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Causal effect of gut microbiota metabolic pathways on CSAG1 expression in chondrosarcoma: a mendelian randomization analysis

Xiaoyun Liu[1*], Sen Yang[2*] and Liyun Liu[3]

Abstract

Background Changes in gut microbiota metabolism might play an important role in the development of some cancers. However, the causal relationships of gut microbiome-related metabolic pathways in chondrosarcomas and the specific pathways affected remain largely unknown.

Methods We used two-sample bidirectional and multivariate Mendelian randomization (MR) to reveal a causal relationship between the gut microbiota metabolic pathway (GMMP) and chondrosarcoma associated gene 1(CSAG1) via the largest available genome-wide association study (GWAS).

Results Univariate MR analysis revealed that tetrapyrrole biosynthesis from glutamate, menaquinol 6 biosynthesis, glycogen degradation II, 8-amino-7-oxononanoate biosynthesis, taxadiene biosynthesis, glycolysis and tRNA charging had a significant causal relationship with CSAG1.Multivariate MR analysis suggested that tetrapyrrole biosynthesis, menaquinol 6 biosynthesis, glycogen degradation II, glycolysis and tRNA charging still had a significant causal effect on CSAG1. According to the results of reverse MR analysis, no significant causal effect of CSAG1 on the GMMP was found.

Conclusions This study offers further insights into the gut microbiota-mediated mechanism of chondrosarcoma development.

Keywords Mendelian randomization, Gut microbiota metabolic pathway, Chondrosarcoma, Chondrosarcoma associated gene 1, Causal relationship

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Introduction

Malignant bone tumors are a rare and heterogeneous group of neoplasms that occur in bone. The incidence of these tumors increased from 0.00069% in 2000 to 0.00749% in 2018, and chondrosarcoma is the second most common primary bone sarcoma [1, 2]. Chondrosarcoma is characterized by the production of a cartilage matrix, which most commonly occurs in adults. Conventional chondrosarcoma is the most common (90%) subtype; the remaining 10% are non-conventional chondrosarcomas, including mesenchymal,

dedifferentiated, and clear cell chondrosarcoma [3]. Low grade chondrosarcomas rarely metastasize. In contrast, high grade chondrosarcomas, which make up 5–10% of all conventional chondrosarcomas, are very aggressive and frequently metastasize to the lung [4]. Most chondrosarcomas are resistant to chemotherapy and radiation therapy [5].Therefore, there are limited treatment options for patients with metastatic or unresectable chondrosarcomas.

However, the specific mechanism of chondrosarcomas has not been elucidated until now.

CSAG1, also known as cancer/testicles antigen 24.1 or CSAGE, is a gene that was first identified as highly expressed in chondrosarcoma [6].Cancer/testicles antigens has been demonstrated to be frequently active in cancer cells [7, 8]. The major role for CSAG1 is to preserve centromere stability during mitosis and can influence the degree of multipolarization of tumor stem cell models by regulating the error rate of mitosis [9]. These antigen genes are closely related to the occurrence and development of tumors, such as head and neck squamous cell carcinoma, prostate cancer and sarcoma [10, 11]. Therefore, taking CSAG1 as the research basis can better discover the specific mechanism of chondrosarcoma.

Recently, an increasing number of studies have demonstrated that the gut microbiota plays an important role in regulating human health and disease by maintaining gut homeostasis [12]. The gut microbiota has been implicated as a risk or preventive factor for a variety of cancers and is closely associated with colorectal, lung, breast, and prostate cancer [13–16]. Gut microbiota can interact with the host through its metabolites to influence the progression of liver cancer, colorectal cancer and gastric cancer [17–19]. The gut microbiota regulates the expression of tumor genes, such as: SOX9, IL-11, and MMP3 [20]. Gut microbiota metabolites have been shown to play a key role in the development and progression of cancer, such as short-chain fatty acids, secondary bile acids, and indole metabolites, which all influence tumor evolution by regulating host cell signaling pathways [21]. Nonetheless, the relationship between the GMMP and CSAG1 in chondrosarcoma is largely unknown.

MR is an efficient approach for investigating the causal relationship between exposure and an outcome in a cross-sectional study while controlling for uncertain confounding effects [22, 23]. The MR analysis applied genetic variation as an instrumental variable (IV) to assess the existence of a causal relationship between exposure and outcomes. To ensure the robustness of causal inference, MR design needs to satisfy the following three important assumptions simultaneously: 1. IVs are strongly associated with exposure; 2. IVs share no common cause with the outcome; 3. IVs do not affect the outcome except through risk factors. In this study, we performed two-sample bidirectional MR analysis and multivariate MR analysis based on public data from a genome-wide association study (GWAS) to reveal the causal effect of the gut microbiota on CSAG1 and its associated risk factors. A schematic diagram of the research design is shown in Fig. 1. Our aim was to identify that the gut microbiome environment may regulate the development of chondrosarcoma by influencing CSAG1, which provides a basis for the development of new treatment strategies.

Methods

Research reporting guidelines and research design

Two-sample MR and open datasets were used to investigate the impact of the GMMP abundance on CSAG1 and the causal relationship between them. Study reporting was performed according to the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (The STROBE-MR Statement) [24].

Data sources

We downloaded the entire sample and the corresponding data from the OpenGWAS [25] database website (https://gwas.mrcieu.ac.uk/). A total of 205 GMMPs provided by Lopera-Maya EA et al. [26] were selected (Table S1). The associated statistics of the GMMPs were obtained and standardized using the R package.

