Clinical Medicine & Pharmacology

press

https://doi.org/10.70731/thgpbr97

# Mendelian Randomization Study Evidence on the Association between Herpes Simplex Virus Antibodies Immune Response and the Risk of Drug-Induced Obesity

Xiaoping Li [a,†], Weikun Zhao [b,†,*], Yanni Li [b], Hong Chen [b]

[a] *Department of Dermatology, Nanxishan Hospital of Guangxi Zhuang Autonomous Region, Guilin,Guangxi, China*
[b] *Premium Healthcare Department, The First Affiliated Hospital of Guilin Medical University, Guilin, Guangxi, China*

KEYWORDS

*Herpes Simplex Virus, Antibody Immune Response, Drug-Induced Obesity, Mendelian Randomization*

ABSTRACT

This study utilized Mendelian randomization (MR) to investigate the causal link between immune response to herpes simplex virus antibodies and drug-induced obesity (DIO). Genome-wide association study (GWAS) data was analyzed using methods like Inverse Variance Weighted (IVW), MR-Egger, and Weighted Median method (WME). Effective instrumental variables were identified, showing a significant increase in DIO risk with Epstein-Barr virus ZEBRA antibody, herpes simplex virus 7 U14 antibody, herpes simplex virus type 1 IgG, and herpes simplex virus type 2 IgG seropositivity. Reverse MR analysis did not reveal a reverse causal relationship. The study provides initial evidence of the relationship between herpes simplex virus antibody immune response and DIO, contributing new theoretical insights for future understanding of DIO.

## INTRODUCTION

Drug-Induced Obesity (DIO) is a metabolic dysregulation syndrome triggered by prolonged use of glucocorticoids, antipsychotics, and other medications, characterized by abnormal adipose accumulation, insulin resistance, and elevated cardiovascular risk [1]. Its prevalence has risen significantly with the widespread application of these pharmaceuticals [2]. Studies suggest that herpesvirus antibody immune responses may contribute to DIO pathogenesis through immunomodulatory mechanisms, though the precise pathways remain unclear [3]. Human herpesviruses (HHVs), a group of latent DNA viruses including Epstein-Barr virus (EBV), HHV-6, and herpes simplex virus types 1/2 (HSV-1/2), are implicated in disrupting adipose metabolic homeostasis via persistent immune activation, potentially linking them to drug-induced adiposity [4-5]. Proposed mechanisms involve antibody-mediated chronic inflammation, aberrant adipocyte differentiation, and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis [6]. Previous research highlights associations between EBV ZEBRA antibody titers and metabolic disorders, yet causal relationships with DIO remain unvalidated [1]. Viral proteins such as HHV-6 U14 have been shown to activate the NF-$\kappa$B signaling pathway, inducing pro-inflammatory cytokines IL-6 and TNF-α [7], which may further drive DIO pathogenesis. Elevated HSV-1/2 IgG seropositivity rates in obese populations suggest latent infection may perturb drug metabolism [4]. However, conventional observational studies are susceptible to confounding bias, limiting causal inference. This study employs a two-sample Mendelian randomization (MR) framework, leveraging genome-wide association study (GWAS) data to systematically investigate causal relationships between HHV antibody immune responses and DIO. Robustness of findings will be assessed through pleiotropy

† These authors contributed equally to this work.
* Corresponding author. E-mail address: zwkguilin0925@163.com

Received 17 April 2025; Received in revised from 13 June 2025; Accepted 18 June 2025; Available online 30 June 2025.

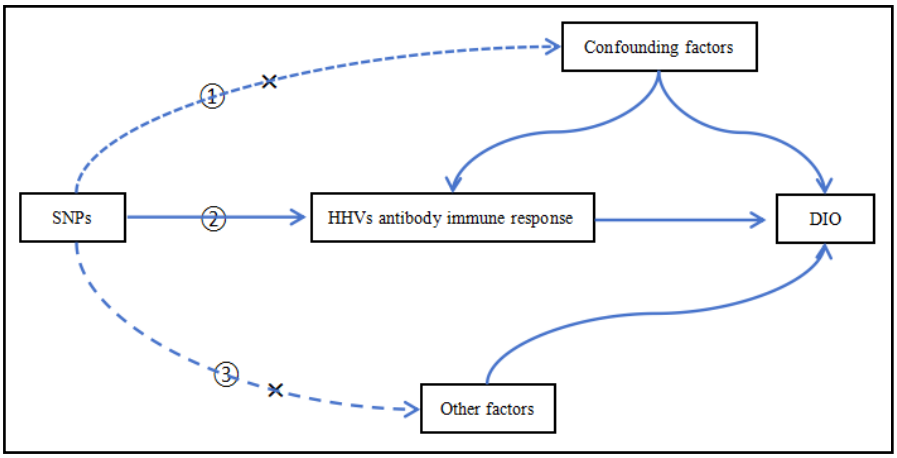


**Figure 1 | Core assumptions of Mendelian randomization.**

**1) Independence assumption;**
**2) Strong association;**
**3) Exclusion restriction)**

testing, heterogeneity analysis, and leave-one-out sensitivity analysis. This work aims to provide genetic evidence elucidating the role of viral immune responses in DIO etiology.

## MATERIALS AND METHODS

### Study Design

Leveraging GWAS summary data, we implemented a bidirectional MR framework to systematically investigate causal relationships between HHV-specific antibody responses and DIO. Single nucleotide polymorphisms (SNPs) fulfilling three core MR assumptions served as genetic instruments [8]: (1) Relevance assumption: Instrumental SNPs demonstrated genome-wide significant associations ($P < 5\times10^{-8}$) with exposure traits (HHV-targeted IgG antibody titers); (2) Exclusion restriction: Genetic variants influenced DIO risk exclusively through antibody-mediated pathways, with horizontal pleiotropy rigorously excluded via sensitivity analyses; (3) Independence assumption: SNPs remained uncorrelated with major confounders (demographic, behavioral, or metabolic covariates). Instrument validity was confirmed through linkage disequilibrium score regression and cross-trait genetic correlation analyses. Weak instrument bias was mitigated by applying F-statistic thresholds ($F > 10$), ensuring robust causal estimates (**Figure 1**).

### Data Sources

Grounded in the UK Biobank population-based cohort, this seroepidemiological investigation led by Guillaume Butler-Laporte et al. was originally reported in Open Forum Infectious Diseases [9]. Serum samples from 8,735 White British participants underwent comprehensive profiling of IgG reactivity against seven herpesviruses: HSV-1/2, EBV, cytomegalovirus (CMV), HHV-6, HHV-7, and varicella-zoster virus (VZV). Viral antibody levels were quantified via multiplex fluorescent immunoassay (MFI), followed by log10 transformation to normalize skewed distributions. The work constitutes the largest genome-wide characterization of HHV humoral immunity, elucidating genetic determinants of herpesvirus-related non-infectious comorbidities. Genetic associations were analyzed using FinnGen R11 (https://www.finngen.fi), a national biorepository combining whole-genome data and longitudinal health registries from 426,310 Finnish participants. Drug-induced obesity GWAS metrics were retrieved from FinnGen's curated repository (https://storage.googleapis.com/finngen-public-data-r11/summary_stats/finngen_R11_E4_OBESITYDRUG.gz), enabling large-scale genetic dissection of pharmacotherapy-related adiposity. As this study involves the analysis of previously collected and published data, no new ethics approval is required.

### Instrument Selection

Instrumental SNPs were selected under genome-wide significance thresholds ($P < 5\times10^{-8}$), with relaxed criteria ($P < 1\times10^{-5}$) applied when candidate variants numbered below 10 to ensure statistical power. Independence between SNPs was enforced through linkage disequilibrium (LD) clumping ($r^2 < 0.001$ within 10,000 kb genomic windows). Instrument strength was quantified using F-statistics: $F=[R^2/(1-R2)]\times(N-K-1)/K$, $R^2=2\times MAF\times(1-MAF)\times(\beta/sd)^2$. Here, $N$ denotes sample size, $K$ the number of instruments, MAF minor allele frequency, $\beta$ effect size, and SD standard deviation. Variants with $F > 10$ were retained to mitigate weak instrument bias [10]. Allelic direction harmonization between exposure and outcome datasets excluded palindromic SNPs and discordant variants. LDlink (https://ldlink.nih.gov) further filtered SNPs showing associations with known confounders, yielding high-specificity instruments for MR analyses [11].

