Causal associations of BAFF-R on IgD+ CD24- immune cell trait with hepatocellular carcinoma and the mediating role of phenylacetylglutamate levels: a Mendelian randomization study

Xuan Zhu
Department of Medical Laboratory, Second Affiliated Hospital of Guangxi Medical University

Zongchao Qiu
Fujian Yuan Shizhuling Community Health Service center

Maochun Yang
Department of Medical Laboratory, Second Affiliated Hospital of Guangxi Medical University

Lingxi Kong
Department of Medical Laboratory, Second Affiliated Hospital of Guangxi Medical University

Limin Li
Department of Medical Laboratory, Second Affiliated Hospital of Guangxi Medical University

Yingting Huang
Department of Medical Laboratory, Second Affiliated Hospital of Guangxi Medical University

Li Xie (*drxieli@126.com*)
Department of Medical Laboratory, Second Affiliated Hospital of Guangxi Medical University

Article

**Keywords:** Mendelian randomization, immune cell, BAFF-R on IgD + CD24-, hepatocellular carcinoma, phenylacetylglutamate

**Posted Date:** February 15th, 2024

**DOI:** [https://doi.org/10.21203/rs.3.rs-3908572/v1](https://doi.org/10.21203/rs.3.rs-3908572/v1)

**License:** [This work is licensed under a Creative Commons Attribution 4.0 International License.](https://creativecommons.org/licenses/by/4.0/) Read Full License

**Additional Declarations:** No competing interests reported.
Abstract

We conducted a bi-directional two-sample Mendelian randomization analysis to investigate the causal associations between immune cell traits and HCC and identify the mediating factor of metabolites. The exposure factors were immune cell traits, the mediators were metabolites, and the outcome variable was HCC. Inverse-variance weighted method (IVW) was the main method. Weighted median, MR-Egger regression, weighted mode, simple mode, and MR pleiotropy residual sum and outlier (MR-PRESSO) methods were used as complementary methods. Subsequently, the potential mediating effect was investigated by conducting a two-step Mediation analysis. We found 7 traits with positive correlations and 19 traits with negative correlations between immune cell traits and HCC. There were no causal correlations between HCC and immune cell traits in the reverse MR analysis. In the mediation analysis, we found a positive causal association between B cell-activating factor receptors (BAFF-R) on IgD+ CD24- and HCC [IVW: odd ratio (OR), 0.845; 95% CI, 0.759-0.942; p = 0.002]. Phenylacetylglutamate (PAG) levels mediated 7.353% of the causal pathway from BAFF-R on IgD+ CD24- and HCC. In conclusion, BAFF-R on IgD+ CD24- lowers risk of HCC, with PAG levels playing a mediating role.

Introduction

Hepatocellular carcinoma (HCC) accounts for more than 90% of primary liver cancers, with a male-to-female ratio of 2.8:1. It ranks sixth among the common cancers but is the fourth leading cause of cancer-related death globally due to its poor prognosis and high recurrence rate. It is predicted that by 2025, new cases of HCC will exceed 1 million per year. The main risk factors of HCC include hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, non-alcoholic fatty liver diseases (NAFLD), chronic alcohol consumption, and dietary toxins, such as aflatoxins and aristolochic acid. Most HCCs develop in the presence of chronic liver inflammation, liver injury, and chronic fibrosis. A number of studies have shown that chronic liver inflammation plays a key role in the development of HCC.

The tumor microenvironment (TME) of HCC is complex and composed of stromal cells, endothelial cells, hepatic stellate cells, and several immune cells. HCC develops in livers with chronic inflammation, where immune cells undergo functional reprogramming, leading to impaire responses and forming a cancer-prone microenvironment. It has been shown that the compositions and distribution of immune cells are significantly different between patients with HCC and healthy controls. Whether tumor-infiltrating lymphocytes (TILs) play a protumor or antitumor role in HCC depends on the TILs subtypes. Many studies have revealed that higher abundances of CD8+ TILs increase the survival rate and lower the recurrence rate in HCC; however, opposite results have also been reported. Similar inconsistency has been reported regarding B cells. Frances et al. observed that B cells protect against the development of HCC. A mouse model of diethylnitrosamine-induced liver cancer demonstrated that B cells play a critical antitumor role. In contrast, Yan et al. found that the CD19+ CD24hiCD38hi phenotype, a specific subset of regulatory B cells, is abundant in the microenvironment of various cancers and involved in HCC progression. These findings indicate that HCC is an immunogenic tumor, which provides a rationale for immunotherapy.