CSAG1 data (prot-a-677) were obtained from a study by Sun BB et al. [27], which analyzed data from both male and female European patients. We downloaded the associated summary statistics for this analysis through the GWAS Catalog [28].

The data for all exposure factors associated with CSAG1 were downloaded from the OpenGWAS database, and univariate MR analysis was subsequently performed on the CSAG1 data. An analysis result of $P < 0.05$ was used for subsequent multivariate MR analysis.

Selection of IVs

In the studies of univariate MR analysis, multivariate MR analysis and reverse causality of screening the GMMP for CSAG1, the screening criteria for IVs were as follows: IVs conformed to the three assumptions of MR analysis, Single nucleotide polymorphisms (SNPs) in the GWAS data were $P < 5 \times 10^{-5}$, and linkage disequilibrium (LD) was eliminated ($r^2 < 0.001$, kb > 10,000).

MR causal effect estimation

A variety of two-sample MR analysis methods, including the inverse variance weighted (IVW), MR–Egger, weighted median (WM) simple and weighted methods, were used to evaluate the causal effect of the GMMP

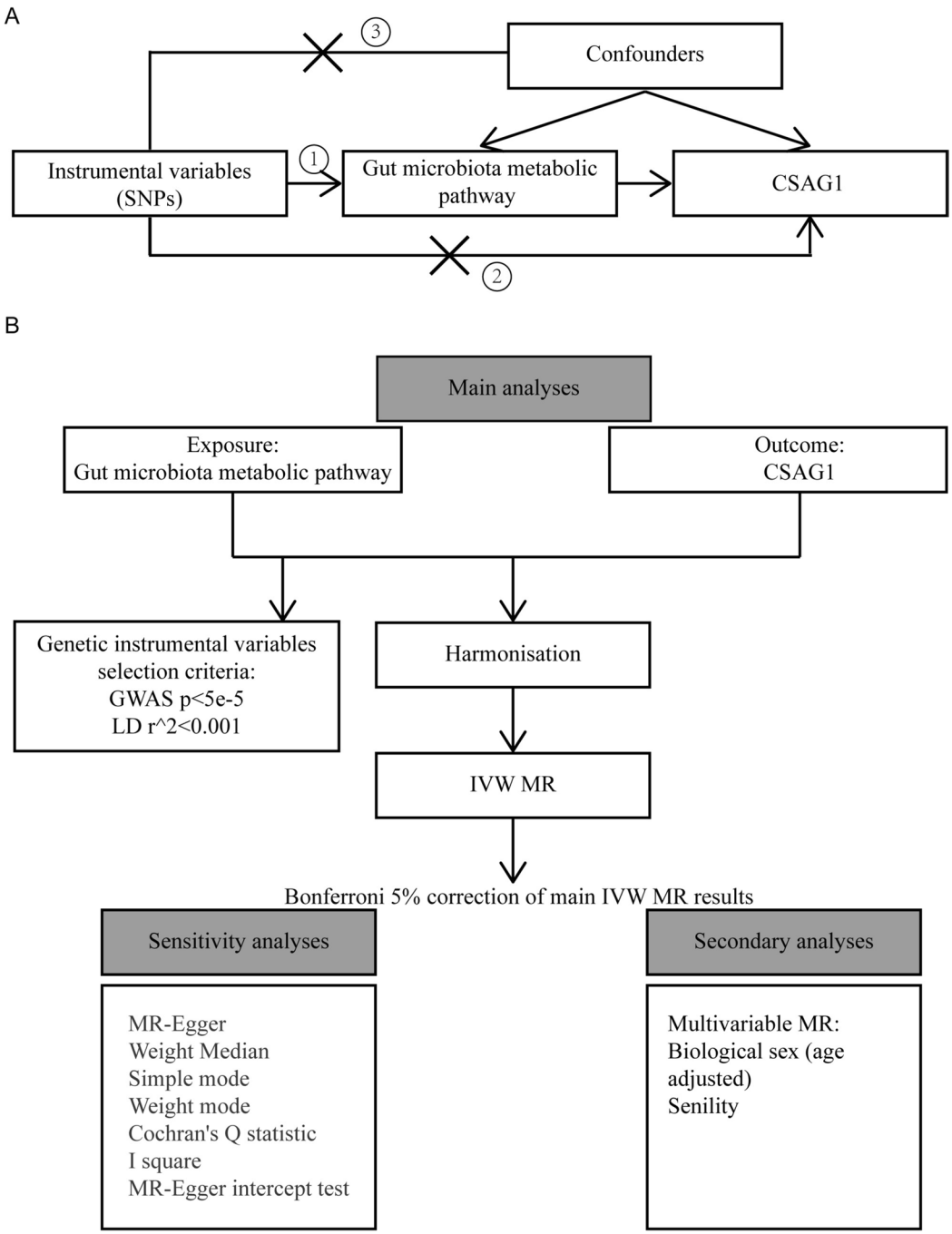

Fig. 1 The whole workflow of MR analysis

on CSAG1. Studies have shown that the IVW is slightly stronger than other methods under certain conditions [29]. Its characteristic is that the existence of the intercept term is not considered during regression, and the reciprocal of the outcome variance is used as the weight for fitting. Therefore, in the absence of pleiotropy, IVW was used as the main MR analysis method in this study regardless of heterogeneity, and the other four methods were used as supplements. When pleiotropy existed, MR–Egger was used to calculate the results.

