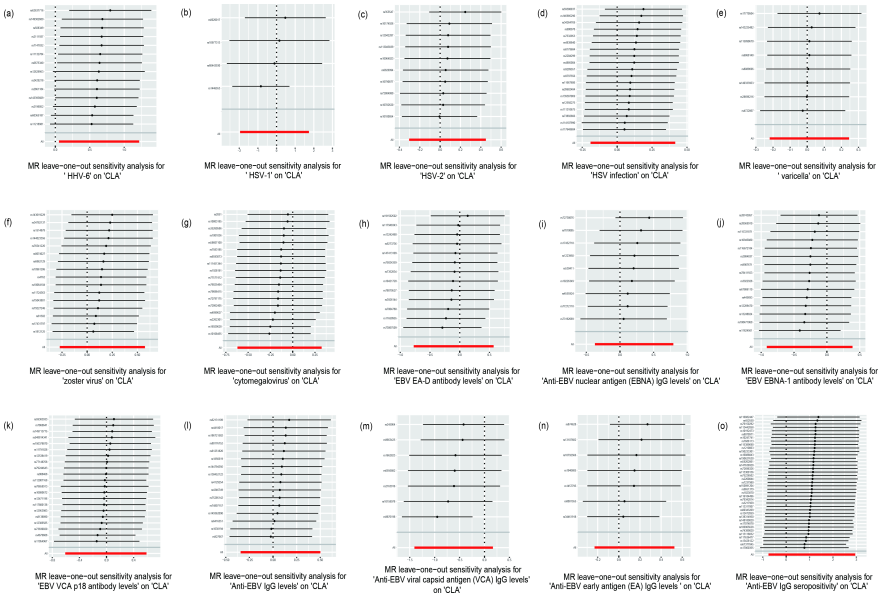


Figure. 5 Leave-One-Out Analysis for Evaluation of Individual SNP Influence on Causal Associations of Herpes Viruses and Infections with CLA. (a)HHV-6, (b)HSV-1, (c)HSV-2, (d)HSV infection, (e-f)VZV, (g)CMV, (h-o)EBV, respectively









(a) MR leave−one−out sensitivity analysis for ' HHV-6' on 'CLA'

(b) MR leave−one−out sensitivity analysis for ' HSV-1' on 'CLA'

(c) MR leave−one−out sensitivity analysis for 'HSV-2' on 'CLA'

(d) MR leave−one−out sensitivity analysis for 'HSV infection' on 'CLA'

(e) MR leave−one−out sensitivity analysis for ' varicella' on 'CLA'

(f) MR leave−one−out sensitivity analysis for 'zoster virus' on 'CLA'

(g) MR leave−one−out sensitivity analysis for 'cytomegalovirus' on 'CLA'

(h) MR leave−one−out sensitivity analysis for 'EBV EA-D antibody levels' on 'CLA'

(i) MR leave−one−out sensitivity analysis for 'Anti-EBV nuclear antigen (EBNA) IgG levels' on 'CLA'

(j) MR leave−one−out sensitivity analysis for 'EBV EBNA-1 antibody levels' on 'CLA'

(k) MR leave−one−out sensitivity analysis for 'EBV VCA p18 antibody levels' on 'CLA'

(l) MR leave−one−out sensitivity analysis for 'Anti-EBV IgG levels' on 'CLA'

(m) MR leave−one−out sensitivity analysis for 'Anti-EBV viral capsid antigen (VCA) IgG levels' on 'CLA'

(n) MR leave−one−out sensitivity analysis for 'Anti-EBV early antigen (EA) IgG levels ' on 'CLA'

(o) MR leave−one−out sensitivity analysis for 'Anti-EBV IgG seropositivity' on 'CLA'