Original Paper

ATXN7 Gene Variants and Expression Predict Post-Operative Clinical Outcomes in Hepatitis B Virus-Related Hepatocellular Carcinoma

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Key Words

ATXN7 • SNPs • Hepatitis B virus • Hepatocellular carcinoma

Abstract

Background/Aims: Hepatocellular carcinoma (HCC) is a lethal disease with nearly equal morbidity and mortality. Thus, the discovery and application of more useful predictive biomarkers for improving therapeutic effects and prediction of clinical outcomes is of crucial significance. **Methods:** A total of 475 HBV-related HCC patients were enrolled. Ataxin 7 (ATXN7) single nucleotide polymorphisms (SNPs) were genotyped by Sanger DNA sequencing after PCR amplification. The associations between ATXN7 SNPs and mRNA expression with the prognosis of HBV-related HCC were analyzed. **Results:** In all, rs3774729 was significantly associated with overall survival (OS) of HBV-related HCC ($P = 0.013$, HR = 0.66, 95% CI: 0.48–0.94). And patients with the AA genotype and a high level of serum alpha fetoprotein (AFP) had significantly worse OS when compared to patients with AG/GG genotypes and a low level of AFP (adjusted $P = 0.007$, adjusted HR = 1.83, 95% CI = 1.18–2.82). Furthermore, low expression of ATXN7 was significantly associated with poor recurrence-free survival (RFS) and OS ($P = 0.007$, HR = 2.38, 95% CI = 1.27–4.45 and $P = 0.025$, HR = 1.75, 95% CI = 1.18–2.62). **Conclusion:** ATXN7 may be a potential predictor of post-operative prognosis of HBV-related HCC.

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Introduction

Liver cancer is a lethal disease and is the fifth most common malignancy in men and the sixth most common in women. Hepatocellular carcinoma (HCC) is the dominant histological type that accounts for 70%–90% of primary liver cancer cases [1]. In 2012, approximately 782,500 new liver cancer cases and 745,500 deaths occurred worldwide, about half of which were found in China [2]. The poor prognosis and high fatality rates of liver cancer lead to incidence and mortality rates that are nearly equal. Nonetheless, progress in surgical techniques and perioperative management can improve survival rates for patients with liver cancer after hepatectomy, but their long-term prognosis remains poor. At present, multiple clinical indicators have been suggested as useful clinical markers to evaluate the prognosis of HCC, such as large tumor size, vascular invasion, positive portal vein tumor thrombosis (PVTT), increased AFP, advanced tumor-node-metastasis stage, tumor tissue mRNA, long noncoding RNAs, serum microRNA levels and circulating tumor cells [3–8]. However, these factors are not satisfactory predictors of HCC outcomes post-lesion resection. Thus, discovery and application of additional predictive biomarkers for improving therapeutic effects and prediction of clinical outcomes is of crucial significance.

Single nucleotide polymorphisms (SNPs), easy to detect by genotyping DNA extracted from blood specimens, have become attractive biomarkers in translational studies. Several studies have shown that specific SNPs are significantly associated with clinical outcomes in many malignant tumors, including HCC. For example, in HCC, SNPs located in telomere maintenance genes are associated with the prognosis of hepatitis C virus-related HCC [9], and the *HIF1A* SNP rs2057482 can be used to predict the prognosis of aggressive HCC cases receiving hepatectomy [10]. In pancreatic cancers, SNPs in the *APC* and *CD24* genes may predict the progress of the disease [11]. In lung cancer, *ERCC1* variant rs2298881 affects the prognosis of lung cancer after surgery by regulating the expression of the gene [12]. Numerous similar reports have been published. Thus, SNPs are potential biomarkers for clinical outcomes and can more accurately predict malignancy than other biomarkers.