Metabolic reactions usually produce abundant small intermediary molecules or end products. These molecules named metabolites regulate tumor growth, proliferation, invasion, metastasis, and immune evasion. Altered metabolic pathways, including metabolites that act as oncogenes or tumor suppressors, are involved in the pathogenesis of HCC. Complex metabolic-immune cross-talk is a characteristic feature of HCC. Therefore, the relationship between metabolites and the function of immune cells in the TME is inseparable. The FDA approved the application of small-molecule metabolites for treating specific tumors, making targeted therapy for metabolites a promising approach for treating HCC. However, the causalities between immune cells and HCC, and whether metabolites play a mediating role are difficult to confirm due to the presence of potential confounders and measurement errors. Furthermore, considering ethical concerns, it is not feasible to measure causal relationships in experimental settings.

Mendelian randomization (MR) analysis is similar to randomized controlled trials, using single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to infer causal relationships between exposure and outcomes. Alleles are randomly separated during meiosis without interference from external factors, and genetic variation occurs before the disease. MR can reduce the bias caused by confounding factors and avoid the interference of reverse causality. This method is also widely used in various clinical causal inferences. However, there is no MR analysis focusing on the association of immune cells, metabolites, and HCC. This study conducted a two-sample MR (TSMR) study to identify whether there is a causal relationship between immune cells and HCC or between metabolites and HCC. We utilized mediation analysis to explore the potential mediating effect of metabolites on the causal associations between immune cells and HCC. Our study can provide a new perspective for exploring the mechanism underlying the development of HCC and provide novel targets for metabolic therapy.

Results

Selection of IVs

All features of SNPs with significant correlations between BAFF-R on IgD+ CD24-, PAG levels, and HCC are shown in Supplementary Tables (see Supplementary Table S3-S6 online). SNPs with LD and ambiguity were eliminated, and MR-PRESSO analysis was used to remove the outlier SNPs. Finally, independent SNPs without proxies were extracted. In total, we found 22 significant SNPs in BAFF-R on IgD+ CD24- immune cell trait as exposure IVs with an F-statistic of 141.123 (see Supplementary Table S3 online). We considered 59 SNPs in HCC as exposure IVs with an F-statistic of...
20.702 (see Supplementary Table S4 online). In total, 23 significant SNPs in PAG levels were considered as exposure IVs with an F-statistic of 24.497 (see Supplementary Table S6 online).

**Associations of immune cell traits with HCC**

The causal effects of immune cell traits on HCC risk were estimated (see Supplementary Table S1 online). There were 26 immunophenotypes that exhibited significant relationship with HCC (P < 0.05), of which 14 were in B panel, 4 in TBNK panel, 2 in the maturation stages of T cell panel, 3 in Treg panel, 2 in cDC panel, and 1 in myeloid cell panel. Several sensitivity analyses were conducted to assess the pleiotropy and heterogeneity in causality estimates (see Supplementary Table S1 online). The results of Cochran's Q-test showed no evidence of heterogeneity in the causal associations among these SNPs. The potential pleiotropy was also not detected by the MR-Egger intercept and MR-PRESSO global test. There were not any causal associations in the reverse TSMR analysis between HCC and immune cell traits (see Supplementary Table S2 online).

Firstly, BAFF-R on IgD- CD24- was identified as the most significant immune cell trait. Then, a series of significant metabolites related to BAFF-R on IgD- CD24- were identified. However, there were no positive associations between these significant metabolites and HCC risk. Then, the immune cell trait of BAFF-R on IgD + CD24- was selected for subsequent analyses.

