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ORIGINAL RESEARCH

Genetic Variants of *UGP2* and *FBP2* in the Glycolysis Pathway Independently Predict Survival of Patients with HBV-Related Hepatocellular Carcinoma

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Purpose: Glycolysis is a group of metabolic processes that may alter tumor microenvironment to have effects on the growth and proliferation of tumor cells, including liver cancer. However, the effect of genetic variants in glycolysis pathway genes in survival of patients with hepatitis B virus-related hepatocellular carcinoma (HBV-HCC) remains unclear.

Methods: We employed multivariable Cox proportional hazards regression analyses to estimate associations between genetic variants in 240 glycolysis pathway genes and overall survival (OS) of 866 patients with HBV-HCC, and we also used false positive report probability for multiple testing corrections.

Results: We found that *UGP2* rs4293553 G allele was significantly associated with a better OS of HBV-HCC patients [hazards ratio (HR) = 0.73, 95% confidence interval (CI) = 0.62–0.86, $P < 0.001$], and that *FBP2* rs635087 G allele was significantly associated with a worse OS in these patients (HR = 1.38, 95% CI = 1.18–1.61, $P < 0.001$). The expression quantitative trait loci analysis using the GTEx database showed that the rs635087 G allele was significantly correlated with reduced *FBP2* mRNA expression levels in normal liver tissues ($P < 0.001$), but such a correlation was not significant for the rs4293553 G allele. Functional annotation results indicate that these two single nucleotide polymorphisms have potential biological functions, providing biological plausibility for the observed associations. In addition, the mRNA expression levels of both *UGP2* and *FBP2* were significantly lower in HCC tissues than in normal liver tissues (both $P < 0.001$), and high expression levels of both *UGP2* and *FBP2* were significantly associated with favorable survival in HCC patients (both $P < 0.001$).

Discussion: Our findings suggested that genetic variants in glycolysis pathway genes may serve as novel prognostic markers for survival of patients with HBV-HCC, especially *FBP2* rs635087, if validated in additional larger studies and functional investigations.

Keywords: glycolysis, hepatitis B virus, hepatocellular carcinoma, single nucleotide polymorphism, over survival

Introduction

Primary liver cancer has become a global health challenge, with more than 8.65 million new cases and 7.58 million deaths worldwide in 2022.[1] Hepatocellular carcinoma (HCC) is the predominant form of primary liver cancer, represents

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75–85% of all the cases.[2] In China, hepatitis B virus (HBV) infection is a significant risk factor, contributing to 84.4% of HCC cases.[3] Although recent improvement in diagnostic and therapeutic measures for HCC, many patients are still diagnosed in the mid- to late-stages,[4] leading to a 5-year survival rate of only 12.1% in China.[5] Indeed, traditional prognostic assessment methods are mainly based on clinical phenotypes, such as alpha-fetoprotein (AFP) levels and tumor stage; however, these metrics usually do not fully reflect the prognostic differences in HCC patients due to the heterogeneity of individual responses and the key role of genetic factors in patient outcomes. Therefore, it is urgent need to develop more biomarkers that help identify those patients with a poor survival so that more appropriate personalized treatment plans could be provided.

Single nucleotide polymorphisms (SNPs) are one of the most common types of genetic variants, some of which are capable of regulating the expression of corresponding genes. Because of the high genetic stability and non-invasive detection of SNPs,[6] more and more studies have indicated that SNPs may serve as potential biomarkers for predicting the risk and prognosis of cancers, including HCC.[7–10] In recent years, the hypothesis-based pathway and functional analysis methods have been widely employed to explore susceptibility loci and survival predictors,[11,12] which may not only improve the study power to effectively identify genetic loci but also take into account their potential biological functions in understanding the disease course.

Glycolysis is a continuous breakdown of glucose processes under anaerobic conditions,[13] which is also an essential pathway for cancers to acquire energy.[14] Most cancer cells have an enhanced glycolysis by promoting glucose uptake and lactate production to meet their energy needs,[15] and increasing evidence suggests that alterations in the glycolysis processes may cause tumor progression.[16] Indeed, studies have reported that an enhanced glycolysis is present in HCC,[17,18] potentially through the promotion of macromolecule synthesis and the alteration of microenvironment during cancer cell proliferation.[19]

To date, although a number of glycolysis-related genes have been found to be strongly associated with HCC survival, the effect of SNPs on patient survival remains largely unknown.[20] Hence, we aimed to identify associations between genetic variants in glycolysis pathway genes and survival of HBV-HCC patients.