### Statistical Analysis

Bidirectional two-sample MR was conducted using R v4.3.1 with the TwoSampleMR package (v0.5.7), modeling herpesvirus antibody responses as exposures and DIO as outcome. Primary causal estimates derived from inverse-variance weighted (IVW) meta-analysis, supplemented by: (1) Weighted median estimator (tolerating ≤50% invalid instruments); (2) MR-Egger regression (intercept test for horizontal pleiotropy); (3) Simple/weighted mode approaches for robustness validation. Heterogeneity was assessed via Cochran's Q test ($P < 0.05$ triggering random-effects IVW). Pleiotropic outliers were identified through MR-Egger intercept tests and MR-PRESSO global test, with subsequent correction. Sensitivity analyses included leave-one-out iterative

**Table 1 | SNPs related to EBV ZEBRA antibody levels and their associations with DIO**

| SNPs | EA | OA | F | EBV ZEBRA antibody levels | | | DIO | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | β | SE | p value | β | SE | p value |
| rs10271963 | A | T | 20 | 0.0752 | 0.0168 | 8.0166E-06 | 0.0091 | 0.0856 | 0.9157 |
| rs1118204 | C | T | 45 | -0.1070 | 0.0160 | 2.03117E-11 | -0.0079 | 0.0861 | 0.9273 |
| rs113103848 | A | G | 22 | -0.1228 | 0.0263 | 3.04318E-06 | 0.0007 | 0.1591 | 0.9965 |
| rs116043458 | G | A | 62 | 0.3762 | 0.0477 | 3.01667E-15 | 0.6551 | 0.3959 | 0.0980 |
| rs116189954 | T | C | 20 | 0.3285 | 0.0726 | 6.08169E-06 | 3.8318 | 2.2066 | 0.0825 |
| rs116302426 | A | G | 25 | 0.3117 | 0.0622 | 5.45706E-07 | 0.9748 | 0.7058 | 0.1673 |
| rs11682654 | C | T | 20 | -0.0701 | 0.0156 | 6.78407E-06 | 0.0675 | 0.0837 | 0.4196 |
| rs11715964 | T | C | 20 | -0.1872 | 0.0419 | 7.77388E-06 | 0.0259 | 0.1540 | 0.8665 |
| rs117187370 | G | A | 21 | 0.3086 | 0.0671 | 4.24959E-06 | 0.3563 | 0.5228 | 0.4956 |
| rs11728735 | T | C | 20 | -0.1477 | 0.0329 | 7.08751E-06 | -0.0176 | 0.1663 | 0.9155 |
| rs11755421 | A | G | 25 | 0.1008 | 0.0201 | 5.57401E-07 | 0.1367 | 0.0955 | 0.1527 |
| rs117875419 | T | G | 20 | -0.2805 | 0.0623 | 6.69217E-06 | -0.4951 | 0.5071 | 0.3289 |
| rs12195665 | A | G | 53 | 0.1269 | 0.0174 | 2.61922E-13 | 0.0284 | 0.1028 | 0.7820 |
| rs1233396 | A | G | 29 | -0.1214 | 0.0224 | 5.6785E-08 | -0.1581 | 0.1625 | 0.3304 |
| rs1265764 | A | G | 28 | -0.1908 | 0.0360 | 1.14716E-07 | 0.0581 | 0.2346 | 0.8045 |
| rs12962837 | G | A | 20 | 0.1023 | 0.0230 | 8.93453E-06 | -0.0404 | 0.1162 | 0.7280 |
| rs140214797 | G | T | 21 | -0.2609 | 0.0573 | 5.29065E-06 | -0.6678 | 0.3531 | 0.0586 |
| rs141016096 | T | C | 22 | -0.3662 | 0.0774 | 2.21438E-06 | -0.1214 | 0.2258 | 0.5908 |
| rs142476835 | C | T | 23 | -0.2304 | 0.0480 | 1.58101E-06 | -0.3230 | 0.3204 | 0.3134 |
| rs1431398 | A | G | 24 | -0.1124 | 0.0231 | 1.14092E-06 | -0.0280 | 0.1139 | 0.8058 |
| rs148347131 | T | C | 37 | 0.4936 | 0.0813 | 1.28875E-09 | 0.3964 | 0.2186 | 0.0697 |
| rs149485984 | A | G | 26 | 0.3801 | 0.0752 | 4.25582E-07 | 0.3195 | 1.1512 | 0.7813 |
| rs150993367 | T | G | 21 | -0.3497 | 0.0758 | 4.00156E-06 | -0.4540 | 0.3093 | 0.1422 |
| rs151302046 | A | G | 20 | -0.3112 | 0.0704 | 9.98621E-06 | -1.0067 | 1.2214 | 0.4098 |
| rs17039421 | C | T | 20 | 0.1659 | 0.0369 | 6.93208E-06 | -0.0654 | 0.1724 | 0.7042 |
| rs17208314 | T | C | 49 | 0.5000 | 0.0712 | 2.20304E-12 | 0.7952 | 0.5647 | 0.1591 |
| rs17219974 | T | A | 30 | 0.3964 | 0.0729 | 5.46238E-08 | -0.3836 | 0.2707 | 0.1564 |
| rs1741740 | G | A | 21 | -0.0948 | 0.0208 | 5.41412E-06 | -0.0358 | 0.1127 | 0.7505 |
| rs17612503 | C | G | 25 | 0.1095 | 0.0219 | 5.47233E-07 | -0.1323 | 0.1217 | 0.2769 |
| rs181204 | G | A | 20 | 0.0742 | 0.0166 | 7.66727E-06 | 0.2002 | 0.0851 | 0.0186 |
| rs187764 | T | C | 31 | 0.1767 | 0.0315 | 2.11218E-08 | 0.6839 | 0.2647 | 0.0098 |
| rs191639797 | T | C | 23 | -0.5017 | 0.1037 | 1.31532E-06 | -0.1124 | 0.2242 | 0.6162 |
| rs206019 | T | C | 21 | 0.1070 | 0.0232 | 3.92756E-06 | 0.1853 | 0.1815 | 0.3071 |
| rs2073520 | G | A | 29 | -0.1308 | 0.0244 | 7.9726E-08 | -0.0004 | 0.1380 | 0.9979 |
| rs2289042 | A | C | 20 | 0.1534 | 0.0343 | 7.51917E-06 | -0.0718 | 0.2334 | 0.7585 |
| rs2516493 | C | T | 47 | -0.1110 | 0.0162 | 8.48696E-12 | -0.0536 | 0.0963 | 0.5778 |
| rs2516670 | A | G | 31 | -0.1174 | 0.0212 | 2.84339E-08 | -0.1827 | 0.1334 | 0.1707 |
| rs2517600 | A | G | 28 | -0.0959 | 0.0183 | 1.52143E-07 | 0.0332 | 0.0925 | 0.7195 |
| rs2523483 | G | T | 34 | 0.1566 | 0.0269 | 5.73191E-09 | -0.1173 | 0.3871 | 0.7619 |
| rs2535332 | T | C | 56 | -0.1619 | 0.0216 | 6.27734E-14 | 0.0539 | 0.1490 | 0.7175 |
| rs2647025 | A | G | 333 | 0.3104 | 0.0170 | 1.61203E-74 | -0.0775 | 0.0901 | 0.3899 |
| rs2735065 | A | C | 25 | 0.1778 | 0.0354 | 5.08363E-07 | 0.0637 | 0.2537 | 0.8018 |
| rs28366157 | G | A | 75 | 0.2751 | 0.0318 | 5.09101E-18 | 0.2764 | 0.4628 | 0.5504 |
| rs28367860 | C | T | 45 | 0.1065 | 0.0159 | 2.02206E-11 | -0.0270 | 0.0850 | 0.7510 |
| rs2844624 | C | T | 28 | -0.0869 | 0.0164 | 1.15356E-07 | -0.0007 | 0.0877 | 0.9936 |
| rs2859090 | A | G | 64 | -0.1494 | 0.0187 | 1.1628E-15 | -0.0874 | 0.1044 | 0.4027 |
| rs28724899 | T | A | 28 | 0.1218 | 0.0230 | 1.13102E-07 | 0.2473 | 0.1280 | 0.0533 |
| rs29220 | G | C | 23 | 0.0858 | 0.0178 | 1.36788E-06 | 0.0710 | 0.0852 | 0.4049 |
| rs3128853 | T | C | 23 | -0.1753 | 0.0367 | 1.7951E-06 | 0.5534 | 0.4781 | 0.2471 |
| rs3129299 | T | C | 53 | 0.1514 | 0.0209 | 4.18197E-13 | -0.1103 | 0.1440 | 0.4436 |
| rs3130177 | A | G | 44 | 0.1144 | 0.0172 | 3.13339E-11 | 0.0278 | 0.0916 | 0.7616 |
| rs3131623 | A | T | 82 | -0.1807 | 0.0199 | 1.26709E-19 | 0.0658 | 0.1384 | 0.6346 |
| rs3134931 | C | T | 42 | 0.1086 | 0.0168 | 1.14595E-10 | 0.0358 | 0.1001 | 0.7208 |
| rs34770282 | G | A | 22 | -0.1221 | 0.0263 | 3.43072E-06 | 0.1066 | 0.1322 | 0.4199 |
| rs35372932 | T | C | 30 | -0.1738 | 0.0320 | 5.42543E-08 | -0.3213 | 0.1841 | 0.0810 |
| rs368652699 | A | T | 20 | -0.1853 | 0.0412 | 7.01223E-06 | 0.0381 | 0.1901 | 0.8412 |
| rs3793017 | G | A | 21 | 0.0758 | 0.0166 | 5.25759E-06 | -0.0374 | 0.0953 | 0.6946 |
| rs419788 | C | T | 66 | 0.1336 | 0.0165 | 5.27627E-16 | -0.0087 | 0.0929 | 0.9254 |
| rs4608857 | A | G | 25 | -0.1950 | 0.0389 | 5.30759E-07 | -0.0144 | 0.1942 | 0.9408 |
| rs507778 | T | C | 92 | -0.1586 | 0.0165 | 6.69324E-22 | 0.0478 | 0.0959 | 0.6179 |
| rs56227443 | T | A | 21 | 0.1523 | 0.0333 | 4.7977E-06 | 0.0835 | 0.2925 | 0.7753 |
| rs61782439 | T | C | 20 | 0.1616 | 0.0361 | 7.43694E-06 | -0.0103 | 0.1595 | 0.9484 |
| rs61914229 | A | G | 20 | 0.0905 | 0.0203 | 8.56017E-06 | -0.0231 | 0.1113 | 0.8353 |
| rs61984726 | G | A | 20 | 0.1980 | 0.0448 | 9.72488E-06 | -0.1176 | 0.2254 | 0.6018 |
| rs62079161 | T | C | 20 | -0.1593 | 0.0356 | 7.883E-06 | -0.0660 | 0.2636 | 0.8024 |