**Causal effects of BAFF-R on IgD + CD24- on HCC**

Table 1 shows the associations between BAFF-R on IgD + CD24- and HCC measured by five methods. All five MR methods consistently supported the significant causal relationship between higher BAFF-R on IgD + CD24- and lower HCC risk (IVW: odd ratio (OR), 0.845; 95% CI, 0.758–0.942; P = 0.002; MR Egger: OR, 0.818; 95% CI, 0.71–0.942; P = 0.012; Weighted median: OR, 0.832; 95% CI, 0.734–0.944; P = 0.004; Weighted mode: OR, 0.842; 95% CI, 0.74–0.958; P = 0.017).

Forest plot and scatter chart in Fig. 3A1 and Fig. 3A2 visualize the causal effects of BAFF-R on IgD + CD24- on HCC risk, respectively. Funnel plots showed no evidence of asymmetry (Fig. 3A3). Leave-one-out analysis indicated that the error line remained relatively stable, suggesting the reliability of our findings. (Fig. 3A4).

Notably, the reverse TSMR analysis showed no causal associations between HCC and BAFF-R on IgD + CD24-.

**Causal effects of BAFF-R on IgD + CD24- on PAG levels**

Table 1 shows the associations between BAFF-R on IgD + CD24- and PAG levels. We found the causal relationship between higher BAFF-R on IgD + CD24- and higher PAG levels (IVW: OR, 1.03; 95% CI, 1.002–1.059; P = 0.037; MR Egger: OR, 1.053; 95% CI, 1.015–1.093; P = 0.012; Weighted median: OR, 1.041; 95% CI, 1.001–1.082; P = 0.044; Weighted mode: OR, 1.041; 95% CI, 1.003–1.08; P = 0.047).

The forest plot and scatter chart in Fig. 3B1 and Fig. 3B2 visualize the causal effects of BAFF-R on IgD + CD24- on HCC risk, respectively. Funnel plots showed no evidence of asymmetry (Fig. 3B3). Leave-one-out analysis showed that the correlation of BAFF-R on IgD + CD24- with PAG levels was not driven by any single SNP (Fig. 3B4).

**Causal effects of PAG levels on HCC**

Table 1 shows the associations between PAG levels on HCC risk. The IVW method identified the causal association between higher PAG levels and lower HCC risk: OR, 0.66; 95% CI, 0.462–0.943; P = 0.022.

The forest plot and scatter chart in Fig. 3C1 and Fig. 3C2 visualize the causal effects of PAG levels on HCC risk, respectively. The Funnel plots showed no evidence of asymmetry (Fig. 3C3). Leave-one-out analysis showed that the correlation of PAG levels with HCC risk was not driven by any single SNP (Fig. 3C4).

**Mediating effect of PAG levels in the relationship between BAFF-R on IgD + CD24- and HCC**

We analyzed the role of PAG levels as a mediator in the pathway from B cell-activating factor receptors (BAFF-R) on IgD + CD24- to HCC (Fig. 2). The results indicated that BAFF-R on D + CD24- was associated with reduced PAG levels, which in turn correlated with a decreased risk of HCC. Figure 4 illustrates that PAG levels contributed to 7.353% of the mediating effect of BAFF-R on IgD + CD24- in reducing the risk of HCC (P value = 0.87, se = 0.076, and Z = -0.163).

**Discussion**

In our study, we presented a characteristic profile of immune cells in HCC. In total, 731 immune cell traits were examined as evidence of HCC risk, with 26 immunophenotypes identified as having potential causal effects on the risk of HCC among 6 panels (TBNK, maturation stages of T-cell, Treg, cDC, myeloid cell, and B cell) and 3 traits (MFI, RC, and AC). The diverse subsets and functions of immune cells in the TME of HCC having roles.
Furthermore, mediation MR analysis also revealed the mediating role of PAG levels in the causal association between BAFF-R on IgD + CD24- and HCC.