Materials and Methods

Study Populations

A total of 866 patients who were HBV surface antigen-positive and underwent hepatectomy were recruited from the Guangxi Medical University Cancer Hospital between 2007 and 2017; Details of study populations have been elucidated in previous publications.[21,22] Briefly, we collected a 5-mL blood sample from each patient to extract DNA for genotyping. Genotyping was performed by using the Illumina Infinium® Global Screening Assay genotyping chip (GSA, GSAMD-24v1-0, Illumina, San Diego) at Genenergy Biotechnology (Shanghai, China) using the Illumina iScan System. Then, we further imputed the genotyping data in the 1000 Genomes Project. Finally, the genotyping data of these 866 patients were merged into a combined dataset and randomly dichotomized into a discovery dataset (n = 433) and a replication dataset (n = 433). Additionally, demographic and clinical information of the patients, including age, sex, smoking status, drinking status, AFP level, cirrhosis, embolus, and Barcelona Clinic Liver Cancer (BCLC) stage was collected and used as covariables in further multivariant analyses.

Postoperative overall survival (OS) we defined as the endpoint, and the last follow-up censored in March 2020. The present study was conducted with the approval of the Institutional Review Board of Guangxi Medical University Cancer Hospital (Approval Number: LW2023138), and each participant signed an informed consent form to allow the use of their biological samples and clinical data in the future research.

Selection of Candidate Genes and SNPs

We retrieved glycolysis pathway genes in the Molecular Signatures database (http://www.gsea-msigdb.org/gsea/msigdb/search.jsp) by using "glycolysis" as a keyword. A total of 240 candidate genes were ultimately retained for further analyses after excluding 18 duplicates, five pseudogenes, and 12 genes on X chromosome (Table S1). All SNPs in these candidate genes and their ±2 kb flanking regions were extracted to cover the promoter region according to the following quality control

criteria: a genotyping rate ≥95%, a minimum allele frequency (MAF) ≥0.05, and the Hardy-Weinberg equilibrium (HWE) P value $\geq 1\times10^{-6}$.

Functional Annotation

Four online bioinformatics tools, ie, RegulomeDB,[23] HaploReg v4.2,[24] SNPinfo,[25] and Encyclopedia of DNA Elements (ENCODE) project,[26] were utilized to predict potential biological functions of the identified statistically significant SNPs. Expression quantitative trait locus (eQTL) analyses were performed to identify the correlation between genotypes of SNPs and mRNA expression levels of the corresponding genes using data of 208 normal liver tissues from the Genotype-Tissue Expression Project (GTEx) database[27] and lymphoblastoid cell lines from 76 Chinese Han Beijing population included in the 1000 Genomes Project.[28] Subsequently, we performed differential expression analysis of genes in HCC and normal liver tissues using TNMplot[29] database and in-house RNA sequencing data. In addition, the available online database Kaplan-Meier plotter[30] was utilized to assess the associations between mRNA expression levels of genes and OS of HBV-HCC patients, and LocusZoom (http://locuszoom.org/)[31] was used to construct regional association plots. Finally, the public database of the cBioPortal for Cancer Genomics (http://www.cbioportal.org/) was used to evaluate the somatic mutation status of the identified genes in HCC tissues.

Statistical Analysis

We utilized multivariable Cox proportional hazards regression analyses with adjustment for the covariables as previously defined to assess associations between SNPs in the glycolysis pathway genes and HBV-HCC OS. Considering the high linkage disequilibrium (LD) between most of the imputed SNPs, false positive report probability (FPRP) with a priori probability of 0.10 and a hazards ratio (HR) of 1.5 was performed to reduce false-positive results,[32] and only SNPs with FPRP < 0.20 in both the discovery and replication datasets were included for further analyses. To identify independent SNPs, we performed multivariable stepwise Cox regression analyses of the validated SNPs with adjustment for the covariables. We then estimated the joint effects of risk genotypes of the identified SNPs on survival of HBV-HCC patients and showed the results with Kaplan-Meier curves. In addition, we performed stratified analysis to determine whether the effect of the combined risk genotypes on HBV-HCC OS was influenced by the covariables. At last, we assessed the ability of prediction models that included both covariables and risk genotypes to predict OS of patients with HBV-HCC by the area under the curve (AUC) of the receiver operating characteristic (ROC) curves.

All statistical analyses were performed in R software (versions 4.0.3 and 4.2.2) and $P < 0.05$ was considered statistical significance.

Results

Associations of SNPs in the Glycolysis Pathway Genes with HBV-HCC OS

The flow of the study is depicted in Figure 1, and the associations of covariables with OS of HBV-HCC patients are summarized in Table S2. In total, we extracted 24069 SNPs (including 1133 genotyped and 23936 imputed) from the 240 glycolysis pathway genes in the discovery dataset by performing multivariable Cox regression analyses in additive models with multiple test correction. We found that 361 SNPs were significantly associated with OS of patients with HBV-HCC ($P < 0.05$, FPRP < 0.20). As shown in Table S3, after further validation of these 361 SNPs in the replication dataset, three SNPs (*UGP2* rs4293553, *FBP2* rs2679604, and *FBP2* rs635087) remained significant difference ($P < 0.05$, FPRP < 0.20), with the *UGP2* rs4293553 G allele being associated with a better OS of HBV-HCC patients ($P < 0.001$ in the combined dataset) and both *FBP2* rs2679604 A and rs635087 G alleles being associated with a poorer OS in HBV-HCC patients (both $P < 0.001$ in the combined dataset). The results are also visualized in Figure S1.

