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Functional variants of autophagy-related genes are associated with the development of hepatocellular carcinoma

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ABSTRACT

Aims: Hepatocellular carcinoma (HCC) is the most common primary liver cancer, and accounts for substantial morbidity and mortality. Autophagy plays an essential role in the development and progression of HCC. This study aims to evaluate whether genetic variants in autophagy-related genes (ATGs) affect the development of HCC.

Materials and methods: We conducted a case-control study with 986 HCC cases and 1000 healthy controls to analyze 14 functional variants of five ATGs (*ATG3, ATG5*, *ATG10*, *ATG12* and *ATG16L1*) among a Chinese population.

Key findings: We found *ATG5* rs17067724 (G vs A: OR = 0.80; 95% CI = 0.65–0.98; $P = 0.031$), *ATG10* rs1864183 (G vs A: OR = 1.29; 95% CI = 1.07–1.57; $P = 0.009$), *ATG10* rs10514231 (C vs T: OR = 1.41; 95% CI = 1.15–1.73; $P = 0.001$), *ATG12* rs26537 (C vs T: OR = 1.16; 95% CI = 1.02–1.33; $P = 0.030$), and *ATG16L1* rs4663402 (T vs A: OR = 1.28; 95% CI = 1.01–1.63; $P = 0.044$) were significantly associated with HCC risk. Specifically, *ATG10* rs10514231 kept significant association even adjusted for Bonferroni correction ($P = 0.001 \times 14 = 0.014$). Bioinformatics analyses showed that allele C of *ATG10* rs10514231 was significantly correlated with higher expression of *ATG10* gene in both HCC tissues and normal liver tissues. Dual-luciferase reporter assay presented that cell lines transfected with vectors containing the risk allele C of rs10514231 showed higher relative luciferase activity compared to that containing the allele T.

Significance: These results suggested that *ATG10* rs10514231 might contribute to an allele-specific effect on the expression of host gene *ATG10* and explain a fraction of HCC genetic susceptibility. Our study would benefit the construction of early warning model, early prevention, screening, even therapeutic target of HCC.

1. Introduction

Live cancer is one of the most common cancers worldwide and accounts for substantial morbidity and mortality [1–3]. According to the report of Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2017, there were about 820 thousand all-age deaths of liver cancer annually in the world [1]. Among those, China account for 466.1 thousand deaths of liver cancer [2]. Hepatocellular carcinoma (HCC) is the most common primary liver cancer and accounts for about 85% to 90% of liver cancers [4]. Chronic hepatitis B virus (HBV) infection, chronic hepatitis C virus (HCV) infection, as well as nonalcoholic fatty liver disease (NAFLD) has been thought to be the most important etiologies of HCC [5]. However, the fact that only a small proportion of subjects with the established risk factors above eventually develop HCC suggests that genetic factors may play an important role in the development of HCC.

Recently, cumulative evidence has indicated that autophagy plays an essential role in multiple stages of HCC development and progression [6–10]. Autophagy is a lysosomal-mediated catabolic process plays a critical role in all eukaryotic life cycles [11,12]. Autophagy-related genes (ATGs) play a key role in autophagy, as well as control autophagic formation and eventually accelerate HCC development, invasion, metastasis, progression and prognosis [6,12,13]. Epidemiological studies also revealed that genetic variants in ATGs might influence the biological function and consequently contribute to development and prognosis of many cancers, including lung cancer, head and neck squamous cell carcinoma, breast cancer, gastric cancer, renal cell carcinoma [14–17]. However, few studies have focused on the role of ATGs in the development of HCC. Inspired by the findings above, we hypothesized that genetic variants of ATGs may influence the susceptibility to HCC. Thus, in current study, we conducted a case-control study in a Chinese population and functional validation experiments to

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establish the relationships, if any, between potentially functional variants of ATGs and development of HCC.

2. Patients and methods

2.1. Study subjects

Totally included in this study were 986 histopathologically confirmed HCC cases and 1000 cancer-free controls, which were consecutively recruited since June 2010. Those who had a history of cancer, metastasized cancer from other organs, radiotherapy or chemotherapy were excluded. Controls were frequency-matched to the HCC cases on age (5-year interval), gender, and residential area (urban or rural). All the participants were face-to-face interviewed by trained interviewers to collect the individual information on demographic data. After that, 5-ml of venous blood was collected from each subject. All participants in the study signed informed consent. Our research sought the consent of the Ethics Committee of the Fourth Affiliated Hospital of China Medical University and conducted following the approved guidelines.

