

Comparative Analysis of the Mutational Profile in HBV-Related and Non-HBV-Related Hepatocellular Carcinoma

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Research Article

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Abstract

Background

Hepatocellular carcinoma (HCC) is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide. The distinct molecular mechanism that regulates cancer progression associated with HBV-related HCC are still unclear.

Methods

The study population included all HCC patients from TCGA lihc_firehose. Mutational signatures of HBV-related HCC samples were calculated with MuSiCa Online website tools. Overall Survival (OS) and disease-free survival (DFS) were estimated according to the log rank in Cox proportional model. Analysis of differential expressed genes was finished with R (version 3.6.3) package DESeq2.

Results

AXIN1 and CACNA2D1 were significantly higher tested in patients with HBV-related HCC. Mutational signature 26,6 and 16 were detected in HBV-related HCC. TMB of non-HBV-related HCC was significantly higher than HBV-related. Mutations of TTN, MUC16, RYR2, DNAH7 and ARID2 had significant effects on OS in HBV-related but were not associated with prognosis of non-HBV-related HCC.

Conclusion

Wnt/ β -catenin and cell cycle signaling pathway may be potentially targets for treatment of HBV-related HCC. Mutation genes of TTN, MUC16, RYR2, DNAH7 and ARID2 may be used as biomarkers of the clinical prognosis and a useful strategy for management of HBV-related HCC.

Introduction

Liver cancer is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide, and hepatocellular carcinoma (HCC) accounts for 75%-85% of the primary liver malignancy[1]. An estimated that 841,000 new cases and 782,000 causalities caused by HCC worldwide in 2018, with Chinese patients making up 50% of the global HCC burden[1]. Multiple risk factors are associated with the onset and progression of HCC, among which hepatitis B virus (HBV) infection is one of the leading risk factors[2]. It was reported that HBV infection may lead to the accumulation of host genomic instability and genetic alterations that confer cell growth advantage, which cause the development of HBV-related HCC[3]. Therefore, revealing the difference of mutational profiling associated with HBV-related HCC facilitates the understanding of the distinct molecular mechanism that regulates cancer progression and the discovery of potential therapeutic targets. For example, KRAS and BRAF mutations is highly associated with prognosis and resistance to targeted therapies of patients with non-small cell lung cancer and colorectal cancer[4]. Somatic mutations caused by HBV involved in HCC initiation and progression, as they are in many other cancers. Mutational

signatures, focusing on the patterns of trinucleotide alterations may analyze the patterns of somatic mutations, the results of which reveal the diversity of mutational processes underlying the development of cancer with potential implications for understanding of cancer etiology, prevention and therapy[5]. Recent research found that a five-gene-signature (CDH10, COL6A3, SMAD4, TMEM132D, VCAN) that predicts survival outcomes for stratifying patients with colorectal cancer independent of TNM staging[6]. Tumor mutational burden (TMB) is the total number of somatic mutations in a defined region of a tumor genome which is currently an emerging, independent biomarker of outcomes with immunotherapy in multiple tumor types[7]. Accumulating evidence of TMB suggests its potential usefulness in lung, melanoma, urothelial cancer and mismatch-repair deficient colorectal tumors[8-10].

Our study aimed to compare the mutational profiles, mutational signature and TMB of HBV-related and non-HBV-related HCC through bioinformatics analysis to reveal the potential key regulators which are responsible for the heterogeneous progression of HCC and identify actionable mutations amenable to targeted therapy.

Materials And Methods

Study population and datasets

The study population included all hepatocellular carcinoma (HCC) patients from cBioportal (http://download.cbioportal.org/lihc_tcga.tar.gz) (N = 377). Mutational signatures of HBV- and non-HBV-related HCC samples were calculated with MuSiCa Online website tools (<http://bioinfo.ciberehd.org:3838/MuSiCa/>).

Clinical and molecular data analyses

Associations of categorical variables were assessed by using t test or the Fisher's exact test. Continuous variable was assessed by wilcox.test. Overall Survival (OS) and disease-free survival (DFS) were estimated according to the log rank in Cox proportional model. $P < 0.05$ was considered as statistically significant. Analysis of differential expressed genes was finished with R (version 3.6.3) package DESeq2.

Results

Patient characteristics

We assessed the characteristics of patients in the global population (N = 377) with HBV-(107) and non-HBV-(270) related HCC. We found that 85.05% of patients with HBV-related HCC were diagnosed in Asia, which was significantly higher compared with other areas ($P < 0.001$). Patients with HBV-related HCC had a higher incidence (82.24% vs. 61.85%) ($P < 0.001$) in male and younger ages (median, maximum and minimum of age were 53, 83, 23 vs. 64, 90, 16) ($P < 0.01$) than non-HBV-related. In addition, the pathological grade [11] of HBV-related HCC was grade 3-4 in common, and have a poor overall survival (OS) and disease-free survival (DFS) than non-HBV-related ($P < 0.001$) (Fig. 1, Table 1).

Mutational profile of HCC patients

Of total 377 HCC patients, 41198 single nucleotide variants (SNV) mutations of 13,668 genes were detected, most of mutations were less than 5, and a total of 440 mutations were more than 10. The most common mutation types of gene were Missense (31925, 77%) and Frameshift mutations (5269, 13%), the rest of which were Stop_gained (1851, 4%), Splice_Site (1709, 4%), Indel (444, 1%) and CN_amp (290, 1%) respectively. The detection rates of mutations more than 10% in HCC patients were TP53, TTN, CTNNB1, MUC16, ALB, MT-NDS, APOB and RYR2 (Table 2, Fig. 2).