Identification of Independent SNPs and Genetic Models Analyses

To determine whether identified SNPs were independent predictors of HBV-HCC OS, multivariable stepwise Cox regression analyses adjusting for the covariables were performed in the combined dataset. We found that *UGP2* rs4293553 A > G (HR = 0.72, 95% CI = 0.61–0.85, $P < 0.001$) and *FBP2* rs635087 A > G (HR = 1.40, 95% CI = 1.19–1.64, $P < 0.001$)

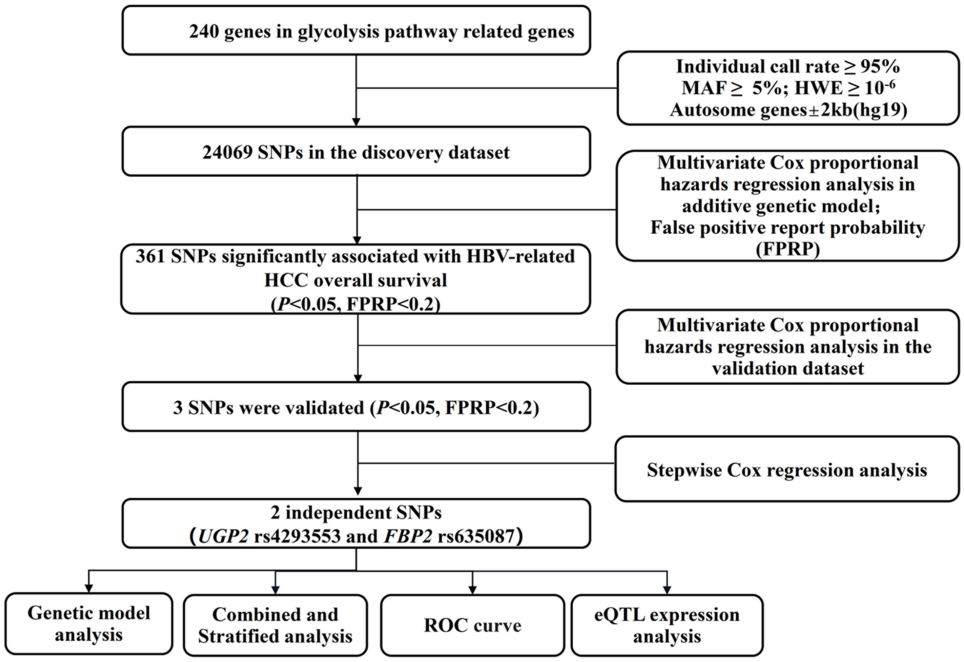

Figure 1 Workflow of the study process.
Abbreviations: MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism; FPRP, false positive report probability; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; ROC, receiver operating characteristic; eQTL, expression quantitative trait loci.

were significantly associated with a better or worse OS of patients with HBV-HCC, respectively (Table 1). The LD among the SNPs of these two genes and their flanking 50-kb regional SNPs are illustrated in the regional association plots (Figure S2).

The results of multivariable Cox regression analyses of two independent SNPs in different genetic models showed that the *UGP2* rs4293553 G allele was associated with a better HBV-HCC OS (P_{trend}< 0.001) and the *FBP2* rs635087 G allele was associated with a worse HBV-HCC OS (P_{trend}< 0. 001) in an additive genetic model. Furthermore, both *UGP2* rs4293553 AA and *FBP2* rs635087 AG+GG genotypes predicted a poor HBV-HCC OS in a dominant model (Table 2).

Table 1 Two Independent Predictors of OS Obtained from Stepwise Cox Regression Analysis in the Combine Dataset

Characteristics	Category	Frequency	HR (95% CI)	P[a]
Age	≤47	434	1.00	
	>47	432	0.82 (0.67–1.00)	0.045
Sex	Female	106	1.00	
	Male	760	1.34 (0.97–1.84)	0.080
AFP level (ng/mL)	≤400	522	1.00	
	>400	344	1.34 (1.09–1.64)	0.005
Embolus	NO	636	1.00	
	Yes	230	1.77 (1.39–2.24)	<0.001
BCLC stage	0/A	427	1.00	
	B/C	439	2.02 (1.59–2.56)	<0.001
UGP2 rs4293553 A > G[b]	AA/AG/GG	466/339/61	0.72 (0.61–0.85)	<0.001
FBP2 rs635087 A > G[b]	AA/AG/GG	479/339/48	1.40 (1.19–1.64)	<0.001

Notes: [a]Multivariate Cox proportional hazards regression analysis was adjusted for age, sex, smoking status, drinking status, AFP level, cirrhosis, embolus and BCLC stage. [b]Additive model.
Abbreviations: OS, overall survival; HR, hazard ratio; CI, confidence interval; AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer.

