scientific reports

Check for updates

OPEN

# The impact of metabolic syndrome on hepatocellular carcinoma: a mendelian randomization study

Chendong Yuan[1,2,5], Xufeng Shu[1,2,5], Xiaoqiang Wang[1,2,5], Wenzheng Chen[1,2], Xin Li[3], Wenguang Pei[1,2], Xujie Su[1,2], Zhenzhen Hu[4]✉ & Zhigang Jie[1,2]✉

**Traditional epidemiological studies are susceptible to confounding factors. To clarify the impact of metabolic syndrome and its diagnostic components on hepatocellular carcinoma, we conducted a preliminary mendelian randomization analysis with metabolic syndrome and its diagnostic components as exposures and hepatocellular carcinoma as the outcome. Another set of genetic data related to hepatocellular carcinoma was used as a validation cohort, repeating the mendelian randomization analysis and combining the two groups for a meta-analysis. Preliminary analysis showed that metabolic syndrome (P-value = 0.002) and waist circumference (P-value = 0.026) are significantly positively correlated with an increased risk of hepatocellular carcinoma. After multiple testing corrections, metabolic syndrome ($P_{FDR}$-value = 0.013) remained significant, although the association between waist circumference ($P_{FDR}$-value = 0.079) and hepatocellular carcinoma was considered suggestive, the meta-analysis further confirmed the impact of metabolic syndrome (P-value = 0.0002) and waist circumference (P-value = 0.0038) in increasing the risk of hepatocellular carcinoma. After adjusting for the genetic predictive effects of all exposures, waist circumference was found to be a key factor significantly influencing the relationship between metabolic syndrome and hepatocellular carcinoma. In summary, our study indicates that metabolic syndrome increases the risk of hepatocellular carcinoma, particularly among individuals with a larger waist circumference.**

**Keywords** Metabolic syndrome, Hepatocellular carcinoma, Waist circumference, Mendelian randomization

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor originating in the liver. Globally, its incidence and prevalence have significantly increased compared to ten years ago, and its mortality rate ranks third among malignant tumors, following only lung cancer and stomach cancer[1,2]. The occurrence of HCC is related to many factors, including hepatitis B and C virus infections, long-term alcohol consumption, aflatoxin exposure, and autoimmune hepatitis. However, about 30% of HCC patients do not have clearly defined risk factors; this group of HCC is termed "cryptogenic liver cancer". Studies indicate that the risk of cryptogenic liver cancer is attributed to metabolic syndrome (MetS)[3].

MetS has now become a global public health issue, particularly in developed countries[4]. According to statistics, the prevalence of MetS in the United States has reached 25%. MetS is a clinical syndrome characterized by the clustering of obesity, dyslipidemia, hyperglycemia, and hypertension. In 2005, the International Diabetes Federation issued a global uniform definition of MetS, which can be diagnosed if any three of the following five diagnostic components are met[5]: (1) central obesity, with waist circumference (WC) as the diagnostic indicator; (2) high triglycerides (TG); (3) low HDL cholesterol (HDL-C); (4) hypertension; (5) abnormal fasting glucose (FG). Recent studies have shown that central obesity and type 2 diabetes can increase the risk of HCC[6]. MetS promotes the development of HCC in the setting of pre-existing liver conditions such as fatty liver disease and cirrhosis, and also in normal livers[7]. However, the connection between MetS and HCC remains controversial; studies have indicated that there is not always a consistent association between them in patients with HCC of different etiologies[8–10].

[1]Medical Innovation Center, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, No. 17, Yongwai Main Street, Nanchang 330006, Jiangxi, China. [2]Department of General Surgery, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330006, Jiangxi, China. [3]Department of Gastroenterology, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330006, Jiangxi, China. [4]Department of Anesthesiology, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, No. 17, Yongwai Main Street, Nanchang 330006, Jiangxi, China. [5]Chendong Yuan, Xufeng Shu and Xiaoqiang Wang contributed equally to this work. ✉email: ndyfyhzz9989@163.com; ndyfy00524@ncu.edu.cn

Mendelian randomization (MR) studies use genetic instrumental variables (IVs) in place of exposures to explore the causal link between exposures and outcomes. Inherent residual confounding and reverse causation problems in traditional epidemiological studies could lead to bias in previous results. Since genes are randomly assigned during meiosis, similar to random assignment in controlled trials, MR can overcome the limitations of traditional epidemiological studies[11]. This study utilizes publicly available genome-wide association study (GWAS) summary data from European populations, employing univariate MR analysis combined with meta-analysis to investigate the causal relationships between MetS, its diagnostic components, and HCC, and using multivariable MR (MVMR) analysis to identify the key diagnostic component influencing the association between MetS and HCC.

## Materials and methods

### Study design

In this study, MetS and its diagnostic components serve as exposures, with HCC as the outcome. We apply MR to investigate the causal interactions between these variables. For genetic IVs to be effective, they must rigorously meet three foundational criteria[12]: (1) a strong association with the exposure, (2) independence from any confounders that may influence both the exposure and the outcome, and (3) no direct relationship with the outcome, influencing it exclusively via the exposure.

In the meanwhile, we adhered to the strengthening the reporting of observational studies in epidemiology-Mendelian randomization (STROBE-MR) guidelines to ensure the integrity and transparency of the Mendelian Randomization study report (Supplementary Table S1). STROBE-MR provides a comprehensive set of reporting standards, ensuring the quality of our research in design, implementation, and analysis.

### Source of genetic instrumental variables

This study selects single nucleotide polymorphisms (SNPs) as genetic IVs, all of which are derived from public GWAS databases. Genetic data related to MetS are sourced from CNCR / CTGlab. Notably, this study stands as the most extensive examination of genetic loci associated with MetS to date. van Walree et al.[13], analyzed data from 461,920 individuals of European ancestry, identifying 235 risk loci linked to MetS. Additionally, GWAS data concerning WC were made available through the IEU Open GWAS Project. Elsworth et al., performed statistical analyses on this dataset, which included 462,166 individuals of European descent, evaluating a total of 9,851,867 SNPs. The genetic data for TG, HDL-C, and hypertension are all sourced from the UK Biobank, with sample sizes ranging from 315,133 to 343,992. The UK Biobank, established as a prospective health database, enrolled around 500,000 participants aged 40 to 69 from 22 assessment centers across the UK during 2006 to 2010. This initiative has compiled comprehensive data encompassing genetics, environment, and health. The GWAS data for FG comes from Chen et al.[14], reported by MAGIC, involving up to 281,416 non-diabetic individuals, 70% of whom are of European descent.

To effectively avoid sample overlap, we used the GWAS summary statistics of the HCC released by FinnGen R10 as the preliminary analysis cohort. This data describes patients with HCC according to ICD-O-3 classification and excludes all cancer patients as controls, with 500 cases and 314,193 controls in the dataset. The genetic data for the replication cohort, examining HCC, were obtained from the GWAS Catalog under accession number GCST90041897. This dataset encompasses 165 cases and 456,111 controls, all of European descent. A comprehensive summary of the data sources is detailed in Table 1.