Ataxin 7 (*ATXN7*), also known as autosomal dominant cerebellar ataxia II (*ADCAII*) or spinocerebellar ataxia type 7 (*SCA7*), contains 15 exons and maps to human chromosome 3p21.1-p12, covering 138.9 kb of genomic sequence. A previous study demonstrated that the normal allele of *ATXN7* contains 7–17 CAG repeats (near the N-terminus), but the disease allele contains 38–130 repeats [13]. *ATXN7* encodes an approximately 95 kD protein, which is involved in the SPT3/TAF9/GCN5 acetyltransferase and TBP-free TAF-containing chromatin remodeling complexes [14]. Hence, *ATXN7* plays an important role in transcriptional regulation. Recently, studies have reported that *ATXN7* is also associated with susceptibility to and prognosis of tumors. Milne et al. found that rs1053338 in the *ATXN7* gene was significantly associated with breast cancer susceptibility [15]. The mRNA expression of *ATXN7* was significantly down-regulated in clear cell renal cell carcinoma (RCC), and RCC patients with lower *ATXN7* expression had a lower rate of cancer-free survival [16].

However, associations between *ATXN7* variants and mRNA expression with prognosis in HBV-associated HCC have not been explored. Thus, in the present study, we investigated the associations between *ATXN7* functional SNPs and mRNA expression with clinical outcomes in Chinese patients with HBV-associated HCC receiving hepatectomy from Guangxi, China.

Materials and Methods

Ethics approval

This study was approved by the Ethical Review Committee of the First Affiliated Hospital of Guangxi Medical University. Written informed consent was obtained from all patients.

Study population

This study includes 475 patients who underwent hepatectomy at the First Affiliated Hospital of Guangxi Medical University (Guangxi, China) and were pathologically diagnosed with HBV-related HCC

from January 2005 to September 2013. The clinicopathological characteristics of patients were obtained from medical records and pathological reports, and included age, gender, smoking status, drinking status, pathological grade features of the cancer, serum AFP level, hepatic cirrhosis, radical resection, adjuvant antiviral therapy, and adjuvant chemotherapy. Tumor status was classified according to the Barcelona Clinic Liver Cancer (BCLC) staging system [17]. Child-Pugh class was defined as previously published [18]. PVT was determined according to previous classifications [19]: (1) vp1, PVT in distal to second order portal branches; (2) vp2, PVT in second order portal branches; (3) vp3, PVT in first order branches; and (4) vp4, PVT in main trunk. Smoking status, drinking status, and radical resection were defined as previously reported [20].

Follow-up information on the patients was obtained through on-site interviews, telephone communication, reviewing medical records, or hospital visits, until death/recurrence or the end of the study in September 2014, whichever came first. The time of overall survival (OS) was defined from hepatectomy to HCC-specific death. The time of recurrence-free survival (RFS) was defined from surgery to the date of the first recurrence or distant metastasis of HCC. Patients alive at the last follow-up were censored.

SNP selection, DNA extraction, and Genotyping

According to a previous publication [21], an online network of SNP screening tools (<http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm>) was used to select candidate SNPs in *ATXN7*. The inclusion criteria were as follows: (i) SNPs in splice sites and CDS regions resulting in amino acid changes; SNPs in transcription factor binding sites of the 5' flanking region; and SNPs in miRNA binding sites of 3' UTR; and (ii) SNPs with minor allele frequency (MAF) $\geq 10\%$ in the Han Chinese population (CHB) from the 1000 Genomes Project (March 2012, build GRCh37/hg19). These criteria resulted in two SNPs (rs3774729 and rs1053338) being identified for assessment in this study, both of which were located in *ATXN7* CDS regions.