Table 2 Associations between Two Functional SNPs and OS of HBV-Related HCC in the Discovery, Validation and Combined Dataset

Genotype	Discovery Dataset (n = 433)				Validation Dataset (n = 433)				Combined Dataset (n = 866)			
	All	Death (%)	HR (95% CI)	*P*[a]	All	Death (%)	HR (95% CI)	*P*[a]	All	Death (%)	HR (95% CI)	*P*[a]
UGP2 rs4293553 A > G												
AA	228	114 (0.50)	1.00		238	130 (0.55)	1.00		466	244 (0.52)	1.00	
AG	177	79 (0.45)	0.75 (0.56–1.01)	0.054	162	75 (0.46)	0.70 (0.52–0.94)	0.016	339	154 (0.45)	0.73 (0.60–0.90)	0.003
GG	28	7 (0.25)	0.47 (0.22–1.01)	0.052	33	14 (0.42)	0.56(0.32–0.98)	0.042	61	21 (0.34)	0.53 (0.34–0.82)	0.005
Trend test				0.010				0.005				<0.001
AA	228	114 (0.50)	1.00		238	130 (0.55)	1.00		466	244 (0.52)	1.00	
AG+GG	205	86 (0.42)	0.71 (0.54–0.95)	0.020	195	89 (0.46)	0.67 (0.51–0.89)	0.005	400	175 (0.44)	0.70 (0.58–0.85)	<0.001
FBP2 rs635087 A > G												
AA	242	106 (0.44)	1.00		237	104 (0.44)	1.00		479	210 (0.44)	1.00	
AG	168	81 (0.48)	1.21 (0.9–1.62)	0.214	171	99 (0.58)	1.43 (1.08–1.89)	0.013	339	180 (0.53)	1.33 (1.08–1.62)	0.006
GG	23	13 (0.57)	2.21 (1.23–3.97)	0.008	25	16 (0.64)	1.90 (1.11–3.25)	0.019	48	29 (0.60)	2.04 (1.38–3.03)	<0.001
Trend test				0.019				0.002				<0.001
AA	242	106 (0.44)	1.00		237	104 (0.44)	1.00		479	210 (0.44)	1.00	
AG+GG	191	94 (0.49)	1.29 (0.97–1.71)	0.079	196	115 (0.59)	1.48 (1.13–1.93)	0.004	387	209 (0.54)	1.39 (1.15–1.69)	<0.001

Note: [a]Multivariate Cox proportional hazards regression analysis was adjusted for age, sex, smoking status, drinking status, AFP level, cirrhosis, embolus and BCLC stage.
Abbreviations: SNP, single nucleotide polymorphism; OS, overall survival; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval.

Combined and Stratified Analyses of Two Independent SNPs

To estimate the combined effect of *UGP2* rs4293553 and *FBP2* rs635087 on HBV-HCC OS, we categorized the patients into 0, 1 and 2 groups according to the number of risk genotypes (rs4293553 AA and rs635087 AG+GG). Multivariable Cox regression analyses with adjustment for the covariables showed a dose-response effect between the number of risk genotypes and HBV-HCC OS (P_{trend}< 0.001, Table S4). Subsequently, we further dichotomized all patients into 0 and 1–2 groups, and the results showed that patients in 1–2 group had a worse survival than those in 0 group (HR = 1.48, 95% CI = 1.17–1.87, P = 0.001), and the results were also depicted by Kaplan-Meier curves in Figure 2.

The results of the stratified analyses using the combined dataset revealed that patients in the 1–2 group exhibited a significantly poorer survival in all subgroups (both P < 0.05) except for age > 47 years, female, smoking, drinking, AFP level > 400 ng/mL, and without cirrhosis; moreover, we did not find a multiplicative interaction between risk genotypes and the covariables (Table S5).

The ROC Curves

We constructed prediction models that included either only covariables or both covariables and risk genotypes to compare the ability of different models in predicting 1-, 3-, and 5-year OS of patients with HBV-HCC. The findings indicated that the AUC of 1-year OS prediction model increased from 71.07% to 71.86% (P = 0.408, Figure S3A). Notably, the models combined risk genotypes significantly improved the ability of predicting 3- and 5-year OS, with the 3-year OS AUC increasing from 72.72% to 74.42% (P = 0.039; Figure S3B) and the 5-year OS AUC increasing from 72.04% to 74.24% (P = 0.020; Figure S3C). Furthermore, we found that the model with combined covariables and risk genotypes had a better ability to predict HBV-HCC OS over the follow-up period (Figure S3D).