| SNPs | EA | OA | F | EBV ZEBRA antibody levels | | | DIO | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | β | SE | p value | β | SE | p value |
| rs62131031 | A | G | 20 | -0.0986 | 0.0218 | 6.16803E-06 | -0.0672 | 0.0942 | 0.4755 |
| rs6457714 | A | T | 36 | -0.1146 | 0.0192 | 2.30008E-09 | -0.0416 | 0.0942 | 0.6588 |
| rs6467 | A | C | 21 | 0.0757 | 0.0164 | 4.11625E-06 | 0.1509 | 0.0845 | 0.0743 |
| rs6904786 | A | C | 36 | 0.1521 | 0.0252 | 1.52135E-09 | -0.0350 | 0.1800 | 0.8458 |
| rs6917363 | A | G | 29 | -0.0838 | 0.0157 | 9.21208E-08 | -0.0105 | 0.0848 | 0.9010 |
| rs72764967 | T | C | 21 | 0.4372 | 0.0953 | 4.49127E-06 | 0.2264 | 1.1557 | 0.8447 |
| rs73403149 | G | A | 24 | 0.1752 | 0.0360 | 1.10362E-06 | -0.1907 | 0.4543 | 0.6746 |
| rs7383287 | G | A | 58 | -0.1433 | 0.0188 | 2.2714E-14 | 0.0196 | 0.1087 | 0.8567 |
| rs75156687 | T | C | 20 | -0.1570 | 0.0349 | 6.78062E-06 | -0.2988 | 0.1680 | 0.0754 |
| rs75571438 | G | C | 20 | 0.1631 | 0.0365 | 7.84161E-06 | 0.2882 | 0.1637 | 0.0783 |
| rs7575 | A | G | 21 | -0.1094 | 0.0238 | 4.17553E-06 | 0.3531 | 0.1800 | 0.0498 |
| rs758778 | C | T | 48 | -0.1265 | 0.0182 | 3.47841E-12 | -0.0357 | 0.1162 | 0.7589 |
| rs7750106 | A | C | 21 | -0.1671 | 0.0365 | 4.60036E-06 | 0.1973 | 0.2038 | 0.3331 |
| rs78793798 | G | A | 22 | 0.1891 | 0.0406 | 3.28592E-06 | -0.0837 | 0.5550 | 0.8802 |
| rs858990 | G | A | 20 | 0.0961 | 0.0218 | 9.98265E-06 | 0.1377 | 0.1320 | 0.2966 |
| rs869836 | G | A | 21 | 0.0740 | 0.0162 | 4.94144E-06 | -0.0138 | 0.0892 | 0.8769 |
| rs9276710 | C | T | 67 | -0.1564 | 0.0191 | 2.62533E-16 | 0.0850 | 0.1027 | 0.4081 |
| rs9276873 | G | C | 31 | -0.0911 | 0.0162 | 2.03609E-08 | -0.0426 | 0.0901 | 0.6368 |
| rs9391892 | A | G | 24 | 0.1932 | 0.0392 | 7.99557E-07 | 0.4748 | 0.2576 | 0.0653 |
| rs9393926 | A | G | 23 | 0.1136 | 0.0236 | 1.51961E-06 | 0.0990 | 0.1898 | 0.6019 |
| rs9505086 | C | T | 21 | -0.0723 | 0.0159 | 5.47732E-06 | 0.0129 | 0.0841 | 0.8778 |
| rs9790601 | G | A | 24 | -0.0813 | 0.0165 | 8.32681E-07 | -0.0178 | 0.0886 | 0.8407 |

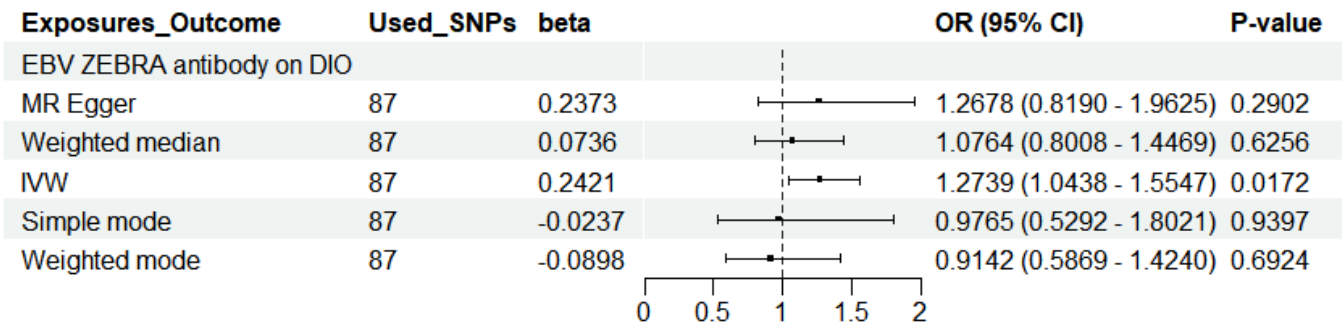


**Figure 2 | Causal Relationship between EBV ZEBRA Antibody Levels and DIO**

testing and palindromic SNP exclusion to prevent strand ambiguity. Results were reported as odds ratios (*OR*s) with 95% confidence intervals (*CI*s), visualized through forest plots and scatter diagrams. Statistical significance threshold was set at $P < 0.05$.

## RESULTS

### Causal Relationship Between EBV ZEBRA Antibody Levels and DIO

Instrumental Variables: Based on the criteria outlined in section "1.3 Instrumental Variable Selection" of this study, a total of 87 SNPs were selected as instrumental variables for subsequent MR analysis (**Table 1**).

MR Analysis and Sensitivity Analysis: Heterogeneity was assessed using the IVW method, with the Cochran's *Q* test yielding a *Q* value of 86 and a *P* value of 0.8060. Therefore, a random-effects model was chosen, indicating that the selected SNPs did not exhibit significant heterogeneity. Furthermore, the funnel plot further supported the conclusion of no heterogeneity (**Figure 5**). The IVW analysis resulted in an *OR* of 1.2739, 95% *CI* (1.0438 ~ 1.5547), $P = 0.0172$ (**Figure 2**). The scatter plot and forest plot also confirmed these results (**Figure 3** and **Figure 4**). The intercept *P*-value of the MR-Egger regression analysis was 0.9806, indicating no horizontal pleiotropy. Sensitivity analysis conducted using the leave-one-out method showed that individual SNPs did not significantly influence the outcome (**Figure 6**). In conclusion, there is a causal effect between EBV ZEBRA antibody levels and DIO. Furthermore, when DIO was considered as the exposure and EBV ZEBRA antibody levels as the outcome, no reverse causal relationship was found.

### Causal Relationship Between HHV-7 U14 Antibodies and DIO

Based on the criteria outlined in section "1.3 Instrumental Variable Selection" of this study, a total of 28 SNPs were selected as instrumental variables for subsequent MR analysis (**Table 2**).

To assess heterogeneity, MR analysis was conducted using the IVW method, and the Cochran's *Q* test yielded a *Q* value