HCC lesion tissues were collected after surgical resection and immediately stored at -80°C until DNA extraction. Genomic DNA of HCC tissue samples was extracted using a TIANamp Genomic DNA Kit (Tiagen Biotech, Beijing, China) according to the manufacturer's protocol. DNA purity was measured with the NanoDrop2000 system (Thermo Fisher Scientific, Waltham, MA, USA), and all DNA quality met the experimental requirements.

rs3774729 and rs1053338 were genotyped by Sanger DNA sequencing after PCR amplification using the following primers: rs3774729, forward: 5'-GGAGCCCTGCTGAATCCATCAAG-3' and reverse: 5'-GAACATCTTCCAAAGGCACCAGAA-3'; rs1053338, forward: 5'-CTCATCCCTCTGGCTCACCATT-3' and reverse: 5'-AAAACCCAAGAGTGAAAAGAACAAAC-3'. PCR was implemented in a 50 μL reaction volume using HotMaster PCR MasterMix (KT208-02, Tiagen Biotech, Beijing, China). According to the manufacturer's protocol, the amplification conditions were as follows: (1) 3 min at 94°C ; (2) 30 cycles of 94°C for 30 s, 62°C for 30 s, 65°C for 60 s; and (3) 65°C for 5 min. All PCR products were sequenced using the ABI Prism 3730XL (Applied Biosystems, California CA, USA) by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China).

ATXN7 expression analyses based on the Gene Expression Omnibus (GEO) database

To investigate the association between *ATXN7* expression and clinical outcomes of HBV-related HCC patients, we searched the GEO database (accession: GSE14520) and obtained a cohort of 221 HBV-related HCC cases from Zhongshan Hospital, Fudan University (Shanghai, China). The 75th percentile of *ATXN7* mRNA expression in the total group of patients was used as the cutoff point to define the lower and higher expression groups.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was calculated by the goodness-of-fit χ^2 test. Univariate and multivariate survival analyses were done using the Cox proportional hazards regression model. Hazard ratios (HR) and their 95% confidence intervals (CI) were calculated from the Cox proportional hazards regression model with adjustment for clinicopathological characteristics such as pathological grade, biobehavior of the cancer, serum AFP level, hepatic cirrhosis, radical resection, adjuvant antiviral therapy, adjuvant chemotherapy, and PVT. The survival curve of different groups of significant SNPs and *ATXN7* mRNA expression were plotted using a Kaplan-Meier survival curve by GraphPad Prism 6. Statistical analysis was performed with SPSS version 18.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Distribution of patient characteristics and clinical outcomes analysis

The distribution of patient characteristics is shown in Table 1. Overall, the median follow-up duration was 41 months (range: 1–125 months), and the median survival time (MST) was 58 months, while the median recurrence time (MRT) was 7 months. The 2-year, 5-year, and 10-year OS (RFS) rates were 70.7% (69.1%), 49.0% (91.4%), and 27.2% (95.3%), respectively. As shown in Table 1, BCLC stage, antiviral therapies, and PVTT were significantly associated with OS and RFS. In addition, adjuvant transcatheter hepatic arterial chemoembolization (TACE) and radical resection were associated with RFS. The biobehavior of HCC, including tumor size (> 5 cm), intrahepatic metastasis, and vascular invasion, had adverse effects on the OS and RFS. Moreover, patients with multiple tumors had a higher risk for shorter OS when compared to patients with a single tumor. There were no significant associations observed between long-term OS or RFS and age, gender, race, body mass index, smoking status, drinking status, cirrhosis, AFP, Child-Pugh class, or pathological grade. Moreover, AFP level was significantly associated with 2-year OS and RFS (Fig. 1). Patients with higher AFP levels (> 400 ng/mL) had a worse 2-year OS and RFS.

Genotyping and association of ATXN7 SNPs with clinical outcomes in HBV-related HCC patients

A total of 475 samples from HBV-related HCC patients were genotyped for rs3774729 and rs1053338 (Fig. 2). There were 100 (21.1%), 240 (50.5%), and 135 (28.4%) carriers of the rs3774729-AA, AG, and GG genotypes, respectively. Additionally, there were 371 (78.1%), 93 (19.6%), and 11 (2.3%) carriers of the rs1053338-AA, AG, and GG genotypes, respectively. The allele frequencies of rs3774729 and rs1053338 conformed to HWE (Table 2).