Functional Annotation Analyses

As shown in Table S6, the functional annotation results suggested potential biological functions of these two SNPs, with rs4293553 being located in the 5'UTR region of *UGP2* and rs635087 being located in the intronic region of *FBP2*. In the RegulomeDB, rs4293553 and rs635087 were ranked of 3a and 5, respectively. In the HaploReg, both SNPs may alter motifs and have an effect on promoter or enhancer histone markers, with the difference that rs4293553 was located in the DNase I hypersensitive site and may alter protein binding activity. In the SNPinfo, rs4293553 was located in transcription factor binding sites (TFBS), which suggests that rs4293553 may alter gene expression through transcriptional regulation. Furthermore, the results of the ENCODE project showed that both SNPs had strong signals of histone modification (eg H3K4Me1, H3K4Me3, and H3K27Ac) acetylation, further suggesting their functions of activating both enhancer and promoter Figure 3A and B).

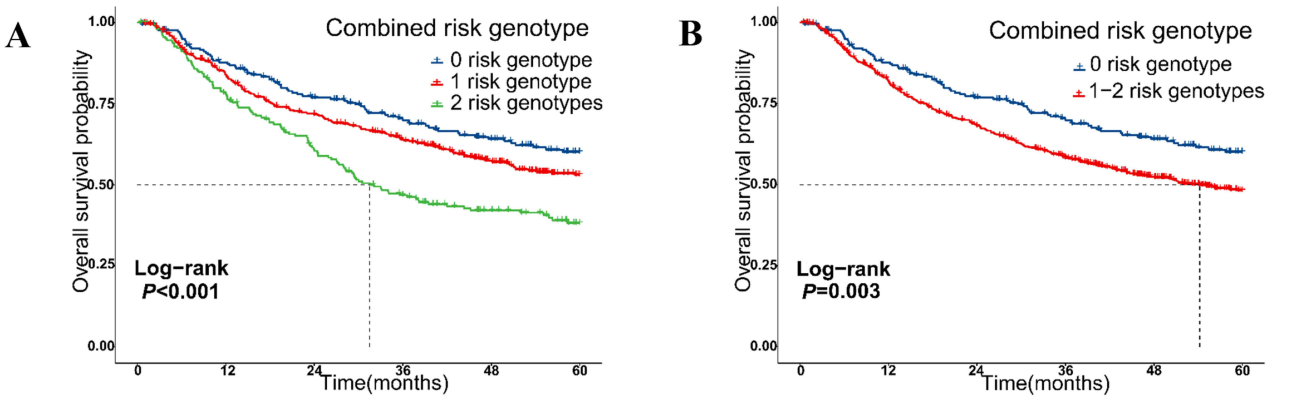

Figure 2 Kaplan–Meier curves for patients with HBV-related HCC by the combined risk genotypes. (**A**) Kaplan–Meier curve for patients with HBV-related HCC by 0, 1 and 2 risk genotypes; (**B**) Kaplan–Meier curve for patients with HBV-related HCC by 0 and 1–2 risk genotypes. The risk genotypes were *UGP2* rs4293553 AA and *FBP2* rs635087 AG/GG.