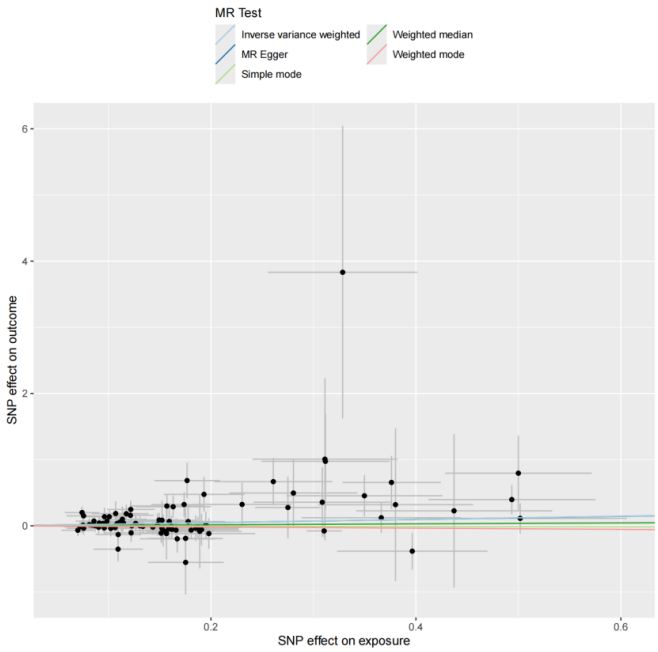


**Figure 3 | Scatter Plot of MR Results**

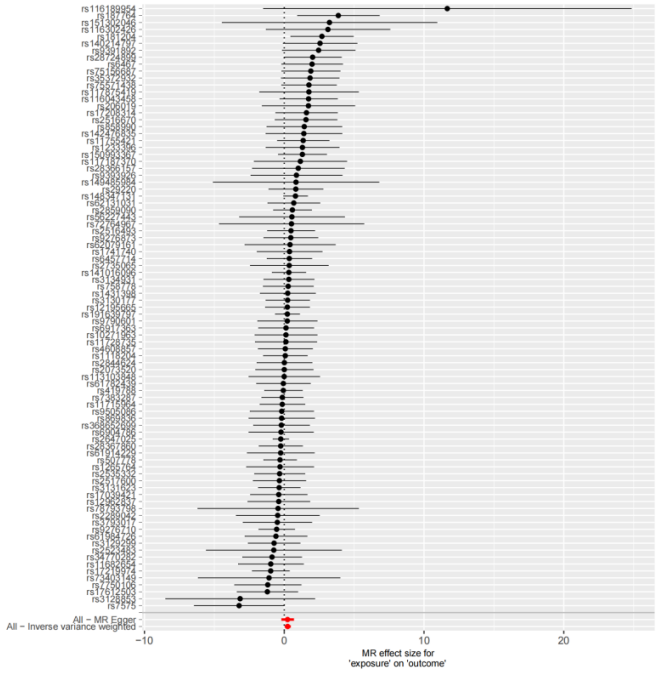


**Figure 4 | Forest Plot of MR Results**

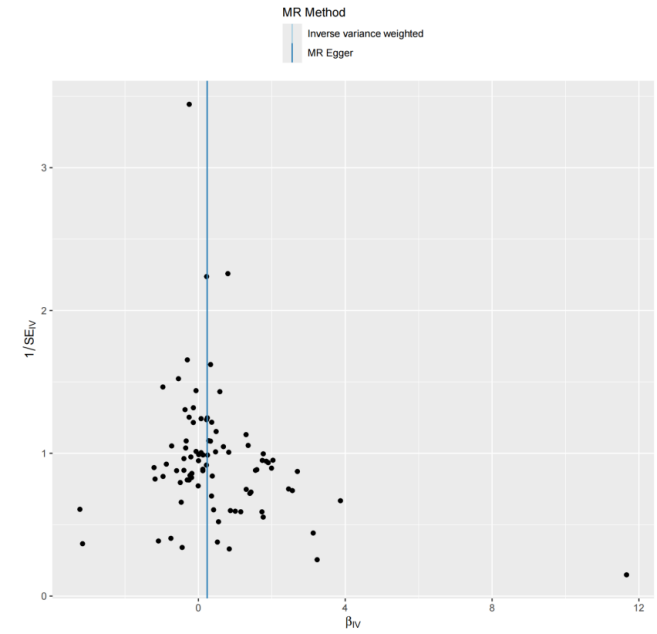


**Figure 5 | Funnel Plot of MR Results**

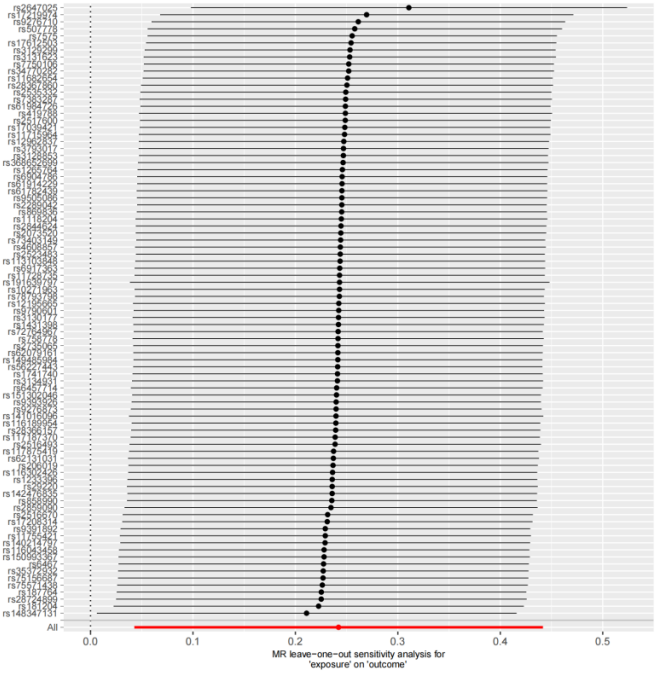


**Figure 6 | MR Results "Leave-One-Out" Analysis**

of 27 and a *P* value of 0.2089, supporting the selection of a random-effects model, indicating no significant heterogeneity among the included SNPs. Furthermore, funnel plot analysis further confirmed the conclusion of no significant heterogeneity (**Figure 10**). The IVW analysis resulted in an OR of 1.7269, 95% CI (1.1175 ~ 2.6684), and a *P* value of 0.0139 (**Figure 7**). The scatter plot and forest plot further validated this conclusion (**Figure 8** and **Figure 9**). Additionally, the intercept *P*-value of the MR-Egger regression was 0.7381, indicating no horizontal pleiotropy. Sensitivity analysis using the leave-one-out method did not reveal any individual SNP significantly influencing the outcome (**Figure 11**). In summary, there is a causal effect between HHV-7 U14 antibody levels and DIO. Furthermore, when DIO was considered as the exposure and HHV-7 U14 antibody levels as the outcome, no reverse causal relationship was found.

**Table 2 | SNPs related to HHV-7 U14 antibodies and their associations with DIO**

| SNPs | EA | OA | F | HHV-7 U14 antibody levels | | | DIO | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | β | SE | p value | β | SE | p value |
| rs1053752 | A | G | 27 | 0.0791 | 0.0152 | 1.8539E-07 | 0.0807 | 0.0861 | 0.3487 |
| rs12440425 | C | T | 21 | 0.1763 | 0.0382 | 3.97035E-06 | -0.0532 | 0.2178 | 0.8069 |
| rs12520297 | C | T | 21 | 0.1664 | 0.0364 | 4.89936E-06 | 0.3384 | 0.1611 | 0.0356 |
| rs139299944 | C | C | 48 | -0.1119 | 0.0161 | 3.94764E-12 | -0.0528 | 0.0838 | 0.5284 |
| rs139382385 | C | C | 20 | 0.1814 | 0.0409 | 9.01817E-06 | -0.1066 | 0.1753 | 0.5433 |
| rs141657353 | G | A | 20 | 0.3194 | 0.0718 | 8.69267E-06 | 0.4045 | 0.3785 | 0.2852 |
| rs17612590 | A | G | 35 | 0.0950 | 0.0161 | 3.52182E-09 | -0.0334 | 0.0835 | 0.6891 |
| rs180067 | A | G | 24 | -0.1959 | 0.0397 | 8.14021E-07 | 0.2199 | 0.2357 | 0.3509 |
| rs1808192 | G | A | 33 | 0.0934 | 0.0164 | 1.14716E-08 | -0.0522 | 0.0851 | 0.5396 |
| rs26515 | T | C | 20 | 0.0703 | 0.0155 | 6.00012E-06 | 0.0894 | 0.0836 | 0.2846 |
| rs2857149 | G | A | 31 | -0.0922 | 0.0167 | 3.18126E-08 | -0.1448 | 0.0850 | 0.0885 |
| rs2900649 | A | G | 23 | -0.1642 | 0.0342 | 1.60444E-06 | -0.1011 | 0.1563 | 0.5178 |
| rs3130192 | T | C | 22 | -0.1252 | 0.0268 | 3.03625E-06 | -0.1362 | 0.1062 | 0.1995 |
| rs33998906 | T | C | 22 | 0.1453 | 0.0310 | 2.8537E-06 | 0.1173 | 0.2297 | 0.6095 |
| rs361725 | C | T | 23 | 0.0759 | 0.0157 | 1.39093E-06 | -0.0882 | 0.0891 | 0.3221 |
| rs4648052 | T | G | 21 | -0.0727 | 0.0157 | 3.57369E-06 | -0.0739 | 0.0857 | 0.3885 |
| rs505533 | C | A | 28 | -0.1017 | 0.0191 | 9.47144E-08 | 0.1104 | 0.0961 | 0.2509 |
| rs7185701 | G | A | 22 | -0.1067 | 0.0226 | 2.25899E-06 | 0.1573 | 0.1146 | 0.1702 |
| rs7347914 | G | C | 22 | 0.1439 | 0.0304 | 2.15981E-06 | 0.6808 | 0.2780 | 0.0143 |
| rs75438046 | A | G | 34 | -0.2759 | 0.0474 | 5.75016E-09 | 0.0293 | 0.3732 | 0.9374 |
| rs75944971 | T | C | 20 | -0.1646 | 0.0364 | 6.05415E-06 | -0.1771 | 0.2128 | 0.4052 |
| rs7773105 | G | A | 37 | -0.1157 | 0.0191 | 1.38075E-09 | -0.0647 | 0.0931 | 0.4867 |
| rs79984539 | G | A | 23 | -0.1622 | 0.0336 | 1.34424E-06 | -0.1266 | 0.2268 | 0.5768 |
| rs805282 | G | T | 26 | -0.0899 | 0.0176 | 3.29366E-07 | -0.2115 | 0.0842 | 0.0120 |
| rs810057 | C | T | 20 | -0.0719 | 0.0162 | 8.87112E-06 | -0.0217 | 0.0838 | 0.7952 |
| rs9313148 | C | G | 20 | -0.0965 | 0.0218 | 9.60263E-06 | -0.2585 | 0.1322 | 0.0506 |
| rs9826169 | T | C | 21 | -0.0930 | 0.0203 | 4.72228E-06 | -0.0084 | 0.1319 | 0.9492 |
| rs9848627 | C | T | 20 | -0.0712 | 0.0161 | 9.29606E-06 | -0.1365 | 0.0897 | 0.1284 |

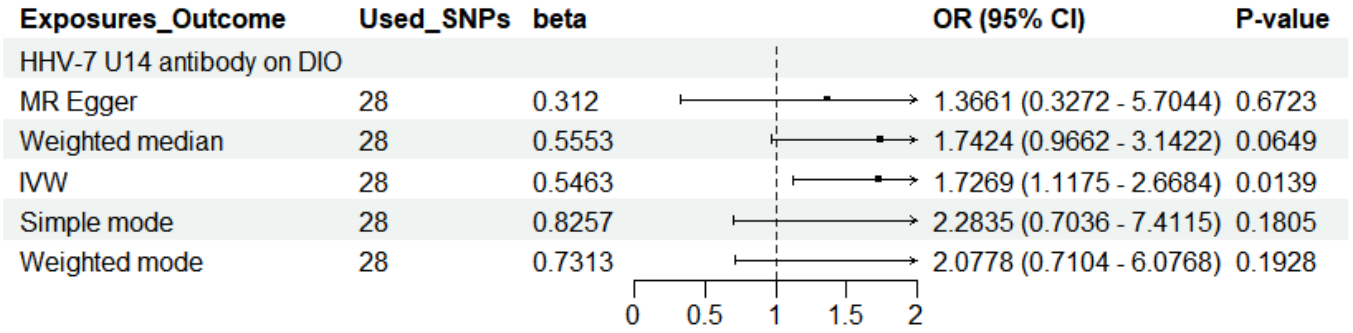


**Figure 7 | Causal Relationship between HHV-7 U14 Antibody Levels and DIO**

## Causal Relationship Between HSV-1 IgG Seropositivity and DIO

Based on the criteria outlined in section "1.3 Instrumental Variable Selection" of this study, a total of 17 SNPs were selected as instrumental variables for subsequent MR analysis (**Table 3**).