2.2. SNP selection and genotyping

The potential functional variants of five ATGs (*ATG3*, *ATG5*, *ATG10*, *ATG12*, and *ATG16L1*) were first selected based on the public HapMap SNP database using the HaploView 4.2 software, with minor allele frequency ≥ 0.05 and an r^2 threshold of 0.80 in the Chinese Han population. Then, the SNPinfo Web Server (http://snpinfo.niehs.nih.gov/) was used to screen the potentially functional SNPs. Finally, 14 variants (*ATG3* rs2705507 and rs7652377, *ATG5* rs17067724, rs3804338, rs510432, and rs688810, *ATG10* rs1864183, rs10514231, and rs10036653, *ATG12* rs26532 and rs26537, *ATG16L1* rs2241880, rs4663402, and rs1045095) were included in the study. The genotyping was performed using the TaqMan methodology and read with the Sequence Detection Software on an ABI-Prism 7900 instrument according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). To assess reproducibility, 5% of the DNA samples were blindly and randomly analyzed in duplicates, and the results revealed a reproducibility of 100%.

2.3. Bioinformatics analyses

The level 3 TCGA gene expression data for 369 liver hepatocellular carcinoma (LIHC) samples were used to assess the mRNA expression levels of the promising genes using UALCAN [18]. GTEx normal liver tissues and TCGA LIHC dataset were used to performed expression quantitative trait loci (eQTL) analyses to examine the effects of promising SNPs on mRNA expression [19,20]. HaploRegv4.1 was used to annotate the functional elements of the selected variants [21].

2.4. Dual luciferase reporter assay

The Hep3B and SK-Hep1 cell lines were purchased from American Type Culture Collection (ATCC; Manassas, VA). Both cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), and kept in a 37 °C humidified incubator in an atmosphere of 5% CO2 in air. About 300-bp DNA sequences centered at rs10514231 (T or C allele) were synthesized and cloned into pGL-3 promoter vectors (Promega, Madison, WI, USA), respectively. Then, cultured cells were transfected with 0.8 μg pGL-3 vectors, while tThe pRL-TK Renilla control vector (0.1 μg) (Promega, Madison, WI, USA) was also co-transfected as an internal control for transfection efficiency. After 48 h transfection, cells were collected and analyzed for luciferase activity with the Dual Luciferase Reporter Assay System (Promega, Wisconsin, USA). All reporter assays were performed in triple at least three independent experiments.

Table 1
Distributions of selected variables in HCC cases and healthy controls.

	Cases (n = 986)	Controls (n = 1000)	P value
Age			
< 50	503 (51.0%)	521 (52.1%)	0.628
≥50	483 (49.0%)	479 (47.9%)	
Gender			
Male	599 (60.8%)	619 (61.9%)	0.599
Female	387 (39.2%)	381 (38.1%)	
Smoking status			
Yes	199 (20.2%)	214 (21.4%)	0.504
No	787 (79.8%)	786 (78.6%)	
Drinking status			
Yes	312 (31.6%)	334 (33.4%)	0.403
No	674 (68.4%)	666 (66.6%)	
HBV infection			
Yes	219 (22.1%)	102 (10.2%)	< 0.001
No	767 (77.9%)	898 (89.8%)	
Family history of cancer			
Yes	99 (10.1%)	38 (3.8%)	< 0.001
No	887 (89.9%)	962 (96.2%)	

P value in bold means statistically significant.

2.5. Statistical analysis

The distributions of general characteristics between HCC cases and controls were performed using x^2 test. Hardy-Weinberg Equilibrium (HWE) in the controls was tested using a x^2 goodness of fit test. Logistic regression models were used to estimate adjusted odds ratio (OR) and 95% confidence interval (CI) adjusted for age, gender, smoking and drinking status, HBV infection, and family history of cancer. The analyses were done first per genotype (additive model) and then per allele (allelic model), which assumes the same effect size for per allele change. All statistical analyses were conducted with SAS version 9.4 (SAS Institute Inc.) $P < 0.05$ was the criterion of statistical significance and all statistical tests were two-tailed. The original data were presented in supplementary file 1.

3. Results

3.1. Characteristics of study subjects

Table 1 presents the distributions of general characteristics of the study population, which includes 986 histopathologically confirmed HCC cases and 1000 cancer-free controls. In brief, there were no statistical differences in the distribution of age, gender, smoking and drinking status between HCC patients and control subjects ($P > 0.05$). However, the HCC patients have higher percentage of HBV infection and family history of cancer, compared with the cancer-free controls ($P < 0.001$).

3.2. Associations of selected functional variants of ATGs with HCC risk

Table 2 presents the distributions of the selected functional variants of ATGs and genetic associations of these variants with risk of HCC. The genotype frequencies of the fourteen SNPs in the controls obeyed HWE ($P > 0.05$) and could represent the general population. Among the 14 variants, significant associations with HCC risk were detected for five variants, including *ATG5* rs17067724 (G vs A: OR = 0.80; 95% CI = 0.65–0.98; $P = 0.031$), *ATG10* rs1864183 (G vs A: OR = 1.29; 95% CI = 1.07–1.57; $P = 0.009$), *ATG10* rs10514231 (C vs T: OR = 1.41; 95% CI = 1.15–1.73; $P = 0.001$), *ATG12* rs26537 (C vs T: OR = 1.16; 95% CI = 1.02–1.33; $P = 0.030$), and *ATG16L1* rs4663402 (T vs A: OR = 1.28; 95% CI = 1.01–1.63; $P = 0.044$) in allelic model. Specifically, *ATG10* rs10514231 kept significant association even

Table 2
Functional variants of the ATGs and occurrence of HCC.