Sensitivity analysis

The sensitivity analysis was carried out by using various methods, such as heterogeneity tests, pleiotropy tests and one-by-one elimination tests.

Cochrane's Q test was used to evaluate the heterogeneity among the SNP estimates, and IVs with $P < 0.05$ were used to indicate that the results were heterogeneous. Cochrane's Q test could only show the presence or absence of heterogeneity; it cannot observe the distribution of heterogeneity. The I^2 statistic was used to reflect the proportion of heterogeneity in the IVs in the total variation. An $I^2 \leq 0$ was set to 0, indicating that no heterogeneity was present; an $I^2 = 0$–25% indicated mild heterogeneity. An I^2 of 25%–50% indicated moderate heterogeneity. An $I^2 > 50\%$ indicated high heterogeneity. The specific calculation formula is as follows:

$$I^2 = \frac{Q-df}{Q} \times 100\%$$

The MR–Egger intercept test was used to test the pleiotropy of the IVs. Horizontal pleiotropy was deemed to exist if $P < 0.05$.

The leave-one-out analysis calculates MR results for the remaining IVs by eliminating individual SNPs one by one to assess whether the SNP affects the association between the GMMP and CSAG1. If there is a large difference between the results of MR effect estimation and the total effect estimation after removing a certain instrumental variable, the MR effect estimation results are sensitive to this SNP.

Multivariate MR analysis

Multivariable MR is an extension of MR analysis that uses genetic variants associated with multiple potentially relevant exposures to estimate the impact of multiple exposures on a single outcome. It can evaluate the direct effect of a single exposure factor on the outcome. Before conducting multivariate MR analysis, we will first perform a univariate MR analysis on the GMMP and CSAG1-related exposure factors that have a significant causal effect on CSAG1. Exposure factors related to CSAG1 were used as exposure factors in subsequent analyses. A multivariable MR model of each the GMMP, CSAG1-related exposure factor and CSAG1 was constructed for multivariable MR analysis.

The direct effects of the GMMP and CSAG1-related exposure factors on CSAG1were determined by multivariate MR analysis.

Statistical analysis

All data calculations and statistical analyses were performed using R (https://www.r-project.org/, version 4.2.2), and MR analysis was carried out using the TwoSampleMR package [30]. Cochrane's Q test and leave-one-out analysis were used to evaluate the robustness and reliability of the results, and the MR–Egger intercept method was used to carry out the horizontal pleiotropy test. All the statistical P values were two-sided tests, and $P < 0.05$ was considered to indicate statistical significance.

Results

Analysis of the causal relationship between the GMMP and CSAG1

We selected 205 SNPs associated with the GMMP from the sorted SNP data in the OpenGWAS database for preliminary causal analysis and verification of the results. The specific information of 205 GMMPS is shown in Table S1. The effect values of all IVs were obtained after matching the 205 SNP sites selected with the GWAS data of CSAG1(prot-a-677). After harmonization, IVs associated with both the GMMP and CSAG1 were finally obtained and included in the MR analysis. The instrumental variable data of specific the GMMP exposure factors are shown in Table 1. Due to the large number of the GMMP exposure factors, only indicators with significant MR analysis results ($P < 0.05$) are shown. The F test statistics of the IVs of these indicators were all greater than 10, indicating that most of the SNPs screened in this study were strong-effect IVs, and the possible bias caused by weak IVs was limited.

MR analysis methods included MR–Egger, WM, IVW, simple mode and weighted mode. According to the IVW results, tetrapyrrole biosynthesis from glutamate (ebi-a-GCST90027546), menaquinol 6 biosynthesis (ebi-a-GCST90027560), glycogen degradation II (ebi-a-GCST90027564), 8-amino-7-oxononanoate biosynthesis (ebi-a-GCST90027582), taxadiene biosynthesis (ebi-a-GCST90027624), glycolysis (ebi-a-GCST90027631) and tRNA charging (ebi-a-GCST90027646) had a significant causal relationship with the high expression of CSAG1 ($p < 0.05$) (Table S2). The MR analysis results are also shown in the form of a forest map (Fig. 2).

The linear relationship between the instrumental effects of the abundance of seven GMMPs on the CSAG1 is shown in Fig. 3. The results showed that the direction of effect values estimated by MR–Egger, WM, simple mode and weighted mode were basically consistent with the direction of the IVW model.

Sensitivity analysis

We used Cochrane's Q test to detect heterogeneity in the IVW model results, and the GMMP abundance in the IVW model was $P > 0.05$, indicating no significant heterogeneity (Table 2).