Comparison of OncoPrint of HBV- and non-HBV-related HCC patients

The results of OncoPrint of HCC show that the mutation detection rate of TTN (19% vs. 30%), SPTA1 (2% vs. 10%) and USH2A (2% vs. 9%) in HBV-related HCC were significantly lower than in non-HBV-related (Fig. 3(A)). In addition, AXIN1, MAST4, CDKN2A, KRT10, CACNA2D1 and COL4A5 were significantly higher tested in patients with HBV-related HCC ($P < 0.01$). TTN, BAP1, CSMD1, SPTA1, USH2A, DNAH17 and MYO18B were significantly higher tested in patients with non-HBV-related HCC ($P < 0.01$) (Fig. 3(A, B, C)).

Mutational signatures of HBV- and non-HBV-related HCC patients

We analyzed mutational gene signatures detected in HBV and non-HBV-related HCC in MutaCa (<http://bioinfo.ciberehd.org:3838/MutaCa/>). The results show that a total of 8545 mutation genes were tested in HBV-related HCC, and 27105 mutation genes were tested in non-HBV-related HCC (Fig. 4(A)). Novel mutational genes compared with enrolled in COSMIC database, which were detected in non-HBV-related HCC were significantly more than HBV-related (4.16% vs. 3.67%, $P = 0.036$) (Fig. 4(B), Table 3). In addition, in HBV-related HCC patients, the mutations of C>T and T>A were more enriched in C>G. In contrast, the mutations of T>C were more enriched in C>C and G>C in non-HBV-related HCC (Fig. 4(C)). Mutational signature 26, 6 and 16 were detected in HBV-related HCC, while were not detected in non-HBV-related. Mutational signature 17, 20 and 12 in non-HBV-related HCC were significantly higher than HBV-related (Fig. 4(D)).

Tumor mutation burden (TMB) and effects on OS of mutation genes between HBV- and non-HBV-related HCC patients.

TMB of non-HBV-related HCC was significantly higher than HBV-related ($P < 0.01$).

TMB of RYR2, MUC16, TTN and TP53 mutations was significantly higher than wild type (WT) in HCC patients ($P < 0.001$) (Fig. 5). Mutations of TTN, MUC16, RYR2, DNAH7 and ARID2 had significant effects on OS in HBV-related but were not associated with prognosis of non-HBV-related HCC. In contrast, mutations of TP53, APOB, ARID1A, LRP1B, SYNE2, CUBN and NBEA had significant effects on OS in non-HBV-related, but were not associated with prognosis of patients with HBV-related HCC (Fig. 6, Fig. 7).

Discussion

HBV infection is at greater increased risk of HCC. Research showed that malignant transformation may occur as a result of continuous or recurrent cycles of hepatocyte necrosis and regeneration when HBV replication is sustained[12]. Our results showed that patients with HBV-related HCC were most (85.05%) diagnosed in Asia, and tend to occur in men (82.24%) and younger ages, which was similar with previous studies[13]. In addition, there are limited data on HBV-related HCC survival. Our results revealed that patients with HBV-related HCC have grade 3–4 of pathology more in common and a poor OS and DFS than non-HBV-related. Detailed molecular mechanisms underlying HBV-related HCC are still obscure. A previous study of mutational profile of HCC patients reported that the most frequently mutated genes occurring in $\geq 10\%$ of 137 samples included TERT (55.6%), CTNNB1 (35.7%) and TP53 (32.5%)[14]. In our study, the detection rates of mutations more than 10% in total 377 HCC patients were TP53 (79.9%), TTN (26.8%) and CTNNB1 (25.7%). We compared the mutational profile of HBV- and non-HBV-related HCC patients, and found that AXIN1, MAST4, CDKN2A, KRT10, CACNA2D1 and COL4A5 were significantly higher tested in patients with HBV-related HCC. Research reported that AXIN1 (serves as a negative regulator of Wnt/ β -catenin signaling) and CDKN2A (a tumor suppressor gene that induces cell cycle arrest in G1 and G2 phases) were both TERT promoter, which represent specific cancer signatures in the pathogenesis of HBV-related HCC[15]. Our results allowed to uncover novel mutations in driver genes of HBV-related HCC and possible pathways including Wnt/ β -catenin and cell cycle signaling pathway, which may be potentially targets for the treatment of HBV-related HCC. The roles and functions of MAST4, KRT10, CACNA2D1 and COL4A5 in HBV-related HCC have not been reported so far, which are needed for more research to clarify.

We analyzed mutational gene signature detected in HBV and non-HBV-related HCC.

Mutational signature 26,6,16 were detected in HBV-related HCC, while were not detected in non-HBV-related. As far as we know, signature 6 and 26 have been associated with post-replicative mismatch repair deficiency (dMMR)[16]. Few research about dMMR in HBV-related HCC was found. The mechanism of dMMR in HBV-related HCC such as methylation of MMR genes (hMLH1, hMSH2 and hMSH3) are necessary to be clarified for early diagnostic marker of HBV-related HCC. Other research show that signature 16 show strong transcriptional strand bias and possibly reflect the involvement of transcription coupled nucleotide excision repair acting on bulky DNA adducts due to exogenous carcinogens[5]. Gene mutations preferentially occurred in the highly methylated and active chromatin areas of the genome when the integration of HBV DNA into the host genome, which included potential therapeutic targets of HBV-related HCC[17].