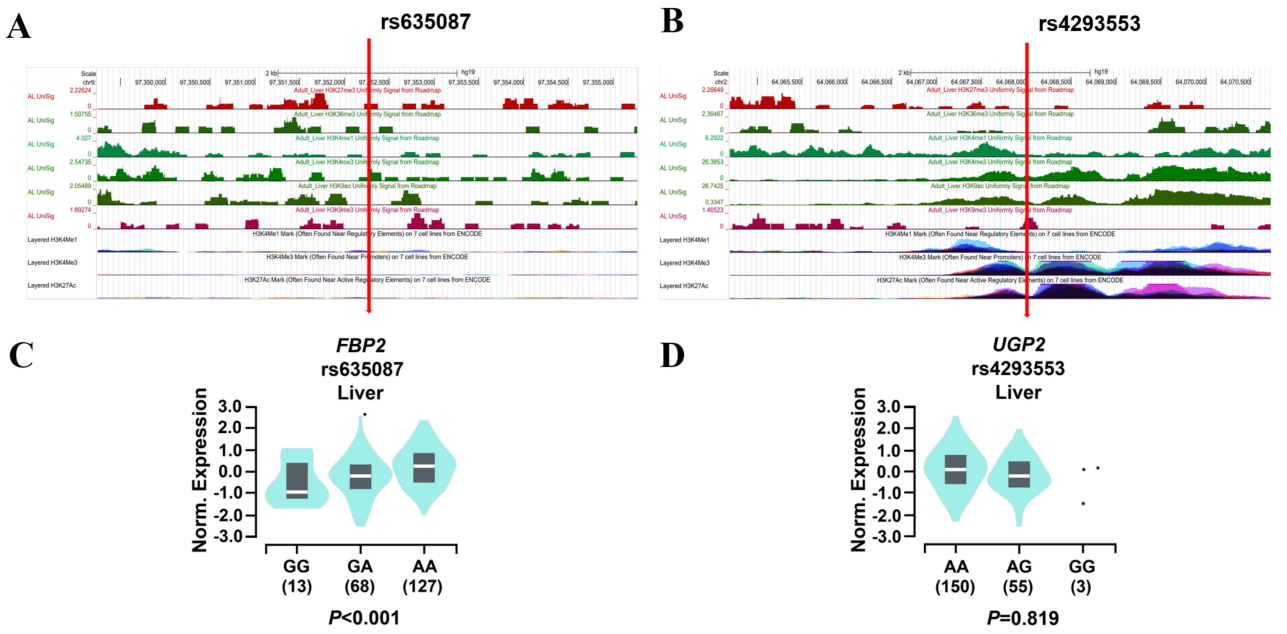

Figure 3 Functional prediction and eQTL analysis of *FBP2* rs635087 and *UGP2* rs4293553. (**A**) Functional prediction of rs635087 in the ENCODE project; (**B**) Functional prediction of rs4293553 in the ENCODE project; (**C**) eQTL analysis of rs635087 (n = 208, $P < 0.001$) in normal liver tissues from the GTEx database; (**D**) eQTL analysis of rs635087 rs4293553 (n = 208, $P = 0.819$) in normal liver tissues from the GTEx database.
Abbreviations: Norm. Expression, normalized gene expression.

eQTL Analyses and Gene Differential Expression Analyses

The results of eQTL analyses revealed that the rs635087 G allele was significantly correlated with reduced mRNA expression levels of *FBP2* in normal liver tissues from the GTEx database ($P < 0.001$, Figure 3C), however, such a correlation was not supported in the 1000 Genomes project ($P = 0.459$, Figure S4A). The correlation between the rs4293553 G allele and mRNA expression levels of *UGP2* was not statistically significant in both GTEx database and 1000 Genomes project ($P = 0.819$, Figure 3D and $P = 0.483$, Figure S4B, respectively). In comparing the mRNA expression levels of *UGP2* and *FBP2* between HCC and normal liver tissues, we found that *UGP2* was significantly lower in HCC tissues than in normal liver tissues in both the TNMplot and in-house RNA sequencing data (both $P < 0.001$, Figure 4A and B); similarly, the TNMplot database showed that *FBP2* was significantly lower in HCC tissues than in normal liver tissues ($P < 0.001$, Figure 4C), though this difference was not apparent in the in-house RNA sequencing data ($P = 0.255$, Figure 4D). Furthermore, higher expression levels of both *UGP2* and *FBP2* were associated with a better HCC OS in the Kaplan-Meier Plotter database (both $P < 0.001$, Figure 4E and F).

Mutation Analysis

Finally, we examined the mutation status of *UGP2* and *FBP2* in HCC tissues using the cBioPortal for Cancer Genomics database. As shown in Figure S5, somatic mutation rates of both genes were extremely low in different HCC datasets. For the *UGP2*, the mutation rates were as follows: 0.87% in the AMC, Hepatology 2014; 0.82% in the MERiC/Basel, Nat Commun, 2022; 0.80% in the TCGA, Firehose Legacy; 0.55% in the TCGA, PanCancer Atlas and 0.41% in the INSERM, Nat Genet 2015. Similarly, for the *FBP2*, the mutation rates were 0.54% in the TCGA, Firehose Legacy and 0.27% in the TCGA, PanCancer Atlas. Since the low somatic mutation frequency of *FBP2* and *UGP2*, functional SNPs may be key factors influencing the mRNA expression levels of these two genes in HCC.

Discussion

Glycolysis is a vital process that not only meets the energy needs of normal cells but also promotes metabolic intermediates essential for macromolecular synthesis in cancer cells.[33] Cancer cells rely primarily on the programmed