Table 1 Instrumental variable screening and instrumental variable strength F test for GMMP and CSAG1

Exposure	Pathway	Number of SNPs	Median of F	Minimum of F	Maximum of F
ebi-a-GCST90027546	Tetrapyrrole biosynthesis from glutamate	50	17.75675	16.47309	25.0195
ebi-a-GCST90027560	Menaquinol 6 biosynthesis	39	18.06635	16.45589	22.64702
ebi-a-GCST90027564	Glycogen degradation II	52	17.77527	16.52922	22.00014
ebi-a-GCST90027582	8 amino 7 oxononanoate biosynthesis	44	17.7721	16.49744	23.24438
ebi-a-GCST90027624	Taxadiene biosynthesis	56	17.78317	16.50707	24.11524
ebi-a-GCST90027631	glycolysis	47	18.31713	16.44694	25.12113
ebi-a-GCST90027646	tRNA charging	59	17.84809	16.52766	29.64234

The robustness of the selected instrumental variables was proved by F test

SNP, single nucleotide polymorphism

ebi-a-GCST90027546, tetrapyrrole biosynthesis from glutamate

ebi-a-GCST90027560, menaquinol 6 biosynthesis

ebi-a-GCST90027564, glycogen degradation II

ebi-a-GCST90027582, 8 amino 7 oxononanoate biosynthesis

ebi-a-GCST90027624, taxadiene biosynthesis

ebi-a-GCST90027631, glycolysis

ebi-a-GCST90027646, tRNA charging

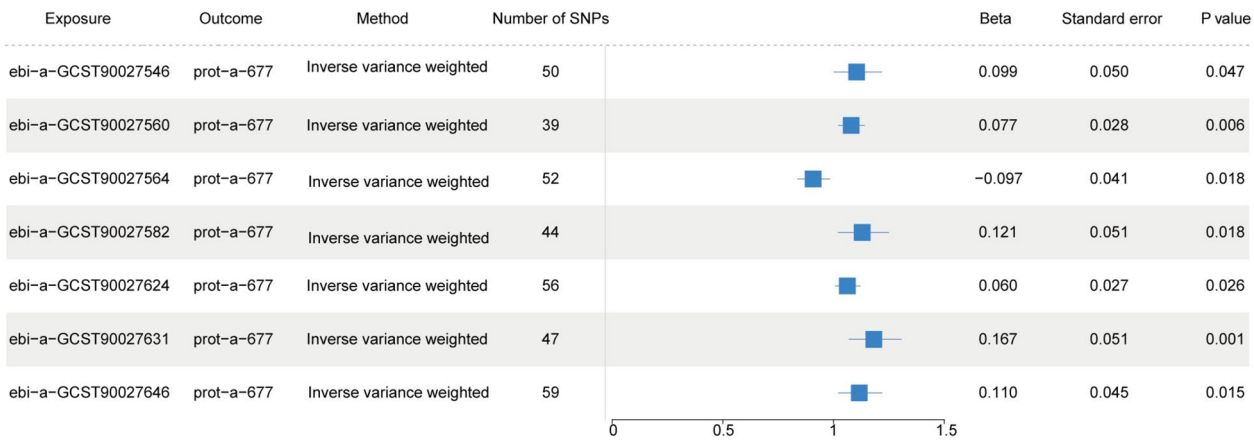

Fig. 2 Forrest plot for summary causal effects of GMMP on CSAG1 based on IVW method for the primary analysis. The forest plot demonstrates that seven pathways have causal effect on CSAG1

We used MR–Egger regression to assess the pleiotropy of IVs to verify the causal relationship between GMMP abundance and CSAG1. As shown in Table 3, the *P* values of the intercept of the statistical hypothesis test for each indicator were greater than 0.05, and the intercept was close to 0, indicating that the tool effect of the 7 GMMPs in this study and the causal effect inference on the CSAG1 were not affected by horizontal pleiotropy. The heterogeneity of the MR analysis between the GMMP and CSAG1 is presented in the form of funnel plots. As shown in Fig. 4, the scattered points of causal relationships are basically symmetrical, indicating no potential bias.

Leave-one-out analysis was performed to eliminate each IV one by one to analyze the influence of GMMP abundance on the causal relationship of CSAG1. Figure S1 shows that the total effect of each set of IVs has not shifted substantially.

Assessment of the impact of CSAG1 on the GMMP

In the GWAS on the risk of CSAG1, we first screened SNPs ($P < 5 \times 10^{-6}$) and excluded LD ($r^2 < 0.001$, and the physical distance between each pair of genes > 10,000 kb) SNP sites. The effect values of all IVs were obtained by matching with GWAS data of GMMP abundance.