Based on the results of MR analysis, BAFF-R on IgD + CD24- B cell protected against HCC. IgD is an immunoglobulin on the surface of mature B cells. BAFF-R on IgD + CD24- cell represented the expression of BAFF-R on mature B cells. It has been reported that compared with non-tumor liver tissues, the abundance of all B cell subsets was decreased in the TME, highlighting the anti-tumor role of B cells in human HCC. BAFF-R, a crucial member of the TNF-receptor superfamily, promotes the maturation, proliferation, and survival of B cells. By binding to BAFF-R, BAFF activates the classical and noncanonical NF-kB signaling pathways, leading to the expression of essential downstream genes necessary for B cell survival. Increased BAFF levels were found in various pathological conditions, including autoimmune diseases, B-cell malignancies, and primary antibody deficiencies (PAD). BAFF has a beneficial effect on autoimmune and malignant cells. The steady-state concentrations of BAFF are mainly represented by the quantity of B cells and the expression of BAFF-binding receptors. The expression of BAFF-R was reduced in B cells of patients with HBV-related HCC. Progressive HCC, large tumor size, and advanced cancer stage may significantly elevate plasma BAFF concentrations and lead to resistance to the biological effects of BAFF. Patients with diffuse large B-cell lymphoma and positive BAFF-R expression had a higher complete response rate after chemotherapy compared with those with diffuse large B-cell lymphoma and negative BAFF-R expression. Our study strongly supports the above conclusions. Identifying new therapeutic targets for BAFF or BAFF-R may be crucial for future treatment of HCC.

BAFF-R on IgD + CD24- cell was causally associated with a decreased risk of HCC, and 7.353% of this effect was mediated through PAG. PAG is a metabolic product that plays an important physiological role in the human body. Ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) showed that PAG is a specific metabolite of ischemic heart disease (IHD). In addition, PAG binds to glutamine to form phenylacetylglutamine (PAGN), a common metabolic product naturally present in human urine. It is metabolized into acetic acid and lactic acid in the intestines, lowering intestinal pH. Furthermore, there is a clinical correlation between PAGN and a series of cardiovascular events. In the phenylalanine metabolism pathway, phenylalanine is first converted to tyrosine by hydroxylase, then tyrosine undergoes a series of reactions to produce phenylpyruvate, which ultimately forms phenylacetyl-CoA. Phenylacetyl-CoA binds to glutamate to form PAGN, which is an important intermediary product in the phenylalanine metabolism pathway. However, no studies reported the role of PAG or PAGN in HCC. A prospective study showed that phenylalanine metabolism is associated with an increased risk of HCC, suggesting that metabolic reprogramming is extensive during HCC development. HCC cells alter their metabolism to grow and spread faster. These changes in metabolism can impact the TME, influencing immune cells, suppressing the immune system, and leading to tumor immune evasion. However, the role of immune cells and their metabolites in HCC is complex. We are the first to reveal the causal association between BAFF-R on IgD + CD24- immune cell trait and the risk of HCC using MR methods, while also illustrating PAG as their mediator. MR can reduce the bias caused by confounding factors and prevent the interference of reverse causality, which is the main strength of this study.

However, there are still several limitations to this study. First, our analysis was conducted using data from the European population; thus, more ethnic groups should be included in the future. Second, the GWAS dataset for HCC had a small number of cases (N_cases = 453); thus, the sample size needs to be expanded. Third, despite our efforts to identify and remove outlier variants, we could not rule out horizontal pleiotropy. Fourth, our study utilized summary statistics rather than individual data, preventing further analysis of causal links in subgroups such as age and sex. Fifth, 7.353% of the mediation proportion is quite low; therefore, other mediators must be quantified in future studies.

Conclusion

In this study, MR analysis was conducted to provide genetic evidence of a causal association between BAFF-R on IgD + CD24- and HCC. Specifically, a higher level of BAFF-R on IgD + CD24- could lower the HCC risk, with PAG acting as a mediator. These findings offer novel insights into the mechanisms underlying the development and progression of HCC and suggest potential immune targets for treatment.