### Selection of genetic instrumental variables

To meet assumption 1, we identified single nucleotide polymorphisms (SNPs) that demonstrate significant associations with the exposure at a genome-wide significance level (P-value $< 5 \times 10^{-8}$), sourced from the previously mentioned databases. We then used the PLINK clumping method to calculate linkage disequilibrium between SNPs for each exposure based on the 1000 Genomes European cohort, excluding SNPs with linkage disequilibrium (settings: $r^2 > 0.001$, clumping distance = 10,000 kb). To exclude bias due to weak SNPs, we calculated the F statistic, which is generally considered strong enough if F statistic > 10.[15] The formula for calculating it is as follows:

| Phenotypes | Ancestry | Simple size | Institution | PMID | Web sources |
|---|---|---|---|---|---|
| MetS | European | 461,920 | CNCR / CTGlab | 35,983,957 | https://cncr.nl/research/summary_statistics/ |
| WC | European | 462,166 | IEU Open GWAS Project | – | https://gwas.mrcieu.ac.uk/datasets/ukb-b-9405/ |
| TG | European | 343,992 | UK biobank | – | https://www.nealelab.is/uk-biobank |
| HDL-C | European | 315,133 | UK biobank | – | https://www.nealelab.is/uk-biobank |
| Hypertension | European | 361,141 | UK biobank | – | https://www.nealelab.is/uk-biobank |
| FG | European | 281,416 | MAGIC | 34,059,833 | https://magicinvestigators.org/downloads/ |
| HCC | European | 314,693 | FinnGen R10 | – | https://www.finngen.fi/en |
| HCC | European | 456,276 | GWAS Catalog | 34,737,426 | https://www.ebi.ac.uk/gwas/downloads/summary-statistics |

**Table 1**. Overview of GWAS data sources related to phenotypes. *MetS* metabolic syndrome, *WC* waist circumference, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *FG* Fasting glucose, *HCC* hepatocellular carcinoma, *CNCR/CTGlab* center for neurogenomics and cognitive research/complex trait genetics lab, *MAGIC* meta-analyses of glucose and insulin-related traits consortium.

$$PVE = \frac{2 \times \beta^2 \times eaf \times (1 - eaf)}{\beta^2 \times eaf \times (1 - eaf) + 2 \times SE^2 \times N \times eaf \times (1 - eaf)}$$

$$F\ statistic = \frac{(N - 2) \times PVE}{1 - PVE}$$

where PVE represents the proportion of variance explained, β is the effect size of the genetic variation on the phenotype, eaf is the allele frequency, SE is the standard error of the effect size, and N is the sample size of the exposure. Subsequently, we obtained SNPs associated with the outcome, harmonized the exposure and outcome, and excluded all palindromic SNPs. To improve the precision and reliability of estimates, we conducted Radial MR to eliminate potentially biased ineffective SNPs[16]. Moreover, to satisfy assumption 3, we performed Steiger filtering to exclude SNPs that might show incorrect directional effects (i.e., those that could directly affect the outcome, not through the exposure). Simultaneously, we conducted the Steiger test to reject reverse causation and prevent erroneous causal inferences[17].

## Preliminary MR analysis

After the screening steps mentioned above, the final SNPs to be used for MR analysis were determined. In this study, three MR analysis methods were used, including inverse variance weighted (IVW), MR-Egger, and weight median methods. The IVW method is an MR analysis method based on a linear model, assuming all SNPs are valid. It uses the effect size of each individual SNP and its standard error to calculate a composite causal estimate, providing an efficient and statistically powerful estimate[18]. The MR-Egger method is similar to Egger's regression in statistics, introducing an intercept to assess whether there is a direct path from genetic variations to the outcome variable, allowing adjustment for the potential invalidity of SNPs, but its statistical power is generally lower than that of the IVW method[19]. The weight median method has a higher tolerance for SNP invalidity and heterogeneity. If at least half of the SNPs are valid, then the weight median method can provide an accurate and reasonable estimate[20]. Therefore, the IVW method was chosen as the primary analysis method in this study, with a P-value < 0.05 considered significant for causal estimation. In the meanwhile, the false discovery rate based on the Benjamini-Hochberg method was used to correct for multiple testing of the causal estimates between exposure and outcome. A P-value < 0.05 but adjusted P-value > 0.05 after Benjamini-Hochberg adjustment is considered suggestive of causal estimation, while an adjusted P-value < 0.05 is considered significant. Finally, scatter plots and forest plots were used to visualize the results.

## Sensitivity analysis

To meet assumption 2 and ensure the robustness of the MR analysis results, we conducted several sensitivity analyses, including Cochran's Q test, MR-Egger, and MR-PRESSO methods[21]. Cochran's Q test is used in MR analysis to evaluate the heterogeneity among multiple SNPs. If the test shows significant heterogeneity, i.e., P-value < 0.05, an IVW random effects model is used instead of a fixed effect model[22]. As previously mentioned, MR-Egger introduces an intercept to provide a detection of potential pleiotropy, with a P-value < 0.05 indicating the presence of pleiotropy[19,23]. The MR-PRESSO method identifies SNPs that might influence the outcome through confounding or other pathways by analyzing residuals and outliers, thus correcting for heterogeneity and potential bias caused by genetic pleiotropy[24]. Additionally, we used the leave-one-out method and funnel plots to visualize the robustness of the results. The leave-one-out method involves sequentially excluding each SNP and recalculating the causal estimates for the remaining SNPs, revealing whether any single SNP has a significant impact on the overall causal estimate[25]. Finally, to reflect the reliability of the causal estimates, we used an online website (https://shiny.cnsgenomics.com/mRnd/) to calculate the statistical power of the MR analysis, which is highly reliable if it is over 80%.[26,27] Specifically, the calculation of statistical power depends on several key parameters: the Type-I error rate (set at $\alpha = 0.05$), the sample size of the outcome, the proportion of cases in the study, the OR value from IVW method, and the PVE related to the exposure.

## MR replication analysis and meta-analysis

To validate the reliability of the MR analysis for causal estimation, we selected additional genetic data related to the outcome from the GWAS Catalog (Table 1) and repeated the analysis process described above. We then merged the IVW results of the two cohorts using meta-analysis methods, calculating the $I^2$ statistic and P-value to compare the heterogeneity between MR results from different genetic data sources. If $I^2 > 50\%$ and P-value < 0.05, significant heterogeneity is observed, and a random effects model is used; otherwise, a fixed effect model is employed. Finally, meta-analysis results with P-value < 0.05 are considered statistically significant.

## Multivariable MR analysis

To accurately assess the impact of each diagnostic component on the causal estimate between MetS and HCC, we employed MVMR analysis methods to adjust for interactions among other diagnostic components with MetS and potential confounding effects. The main methods included are IVW, MR-Egger, and MR-LASSO, with IVW as the primary analysis method, and P-value < 0.05 is considered to indicate a statistically significant causal relationship. Notably, to avoid multicollinearity caused by exposures, we utilized the MR-LASSO method to eliminate exposure factors in the MVMR analysis that have minimal impact or are statistically insignificant, thereby enhancing the model's predictive accuracy and statistical power[28]. Specifically, the IVW method provides a baseline estimate, the MR-Egger method checks for bias due to direct effects, and LASSO is used to optimize variable selection[28,29]. Forest plots visualize the results.