To assess heterogeneity, an MR analysis was conducted using the IVW method, and the Cochran's *Q* test yielded a *Q* value of 16 and a *P* value of 0.6401, supporting the selection of a random-effects model, indicating no significant heterogeneity among the included SNPs. Furthermore, funnel plot analysis further confirmed the conclusion of no significant heterogeneity (**Figure 15**). The IVW an

alysis resulted in an *OR* of 1.3312, 95% *CI* (1.0332 ~ 1.7153), and a *P* value of 0.0270 (**Figure 12**). The scatter plot and forest plot further validated this conclusion (**Figure 13** and **Figure 14**). Additionally, the intercept *P*-value of the MR-Egger regression was 0.3523, indicating no horizontal pleiotropy. Sensitivity analysis using the leave-one-out method did not reveal any individual SNP significantly influencing the outcome (**Figure 16**). In summary, there is a causal effect between HSV-1 IgG significantly and DIO. Furthermore, when DIO was considered as the exposure and HSV-1 IgG significantly as the outcome, no reverse causal relationship was found.

## Causal Relationship Between HSV-2 IgG Seropositivity and DIO

Based on the criteria outlined in section "1.3 Instrumental Variable Selection" of this study, a total of 16 SNPs were selected as instrumental variables for subsequent MR analysis (**Table 4**).

To assess heterogeneity, an MR analysis was conducted using the IVW method, and the Cochran's *Q* test yielded a *Q* value of 15 and a *P* value of 0.5386, supporting the selection of a random-effects model, indicating no significant hetero-

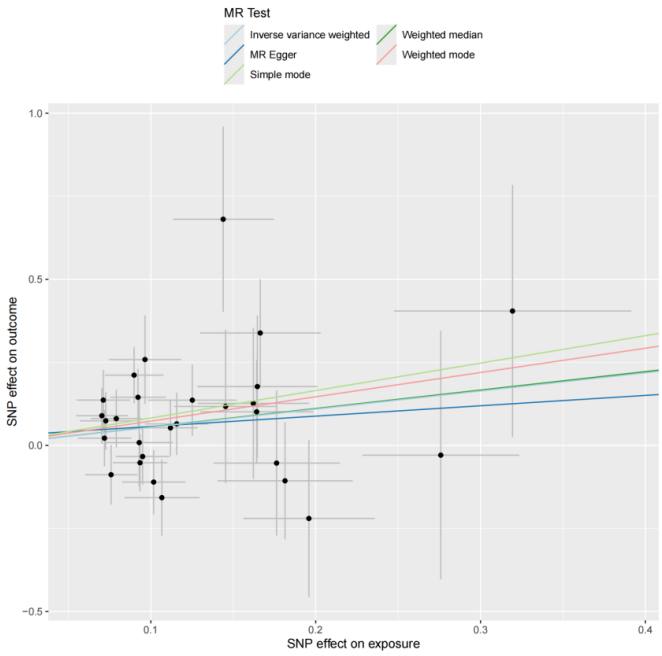


**Figure 8 | Scatter Plot of MR Results**

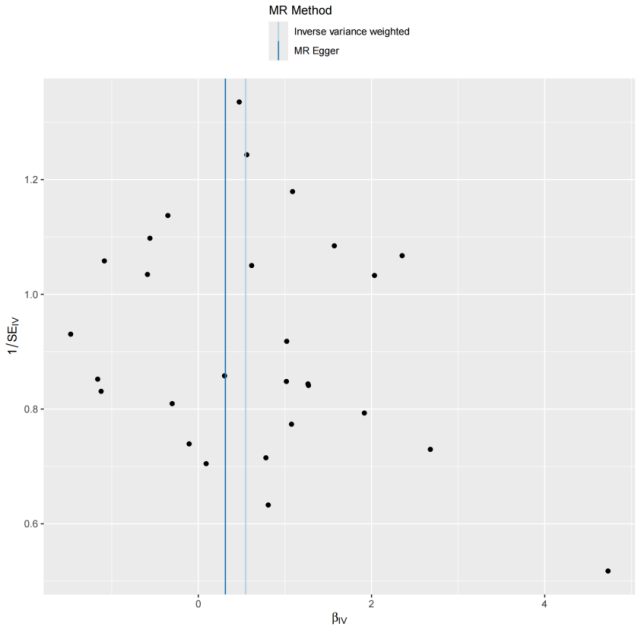


**Figure 10 | Funnel Plot of MR Results**

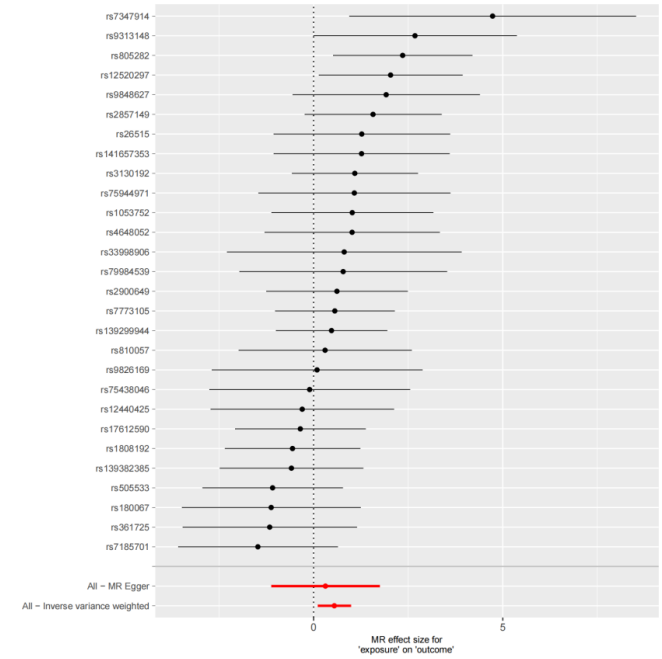


**Figure 9 | Forest Plot of MR Result**

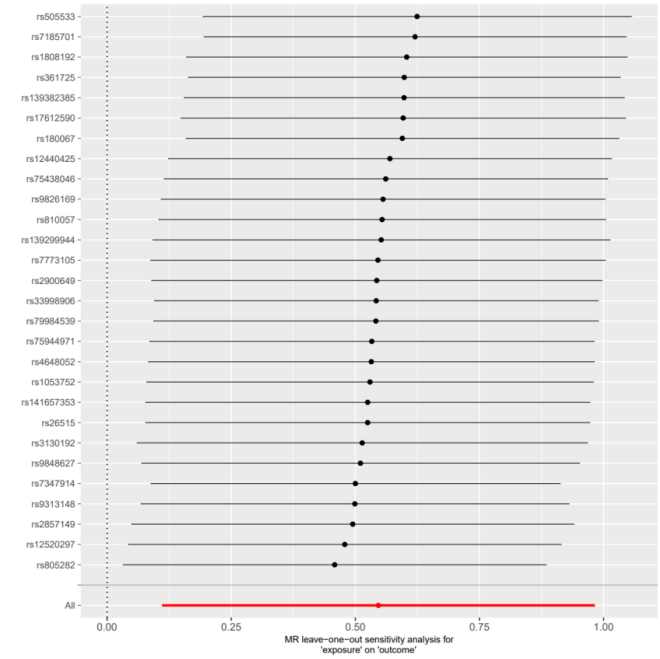


**Figure 11 | MR Results "Leave-One-Out" Analysis**

geneity among the included SNPs. Furthermore, funnel plot analysis further confirmed the conclusion of no significant heterogeneity (**Figure 20**). The IVW analysis resulted in an *OR* of 1.7269, 95% *CI* (1.1175 ~ 2.6684), and a *P* value of 0.0139 (**Figure 17**). The scatter plot and forest plot further validated this conclusion (**Figure 18** and **Figure 19**). Additionally, the intercept *P*-value of the MR-Egger regression was 0.4080, indicating no horizontal pleiotropy. Sensitivity analysis using the leave-one-out method did not reveal any individual SNP significantly influencing the outcome (**Figure 21**). In summary, there is a causal effect between HSV-2 IgG seropositivity and DIO. Furthermore, when DIO was considered as the exposure and HSV-2 IgG seropositivity as the outcome, no reverse causal relationship was found.