Multivariate survival analysis showed that rs3774729 was significantly associated with OS after adjustment for clinicopathological characteristics (Table 2, 3 and Fig. 2). However, rs3774729 was not significantly associated with RFS, and no significant associations were observed between rs1053338 genotype and clinical outcomes in HBV-related HCC patients. Table 3 shows that compared to patients with the AA genotype of rs3774729, patients with the AG or GG genotypes had a better OS (adjusted $P = 0.013$, adjusted HR = 0.66, 95% CI: 0.48–0.94). Moreover, in the multivariate Cox proportional hazards regression model, only the following clinical factors were significantly with overall survival: BCLC ($P < 0.001$, HR = 1.53, 95% CI = 1.23–1.91), PVTT ($P = 0.019$, HR = 1.20, 95% CI = 1.03–1.40) and antiviral therapy ($P = 0.047$, HR = 0.71, 95% CI = 0.51–0.99).

Stratification analysis of ATXN7 rs3774729 genotypes associated with OS

We carried out further stratified analysis by age, smoking and drinking status, preoperative AFP, radical resection, intrahepatic metastasis, BCLC stage, antiviral therapies, Child-Pugh class, and vascular invasion to evaluate associations between rs3774729 and OS of patients with HBV-related HCC. As shown in Fig. 3, we observed that the risk of death was significantly increased in patients with the rs3774729-AA genotype when they were older than 46 years (HR = 2.2, 95% CI: 1.04–4.67) or had Child-Pugh class B (HR = 1.74, 95% CI: 0.72–4.18), AFP level > 400 ng/mL (HR = 1.62, 95% CI: 1.03–2.55), the presence of PVTT (HR = 1.54, 95% CI: 0.74–3.18), or regional invasion (HR = 1.56, 95% CI: 0.75–3.22).

The combined effect of rs3774729 and serum AFP on OS

Furthermore, we assessed the combined effect of rs3774729 and serum AFP on OS (Table 4, Fig. 4). According to genotype and serum AFP level, the patients were classified into four subgroups as follows: (1) low level of AFP (≤ 400 ng/mL) with AA genotype; (2) low level of AFP with AG/GG genotype; (3) high level of AFP (> 400 ng/mL) with AA genotype; and (4) high level of AFP with AG/GG genotype. The results showed that in patients with the same genotype who had high levels of AFP had a higher risk for death than those with a low level of AFP (Table 4). When compared to patients carrying AG/GG genotypes and a low level

Table 1. Demography and clinical outcomes of 475 HCC patients. Note: *HR and *P* value for univariate survival analysis of Cox proportional hazard regression model. Abbreviations: AFP, alpha-fetoprotein; TACE, transarterial chemoembolization; BMI, body mass index; PVTT, portal vein tumor thrombus; MST, median survival time; MRT, median recurrence time; HR, hazard ratio; 95% CI, 95% confidence interval; Ref., reference