Immunotherapeutics are very promising therapeutic tools in many advanced cancers including HCC. Immunotherapy with nivolumab, which targeting programmed cell death protein 1 (PD-1) led to promising response rates and survival durations in a phase I–II study involving advanced HCC patients[18]. It was reported that there was benefit of cabozantinib and nivolumab treatment in a patient with HCC and RET amplification, high TMB, and positive PD-L1 expression[19]. Our results revealed that

TMB of RYR2, MUC16, TTN and TP53 mutations was significantly higher than wild type (WT) in HCC patients, and TMB of non-HBV-related HCC was significantly higher than HBV-related. It may mean that the better efficacy of PD-1 immune checkpoint inhibitor in mutations of RYR2, MUC16, TTN and TP53 mutations of patients with HCC than WT, and the same non-HBV-related HCC is more effective than HBV-related. Interestingly, we also found that mutations of TTN, MUC16, RYR2, DNAH7 and ARID2 had significant effects on OS in HBV-related but were not associated with prognosis of non-HBV-related HCC. In contrast, mutations of TP53, APOB, ARID1A, LRP1B, SYNE2, CUBN and NBEA had significant effects on OS in non-HBV-related, but were not associated with prognosis of patients with HBV-related HCC. Our results suggest that the mutated genes, or combined with mutated genes could be used as biomarkers of the clinical prognosis and a useful strategy for management of HBV-related HCC. Research on the mutated genes associated with prognosis of patients with non-HBV-related HCC may help understand different mechanisms lead to HCC between HBV infection and other risk factors.

Conclusions

In conclusion, via integrative bioinformatics analysis of the mutation data from HBV-related and non-HBV-related HCC, we found highly mutated genes including AXIN1 and CACNA2D1 were significantly higher tested in patients with HBV-related HCC which suggest that Wnt/ β -catenin and cell cycle signaling pathway may be potentially targets for the treatment of HBV-related HCC. Mutational signature 26,6,16 were detected in HBV-related HCC, while were not detected in non-HBV-related, which suggest that gene mutations preferentially occurred in the highly methylated and active chromatin areas of the genome when the integration of HBV DNA into the host genome, which may include potential therapeutic targets of HBV-related HCC. TMB of non-HBV-related HCC was significantly higher than HBV-related, which suggest that the worse efficacy of PD-1 immune checkpoint inhibitor in HBV-related HCC than non-HBV-related. Mutations of TTN, MUC16, RYR2, DNAH7 and ARID2 had significant effects on OS in HBV-related but were not associated with prognosis of non-HBV-related HCC, which suggest that mutation genes, or combined with mutation genes tested may be used as biomarkers of the clinical prognosis and a useful strategy for management of HBV-related HCC.

Abbreviations

HCC: Hepatocellular carcinoma

OS: Overall Survival

DFS: Disease-free survival

TMB: Tumor Mutation Burden

SNV: Single nucleotide variants

TCGA: The cancer genome atlas

Declarations

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Conflict of interest statement

The authors declare no conflict of interest in preparing this article.

Ethics approval and consent to participate

Ethical permission and written informed consent of the patient in this study was not applied because this study population included all hepatocellular carcinoma (HCC) patients from cBioportal (http://download.cbioportal.org/lihc_tcga.tar.gz).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

The interpretation and analysis of data were by Bingqing Du and Haifeng Wang.

The preparation of the manuscript was by Jie Yang. The revision for important intellectual content was by Yisheng Wei. The supervision was by Zili Shao.

Acknowledgements

Not applicable.

Author information

Bingqing Du and Haifeng Wang contributed equally to this study.