## DISSCUSION

This study explores the causal relationship between HHVs antibody immune response and DIO. The research findings indicate a significant positive correlation between EBV ZE-

**Table 3 | SNPs related to HSV-1 IgG seropositivity and their associations with DIO**

| SNPs | EA | OA | F | HSV-1 IgG seropositivity | | | DIO | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | β | SE | p value | β | SE | p value |
| rs10283891 | C | T | 23 | 0.1938 | 0.0406 | 1.83483E-06 | 0.0717 | 0.1035 | 0.4885 |
| rs10898480 | G | A | 20 | -0.3058 | 0.0690 | 9.32289E-06 | -0.0693 | 0.2123 | 0.7442 |
| rs111494201 | A | G | 20 | -0.5352 | 0.1210 | 9.64078E-06 | -0.3082 | 0.3777 | 0.4146 |
| rs1117513 | G | T | 20 | -0.2586 | 0.0572 | 6.24857E-06 | -0.3213 | 0.1494 | 0.0315 |
| rs143000632 | T | T | 20 | -0.4225 | 0.0933 | 6.00378E-06 | -0.2451 | 0.2235 | 0.2728 |
| rs148961785 | A | A | 24 | -0.7739 | 0.1569 | 8.0944E-07 | -0.3869 | 0.4375 | 0.3765 |
| rs199741519 | T | T | 27 | -0.6142 | 0.1193 | 2.61146E-07 | -0.2988 | 0.3279 | 0.3621 |
| rs28677033 | T | G | 20 | 0.1725 | 0.0386 | 7.64934E-06 | 0.2279 | 0.0881 | 0.0096 |
| rs34811474 | A | G | 23 | -0.1890 | 0.0393 | 1.52578E-06 | -0.0687 | 0.1001 | 0.4924 |
| rs4792444 | A | G | 21 | 0.2442 | 0.0535 | 5.08676E-06 | -0.0053 | 0.1862 | 0.9771 |
| rs4801449 | C | G | 24 | -0.2129 | 0.0437 | 1.12621E-06 | 0.1559 | 0.1247 | 0.2114 |
| rs57069894 | T | C | 20 | -0.4089 | 0.0903 | 5.97173E-06 | -0.0506 | 0.1737 | 0.7709 |
| rs61940663 | T | G | 25 | -0.5464 | 0.1083 | 4.55367E-07 | -0.1269 | 0.2644 | 0.6314 |
| rs72681141 | G | A | 23 | -0.3581 | 0.0747 | 1.61905E-06 | -0.0426 | 0.1689 | 0.8010 |
| rs73209430 | T | C | 20 | -0.3677 | 0.0820 | 7.31895E-06 | -0.0694 | 0.2152 | 0.7470 |
| rs78861723 | G | A | 23 | -0.3490 | 0.0728 | 1.63933E-06 | 0.0394 | 0.1473 | 0.7893 |
| rs9616098 | A | G | 23 | -0.5830 | 0.1217 | 1.66167E-06 | 0.1717 | 0.2874 | 0.5502 |

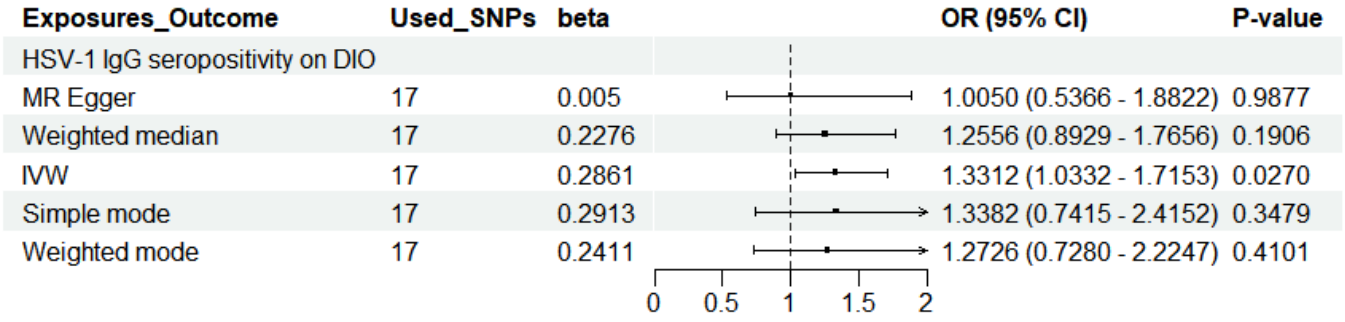


**Figure 12 | Causal Relationship between HSV-1 IgG significantly and DIO**

BRA antibody, HHV-7 U14 antibody, HSV-1 IgG positive, and HSV-2 IgG positive, and the elevated risk of DIO. This finding provides new evidence to some extent for a deeper understanding of the potential pathophysiological mechanisms of HHVs antibody immune response in DIO.

Members of the HHVs family, such as EBV, HHV-7, and HSV-1/2, may play a crucial role in the pathophysiological processes of various diseases because of their latency and sustained activation of the host immune system. The study suggests that these viruses not only directly lead to tissue damage and clinical diseases, but also impact the host's health status through indirect pathways such as immune regulation and metabolic reprogramming [12].

EBV's encoded ZEBRA protein regulates DNA methylation-dependent transcriptional activation [13] and mediates DNA damage signals, governing the virus' latent-lytic cycle switch and serving as a key molecular switch in EBV pathogenesis. In DIO, ZEBRA antibodies may induce host immune metabolic disorders [14] and EBERs-mediated innate immune signal dysregulation, exacerbating adipose tissue inflammation and metabolic abnormalities. Additionally, as a biomarker for EBV-related tumors such as nasopharyngeal carcinoma [15], the persistent presence of EBV antibodies may promote tumor microenvironment reshaping through immune editing. Therefore, the EBV ZEBRA antibody response may be involved in the pathogenesis of DIO through multiple mechanisms. However, there is limited direct research on the association between EBV ZEBRA antibodies and DIO domestically and internationally. This study confirms that EBV ZEBRA antibodies are one of the risk factors for DIO.

Furthermore, this study also found a positive correlation between HHV-7 U14 antibodies and DIO. HHV-7 is associated with various human diseases, including febrile syndromes, skin lesions, neurological defects, and transplant complications [16]. Despite being a com

mon human virus, the specific role of HHV-7 in the pathogenesis of DIO remains unclear. It is speculated to be related to the following mechanisms: (1) activation of the NF-$\kappa$B signaling pathway leading to the upregulation of pro-inflammatory cytokines (such as IL-6, TNF-α), inducing chronic inflammation in adipose tissue and interfering with lipid/glucose metabolism, directly driving obesity-related metabolic imbalance [6, 17]; (2) synergistic infection with HHV-6 exacerbating immune-metabolic dysregulation, forming a virus-host interaction network that promotes obesity [18-19]; (3) HHV-7-mediated abnormal activation of the HPA axis may disrupt energy homeostasis through neuroendocrine pathways, promoting increased appetite and fat accumulation [20].

Additionally, the association between HSV infection and obesity involves multiple pathological mechanisms: the seropositivity of HSV-1 is significantly higher in obese adults, and its infection can induce neuroinflammation and