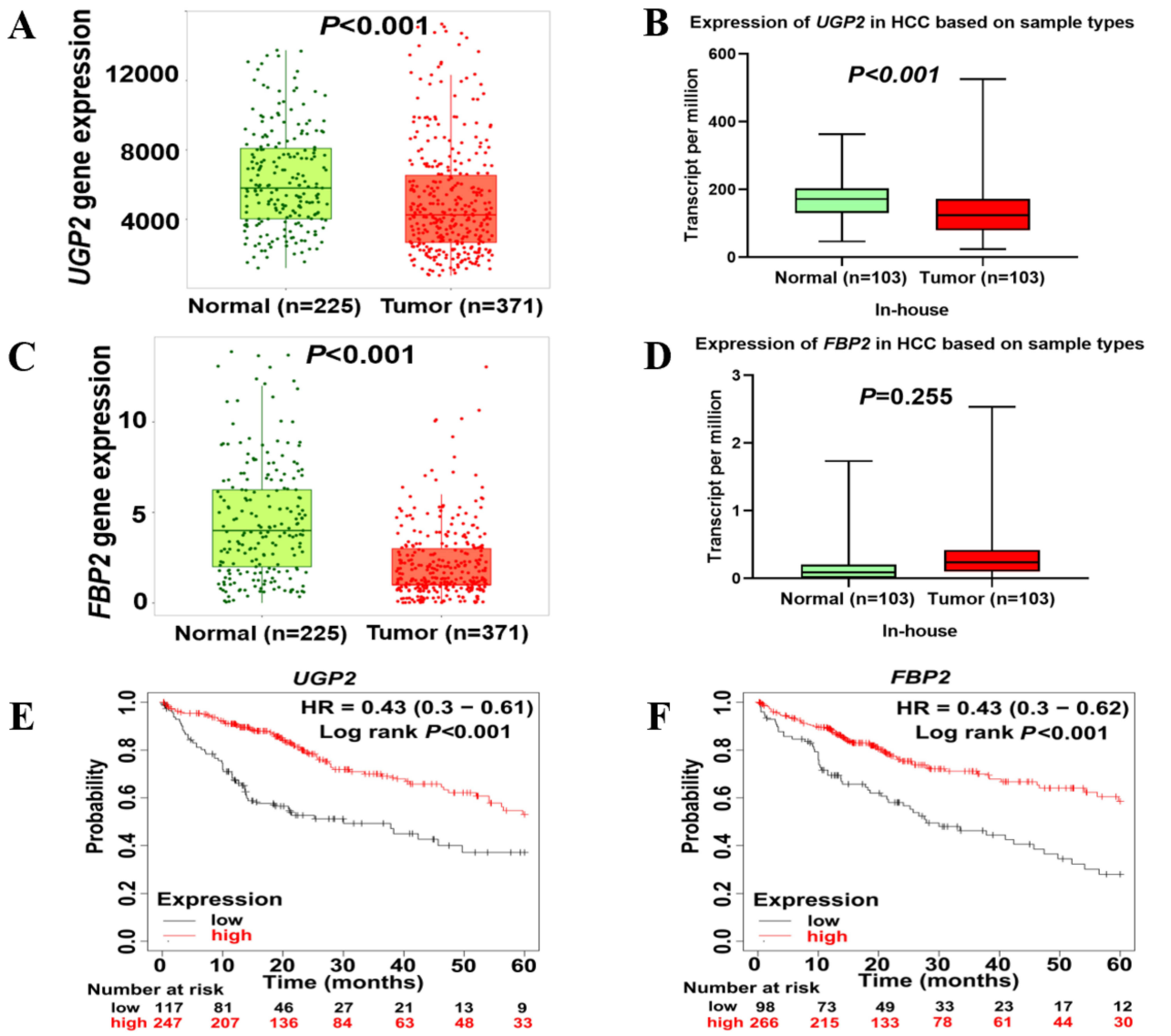

Figure 4 Differential mRNA expression analysis in the TNMplot database (https://tnmplot.com/analysis/) and in-house RNA sequencing data and survival analysis in the Kaplan-Meier plotter database (http://kmplot.com/analysis/). (**A**) *UGP2* mRNA expression levels were down-regulated in HCC tissues from the TNMplot database; (**B**) *UGP2* mRNA expression levels were down-regulated in HCC tissues from the in-house data; (**C**) *FBP2* mRNA expression levels were down-regulated in HCC tissues from the TNMplot database; (**D**) *FBP2* mRNA expression levels were not statistically different between HCC tissues and in normal liver tissues; (**E**) higher mRNA expression levels of *UGP2* had better OS of HCC patients. (**F**) higher mRNA expression levels of *FBP2* had better OS of HCC patients.
Abbreviations: HCC, hepatocellular carcinoma; HR, hazards ratio.

glycolysis metabolism to support their rapid growth and division.[34] Considering many genes involved in the glycolysis pathway, we comprehensively investigated associations between 24,069 SNPs in 240 glycolysis pathway genes and survival of patients with HBV-HCC. We found that *UGP2* rs4293553 G and *FBP2* rs635087 G alleles were significantly associated with a better and a worse OS of patients, respectively. Additional eQTL analyses revealed that the rs635087 G allele was significantly associated with mRNA expression levels of *FBP2* in normal liver tissues. Moreover, the mRNA expression levels of *UGP2* and *FBP2* were significantly higher in normal liver tissues than in HCC tissues, and higher mRNA expression levels were significantly associated with better survival in HCC patients. These findings are consistent with the results of survival analyses using risk alleles for two significant SNPs.