Variables	Patients (n=475)	MST (months)	Overall survival HR* (95% CI)	<i>P</i> *	Recurrence-free survival MRT (months)	HR* (95% CI)	<i>P</i> *
Age (yr)							
≤ 46	259	61	Ref.		6	Ref.	
> 46	216	58	0.90 (0.69-1.17)	0.431	12	0.76 (0.58-1.01)	0.055
Gender							
Male	420	57	Ref.		6	Ref.	
Female	55	80	0.78 (0.49-1.23)	0.285	14	0.86 (0.54-1.36)	0.509
Race							
Han	300	71	Ref.		7	Ref.	
Minority	175	51	1.14 (0.86-1.50)	0.358	7	1.18 (0.89-1.57)	0.247
BMI							
≤ 25	391	61	Ref.		7	Ref.	
> 25	84	57	0.99 (0.71-1.38)	0.94	6	1.13 (0.79-1.61)	0.513
Smoking status							
None	313	71	Ref.		10	Ref.	
Ever	162	44	1.19 (0.90-1.56)	0.228	5	1.27 (0.95-1.70)	0.106
Drinking status							
None	287	71	Ref.		8	Ref.	
Ever	188	48	1.24 (0.95-1.63)	0.108	6	1.24 (0.94-1.64)	0.134
Adjuvant TACE ^a							
No	203	93	Ref.		14	Ref.	
Yes	272	47	1.27 (0.96-1.68)	0.095	6	1.52 (1.11-2.08)	0.008
BCLC stage							
A	279	96	Ref.	< 0.001	13	Ref.	< 0.001
B	75	71	1.80 (1.25-2.59)	0.002	7	1.01 (0.68-1.50)	0.971
C	121	24	3.27 (2.43-4.39)	< 0.001	3	1.86 (1.37-2.54)	< 0.001
Child-Pugh score							
A	394	63	Ref.		7	Ref.	
B	81	39	1.26 (0.86-1.83)	0.236	6	1.31 (0.85-2.02)	0.224
Cirrhosis							
No	58	82	Ref.		13	Ref.	
Yes	417	52	1.27 (0.83-1.95)	0.265	6	1.39 (0.88-2.21)	0.163
Antiviral therapy ^b							
No	306	50	Ref.		6	Ref.	
Yes	169	81	0.72 (0.53-0.98)	0.042	13	0.74 (0.55-0.99)	0.042
AFP (ng/ml)							
≤ 400	243	68	Ref.		12	Ref.	
> 400	198	50	1.32 (0.99-1.74)	0.052	5	1.18 (0.88-1.58)	0.263
NA	34						
Radical resection							
No	201	47	Ref.		5	Ref.	
Yes	264	74	0.77 (0.59-1.01)	0.056	11	0.70 (0.53-0.93)	0.012
NA	10						
Pathological grade							
Well	26	79	Ref.	0.583	6	Ref.	0.209
Moderately	366	51	1.34 (0.71-2.54)	0.367	6	1.36 (0.71-2.61)	0.348
Poorly	12	NA	1.15 (0.32-3.25)	0.976	3	2.67 (0.90-7.93)	0.077
NA	71						
Oncological features							
Tumor size							
≤ 5 cm	152	123	Ref.		23	Ref.	
> 5 cm	323	41	2.11 (1.53-2.93)	< 0.001	5	1.76 (1.28-2.40)	< 0.001
No. of tumors							
Single (n = 1)	351	68	Ref.		9	Ref.	
Multiple (n > 1)	124	35	1.56 (1.18-2.07)	0.002	5	1.13 (0.834-1.54)	0.427
Regional invasion							
Absence	403	71	Ref.		11	Ref.	
Presence	72	37	1.73 (1.21-2.47)	0.002	2	1.66 (1.18-2.36)	0.004
Intrahepatic metastasis							
Absence	258	81	Ref.		11	Ref.	
Presence	217	38	1.84 (1.41-2.41)	< 0.001	5	1.67 (1.18-2.36)	0.004
Vascular invasion							
Absence	389	80	Ref.		10	Ref.	
Presence	86	18	3.45 (2.55-4.65)	< 0.001	2	1.68 (1.21-2.34)	0.002
PVTT							
No	399	79	Ref.	< 0.001	11	Ref.	0.002
vp1	11	28	2.59 (1.21-5.53)	0.014	2	1.42 (0.53-3.83)	0.491
vp2	17	17	3.74 (2.15-5.49)	< 0.001	2	2.21 (1.12-4.35)	0.021
vp3	40	17	3.12 (2.11-4.62)	< 0.001	3	1.52 (0.97-2.38)	0.065
vp4	8	8	6.60 (3.07-14.20)	< 0.001	1	3.82 (1.66-8.76)	0.002

Fig. 1. Kaplan-Meier plots of survival by AFP levels in clinical outcomes of HBV-related hepatocellular carcinoma (HCC) patients. (A) Kaplan-Meier plot for long-term (10-year) overall survival (OS) of HBV-related HCC patients; (B) Kaplan-Meier plot for 2-year OS of HBV-related HCC patients; (C) Kaplan-Meier plot for long-term (10-year) recurrence-free survival (RFS) of HBV-related HCC patients; (D) Kaplan-Meier plot for 2-year RFS of HBV-related HCC patients.