## Statistical analysis and ethical statement

This study used the "RadialMR", "TwoSampleMR", and "MRPRESSO" packages for MR analysis, the "meta" package for meta-analysis, and the "MendelianRandomization" package for multivariable MR analysis, all within R version 4.1.3.

Finally, this study utilized publicly accessible datasets, and each of the GWAS datasets involved has been approved by the relevant ethical review committees. We strictly adhered to relevant ethical standards to safeguard participants' privacy and the security of the data.

## Results

### MR preliminary analysis

After stringent selection of genetic instrumental variables, we obtained a variable number of SNPs ranging from 54 to 303 (Supplementary Table S2, Supplementary Fig S1). Previously, through Radial MR, we identified and removed between 7 and 16 ineffective SNPs associated with exposure (Supplementary Table S3). Steiger filtering did not identify any SNPs with incorrect directional effects (Supplementary Table S4). All SNPs had an F statistic greater than 10. Subsequent MR analysis using IVW showed a significant association between the genetic susceptibility of MetS (OR [95% CI] = 2.72 [1.44–5.13], P-value = 0.002) and increased risk of HCC, and the weight median method further confirmed this result (OR [95% CI] = 4.39 [1.56–12.35], P-value = 0.005). Similar results were observed for the diagnostic component WC using IVW (OR [95% CI] = 1.82 [1.07–3.08], P-value = 0.026), MR-Egger (OR [95% CI] = 6.30 [1.45–27.37], P-value = 0.015), and weight median methods (OR [95% CI] = 2.43 [1.01–5.80], P-value = 0.046) (Fig. 1). Scatter plots showed consistency in the direction and magnitude of the three MR methods (Fig. 2). Additionally, the Steiger test rejected the reverse causation between MetS and its diagnostic components and HCC (Supplementary Table S5). However, after correction using the Benjamini-Hochberg method, the genetic susceptibility of WC to increased risk of HCC was considered

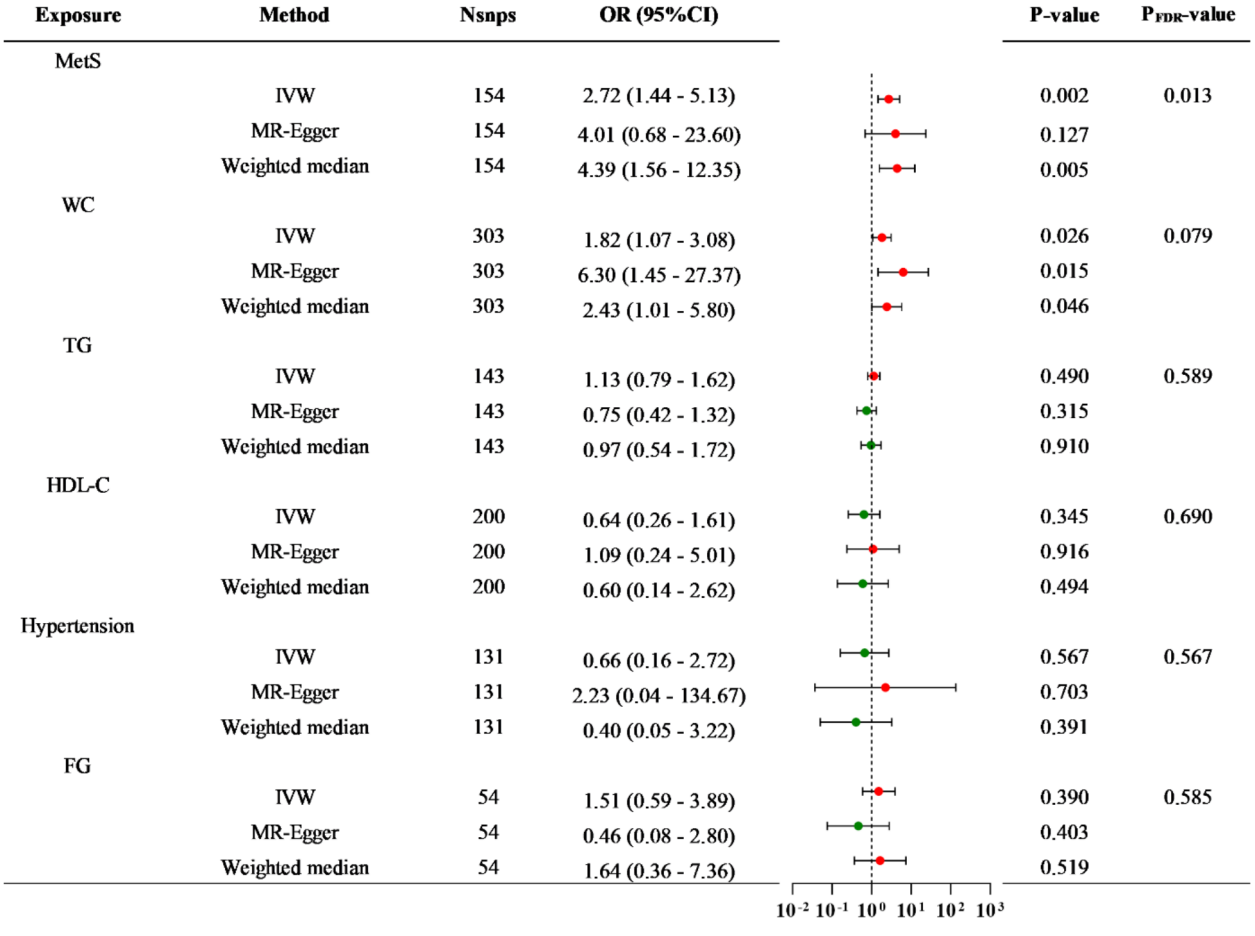


**Fig. 1**. In the preliminary MR analysis, causal estimates were made for metabolic syndrome and its diagnostic components and hepatocellular carcinoma. $P_{FDR}$-value indicates the P-value of the IVW method after correction using the Benjamini-Hochberg method. *MetS* metabolic syndrome, *WC* waist circumference, *TG* triglycerides, *HDL-C* HDL cholesterol, *FG* fasting glucose, *IVW* inverse variance weighted, *Nsnps* number of single nucleotide polymorphisms.