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

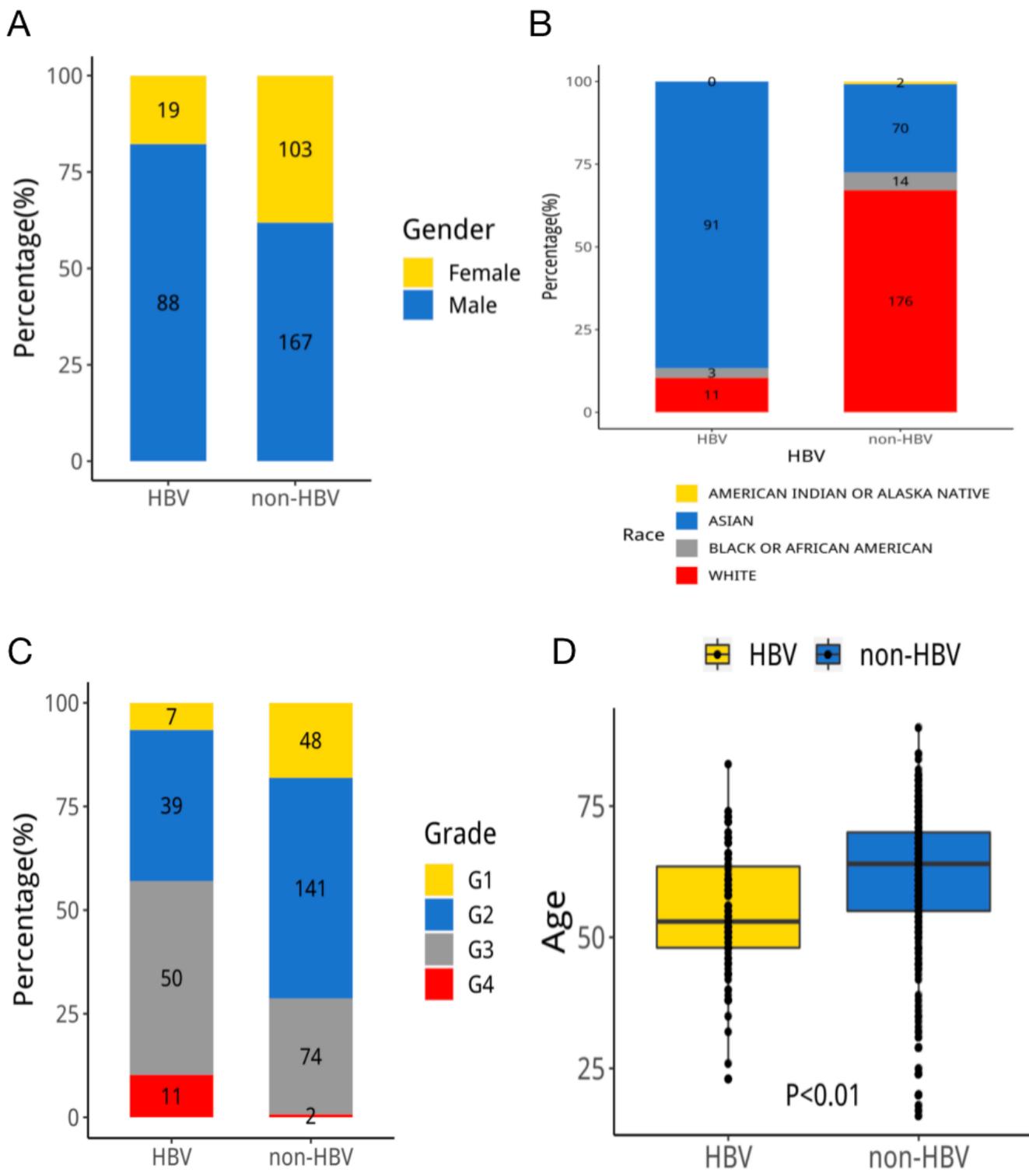


Figure 1

Characteristics of HBV- and non-HBV-related HCC patients in the global population.(A) Gender of HBV- and non-HBV-related HCC,(B)Race of HBV- and non-HBV-related HCC,(C) Pathological grade of HBV-and non-HBV-relate HCC ,(D) Age of HBV- and non-HBV-related HCC.