Table 2. Association of *ATXN7* rs3774729 and rs1053338 with clinical outcomes of HCC patients. Note: **P* adjustment for age, gender, BMI, race, smoking status, drinking status, child-pugh class, cirrhosis, BCLC stage, pathological grade, adjuvant TACE, adjuvant antiviral therapy, radical resection, AFP level, intrahepatic metastasis, vascular invasion, regional invasion and PVT. Abbreviations: SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; TFBS, Transcription Factor Binding Site; ESE, exonic splicing enhancer; ESS, exonic splicing silencer; HR, hazard ratio; 95% CI, 95% confidence interval

SNP	Chr	Position	Gene	Allele	Function	MAF	HWE	Overall survival		Recurrence-free survival	
								<i>P</i> *	HR* (95% CI)	<i>P</i> *	HR* (95% CI)
rs3774729	3	63982082	<i>ATXN7</i>	A/G	Nonsynonymous	0.46	0.726	0.035	0.79 (0.65-0.98)	0.889	1.02 (0.83-1.24)
rs1053338	3	63967900	<i>ATXN7</i>	A/G	Nonsynonymous	0.12	0.082	0.707	1.18 (1.01-1.38)	0.227	1.25 (0.87-1.79)

Table 3. Genotypes of *ATXN7* rs3774729 and overall survival of HCC patients. Note: **P* adjustment for age, gender, BMI, race, smoking status, drinking status, child-pugh class, cirrhosis, BCLC stage, pathological grade, adjuvant TACE, adjuvant antiviral therapy, radical resection, AFP level, intrahepatic metastasis, vascular invasion, regional invasion and PVT. In the multivariate Cox proportional hazards regression model, only the following clinical factors were significantly with overall survival: BCLC, PVT and antiviral therapy. Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval, MST, median survival time

SNP	Patients (n=475)	MST(months)	Crude HR (95% CI)	Overall survival		
				Crude <i>P</i>	Adjusted HR* (95%CI)	Adjusted <i>P</i> *
rs3774729						
AA	100	40	Ref.	0.035	Ref.	0.043
AG	240	75	0.67 (0.48-0.92)	0.014	0.67 (0.48-0.95)	0.023
GG	135	71	0.68 (0.47-0.98)	0.038	0.64 (0.43-0.96)	0.030
AG+GG	375	71	0.67 (0.49-0.91)	0.010	0.66 (0.48-0.94)	0.013

Fig. 2. Kaplan-Meier survival curves for *ATXN7* variant rs3774729 and mRNA expression. (A) rs3774729 (AA vs. AG and GG). (B) rs3774729 (AA vs. AG/GG). (C-D) Kaplan-Meier plot for clinical outcomes of HCC patients by *ATXN7* mRNA expression. The 75th percentile of *ATXN7* mRNA expression in the total population was used as the cutoff point to define lower and higher expression groups.

Fig. 3. Stratification analysis of the association of rs3774729 with OS of HBV-related HCC patients. Stratified by favorable and adverse strata. *Intrahepatic metastasis.

of AFP, patients with the AA genotype and a high level of AFP had a significantly worse OS (adjusted $P = 0.007$, adjusted HR = 1.83, 95% CI = 1.18–2.82).

Association of *ATXN7* expression with clinical outcomes in HBV-related HCC patients

To confirm the expression pattern and prognostic value of *ATXN7* in HBV-related HCC, we retrieved data on another HBV-related HCC cohort from GEO. Compared with adjacent normal tissues, down-regulated expression of *ATXN7* was observed in HCC tissues (Fig. 5). Furthermore, we analyzed the association of *ATXN7* expression with clinical outcomes in HBV-related HCC patients. We observed that the expression of *ATXN7* was significantly associated with OS and RFS (Table 5, Fig. 2 C-D). Patients with higher *ATXN7* expression had