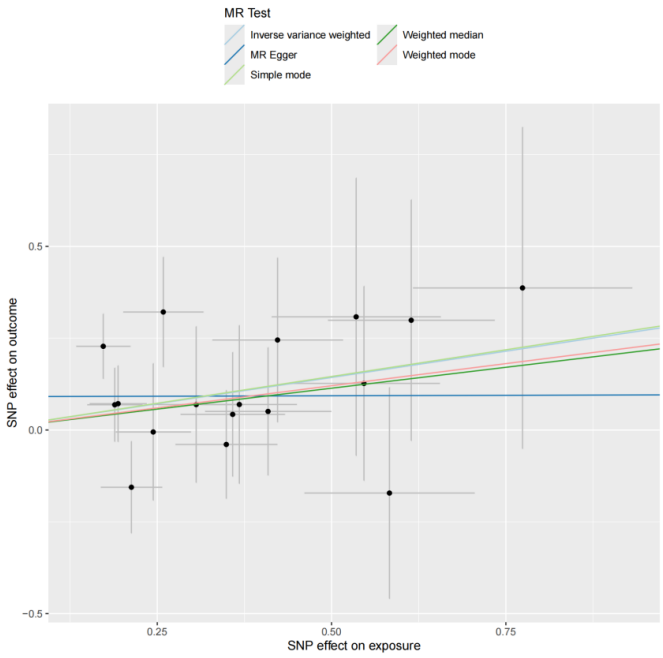


**Figure 13 | Scatter Plot of MR Results**

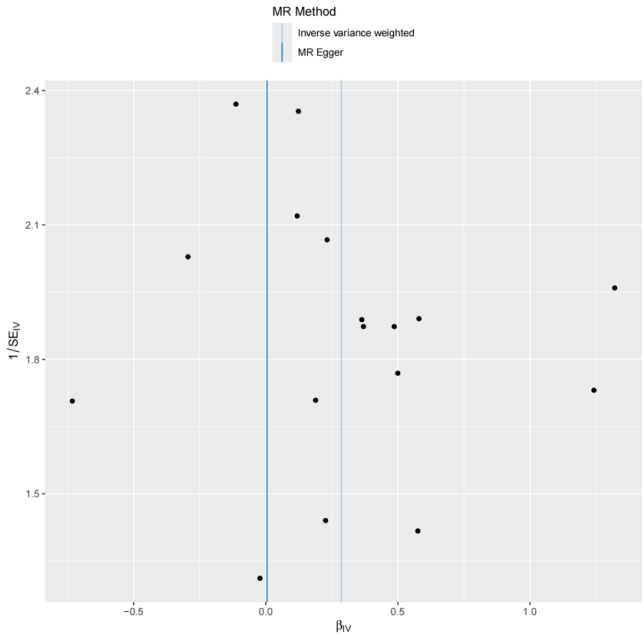


**Figure 15 | Funnel Plot of MR Results**

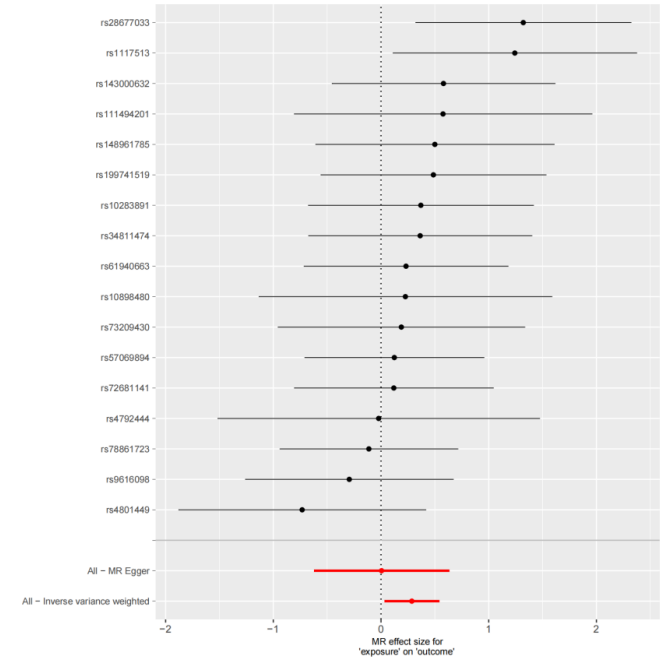


**Figure 14 | Forest Plot of MR Results**

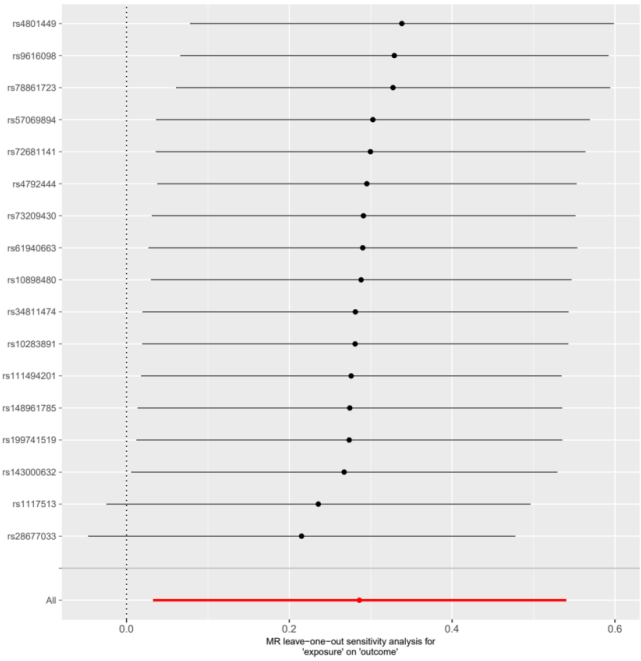


**Figure 16 | MR Results "Leave-One-Out" Analysis**

oxidative stress to promote the progression of Alzheimer's disease [21-23], as well as interfere with cholesterol metabolism leading to lipid accumulation, creating an obesogenic microenvironment [4, 21]; while HSV-2 infection exacerbates immune-metabolic dysregulation through ASC-dependent inflammasome activation [24] and autophagy abnormalities [25], contributing to a vicious cycle of obesity-related chronic low-grade inflammation. Research on the association of HSV-1 and HSV-2 IgG positivity with DIO is relatively limited. However, this study found a significant correlation between HSV-1 IgG and HSV-2 IgG positivity and the risk of DIO, pointing towards directions for further in-depth research.

This study, based on a two-sample MR design, utilized genetic data from GWAS public databases to select SNPs strongly associated with the exposure factors and meeting the assumptions of independence and exclusion restrictions as instrumental variables. This approach effectively controlled potential confounding biases related to environmental exposures and social behaviors, ensuring the robustness of causal effect estimates. The study employed the IVW method as the

**Table 4 | SNPs related to HSV-2 IgG seropositivity and their associations with DIO**

| SNPs | EA | OA | F | HSV-2 IgG seropositivity | | | DIO | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | β | SE | p value | β | SE | p value |
| rs113345377 | G | A | 21 | 0.9950 | 0.2185 | 5.27712E-06 | 0.4384 | 0.2124 | 0.3516 |
| rs114258283 | A | G | 23 | 0.8091 | 0.1674 | 1.34731E-06 | 0.0507 | 0.8898 | 0.3660 |
| rs11568050 | C | T | 20 | 0.6422 | 0.1439 | 8.05438E-06 | -0.1348 | 0.6248 | 0.2757 |
| rs118014610 | A | C | 20 | 0.7740 | 0.1728 | 7.45298E-06 | -1.0351 | 0.1616 | 0.7395 |
| rs12514634 | G | A | 21 | 0.1919 | 0.0420 | 4.89137E-06 | 0.2240 | 0.0052 | 0.0801 |
| rs12515138 | C | T | 22 | -0.3457 | 0.0745 | 3.45346E-06 | -0.0638 | 0.6791 | 0.1542 |
| rs148736746 | T | C | 21 | 1.1821 | 0.2599 | 5.43251E-06 | 0.3022 | 0.5398 | 0.4929 |
| rs201862459 | A | A | 20 | 0.4951 | 0.1101 | 6.86614E-06 | 0.1038 | 0.6740 | 0.2468 |
| rs4322800 | G | T | 20 | -0.2214 | 0.0500 | 9.46708E-06 | -0.1290 | 0.1974 | 0.1001 |
| rs4683063 | T | C | 20 | 0.2280 | 0.0509 | 7.54209E-06 | 0.0216 | 0.8248 | 0.0976 |
| rs56368930 | C | G | 21 | 1.1608 | 0.2520 | 4.10794E-06 | 0.5372 | 0.1660 | 0.3878 |
| rs62406531 | G | C | 21 | -0.2269 | 0.0495 | 4.58868E-06 | -0.0410 | 0.6921 | 0.1036 |
| rs72880063 | G | T | 20 | 0.3353 | 0.0752 | 8.33249E-06 | 0.1018 | 0.4637 | 0.1389 |
| rs7503464 | A | G | 35 | -0.2489 | 0.0422 | 3.75456E-09 | 0.0816 | 0.3320 | 0.0841 |
| rs7979797 | T | C | 22 | 0.2090 | 0.0449 | 3.33173E-06 | 0.1042 | 0.3026 | 0.1011 |
| rs816208 | A | G | 20 | -0.1968 | 0.0437 | 6.65925E-06 | -0.0971 | 0.2637 | 0.0869 |

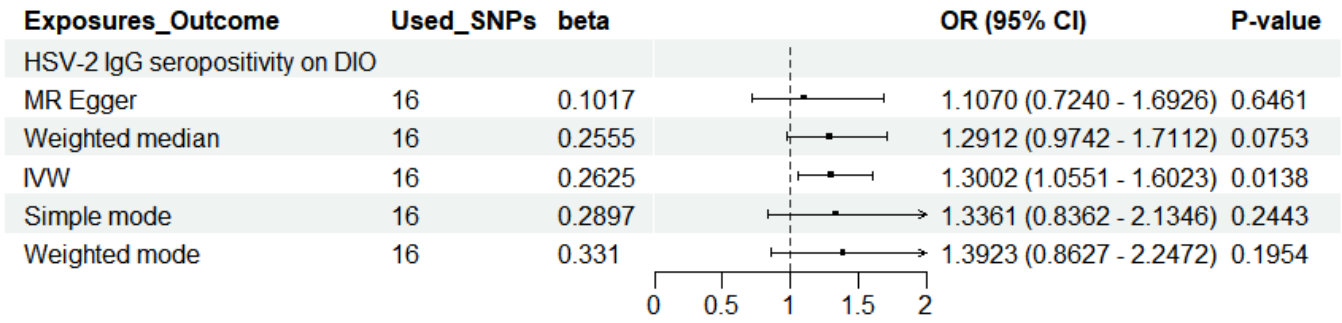


**Figure 17 | Causal Relationship between HSV-2 IgG seropositivity and DIO**

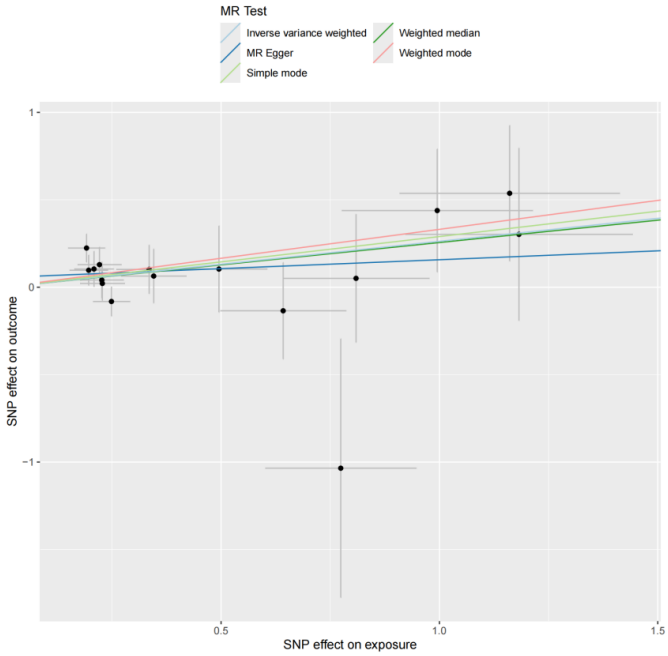


**Figure 18 | Scatter Plot of MR Results**

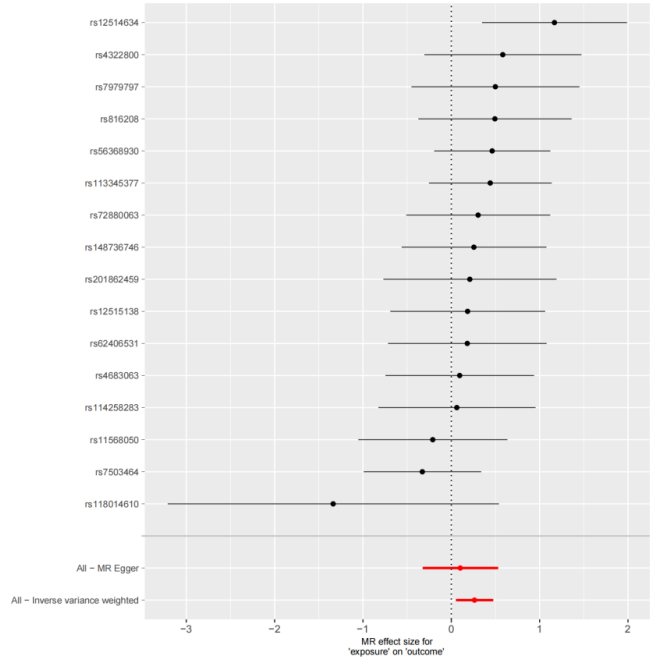


**Figure 19 | Forest Plot of MR Results**

main analytical approach, complemented by methods such as the weighted median method and MR-Egger regression for multidimensional sensitivity analysis, significantly enhancing the statistical power and biological plausibility of causal inference.