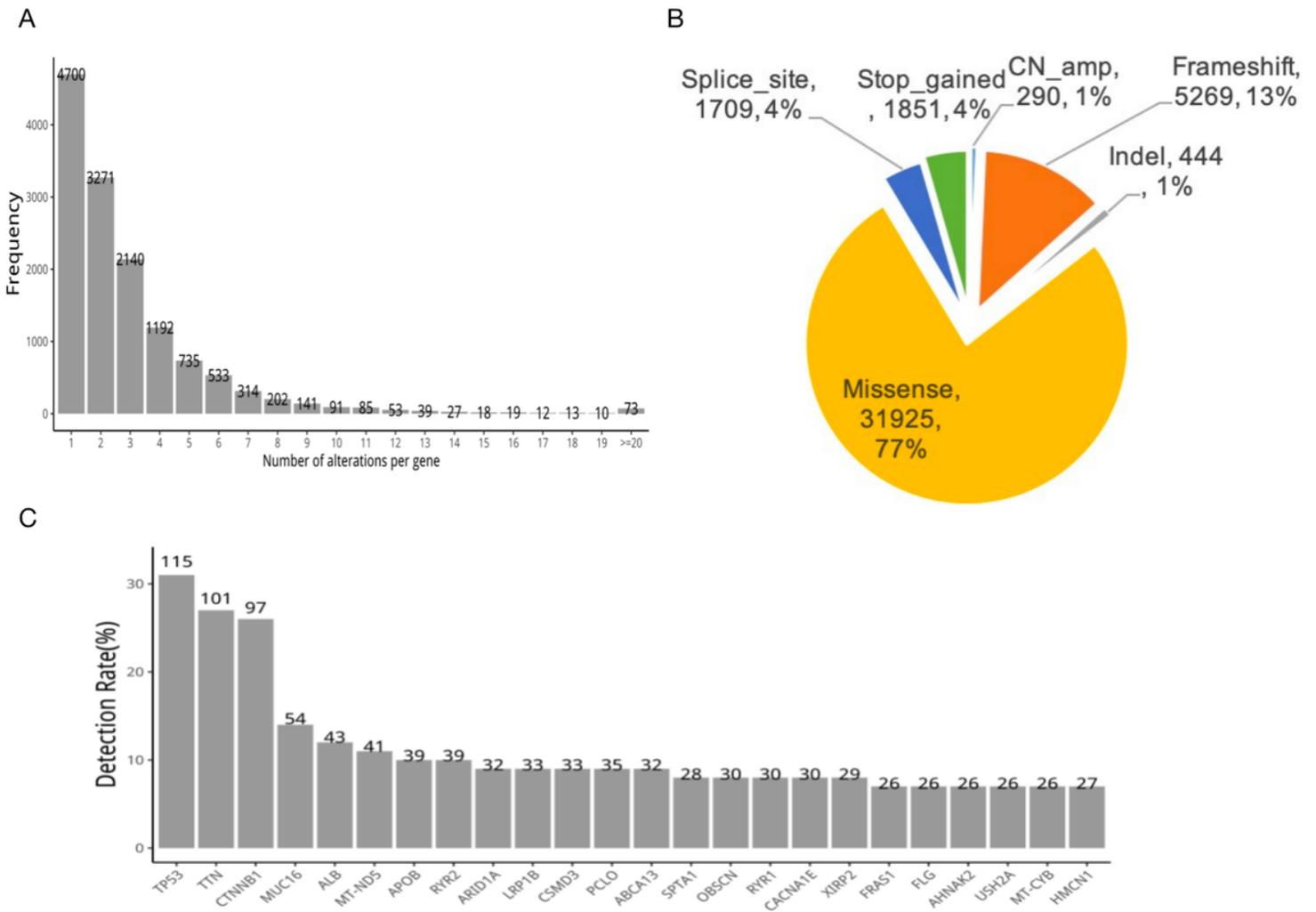


Figure 2

Mutational profile of HCC patients.(A)Distribution of mutatioal gene.X-axis denotes the number of alterations per gene, Y-axis denotes total number of genes tested. (B)Major mutation types of genes in HCC patients.(C) The detection rates of mutations in HCC patients.

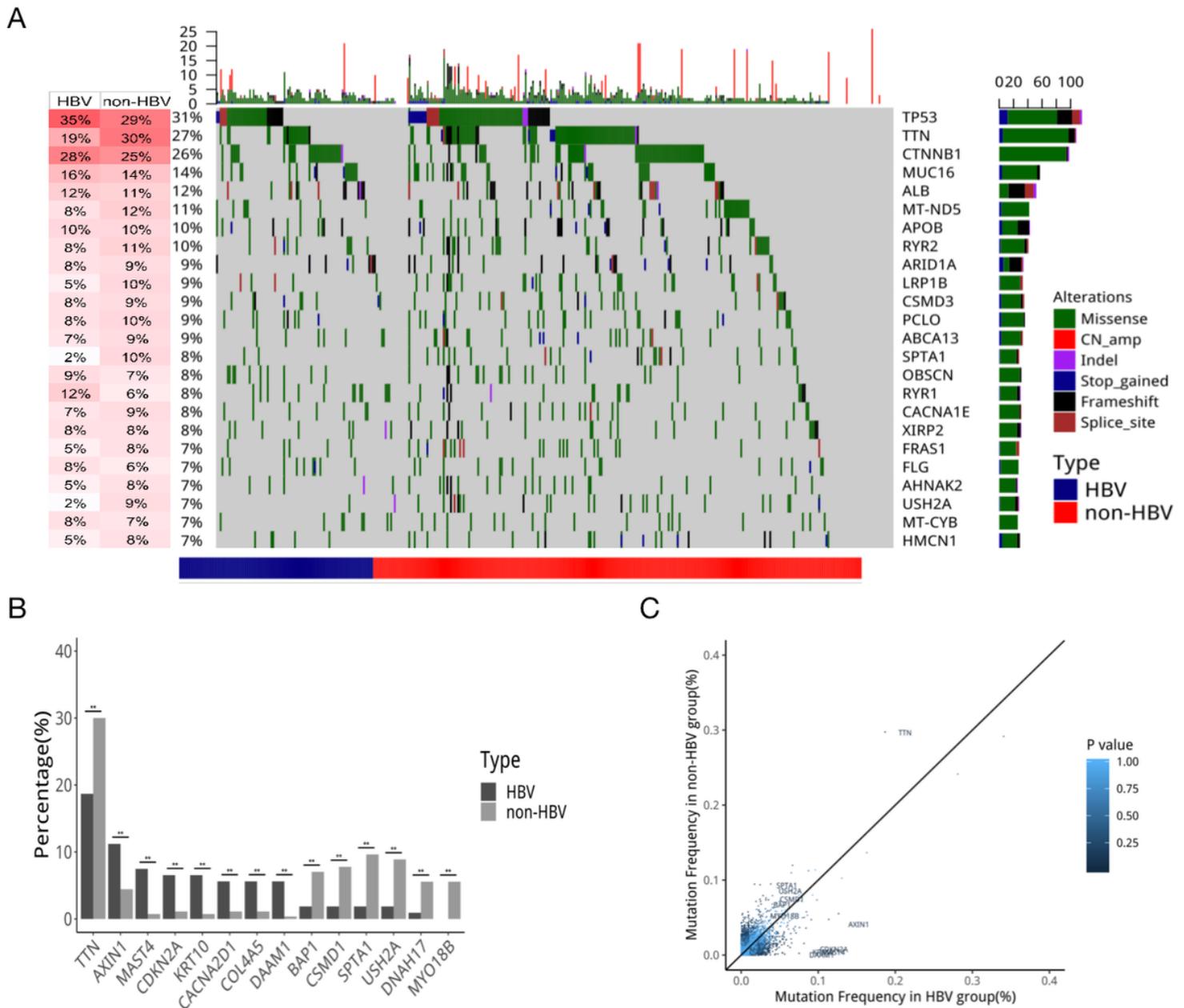


Figure 3

OncoPrint of HBV- and non-HBV-related HCC patients.(A)Each column represents a sample and each row represents a gene. Figure on the left represents the mutation frequency more than 7% of gene that corresponds to either HBV- or non-HBV-related HCC.Top plot represents the overall number of mutations a patient carried.Bottom blue and red colors denote HBV- or non-HBV-related HCC samples. Different colors on the right of figure denote different types of mutation.(B,C)Genetic difference analysis of HBV- and non-HBV-related HCC.** denotes $P < 0.01$.