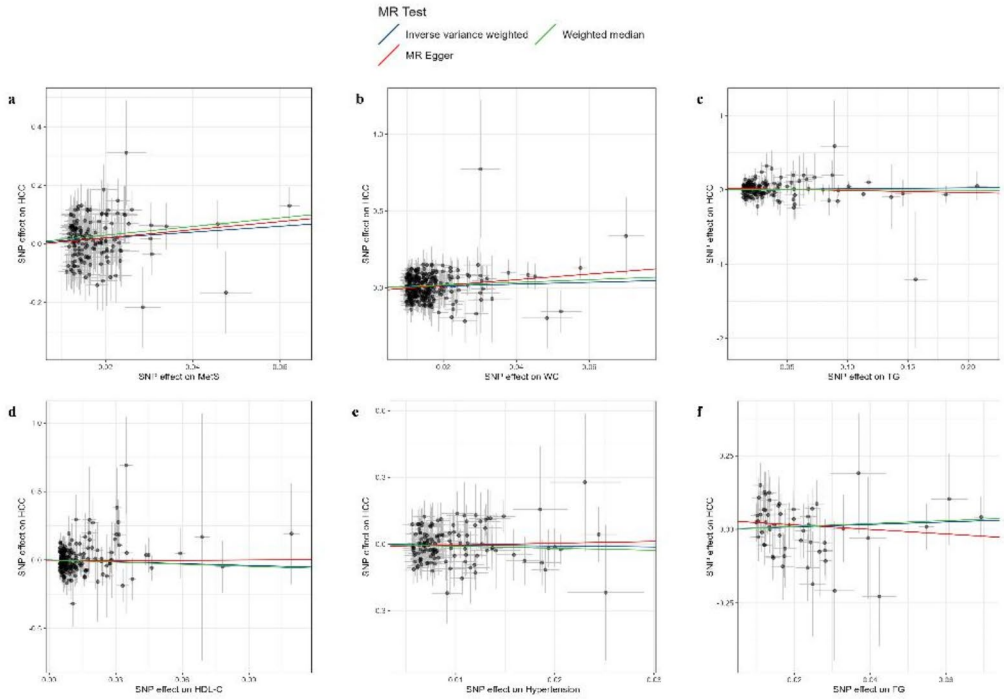


**Fig. 2**. In preliminary MR analysis, (**a**) Scatter plot of metabolic syndrome (MetS) and hepatocellular carcinoma (HCC); (**b**) Scatter plot of waist circumference (WC) and HCC; (**c**) Scatter plot of triglycerides (TG) and HCC; (**d**) Scatter plot of HDL cholesterol (HDL-C) and HCC; (**e**) Scatter plot of hypertension and HCC; (**f**) Scatter plot of fasting glucose (FG) and HCC. *SNP* single nucleotide polymorphism, *MR* Mendelian randomization.

| | Heterogeneity | | | | Pleiotropy | | MR-PRESSO | | |
|---|---|---|---|---|---|---|---|---|---|
| | MR-Egger | | IVW | | MR-Egger | | Global test | | |
| Exposure/outcome | Q | *P*-value | Q | *P*-value | Intercept | *P*-value | RSSobs | *P*-value | Power |
| MetS/HCC | 125.161 | 0.945 | 125.373 | 0.950 | -0.007 | 0.645 | 127.044 | 0.948 | 100% |
| WC/HCC | 253.811 | 0.978 | 256.961 | 0.972 | -0.021 | 0.077 | 258.835 | 0.969 | 95% |
| TG/HCC | 121.235 | 0.884 | 124.661 | 0.849 | 0.020 | 0.066 | 126.531 | 0.853 | 10% |
| HDL-C/HCC | 163.476 | 0.965 | 164.180 | 0.966 | -0.008 | 0.402 | 165.601 | 0.967 | 59% |
| Hypertension/HCC | 96.120 | 0.986 | 96.500 | 0.988 | -0.012 | 0.538 | 99.992 | 0.992 | 20% |
| FG/HCC | 42.901 | 0.812 | 45.205 | 0.768 | 0.031 | 0.135 | 46.382 | 0.792 | 49% |

**Table 2**. Results of sensitivity analysis in preliminary MR analysis. *IVW* inverse variance weighted, *RSSobs* residual sum of squares observed, *MetS* metabolic syndrome, *WC* waist circumference, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *FG* Fasting glucose, *HCC* hepatocellular carcinoma.

suggestive of causal estimation ($P_{FDR}$-value = 0.079), while MetS remained significant ($P_{FDR}$-value = 0.013). No significant causal associations were observed between other diagnostic components and HCC. Notably, only MetS and WC had power values greater than 80%, with all others below 80%, thus not ruling out the risk of Type II error (Table 2). Lastly, in the sensitivity analysis, Cochran's Q test found no heterogeneity, no horizontal pleiotropy was observed in the MR-Egger intercept, and the MR-PRESSO method did not detect any outliers (Table 2). Leave-one-out method confirmed that individual SNPs do not cause bias in MR results (Supplementary Fig S2), and funnel plots demonstrated the robustness of the results (Supplementary Fig S3).

## Replication MR analysis and meta-analysis

To enhance the credibility of the MR results, we validated the MR analysis with another HCC GWAS dataset. As expected, similar trends were observed in the replication cohort (Supplementary Table S6 – S11, Supplementary

Fig S4 – S7). Meta-analysis further confirmed a significant association between the genetic susceptibility of MetS (OR [95% CI] = 2.89 [1.66–5.04], P-value = 0.0002) and increased risk of HCC, with similar results observed for the diagnostic component WC (OR [95% CI] = 1.97 [1.24–3.12], P-value = 0.0038); no significant associations were found between other diagnostic components and HCC (Fig. 3). Although an $I^2$ of 63% was found in the hypertension group, suggesting possible heterogeneity, the P-value was 0.10, indicating that the heterogeneity was not significant. Therefore, we continued to use the fixed effect model.

### Multivariable MR analysis

After adjusting for the interactions between all diagnostic components and MetS, the IVW (OR [95% CI] = 0.13 [$7.84 \times 10^{-4}$ – 23.06], P-value = 0.445), MR-Egger (OR [95% CI] = 0.22 [$1.31 \times 10^{-3}$ – 38.55], P-value = 0.569), and MR-LASSO (OR [95% CI] = 0.28 [$2.73 \times 10^{-3}$ – 29.51], P-value = 0.595) methods all showed that the association between MetS and HCC significantly disappeared (Fig. 4). However, after excluding the diagnostic component WC and adjusting for the interactions of other remaining diagnostic components with MetS, the association between MetS and HCC did not weaken. Specifically, the IVW (OR [95% CI] = 4.41 [1.33–14.66], P-value = 0.015), MR-Egger (OR [95% CI] = 4.80 [1.44–16.02], P-value = 0.011), and MR-LASSO (OR [95% CI] = 3.39 [1.09–10.54], P-value = 0.035) methods all showed consistent results (Fig. 5). These results suggest that WC may be a key factor, significantly influencing the relationship between MetS and HCC.

## Discussion

This study integrates two large-scale GWAS datasets related to HCC and explores the causal impact of MetS and its diagnostic components on HCC through a rigorous MR design. We found that the genetic susceptibility of MetS is associated with an increased risk of HCC. Notably, there is a suggestive association between the genetic prediction level of the diagnostic component WC and HCC. There is no evidence that other diagnostic components, excluding WC, affect the risk of HCC. However, MVMR analysis adjusted findings suggest that WC may significantly influence the association between MetS and HCC. To our knowledge, this is the first study to date to use MR analysis to evaluate the causal relationship between MetS and its diagnostic components and HCC.