Table 4. Combined effects of *ATXN7* rs3774729 and serum AFP level. Note: **P* adjustment for age, gender, BMI, race, smoking status, drinking status, child-pugh class, cirrhosis, BCLC stage, pathological grade, adjuvant TACE, adjuvant antiviral therapy, radical resection, intrahepatic metastasis, vascular invasion, regional invasion and PVTT. Abbreviations: AFP, alpha-fetoprotein; HR, hazard ratio; 95% CI, 95% confidence interval, MST, median survival time

Groups	MST (months)	Crude HR (95%CI)	Crude <i>P</i>	HR*(95%CI)	<i>P</i> *
AFP ≤ 400 (ng/mL) + AG/GG	75	Ref.	0.014	Ref.	0.042
AFP > 400 (ng/mL) + AG/GG	74	1.27 (0.92-1.74)	0.151	1.08 (0.77-1.50)	0.664
AFP ≤ 400 (ng/mL) + AA	47	1.46 (0.92-2.32)	0.111	1.32 (0.83-2.11)	0.248
AFP > 400 (ng/mL) + AA	27	1.98 (1.30-3.04)	0.002	1.83 (1.18-2.82)	0.007

Table 5. Association between *ATXN7* mRNA expression and clinical outcomes of HCC patients. Note: The 75th percentile of mRNA expression in the total population was used as the cut-off point to define lower and higher expression groups. *Adjustment for age, gender, cirrhosis, BCLC stage, serum AFP levels. Abbreviations: MST, median survival time; MRT, median relapse time; HR, hazard ratio; 95% CI, 95% confidence interval; Ref., reference

Gene	Patients (n=221)	MST(months)	Overall survival HR* (95%CI)	<i>P</i> *	Recurrence-free survival MRT(months)	HR* (95%CI)	<i>P</i> *
<i>ATXN7</i>							
Higher	56	NA	Ref.		NA	Ref.	
Lower	165	60.5	2.38 (1.27-4.45)	0.007	32.6	1.75 (1.18-2.62)	0.025

Fig. 4. Kaplan-Meier survival curves for the combined effects of *ATXN7* variant rs3774729 and serum AFP levels.

Fig. 5. The mRNA expression of *ATXN7* in HCC tissues and adjacent normal tissues. There was a significant difference between HCC tissues and adjacent normal tissues ($P < 0.001$). Data are expressed as mean $\pm$ standard deviation. Student's *t*-test was used to examine the statistical difference.

a better OS and RFS than those with lower *ATXN7* expression ($P = 0.007$, HR = 2.38, 95% CI = 1.27–4.45 and $P = 0.025$, HR = 1.75, 95% CI = 1.18–2.62, respectively).

Discussion

In the present study, we investigated associations between *ATXN7* SNPs and mRNA expression with clinical outcomes in HBV-related HCC patients. We identified that rs3774729 was significantly associated with long-term (10-year) OS in HBV-related HCC. Patients with the rs3774729-AA genotype had a higher risk of death compared with patients who carried the AG/GG genotypes. The expression of *ATXN7* mRNA was also tied to OS and RFS, and patients had worse OS and RFS when they had lower *ATXN7* mRNA expression. Therefore, *ATXN7* may be a potential biomarker for predicting clinical outcomes in HBV-related HCC.

Previously, *ATXN7* has mostly been reported in neurodegenerative disorders. Ajayi et al. [22] reported that mutant *ATXN7* can inhibit TP53 activity and enhance NOX1 activity, resulting in metabolic disorder and mitochondrial dysfunction in SCA7 disease. Mutant *ATXN7* protein formed a co-polymer with TP53, and then reduced soluble TP53 levels and inhibited TP53 activity. In addition, Gatchel et al. [23] found that insulin-like growth factor (IGF) pathway was involved in SCA7 disease. There have been many studies that reported that both TP53 and IGF play a critical role in the occurrence and prognosis of HCC [24-28]. Recent studies demonstrated that *ATXN7* was associated with breast cancer susceptibility and prognosis of RCC [15, 16]. Unfortunately, these studies did not further explore the mechanism of how *ATXN7* affects the prognosis of patients with these tumors. Currently, based on limited evidence, we suspect that *ATXN7* may affect clinical outcomes via disrupting the activity of TP53 and the function of the IGF pathway.