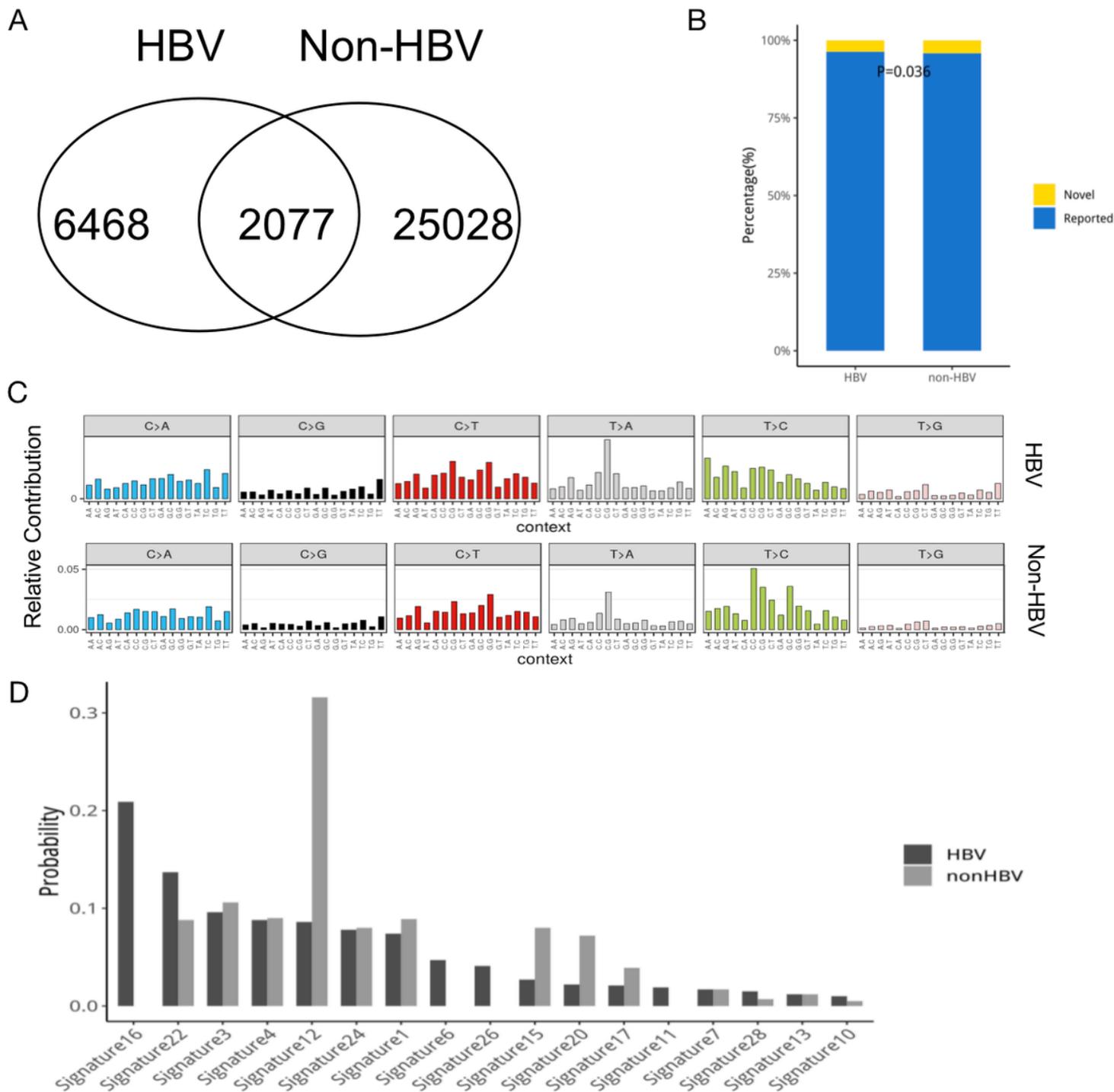


Figure 4

Mutational profile comparison between HBV and non-HBV(A) Venn plot of mutational gene detected in patients with HBV and non-HBV-related HCC.(B)Mutational gene compared with enrolled in COSMIC database which were detected in HBV and non-HBV-related HCC.(C)Mutational context distribution of HBV and non-HBV samples.(D)Mutation signatures of HBV- and non-HBV-related HCC patients