In recent years, the incidence of MetS has significantly increased globally. Many studies indicate an epidemiological link between MetS and the risk of HCC. Non-alcoholic fatty liver disease, a manifestation of MetS in the liver, leads to cirrhosis and subsequently promotes the development of HCC[30]. A meta-analysis by Ren, H, et al.[7], demonstrated that MetS significantly increases the risk of HCC (risk ratio: 1.76, 95% CI: 1.33–2.33), consistent with our study results. However, this association remains controversial. For instance, men with MetS have a higher risk of developing HCC, but no similar association is found in women[10]. Research by Yang, DL, et al.[8], indicates that the association between MetS and HCC risk is not always consistent among patients with HCC of different etiologies. We speculate that the observed differences might originate from inherent confounding factors typically encountered in epidemiological study designs. Importantly, MR analysis,

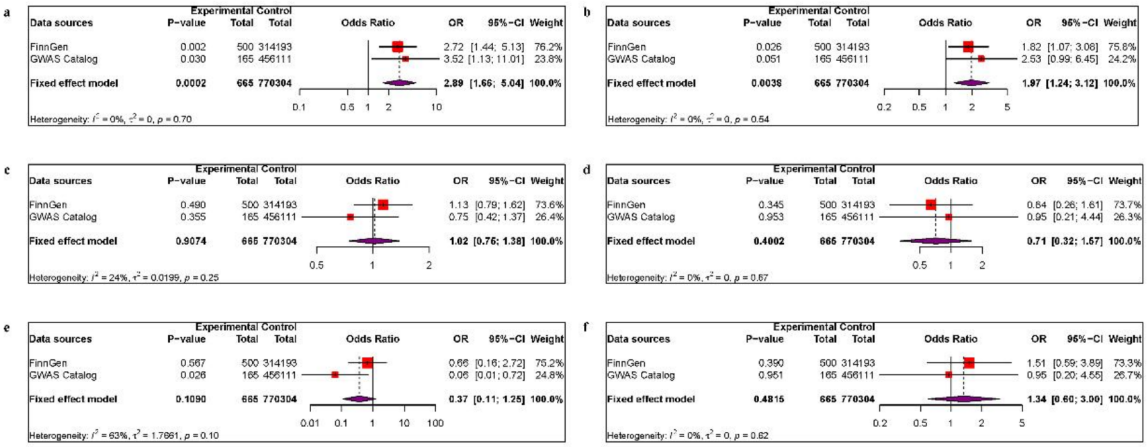


**Fig. 3**. (**a**) Meta-analysis results of metabolic syndrome; (**b**) Meta-analysis results of waist circumference; (**c**) Meta-analysis results of triglycerides; (**d**) Meta-analysis results of HDL cholesterol; (**e**) Meta-analysis results of hypertension; (**f**) Meta-analysis results of fasting glucose.

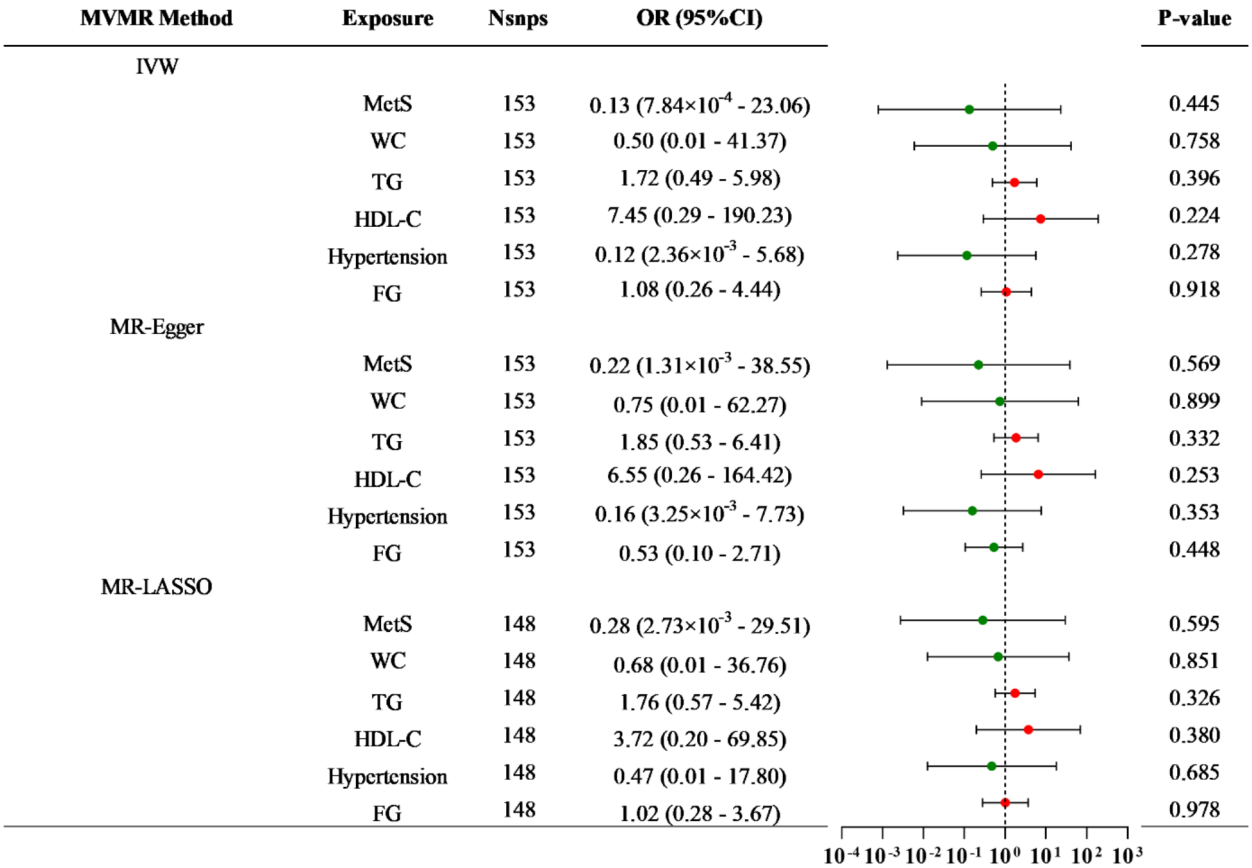


**Fig. 4**. Using multivariable MR analysis for direct causal estimation of genetically predicted metabolic syndrome and its diagnostic components with hepatocellular carcinoma. *MetS* metabolic syndrome, *WC* waist circumference, *TG* triglycerides, *HDL-C* HDL cholesterol, *FG* fasting glocuse, *IVW* inverse variance weighted, *MVMR* multivariable MR, *Nsnps* number of single nucleotide polymorphisms.

which utilizes genetic variants as instruments assigned at conception, substantially mitigates the effects of these confounders, thereby bolstering the reliability of our findings.