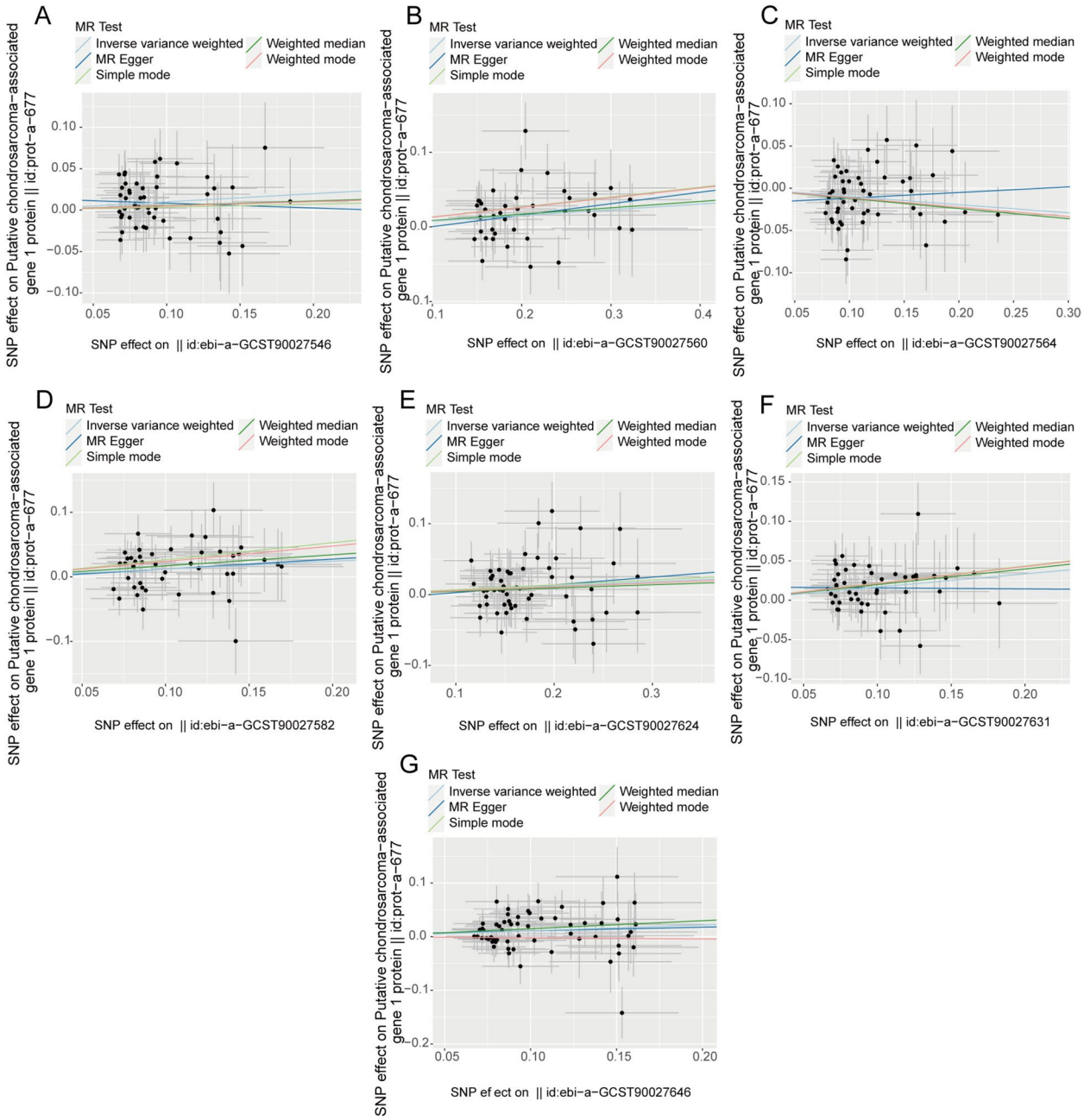

Fig. 3 Scatter plots for the causal association between GMMP and CSAG1

After harmonization, 7 exposure factors were included in the MR analysis.

A total of 5 models were adopted for analysis, including MR–Egger, WM, IVW, simple and weighted models. The IVW model results showed that there was no significant causal relationship between CSAG1and the GMMP (Table S3). The expression of CSAG1 does not cause changes in the GMMP. The results of MR analysis of CSAG1and the GMMP are shown in the forest plot (Fig. 5).

Multivariate MR analysis of the effect of the GMMP on the CSAG1

To assess the direct effect of significant the GMMP on CSAG1, we performed multivariate MR analyses of 2 exposure factors associated with CSAG1 (biological

Table 2 Heterogeneity of the Cochran Q test for MR analysis of GMMP on CSAG1

Exposure	Outcome	Method	Cochrane's Q	Cochrane's Q df	Cochrane's Q *p*-value	I^2 (%)
ebi-a-GCST90027546	prot-a-677	IVW	36.846	49	0.899	0
ebi-a-GCST90027560	prot-a-677	IVW	41.326	38	0.327	8.048
ebi-a-GCST90027564	prot-a-677	IVW	42.460	51	0.797	0
ebi-a-GCST90027582	prot-a-677	IVW	40.121	43	0.597	0
ebi-a-GCST90027624	prot-a-677	IVW	55.812	55	0.444	1.455
ebi-a-GCST90027631	prot-a-677	IVW	31.131	46	0.954	0
ebi-a-GCST90027646	prot-a-677	IVW	49.633	58	0.775	0

df, the degrees of freedom; *MR*, mendelian randomization; *IVW*, Inverse variance weighted

ebi-a-GCST90027546, tetrapyrrole biosynthesis from glutamate

ebi-a-GCST90027560, menaquinol 6 biosynthesis

ebi-a-GCST90027564, glycogen degradation II

ebi-a-GCST90027582, 8 amino 7 oxononanoate biosynthesis

ebi-a-GCST90027624, taxadiene biosynthesis

ebi-a-GCST90027631, glycolysis

ebi-a-GCST90027646, tRNA charging

prot-a-677, CSAG1

The I^2 statistic was used to reflect the proportion of heterogeneity in the IVs in the total variation. An $I^2 \leq 0$ was set to 0, indicating that no heterogeneity was present; an $I^2 = 0$–25% indicated mild heterogeneity

Table 3 Horizontal pleiotropy test for MR analysis of GMMP on CSAG1

Exposure	Outcome	MR-Egger intercept	SE	*p*-value
ebi-a-GCST90027546	prot-a-677	0.014	0.018	0.436
ebi-a-GCST90027560	prot-a-677	−0.015	0.024	0.542
ebi-a-GCST90027564	prot-a-677	−0.018	0.016	0.258
ebi-a-GCST90027582	prot-a-677	−0.003	0.021	0.876
ebi-a-GCST90027624	prot-a-677	−0.008	0.021	0.697
ebi-a-GCST90027631	prot-a-677	0.017	0.019	0.374
ebi-a-GCST90027646	prot-a-677	0.004	0.018	0.818