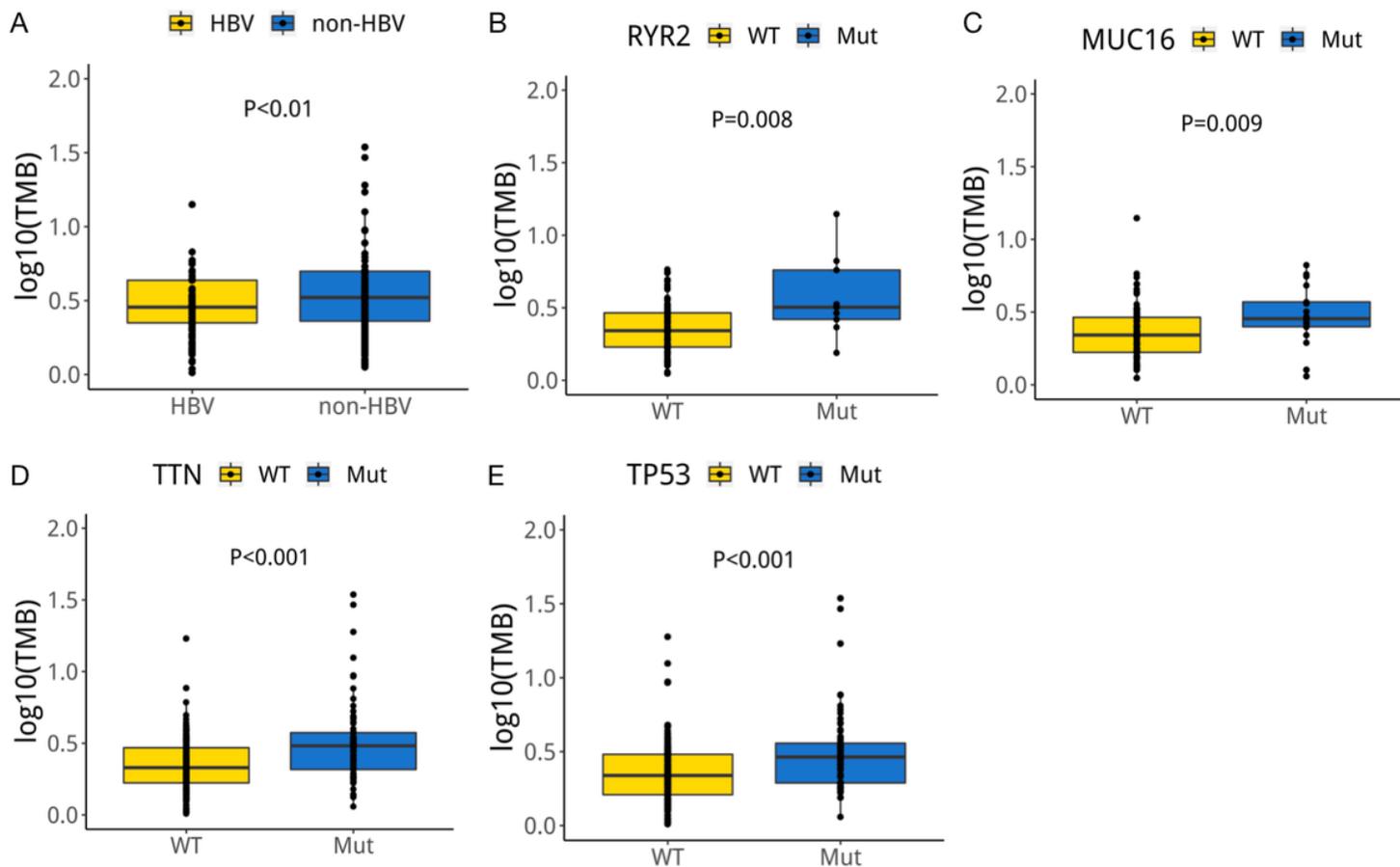


Figure 5

Comparative of TMB in HCC patients.(A) TMB of HBV- and non-HBV-related HCC patients,(B,C,D,E) TMB of RYR2,MUC16,TTN and TP53 mutations in HCC patients. TMB = sum(non synonymous mutation)/(total Exon length).