Furthermore, after correction with the Benjamini-Hochberg method, the association between WC and HCC risk was suggestive. The meta-analysis results, however, confirmed a significant association between WC and HCC risk. In the subsequent MVMR analysis, adjusting for the interaction of WC with MetS caused the association between MetS and HCC to disappear, suggesting that WC is a key factor contributing to the increased risk of HCC associated with MetS. WC, as a reliable indicator of central obesity and visceral fat[31], is associated with an increased risk of cancers of the digestive system, particularly HCC, as reported in different populations and regions[32]. Central obesity is also associated with genetic predispositions affecting HCC risk; for instance, gene variants related to lipid metabolism can influence the severity of liver damage in obese individuals, increasing the risk of HCC[33]. However, the exact mechanisms linking central obesity with HCC risk are not yet clear. Research indicates that excessive visceral fat, especially abdominal fat, leads to metabolic disorders such as increased free fatty acids, which in turn induce lipotoxicity, oxidative stress, and endoplasmic reticulum stress, further promoting liver damage and fibrosis, a key precursor to HCC[34]. Accumulation of abdominal fat affects various hormones and signaling pathways, including IGF, mTOR, NF-kB, and MAPK, which are involved in the progression of HCC[35]. These findings suggest that controlling obesity, especially central obesity, may be a key factor in reducing the risk of HCC.

This study found no evidence of an association between HCC and the remaining diagnostic components of MetS, apart from WC, but the statistical power of these findings is below 80%, which means we cannot dismiss the risk of false negatives. Lipid metabolism disorder is a crucial component of MetS, and there are few studies specifically addressing the correlation between hyperlipidemia and HCC. Kitahara et al.[36], have found that the risk of HCC decreases with increasing serum cholesterol levels, and that reduced high-density lipoprotein is an independent risk factor for the occurrence of HCC. It is noteworthy that lipid metabolism abnormalities, such as high triglycerides and low high-density lipoprotein, are often associated with obesity, particularly central obesity[37]. Lipid metabolic reprogramming, including changes in lipid synthesis, β-oxidation, and intracellular lipid components, is associated with the occurrence and progression of HCC[38,39]. Studies have confirmed that

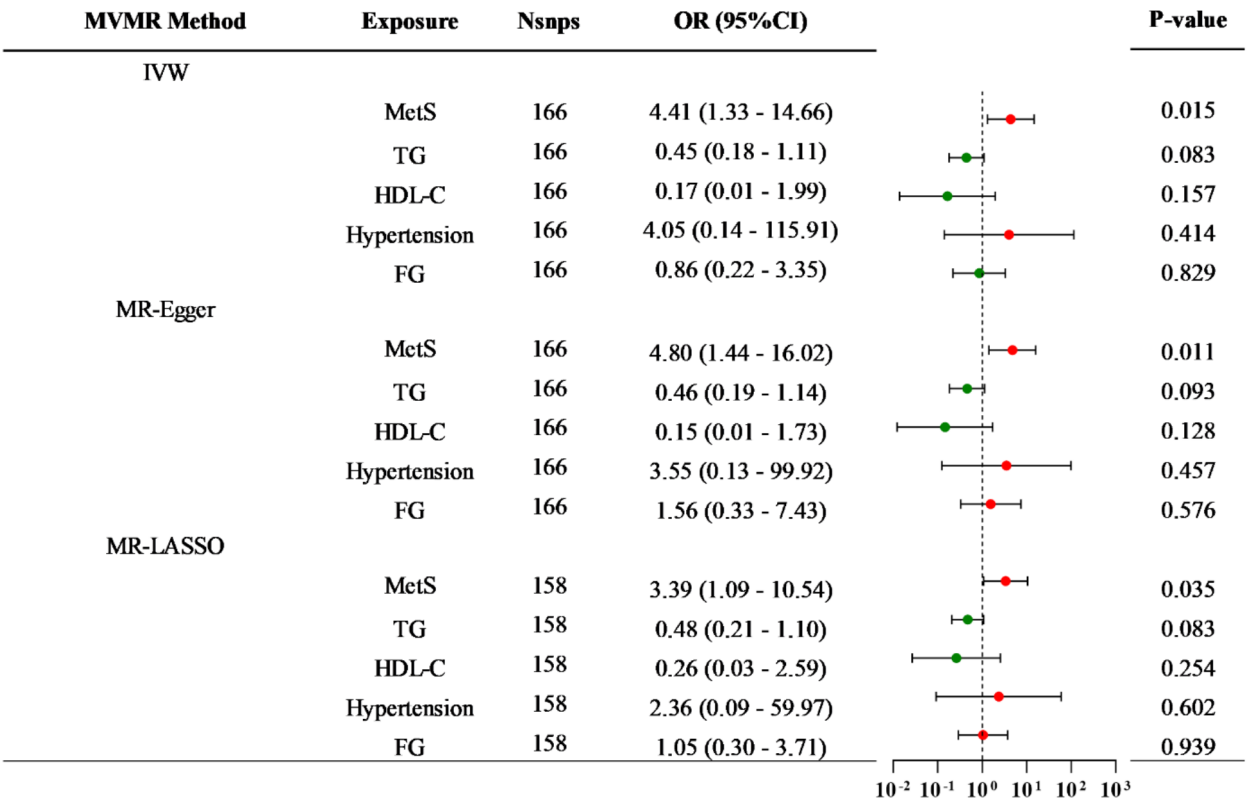


**Fig. 5**. After excluding waist circumference, direct causal estimation of genetically predicted metabolic syndrome and its remaining diagnostic components with hepatocellular carcinoma using multivariable MR analysis. *MetS* metabolic syndrome, *TG* triglycerides, *HDL-C* HDL cholesterol, *FG* fasting glocuse, *IVW* inverse variance weighted, *MVMR* multivariable MR, *Nsnps* number of single nucleotide polymorphisms.

hypertension is closely related to tumors, but there are limited reports regarding its association with HCC. An analysis based on the SEER database by Welzel TM et al.[40], reviewing clinical data of HCC patients from 1993 to 2005, identified hypertension as a potential risk factor for HCC, but a further study from India did not find a correlation between them[41]. Some studies have confirmed that diabetes is an independent risk factor for HCC. However, in recent years, insulin resistance has been considered a core component of MetS, and an increase in visceral fat exacerbates insulin resistance[42]. Insulin resistance can lead to hyperinsulinemia, activating the ERK/PI3K pathways that promote mitosis and inhibit apoptosis, thereby indirectly enhancing IGF expression, which promotes cell migration and proliferation[43]. Current MR studies have demonstrated that elevated fasting insulin levels are associated with an increased risk of HCC, a connection that is more significant than elevated fasting glucose levels or type 2 diabetes[44]. Furthermore, studies suggest that WC, in conjunction with other components of MetS such as hyperglycemia, hypertension, and dyslipidemia, acts through multiple metabolic and inflammatory pathways to affect liver health. Therefore, we infer that WC may play a key role in the relationship between MetS, its diagnostic components, and HCC.