However, this study has certain limitations. Firstly, due to the limited coverage of genetic loci in existing GWAS data, the selection criteria for some exposure-related SNPs were relaxed to $P < 1\times10^{-5}$, potentially introducing weak instrument bias or horizontal pleiotropy effects. Secondly, the analysis data predominantly originated from individuals of

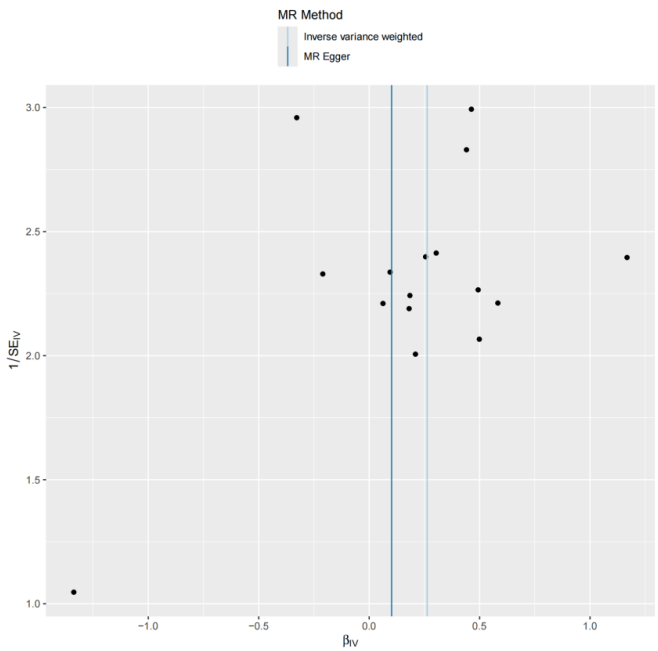


**Figure 20 | Funnel Plot of MR Results**

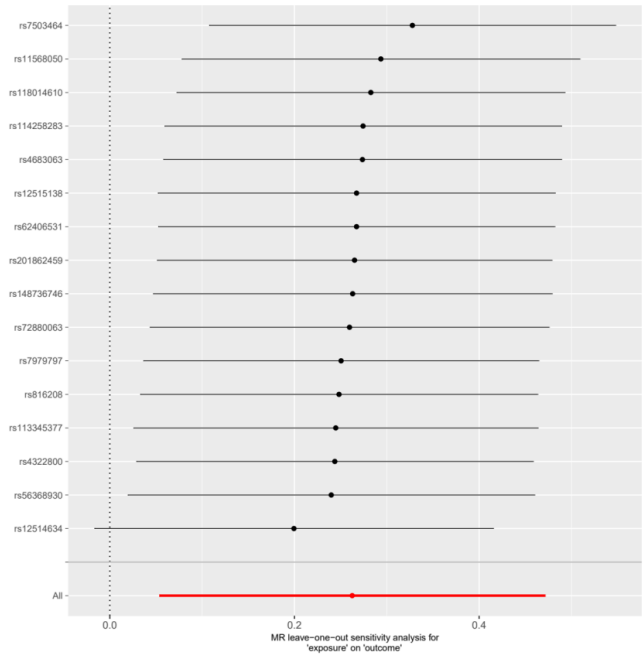


**Figure 21 | MR Results "Leave-One-Out" Analysis**

European descent, not encompassing genetic heterogeneity across different races or regions, thus requiring further validation for the generalizability of conclusions. Additionally, the study did not explore potential moderating effects of factors such as gender and age on causal associations, which may impact the depth of mechanistic analysis in precision medicine scenarios.

In conclusion, there is a positive causal relationship between HHVs-specific antibody immune responses and DIO, with EBV ZEBRA antibody, HHV-7 U14 antibody, HSV-1 IgG, and HSV-2 IgG seropositivity potentially serving as risk factors for DIO. This suggests that virus-mediated chronic immune activation may be involved in the pathogenesis of DIO.

**Funding** This work was supported by the Guangxi Natural Science Foundation (2025GXNSFHA069077), and the Guangxi Zhuang Autonomous Region Administration of Traditional Chinese Medicine Self-funded Research Project (GXZYC20240258; GXZYC20240401).

**References**

1. Verhaegen, A. A., & Van Gaal, L. F. Drug-induced obesity and its metabolic consequences: a review with a focus on mechanisms and possible therapeutic options. *J. Endocrinol. Invest*. **40**, 1165–1174 (2017).
2. Müller, T. D., Blüher, M., Tschöp, M. H., & DiMarchi, R. D. Anti-obesity drug discovery: advances and challenges. *Nat. Rev. Drug. Discov.* **21**, 201–223 (2022).
3. Krawczyk, A. *et al*. Overcoming drug-resistant herpes simplex virus (HSV) infection by a humanized antibody. *Proc. Natl. Acad. Sci. U. S. A*. **110**, 6760–6765 (2013).
4. Hasan, M. R. *et al*. Virome-wide serological profiling reveals association of herpesviruses with obesity. *Sci. Rep.* **11**, 2562 (2021).
5. Freuer, D. *et al*. Seropositivity of selected chronic infections and different measures of obesity. *PloS. One*. **15**, e0231974 (2020).
6. Bassols, J., Moreno, J. M., Ortega, F., Ricart, W., & Fernandez-Real, J. M. Characterization of herpes virus entry mediator as a factor linked to obesity. *Obesity. (Silver. Spring.)*. **18**, 239–246 (2010).
7. Aktar, S., Arii, J., Tjan, L. H., Nishimura, M., & Mori, Y. Human herpesvirus 6A tegument protein U14 induces NF-κB signaling by interacting with p65. *J. Virol.* **95**, e0126921 (2021).
8. Burgess, S. *et al*. Guidelines for performing Mendelian randomization investigations: update for summer 2023. *Wellcome. Open. Res.* **4**, 186 (2023).
9. Butler-Laporte, G., Kreuzer, D., Nakanishi, T., Harroud, A., Forgetta, V., & Richards, J. B. Genetic determinants of antibody-mediated immune responses to infectious diseases agents: a genome-wide and hla association study. *Open. Forum. Infect. Dis.* **7**, ofaa450 (2020).
10. Palmer, T. M. *et al*. Using multiple genetic variants as instrumental variables for modifiable risk factors. *Stat. Methods. Med. Res*. **21**, 223–242 (2012).
11. Lin, S. H., Brown, D. W., & Machiela, M. J. LDtrait: an online tool for identifying published phenotype associations in linkage disequilibrium. *Cancer. Res*. **80**, 3443–3446 (2020).
12. Zamora M. R. DNA viruses (CMV, EBV, and the herpesviruses). *Semin. Respir. Crit. Care. Med.* **32**, 454–470 (2011).
13. Bernaudat, F., *et al*. Structural basis of DNA methylation-dependent site selectivity of the Epstein-Barr virus lytic switch protein ZEBRA/Zta/BZLF1. *Nucleic. Acids. Res.* **50**, 490–511 (2022).
14. Zhang L. A common mechanism links Epstein-Barr virus infections and autoimmune diseases. *J. Med. Virol.* **95**, e28363 (2023).
15. Song, L. *et al*. Identification of anti-Epstein-Barr virus (EBV) antibody signature in EBV-associated gastric carcinoma. *Gastric. Cancer.* **24**, 858–867 (2021).
16. Verbeek, R., Vandekerckhove, L., & Van Cleemput, J. Update on human herpesvirus 7 pathogenesis and clinical aspects as a roadmap for future research. *J. Virol.* **98**, e0043724 (2024).
17. Zhang, Y. *et al*. Neuregulin4 acts on hypothalamic ErBb4 to excite oxytocin neurons and preserve metabolic homeostasis. *Adv. Sci. (Weinh)*. **10**, e2204824 (2023).
18. Cao, L. *et al*. Immune mechanisms in multiple sclerosis: CD3 levels on CD28+ CD4+ T cells link antibody responses to human herpesvirus 6. *Cytokine*. **187**, 156866 (2025).
19. Darvish Molla, Z., Kalbasi, S., Kalantari, S., Bidari Zerehpoosh, F., Shayestehpour, M., & Yazdani, S. Evaluation of the association between human herpes virus 6 (HHV-6) and Hashimoto's thyroiditis. *Iran. J. Microbiol.* **14**, 563–567 (2022).
20. Kobayashi, N. *et al*. Human herpesvirus 6B greatly increases risk of depression by activating hypothalamic-pituitary -adrenal axis during latent phase of infection. *iScience*. **23**, 101187 (2020).

21. Salgado, B. *et al*. Cholesterol modulation attenuates the AD-like phenotype induced by herpes simplex virus type 1 infection. *Biomolecules*. **14**, 603 (2024).
22. Piacentini, R., & Grassi, C. Interleukin 1β receptor and synaptic dysfunction in recurrent brain infection with Herpes simplex virus type-1. *Neural. Regen. Res.* **20**, 416–423 (2025).
23. Asakura, M. *et al*. Elevated cerebrospinal fluid IgG index in herpes simplex encephalitis post-HSV-1 clearance: A preliminary study. *J. Med. Virol.* **96**, e29850 (2024).
24. Hayes, C. K., Wilcox, D. R., Yang, Y., Coleman, G. K., Brown, M. A., & Longnecker, R. ASC-dependent inflammasomes contribute to immunopathology and mortality in herpes simplex encephalitis. *PLoS. Pathog.* 17(2), e1009285 (2021).
25. Hait, A. S. *et al*. Defects in LC3B2 and ATG4A underlie HSV2 meningitis and reveal a critical role for autophagy in antiviral defense in humans. *Sci. Immunol.* **5**, eabc2691 (2020).