rs3774729 (G>A, Val862Met) is located in exon 13 of *ATXN7*. A previous study reported that this SNP was significantly associated with cerebellar grey matter volume in healthy adults [29]. However, there have been no studies to evaluate the association between rs3774729 and prognosis and risk in malignant tumors. In our study, the rs3774729-AA genotype was associated with a shorter MST (40 months) when compared with patients carrying rs3774729 AG/GG genotypes (71 months). rs1053338 (A>G, Lys264Arg) is located in exon 8 of *ATXN7*. One previous study reported that rs1053338 was closely related to the susceptibility to breast cancer [15]. However, in our study, we did not observe an association between rs1053338 genotype and OS or RFS in HBV-related HCC patients. Gotoh et al. [16] reported that *ATXN7* mRNA expression was significantly reduced in tumor tissue of RCC, and lower expression of *ATXN7* was associated with a poor rate of cancer-free survival. In our study, we observed similar results. Furthermore, in HBV-related HCC patients, compared to patients with higher *ATXN7* expression, lower expression of *ATXN7* was an indicator that predicted poor OS and RFS. However, at present, the mechanism of how *ATXN7* affects clinical outcomes of HBV-related HCC remains unknown. According to the above evidence, we hypothesize that rs3774729 may influence the prognosis of HBV-related HCC by affecting the expression or function of *ATXN7*, but further *in vitro* experiments are necessary.

AFP is one of the most commonly used biomarkers for diagnosing and evaluating the clinical outcomes of HCC, and the contribution of AFP to oncogenic and ontogenetic growth has been recognized [30, 31]. However, the prognostic value of AFP remains controversial. Some studies have suggested using AFP as an indicator to predict clinical outcomes in HCC [32, 33]. However, other studies have suggested that serum AFP is not an effective predictor of prognosis in HCC [34-36]. In this report, 400 ng/mL was used as the cut-off value to define lower and higher levels of AFP, and we found that a higher AFP level (> 400ng/mL) was significantly associated with shorter (within 2 years) OS and RFS, but not with long-term clinical outcomes. This result is similar to previous research [37]. Moreover, when rs3774729 genotypes were analyzed in combination with AFP levels, we defined four subgroups ((1) low level of AFP ($\leq$ 400 ng/mL) with AA genotype; (2) low level of AFP with AG/GG genotype; (3) high level of AFP (> 400 ng/mL) with AA genotype; (4) high level of AFP with AG/GG genotype), and the MST for each group was 47, 75, 27, and 74 months, respectively (adjusted $P = 0.042$). We found that patients with the same level of AFP who carried the AA genotype had the worst OS. Hence, our results indicate that rs3774729 not only is an independent

predictor for poor OS of HBV-related HCC patients, but also that, in combination with AFP levels, it gave a more accurate clinical outcomes prediction.

However, this study presents several limitations. Multicenter studies are needed to confirm the association of rs3774729 with OS and to determine whether it is a reliable biomarker for predicting clinical outcomes in HBV-related HCC. Additionally, rs3774729 was not functionally characterized to reveal its effects on *ATXN7* expression and prognosis of HBV-related HCC. Therefore, *in vitro* studies are required to clarify the underlying mechanism of how *ATXN7* is involved in influencing clinical outcomes.

In conclusion, our results demonstrate that the *ATXN7* variant rs3774729 is significantly associated with post-operative OS in HBV-related HCC. rs3774729 is an independent indicator for poor OS, and when combined with AFP levels can improve the accuracy of clinical outcomes prediction. *ATXN7* mRNA expression is also significantly correlated with OS and RFS of HBV-related HCC patients receiving hepatectomy. Hence, *ATXN7* may be a potential predictor of post-operative prognosis of HBV-related HCC.

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Disclosure Statement

All authors declare no conflict of interest.

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