MR-Egger, Mendelian randomization-Egger; *SE*, Standard error

ebi-a-GCST90027546, tetrapyrrole biosynthesis from glutamate

ebi-a-GCST90027560, menaquinol 6 biosynthesis

ebi-a-GCST90027564, glycogen degradation II

ebi-a-GCST90027582, 8 amino 7 oxononanoate biosynthesis

ebi-a-GCST90027624, taxadiene biosynthesis

ebi-a-GCST90027631, glycolysis

ebi-a-GCST90027646, tRNA charging

prot-a-677, CSAG1

sex: ebi-a-GCST90013474, senility: finn-b-R18) and 7 GMMPs. As shown in Table S4, after correcting for the effects of biological sex and senility, tetrapyrrole biosynthesis (Model 1), menaquinol 6 biosynthesis (Model2), glycogen degradation II (Model3), glycolysis (Model 6) and tRNA charging (Model 7) still had a significant causal effect on CSAG1 ($P < 0.05$).

Discussion

CSAG1 is highly expressed in chondrosarcoma, but its specific function in the development of the disease remains to be further investigated. This is the first study to investigate the causal relationships between the GMMP and CSAG1 by using bidirectional two-sample MR analyses. In this bidirectional MR study, no reverse causal relationship was observed between CSAG1 and the GMMP, but a strong positive causal relationship was found between the GMMP and CSAG1. These results were examined through several analyses, such as Cochrane's Q test, the MR–Egger intercept test, and leave-one-out analysis, which showed consistent findings and suggested no horizontal pleiotropy. Multivariate MR analysis also revealed a direct causal relationship between the GMMP and CSAG1, including tetrapyrrole biosynthesis, Menaquinol6 biosynthesis, glycogen degradation II, glycolysis and tRNA charging.

As metabolic organs of the body, gut bacteria play important roles in inflammation and immune regulation in the host. Previous studies have described a pathway by which Clostridium sporogenes generates aromatic amino acid metabolites and revealed that these metabolites affect intestinal permeability and systemic immunity [31]. Gut microbiota dysbiosis and microbiota metabolites are key pathways that drive cancer-promoting liver inflammation, fibrosis and genotoxicity [18]. In the study of osteosarcoma, transcriptomics revealed specific gene expression signatures and changes in the abundance of intestinal flora [32–35]. However, the effect of the