UGP2, known as UDP-glucose pyrophosphorylase 2, is located at 2p15 and encodes an enzyme crucial for converting glucose 1-phosphate into UDP-glucose,[35] which meets the energy demands for cancer cell proliferation primarily by

promoting glycogen biosynthesis.[36] Similarly, previous studies have reported that *UGP2* as a significant factor in the metabolic regulation and post-translational modifications of cancer,[37,38] and its expression is reduced in a variety of cancers, including cancer of the pancreas,[39] gallbladder,[40] and colorectum,[41] suggesting that *UGP2* may serve as an important prognosis predictor. In the present study, we identified that *UGP2* rs4293553 G allele was associated with a favorable survival of HBV-HCC patients. In addition, *UGP2* mRNA expression was found to be significantly higher in normal liver tissues than in HCC tissues, and patients with a higher expression level had a better survival, which is consistent with previous findings. Unfortunately, our data did not support the correlation between the rs4293553 G allele and the mRNA expression levels of *UGP2*, but functional annotation indicated that rs4293553 is located at TFBS, DNase I hypersensitive sites, and marker of promoter histones, which suggests that rs4293553 may have an effect on transcription factor activity.

FBP2 (fructose-bisphosphatase 2), located at 9q22.32, mainly encodes a gluconeogenesis regulatory enzyme and acts as an important player in the glycogen synthesis.[42] Gluconeogenesis is essentially the reverse of glycolysis and can inhibit glycolysis in cancer cells.[43] The up-regulation of *FBP2* may lead to suppression of glucose metabolism, cell proliferation and tumor growth;[44] conversely, down-regulation of *FBP2* can promote tumorigenesis by enhancing glycolysis.[45] In the present study, we found that rs635087 G allele was significantly associated with reduced mRNA expression levels of *FBP2* and poorer survival of HBV-HCC patients, respectively. Furthermore, we observed that reduced expression of *FBP2* in HCC tissues was associated with a worse survival of HCC patients. These findings indicate that *FBP2* may act as an oncogene and that rs635087 G allele may affect the survival of HCC patients by reducing *FBP2* expression levels.

The present study exhibits several significant strengths. For SNPs significantly associated with survival of HBV-HCC patients, we used a rigorous multiple correction method (FPRP < 0.20) to reduce the possibility of false discovery and employed an internal replication strategy to bolster the reliability of findings. Furthermore, we found that SNPs combined with covariables significantly improved the ability to predict survival of HBV-HCC patients, emphasizing the importance of SNPs in optimizing prognostic assessment of patients, as well as potential clinical application of SNPs. For example, patients carrying more risk genotypes of SNPs may be categorized as high-risk and thus require a more aggressive treatment approach or closer monitoring. However, it is important to acknowledge the limitations inherent in the present study. First, prognostic assessment methods based on single genotype, phenotype or clinical information usually do not fully reflect the prognostic differences in HCC patient. Therefore, it is necessary to further explore their combined effects to provide a more comprehensive assessment in future studies. Second, all study population was recruited from a single institution in Guangxi, China, which may limit the applicability of our findings to other populations or ethnic groups. Third, we failed to collect more comprehensive clinical information about patients, such as treatment information of patients underwent hepatectomy, which may influence our findings. At last, the exact biological mechanisms underlying the observed associations are not fully understood.

Conclusions

We identified two independent and potentially functional SNPs (*UGP2* rs4293553 A > G and *FBP2* rs635087 A > G) that are associated with OS of HBV-HCC patients. Given that these two SNPs play a role in the glycolysis pathway and effectively predicting 3- and 5-year OS, once validated by additional studies, our results may provide some information about new biomarkers for predicting survival of HBV-HCC patients underwent surgery and new insights for further functional studies in the future.

Abbreviations

HCC, Hepatocellular Carcinoma; HBV, Hepatitis B Virus; SNPs, Single Nucleotide Polymorphisms; AFP, Alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; OS, Over Survival; MAF, Minimum Allele Frequency; HWE, Hardy-Weinberg Equilibrium; eQTL, Expression Quantitative Trait Locus; GTEx, Genotype-Tissue Expression Project; LD, Linkage Disequilibrium; FPRP, False Positive Report Probability; ROC, Receiver Operating Characteristic; AUC, Area Under Curve; TFBS, Transcription Factor Binding Sites.

Data Sharing Statement

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

Informed Consent Statement

Informed consent was obtained from all individual participants included in the study.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of Guangxi Medical University Cancer Hospital (Approval Number: LW2023138).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no relevant financial or non-financial interests to disclose in this work.

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