Materials and methods

Study design

A TSMR method was used (Fig. 1A) to assess the causality of 731 immune cell traits on the risk of HCC and a two-step mediation analysis was used to investigate whether this relationship could be mediated by 1400 metabolites. MR studies must follow three fundamental assumptions: I) Relevance assumption: the instrumental variables (IVs) should be closely associated with exposure. II) Independence assumption: IVs should be independent of potential confounders. III) Exclusivity assumption: IVs impact outcomes only via exposure. The analysis was performed in four steps. We first investigated the overall causal association between immune cell traits and HCC using bidirectional two-sample MR (TSMR) analyses, then identified the significant immune cell traits causally associated with HCC. Next, we utilized the most significant immunophenotypes as exposures to identify significant mediating factors among 1400 metabolites. Then, the most significant metabolites related to immune cell traits were used as mediating factors to analyze the causality of HCC. Finally, we performed mediation analysis to calculate the mediating effect of phenylacetylglutamate (PAG) levels in the causal pathway from BAFF-R on IgD + CD24- to HCC.
Data sources

The accession numbers of immune cells data were from GCST0001391 to GCST0002121, obtained from the GWAS Catalog provided by Valeria Orrù's group. In this study, they profiled 731 immune cell traits and genotyped 22 million SNPs from 3,757 European individuals of unique family-based cohort, based on the high-density arrays or imputed through sequence-based reference panel. The whole 731 cell traits included 389 median fluorescence intensities (MFI) of surface antigens, 118 absolute cell (AC) counts, and 32 morphological parameters (MP). Data was acquired through 7 panels: TBNK (T cells, B cells, natural killer cells) panel, regulatory T cell (Treg) panel, maturation stages of T-cell panel, dendritic cell panel, myeloid cell panel, monocyte cell panel, and B cell panel. The MP feature contains CDC and TBNK panels. Data on metabolites were obtained from the GWAS Catalog with accession numbers from GCST90199621 to GCST90201020, comprising 1,091 metabolites and 309 metabolite ratios from 8,299 individuals of the Canadian Longitudinal Study on Aging (CLSA) cohort (published PMID: 36635386). HCC data was obtained from the FinnGen database, including 456,348 individuals (453 cases with European ancestry and 287,137 controls with European ancestry). The number of SNPs was 20167509. Diagnostic and inclusion criteria were based on the original literature.

The bias caused by confounding factors was minimized since no participants had sample overlap between the exposure and outcome traits. As this study was conducted based on publicly available data, no ethics approval was needed.

Instrument selection

The genetic variation was selected as an instrumental variable using the following steps to choose the best SNPs correlated with exposure factors. First, all P values were set to 5×10^-8 to identify independent SNPs. The statistical significance threshold was adjusted to P < 1×10^-5 to identify more SNPs of both immune traits and metabolites as exposures in the forward MR analysis and prevent inaccurate results due to insufficient SNPs. The significance level was also adjusted to P < 5×10^-5 to identify more SNPs of HCC as exposures in the reverse MR analysis. The threshold was set to r^2 < 0.001 within a 10,000 kb distance to avoid linkage disequilibrium (LD) between the selection of SNPs. The IVs were screened in the Phenoscanner database (http://www.phenoscanner.medschl.cam.ac.uk/) to identify eligible SNPs and exclude confounders related to HCC risk, including cirrhosis, hepatitis B infection, and hepatitis D infection, NAFLD, chronic ingestion of aflatoxin-contaminated foodstuffs, alcohol addiction, obesity, smoking, and type II diabetes. The threshold of LD was set to r^2 < 0.001 within a 10,000 kb distance. The F statistic was calculated using the approximation method to assess the statistical strength between the IVs and the exposure. An F value less than 10 suggests a weak correlation within the IVs, which means that a gene variant with low decoding ability to exposure provides limited statistical power for testing the hypothesis. It may lead to inaccurate estimates of causal effects and an elevated likelihood of type I errors. SNPs with F ≤ 10 were automatically excluded. r^2 is the explained variance of IVs.