However, our study still has certain limitations. Firstly, our GWAS data are sourced from individuals of European descent, which limits the generalizability of our findings to other ethnic groups. Secondly, the GWAS data we used do not allow for stratified analysis, such as by gender differences, subtypes of HCC, etc. Future subgroup analyses should be conducted to further explore the causal relationship between MetS and HCC. Additionally, we observed that in the preliminary MR analysis, the significance of WC disappeared after FDR correction, likely due to the conservative nature of the correction method, which reduces false positives but increases the risk of false negatives, especially in cases of multiple comparisons. However, the meta-analysis, by incorporating additional datasets and increasing statistical power, restored the significance of WC and provided stronger evidence for its causal role in HCC. As mentioned previously, we did not observe an association between HCC and the remaining diagnostic components of MetS, excluding WC, but the statistical power of these findings is below 80%, which does not allow us to reject the risk of false negatives. This limitation may be attributed to several factors, including the relatively small number of genetic variants available for these components, the potential for weaker genetic instruments to explain less phenotypic variance, and insufficient sample sizes in the GWAS datasets used for these components. Additionally, the inherent variability in the genetic architecture of different diagnostic components may reduce the precision of causal estimates. Therefore,

caution should be exercised in interpreting some of our results, and further studies with larger sample sizes and stronger instruments are warranted to validate these findings. Finally, our research may exhibit a certain level of survivorship bias. Given the delayed onset of HCC, the publicly accessible GWAS datasets might exclude individuals who passed away or remained undiagnosed throughout the duration of the study, thus potentially leading to bias in the genetic association estimates. By conducting meta-analyses summarizing multiple datasets, we provide more stable estimates and minimize the impact of survivorship bias as much as possible.

## Conclusion

In summary, this study provides reliable evidence that MetS and WC are associated with an increased risk of HCC, with WC potentially being a key factor driving the heightened HCC risk associated with MetS. Early identification and management of MetS, particularly in patients with larger WC, could inform targeted prevention strategies and improve patient outcomes. Future research should investigate the efficacy of targeted interventions, such as weight control or metabolic therapy, in reducing HCC risk, and validate these findings across diverse populations. Moreover, incorporating these insights into public health policies may help reduce the global burden of HCC by promoting early risk detection and prevention efforts.

## Data availability

In the "Source of genetic instrumental variables" section (Table 1) of our Materials and Methods, we have indicated the sources of all original data. If needed, please contact the original authors to obtain it. The results of this study can be obtained by contacting the corresponding author.

Received: 12 August 2024; Accepted: 9 January 2025
Published online: 14 January 2025

## References

1. Bray, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Cancer J. Clin.* **68**, 394–424. https://doi.org/10.3322/caac.21492 (2018).
2. Ferlay, J. et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int. J. Cancer*. **144**, 1941–1953. https://doi.org/10.1002/ijc.31937 (2019).
3. Siegel, A. B. & Zhu, A. X. Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. *Cancer* **115**, 5651–5661. https://doi.org/10.1002/cncr.24687 (2009).
4. Lemieux, I. & Després, J. P. Metabolic Syndrome: Past, Present and Future. *Nutrients* 12. (2020). https://doi.org/10.3390/nu12113501
5. Zimmet, P., Magliano, D., Matsuzawa, Y., Alberti, G. & Shaw, J. The metabolic syndrome: a global public health problem and a new definition. *J. Atheroscler. Thromb.* **12**, 295–300 (2005).
6. Sohn, W. et al. Obesity and the risk of primary liver cancer: a systematic review and meta-analysis. *Clin. Mol. Hepatol.* **27**, 157–174. https://doi.org/10.3350/cmh.2020.0176 (2021).
7. Ren, H., Wang, J., Gao, Y., Yang, F. & Huang, W. Metabolic syndrome and liver-related events: a systematic review and meta-analysis. *BMC Endocr. Disord.* **19**, 40. https://doi.org/10.1186/s12902-019-0366-3 (2019).
8. Yang, D. L. et al. Prevalence of metabolic syndrome among patients with hepatocellular carcinoma of different etiologies: a retrospective study. *Infect. Agent Cancer*. **19**, 21. https://doi.org/10.1186/s13027-024-00575-6 (2024).
9. Cho, Y. et al. The importance of metabolic syndrome status for the risk of non-viral Hepatocellular Carcinoma: a Nationwide Population-based study. *Front. Oncol.* **12**, 863352. https://doi.org/10.3389/fonc.2022.863352 (2022).
10. Xia, B. et al. Metabolic syndrome and its component traits present gender-specific association with liver cancer risk: a prospective cohort study. *BMC Cancer*. **21**, 1084. https://doi.org/10.1186/s12885-021-08760-1 (2021).
11. Richmond, R. C. & Davey Smith, G. Mendelian randomization: concepts and scope. *Cold Spring Harb Perspect. Med.* **12** https://doi.org/10.1101/cshperspect.a040501 (2022).
12. Boef, A. G. C., Dekkers, O. M. & le Cessie, S. Mendelian randomization studies: a review of the approaches used and the quality of reporting. *Int. J. Epidemiol.* **44**, 496–511. https://doi.org/10.1093/ije/dyv071 (2015).
13. van Walree, E. S. et al. Disentangling genetic risks for metabolic syndrome. *Diabetes* **71**, 2447–2457. https://doi.org/10.2337/db22-0478 (2022).
14. Chen, J. et al. The trans-ancestral genomic architecture of glycemic traits. *Nat. Genet.* **53**, 840–860. https://doi.org/10.1038/s41588-021-00852-9 (2021).
15. Pierce, B. L., Ahsan, H. & Vanderweele, T. J. Power and instrument strength requirements for mendelian randomization studies using multiple genetic variants. *Int. J. Epidemiol.* **40**, 740–752. https://doi.org/10.1093/ije/dyq151 (2011).
16. Bowden, J. et al. Improving the visualization, interpretation and analysis of two-sample summary data mendelian randomization via the Radial plot and radial regression. *Int. J. Epidemiol.* **47**, 2100. https://doi.org/10.1093/ije/dyy265 (2018).
17. Hemani, G., Tilling, K. & Davey Smith, G. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Genet.* **13**, e1007081. https://doi.org/10.1371/journal.pgen.1007081 (2017).
18. Hemani, G. et al. The MR-Base platform supports systematic causal inference across the human phenome. *Elife* **7** https://doi.org/10.7554/eLife.34408 (2018).
19. Bowden, J., Davey Smith, G. & Burgess, S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int. J. Epidemiol.* **44**, 512–525. https://doi.org/10.1093/ije/dyv080 (2015).
20. Pierce, B. L. & Burgess, S. Efficient design for mendelian randomization studies: subsample and 2-sample instrumental variable estimators. *Am. J. Epidemiol.* **178**, 1177–1184. https://doi.org/10.1093/aje/kwt084 (2013).
21. Yuan, C., Shu, X., Hu, Z. & Jie, Z. Genetic prediction of the relationship between metabolic syndrome and colorectal cancer risk: a mendelian randomization study. *Diabetol. Metab. Syndr.* **16**, 109. https://doi.org/10.1186/s13098-024-01351-7 (2024).
22. Cohen, J. F. et al. Cochran's Q test was useful to assess heterogeneity in likelihood ratios in studies of diagnostic accuracy. *J. Clin. Epidemiol.* **68**, 299–306. https://doi.org/10.1016/j.jclinepi.2014.09.005 (2015).
23. Burgess, S. & Thompson, S. G. Interpreting findings from mendelian randomization using the MR-Egger method. *Eur. J. Epidemiol.* **32**, 377–389. https://doi.org/10.1007/s10654-017-0255-x (2017).
24. Verbanck, M., Chen, C. Y., Neale, B. & Do, R. Detection of widespread horizontal pleiotropy in causal relationships inferred from mendelian randomization between complex traits and diseases. *Nat. Genet.* **50**, 693–698. https://doi.org/10.1038/s41588-018-0099-7 (2018).
25. Cheng, H., Garrick, D. J. & Fernando, R. L. Efficient strategies for leave-one-out cross validation for genomic best linear unbiased prediction. *J. Anim. Sci. Biotechnol.* **8**, 38. https://doi.org/10.1186/s40104-017-0164-6 (2017).