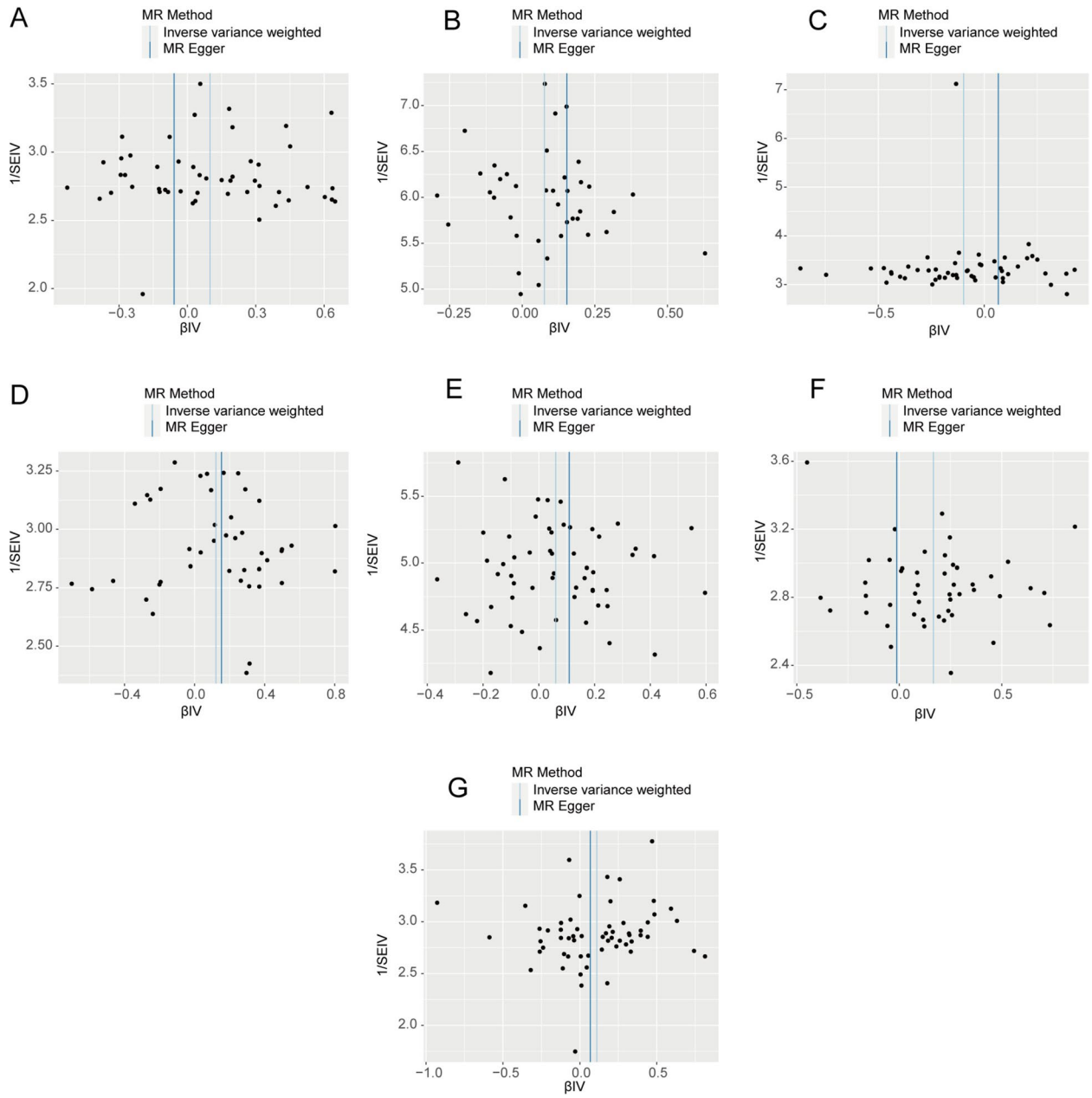

Fig. 4 Funnel plots evaluated the validity of the causal relationship between GMMP and CSAG1

GMMP on chondrosarcoma has not been reported thus far.

In our study, tetrapyrrole biosynthesis was found to be a factor for the development of chondrosarcoma. Tetrahydropyrrole is a macrocyclic molecule with various structural variants and multiple functions in prokaryotes and eukaryotes, and it can either absorb visible light or accept different redox states [36]. There are many studies on the pathway mechanism in plants, and it is described as"the pigments of life" [37]. In the study of inflammatory bowel disease, in addition to changes in the structure of the gut microbiota, there are also changes in the characteristics of metabolites, such as tetrahydropyrrole consumption [38]. Menaquinol 6 biosynthesis is also factor for chondrosarcoma. Menaquinol synthesis is highly enriched in the GMMP in carotid atherosclerosis [39] and atopic dermatitis [40]. According to the metaCycle metabolic pathway prediction analysis, the abundance of glycogen degradation I (bacterial) was lower than that in the healthy

Exposure	Outcome	Method	Number of SNPs	Beta	Standard error	P value
prot-a-677	ebi-a-GCST90027546	Inverse variance weighted	55	0.029	0.021	0.166
prot-a-677	ebi-a-GCST90027546	MR Egger	55	−0.031	0.091	0.731
prot-a-677	ebi-a-GCST90027546	Simple mode	55	−0.036	0.068	0.601
prot-a-677	ebi-a-GCST90027546	Weighted median	55	0.016	0.030	0.600
prot-a-677	ebi-a-GCST90027546	Weighted mode	55	−0.020	0.063	0.757
prot-a-677	ebi-a-GCST90027560	Inverse variance weighted	55	−0.062	0.047	0.186
prot-a-677	ebi-a-GCST90027560	MR Egger	55	0.065	0.208	0.755
prot-a-677	ebi-a-GCST90027560	Simple mode	55	−0.280	0.170	0.105
prot-a-677	ebi-a-GCST90027560	Weighted median	55	−0.088	0.064	0.168
prot-a-677	ebi-a-GCST90027560	Weighted mode	55	−0.096	0.152	0.531
prot-a-677	ebi-a-GCST90027564	Inverse variance weighted	54	0.005	0.025	0.828
prot-a-677	ebi-a-GCST90027564	MR Egger	54	0.043	0.111	0.699
prot-a-677	ebi-a-GCST90027564	Simple mode	54	−0.073	0.085	0.391
prot-a-677	ebi-a-GCST90027564	Weighted median	54	−0.044	0.035	0.207
prot-a-677	ebi-a-GCST90027564	Weighted mode	54	−0.073	0.081	0.371
prot-a-677	ebi-a-GCST90027582	Inverse variance weighted	54	0.002	0.023	0.920
prot-a-677	ebi-a-GCST90027582	MR Egger	54	0.124	0.103	0.233
prot-a-677	ebi-a-GCST90027582	Simple mode	54	−0.073	0.074	0.326
prot-a-677	ebi-a-GCST90027582	Weighted median	54	−0.037	0.032	0.246
prot-a-677	ebi-a-GCST90027582	Weighted mode	54	−0.071	0.075	0.351
prot-a-677	ebi-a-GCST90027624	Inverse variance weighted	55	−0.014	0.042	0.738
prot-a-677	ebi-a-GCST90027624	MR Egger	55	0.016	0.185	0.930
prot-a-677	ebi-a-GCST90027624	Simple mode	55	−0.174	0.142	0.224
prot-a-677	ebi-a-GCST90027624	Weighted median	55	−0.080	0.055	0.148
prot-a-677	ebi-a-GCST90027624	Weighted mode	55	−0.162	0.120	0.181
prot-a-677	ebi-a-GCST90027631	Inverse variance weighted	55	0.003	0.026	0.907
prot-a-677	ebi-a-GCST90027631	MR Egger	55	0.057	0.114	0.618
prot-a-677	ebi-a-GCST90027631	Simple mode	55	−0.020	0.069	0.777
prot-a-677	ebi-a-GCST90027631	Weighted median	55	−0.002	0.031	0.948
prot-a-677	ebi-a-GCST90027631	Weighted mode	55	−0.020	0.062	0.751
prot-a-677	ebi-a-GCST90027646	Inverse variance weighted	55	−0.009	0.022	0.672
prot-a-677	ebi-a-GCST90027646	MR Egger	55	0.079	0.096	0.413
prot-a-677	ebi-a-GCST90027646	Simple mode	55	−0.055	0.075	0.469
prot-a-677	ebi-a-GCST90027646	Weighted median	55	−0.021	0.031	0.498
prot-a-677	ebi-a-GCST90027646	Weighted mode	55	−0.049	0.074	0.513