Mendelian randomization and statistical analysis

TSMR study was performed to assess the causal relationship between 731 immune cell traits (exposure) and HCC (outcome) using the "TwoSampleMR" (version 0.5.7) package of R (version 4.3.1). In this study, the inverse variance weighted (IVW) method was the main method with ORs to describe the increase in risk levels per standard deviation (SD). Considering the possible heterogeneity and pleiotropy of SNPs, MR-Egger, weighted median, and MR-PRESSO methods were used as supplements to verify the causal effect. The weighted median is the best choice in the presence of heterogeneity and the absence of horizontal pleiotropy. It provides an accurate causal estimate when < 50% of the instruments are invalid. The MR-Egger method provides reliable results when there is potential horizontal pleiotropy, but it is less effective compared with the IVW method. The M-PRESSO method was used to detect and, if needed, correct for horizontal pleiotropy and potential outliers. Heterogeneous effects among instrumental SNPs in the IVW method were assessed using Cochran's Q test. Finally, leave-one-out analysis was employed in which single variants were successively removed. It assessed whether a single genetic variant had a disproportionate impact on the over-MR estimate. Genetic variation effect estimates were assessed using forest plots. Publication bias was assessed by checking the symmetry of the funnel plots. P value was set to 0.05.

Mediation analysis

In mediation analysis, the total effects of the exposures on HCC were estimated using univariable MR. The direct (without mediators) and mediating effects (through mediators) of mediators between the exposure and the outcome were decomposed using TSMR analysis. The mediating effect was calculated using the following formula: beta12 = beta1*beta2. The proportion of mediating effect in the total effect was calculated using the following formula: beta12_p = beta12/beta_all*100%. The effect of exposure on outcome was considered to be a direct effect and was calculated using the following formula: beta_dir = beta_all - beta12 (Fig. 1B).

 declarations

Acknowledgements

The authors would like to express their gratitude to EditSprings (https://www.editsprings.cn) for the expert linguistic services provided.

Author contributions
X.Z. wrote the main manuscript text. ZC.Q. performed the statistical analysis. L.X. coordinated the paper. All authors critically reviewed the manuscript.

Funding

This work was financially supported by National Natural Science Foundation of China (No. 82360412).

Data availability statement

Data for the present study can be downloaded in GWAS (GWAS ID included in the article), and further inquiries can be directed to the corresponding author.

Competing interests

The author(s) declare no competing interests.

Ethical approval

Data were publicly available, no ethics approval was needed.