26. Brion, M. J. A., Shakhbazov, K. & Visscher, P. M. Calculating statistical power in mendelian randomization studies. *Int. J. Epidemiol.* **42**, 1497–1501. https://doi.org/10.1093/ije/dyt179 (2013).
27. Burgess, S. Sample size and power calculations in mendelian randomization with a single instrumental variable and a binary outcome. *Int. J. Epidemiol.* **43**, 922–929. https://doi.org/10.1093/ije/dyu005 (2014).
28. Grant, A. J. & Burgess, S. Pleiotropy robust methods for multivariable mendelian randomization. *Stat. Med.* **40**, 5813–5830. https://doi.org/10.1002/sim.9156 (2021).
29. Burgess, S. & Thompson, S. G. Multivariable mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. *Am. J. Epidemiol.* **181**, 251–260. https://doi.org/10.1093/aje/kwu283 (2015).
30. Rahman, R., Hammoud, G. M., Almashhrawi, A. A., Ahmed, K. T. & Ibdah, J. A. Primary hepatocellular carcinoma and metabolic syndrome: an update. *World J. Gastrointest. Oncol.* **5**, 186–194. https://doi.org/10.4251/wjgo.v5.i9.186 (2013).
31. Després, J. P. & Lemieux, I. Prud'homme, D. Treatment of obesity: need to focus on high risk abdominally obese patients. *BMJ* **322**, 716–720 (2001).
32. Li, X. et al. Abdominal obesity and digestive system cancer: a systematic review and meta-analysis of prospective studies. *BMC Public. Health*. **23**, 2343. https://doi.org/10.1186/s12889-023-17275-2 (2023).
33. Reeves, H. L., Zaki, M. Y. W. & Day, C. P. Hepatocellular Carcinoma in Obesity, type 2 diabetes, and NAFLD. *Dig. Dis. Sci.* **61**, 1234–1245. https://doi.org/10.1007/s10620-016-4085-6 (2016).
34. Rajesh, Y. & Sarkar, D. Molecular mechanisms regulating obesity-Associated Hepatocellular Carcinoma. *Cancers (Basel)*. **12** https://doi.org/10.3390/cancers12051290 (2020).
35. Chen, Y., Wang, W., Morgan, M. P., Robson, T. & Annett, S. Obesity, non-alcoholic fatty liver disease and hepatocellular carcinoma: current status and therapeutic targets. *Front. Endocrinol. (Lausanne)*. **14**, 1148934. https://doi.org/10.3389/fendo.2023.1148934 (2023).
36. Kitahara, C. M. et al. Total cholesterol and cancer risk in a large prospective study in Korea. *J. Clin. Oncol.* **29**, 1592–1598. https://doi.org/10.1200/JCO.2010.31.5200 (2011).
37. Orabi, D., Berger, N. A. & Brown, J. M. Abnormal metabolism in the progression of nonalcoholic fatty liver disease to Hepatocellular Carcinoma: mechanistic insights to Chemoprevention. *Cancers (Basel)*. 13. https://doi.org/10.3390/cancers13143473 (2021).
38. Cheng, Y., He, J., Zuo, B. & He, Y. Role of lipid metabolism in hepatocellular carcinoma. *Discov Oncol.* **15**, 206. https://doi.org/10.1007/s12672-024-01069-y (2024).
39. Sangineto, M. et al. Lipid metabolism in Development and Progression of Hepatocellular Carcinoma. *Cancers (Basel)*. **12** https://doi.org/10.3390/cancers12061419 (2020).
40. Welzel, T. M. et al. Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the SEER-Medicare database. *Hepatology* **54**, 463–471. https://doi.org/10.1002/hep.24397 (2011).
41. Turati, F. et al. Metabolic syndrome and hepatocellular carcinoma risk. *Br. J. Cancer*. **108**, 222–228. https://doi.org/10.1038/bjc.2012.492 (2013).
42. Gautier, A. et al. Increases in waist circumference and weight as predictors of type 2 diabetes in individuals with impaired fasting glucose: influence of baseline BMI: data from the DESIR study. *Diabetes Care*. **33**, 1850–1852. https://doi.org/10.2337/dc10-0368 (2010).
43. Jia, G. & Sowers, J. R. Targeting CITED2 for angiogenesis in obesity and insulin resistance. *Diabetes* **65**, 3535–3536 (2016).
44. Yuan, S. et al. Is type 2 diabetes causally Associated with Cancer Risk? Evidence from a two-sample mendelian randomization study. *Diabetes* **69**, 1588–1596. https://doi.org/10.2337/db20-0084 (2020).


## Acknowledgements

We thank the participants and researchers from CNCR/CTGlab, IEU Open GWAS Project, UK Biobank, MAGIC, FinnGen, and GWAS Catalog for their contributions to this study. We are grateful to Dr. Lu Yuan and Dr. Jim Zhong from the University of Leeds for their revisions to the manuscript.

## Author contributions

C.Y., X.S., X.W., W.C., X.L., W.P., X.S., Z.H., and Z.J. were involved in the conception and design of the study. Material preparation, data collection, and analysis were performed by C.Y., Z.H., X.W., and X.S. The first draft of the manuscript was written by C.Y. All authors commented on and revised the manuscript. C.Y., X.S., X.W., W.C., X.L., W.P., X.S., Z.H., and Z.J. have read and approved the final manuscript.

## Funding

This study was supported by grants from the Ganpo Talent 555 Project of Jiangxi Province, Jiangxi Provincial Health Technology Project (Grant No. 202310020) and the First Affiliated Hospital of Nanchang University Young Talent Research and Cultivation Project (Grant No. YFYPY202272).

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-86317-z.

**Correspondence** and requests for materials should be addressed to Z.H. or Z.J.