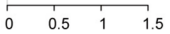

Fig. 5 Reverse MR analysis showed that there was no causal relationship between CSAG1 and GMMP

control group [41]. However, bacterial functional analysis revealed increased glycogen degradation pathway activity in colorectal adenomas with low-grade dysplasia [42]. Therefore, the abundance of genes involved in the glycogen degradation pathway varies in different disease states. In our study, we found that the glycogen degradation pathway can directly affect the expression of CSAG1 and thus regulate the development of chondrosarcoma. As a pathway of vitamin metabolism, 8-amino-7-oxononanoate biosynthesis was found in our study to be an influential factor for the development of chondrosarcoma. In animal models, adult zebrafish acutely exposed to microplastics showed a synthetic downregulation of 8 amino 7 oxononanoate [43]. In a mouse model of acute colitis induced by dextran sulfate sodium salt, 8-amino-7-oxononanoate biosynthesis in liver tissue was significantly reduced, and that of curcumin was reversed [44], suggesting that the 8-amino-7-oxononanoate biosynthesis pathway might play a protective role in the development of diseases in tissues. Taxadiene is an important precursor for the biosynthesis of the highly effective anticancer drug paclitaxel and can be produced in *Escherichia coli* and *Yarrowia lipolytica* [45, 46]. The specific regulatory mechanism of the taxadiene biosynthesis pathway in the disease state has not been reported. We found that taxadiene biosynthesis in the gut affected the expression of CSAG1. Glycolysis, an important short-chain fatty acid fermentation pathway in the gut microbiota [47, 48], was reduced in arterial stiffness patients compared to normal controls [49]. The gut microbiome of obese adolescents is significantly enriched in KEGG pathways involved in glycolysis [50]. Enrichment of the intestinal flora glycolytic pathway in the disease state has also been demonstrated in our study of chondrosarcoma. Aminoacyl-tRNA synthetases (ARSs) couple amino acids to their respective tRNAs in a process known as tRNA charging. Increased expression of tRNA synthetases has been found to play an active role in promoting tumor growth in a variety of human

cancers [51, 52]. Our study revealed that enrichment of the tRNA charging pathway plays an important role in the progression of chondrosarcoma by CSAG1.

Our study has several advantages. First, we used an MR approach to study the GMMP and CSAG1, providing key advantages in terms of establishing causality while reducing the risk of reverse causation bias and the effect of confounders. Second, we conducted a bidirectional study on the causal relationship between the GMMP and CSAG1 and added multivariate analysis to ensure that the MR analysis results were reliable. Third, the F statistic of each instrument was greater than 10, indicating that there was negligible weak IV bias. Finally, sensitivity analyses suggested that there was no heterogeneity or horizontal pleiotropy.

Our study also has several shortcomings that must be considered. First, although the SNPs that showed an association with CSAG1 through known confounding factors were eliminated in this study, we could not completely rule out weak IV bias due to other confounders; therefore, we could not determine with certainty that IVs influenced the expression of CSAG1 through the GMMP and not through other pathways. Second, data from European patients and healthy subjects were used for MR analysis, and the results might not be generalizable to other populations. Fourth, in the study of disease mechanism, both gene expression and gene variation have an important impact on clinical outcomes [53–55], but this analysis was not involved in our study. Finally, the underlying mechanisms of the association between the GMMP and chondrosarcoma are totally unknown, and the role of gut bacteria and CSAG1 needs to be explored further.

In conclusion, by conducting a two-sample MR analysis using publicly available GWAS summary data, we evaluated the causal link between the GMMP and CSAG1 and identified a potential causal pathway for chondrosarcoma carcinogenesis. Although our approach provides strong genetic evidence to support causation, future studies combining in vitro and in vivo experiments will be a necessary step to further validate our findings and elucidate the underlying mechanisms. This study might provide new insights into the mechanisms of gut microbiota-mediated chondrosarcoma development.

Abbreviations

MR	Mendelian randomization
GMMP	Gut microbiota metabolic pathway
CSAG1	Chondrosarcoma associated gene 1
GWAS	Genome-wide association study
IV	Instrumental variable
SNP	Single nucleotide polymorphism
IVW	Inverse variance weighted
WM	Weighted median
LD	Linkage disequilibrium

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-14281-y.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.

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Authors' contributions

S.Y., and X.Y.L.conducted statistical analyses. All authors contributed to the design of the study and wrote the manuscript. All authors reviewed the manuscript.

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Data availability

All GWAS data are available on OpenGWAS (https://gwas.mrcieu.ac.uk/). Other data, including GMMPs and CSAG1, are available in the article/Supplementary Material.

Declarations

Ethics approval and consent to participate

This study only used publicly available data. No original data were collected. Ethical approval for each of the studies included in the investigation can be found in the original publications. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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