References


### Table 1
The mediated effect of PAG between BAFF-R on IgD+ CD24- and hepatocellular carcinoma.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Outcome</th>
<th>Methods</th>
<th>N</th>
<th>Beta</th>
<th>P-value</th>
<th>OR</th>
<th>95% CI (OR)</th>
<th>Pleiotropy</th>
<th>Heterogeneity</th>
<th>Horizontal pleiotropy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAFF-R on IgD+ CD24-</td>
<td>HCC</td>
<td>IVW</td>
<td>22</td>
<td>-0.168</td>
<td>0.002</td>
<td>0.845</td>
<td>0.758-0.942</td>
<td>0.024 0.485</td>
<td>26.075 0.204</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MR Egger</td>
<td></td>
<td>-0.201</td>
<td>0.012</td>
<td>0.818</td>
<td>0.71-0.942</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weighted median</td>
<td></td>
<td>-0.184</td>
<td>0.004</td>
<td>0.832</td>
<td>0.734-0.944</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Simple mode</td>
<td></td>
<td>-0.228</td>
<td>0.128</td>
<td>0.796</td>
<td>0.601-1.055</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weighted mode</td>
<td></td>
<td>-0.171</td>
<td>0.017</td>
<td>0.842</td>
<td>0.74-0.958</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCC</td>
<td>BAFF-R on IgD+ CD24-</td>
<td>IVW</td>
<td>57</td>
<td>0.013</td>
<td>0.244</td>
<td>1.013</td>
<td>0.991-1.036</td>
<td>0.016 0.169</td>
<td>48.574 0.749</td>
<td>0.764</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MR Egger</td>
<td></td>
<td>-0.02</td>
<td>0.454</td>
<td>0.981</td>
<td>0.932-1.032</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weighted median</td>
<td></td>
<td>-0.004</td>
<td>0.809</td>
<td>0.996</td>
<td>0.963-1.032</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Simple mode</td>
<td></td>
<td>-0.01</td>
<td>0.781</td>
<td>0.99</td>
<td>0.921-1.064</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weighted mode</td>
<td></td>
<td>-0.013</td>
<td>0.639</td>
<td>0.987</td>
<td>0.937-1.041</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAFF-R on IgD+ CD24-</td>
<td>PAG</td>
<td>IVW</td>
<td>22</td>
<td>0.030</td>
<td>0.037</td>
<td>1.030</td>
<td>1.002-1.059</td>
<td>-0.014 0.082</td>
<td>19.776 0.535</td>
<td>0.582</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MR Egger</td>
<td></td>
<td>0.052</td>
<td>0.012</td>
<td>1.053</td>
<td>1.015-1.093</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weighted median</td>
<td></td>
<td>0.040</td>
<td>0.044</td>
<td>1.041</td>
<td>1.001-1.082</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Simple mode</td>
<td></td>
<td>0.018</td>
<td>0.594</td>
<td>1.018</td>
<td>0.955-1.085</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weighted mode</td>
<td></td>
<td>0.040</td>
<td>0.047</td>
<td>1.041</td>
<td>1.003-1.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAG</td>
<td>HCC</td>
<td>IVW</td>
<td>22</td>
<td>-0.416</td>
<td>0.022</td>
<td>0.660</td>
<td>0.462-0.943</td>
<td>-0.026 0.696</td>
<td>20.742 0.475</td>
<td>0.511</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MR Egger</td>
<td></td>
<td>-0.250</td>
<td>0.591</td>
<td>0.779</td>
<td>0.318-1.911</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weighted median</td>
<td></td>
<td>-0.457</td>
<td>0.105</td>
<td>0.633</td>
<td>0.364-1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Simple mode</td>
<td></td>
<td>-0.454</td>
<td>0.362</td>
<td>0.635</td>
<td>0.245-1.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weighted mode</td>
<td></td>
<td>-0.501</td>
<td>0.161</td>
<td>0.606</td>
<td>0.308-1.19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: PAG, phenylacetylglutamate levels; IVW, inverse variance weighted.
Figures

Figure 1

Study design overview. A Three critical assumptions of MR analysis. B Mediation analysis. All effects were beta_all, mediation effect was beta12, direction effect was beta_dir, the proportion of mediating effect in the total effect was beta12_p.

Figure 2

Forest plot to show the causal effects of phenylacetylglutamate levels with BAFF-R on IgD+ CD24- and HCC. HCC, hepatocellular carcinoma; PAG, phenylacetylglutamate.
Figure 3

A. Plots of MR mediation analysis of BAFF-R on IgD+ CD24- and HCC risk; B. Plots of MR mediation analysis of BAFF-R on IgD+ CD24- and phenylacetylglutamate levels; C. Plots of MR mediation analysis of phenylacetylglutamate levels and HCC risk; A1, B1, C1. Forest plot; A2, B2, C2. Funnel plot; A3, B3, C3. Scatter plot; A4, B4, C4. Leave-one-out plot. HCC, hepatocellular carcinoma.
Figure 4

Mediation effect of PAG levels in the association between BAFF-R on IgD+ CD24- and HCC. BAFF-R, BAFF-R on IgD+ CD24-; PAG, phenylacetylglutamate; HCC, hepatocellular carcinoma.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.xlsx