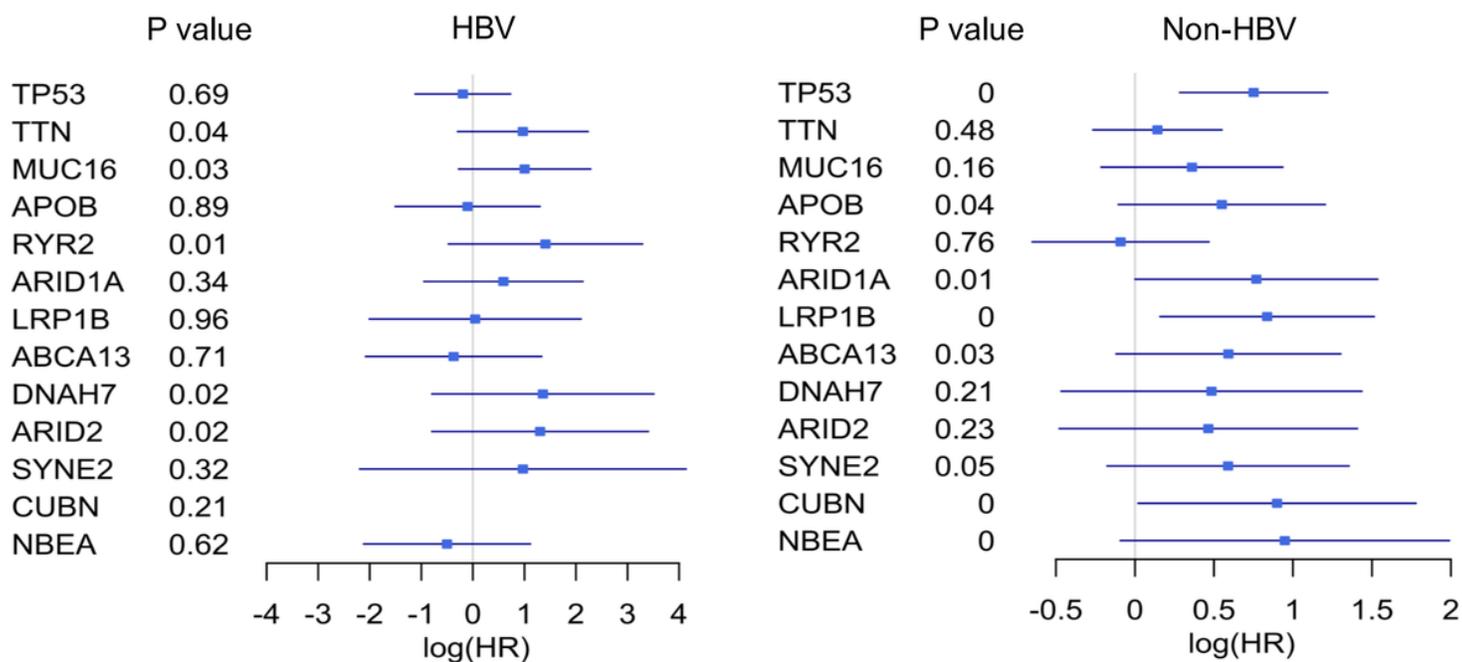


Figure 6

Comparative of prognosis of patients with HBV-related and non-HBV-related HCC.

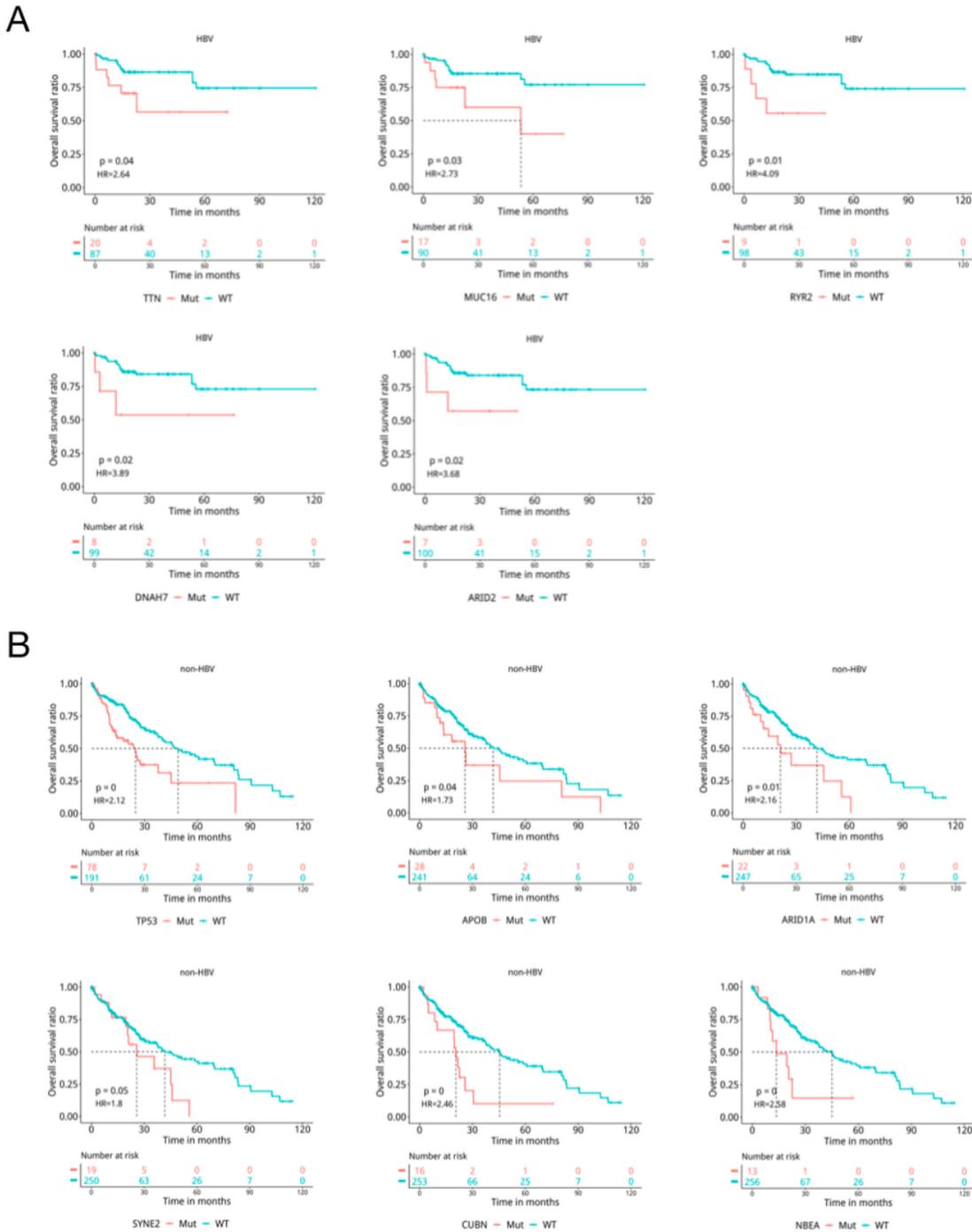


Figure 7

Effects on OS of mutation genes between HBV- and non-HBV-related HCC patients.(A)K-M plots of mutation genes in HBV-related HCC patients.(B)K-M plots of mutation genes in non-HBV-related HCC patients.

Supplementary Files

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