SNP	Cases	Controls	OR (95% CI)[a]	*P* value
ATG3				
Rs2705507				
CC	638	642	1.00	
CA	318	323	0.99 (0.81–1.20)	0.895
AA	30	35	0.87 (0.52–1.45)	0.595
A vs C			0.97 (0.82–1.14)	0.696
rs7652377				
CC	667	693	1.00	
CA	292	283	1.08 (0.88–1.32)	0.458
AA	27	24	1.11 (0.62–1.97)	0.725
A vs C			1.07 (0.90–1.27)	0.436
ATG5				
rs17067724				
AA	801	780	1.00	
AG	174	201	0.83 (0.66–1.04)	0.111
GG	11	19	0.52 (0.24–1.13)	0.099
G vs A			0.80 (0.65–0.98)	0.031
rs3804338				
GG	686	691	1.00	
AG	267	279	0.96 (0.78–1.17)	0.684
AA	33	30	1.17 (0.70–1.96)	0.553
A vs G			1.00 (0.85–1.19)	0.980
rs510432				
GG	376	371	1.00	
AG	488	499	0.95 (0.78–1.15)	0.608
AA	122	130	0.95 (0.71–1.28)	0.750
A vs G			0.97 (0.85–1.11)	0.650
rs688810				
AA	396	399	1.00	
AG	471	478	1.00 (0.83–1.22)	0.975
GG	119	123	0.96 (0.71–1.29)	0.785
G vs A			0.99 (0.86–1.13)	0.845
ATG10				
rs1864183				
AA	751	806	1.00	
AG	209	179	1.27 (1.01–1.59)	0.041
GG	26	15	1.81 (0.94–3.49)	0.077
G vs A			1.29 (1.07–1.57)	0.009
rs10514231				
TT	763	827	1.00	
TC	202	165	1.32 (1.05–1.67)	0.019
CC	21	8	2.98 (1.30–6.84)	0.010
C vs T			1.41 (1.15–1.73)	0.001
rs10036653				
AA	450	449	1.00	
AT	439	445	0.98 (0.81–1.18)	0.823
TT	97	106	0.88 (0.65–1.21)	0.432
T vs A			0.95 (0.83–1.09)	0.495
ATG12				
rs26532				
AA	396	403	1.00	
AC	461	459	1.03 (0.85–1.25)	0.778
CC	129	138	0.90 (0.68–1.19)	0.458
C vs A			0.97 (0.85–1.11)	0.637
rs26537				
TT	421	475	1.00	
TC	452	431	1.16 (0.96–1.41)	0.121
CC	113	94	1.35 (0.99–1.85)	0.056
C vs T			1.16 (1.02–1.33)	0.030
ATG16L1				
rs2241880				
AA	389	394	1.00	
AG	463	465	1.00 (0.82–1.21)	0.974
GG	134	141	0.97 (0.73–1.29)	0.842
G vs A			0.99 (0.87–1.13)	0.865
rs4663402				
AA	844	883	1.00	
AT	127	111	1.17 (0.89–1.55)	0.270
TT	15	6	2.75 (1.05–7.21)	0.040
T vs A			1.28 (1.01–1.63)	0.044
rs1045095				
CC	302	305	1.00	
CT	482	492	1.00 (0.81–1.23)	0.977
TT	202	203	0.98 (0.76–1.27)	0.902
T vs C			0.99 (0.87–1.13)	0.909

P value in bold means statistically significant.
[a] Adjusted for age, gender, smoking and drinking status, HBV infection, and family history of cancer.

adjusted for Bonferroni correction ($P = 0.001 \times 14 = 0.014$). Compared the subjects with major homozygote genotype TT, those with heterozygote genotype TC (OR = 1.32; 95% CI = 1.05–1.67; $P = 0.019$), as well as minor homozygote genotype CC (OR = 2.98; 95% CI = 1.30–6.84; $P = 0.010$), had significantly higher susceptibility of HCC.

3.3. Bioinformatics analyses of the significant loci

As shown in Fig. 1, expression of the *ATG5, ATG10, ATG12,* and *ATG16L1* genes in HCC tissues were evidently higher than that in normal tissues of TCGA samples ($P < 0.001$), which indicates the potential oncogene effect of these four genes. We searched the eQTL evidence based on the public GTEx database and the TCGA database of LIHC, which the genotype of rs17067724 was significantly correlated with the expression of *ATG5* in HCC tissues (Fig. 2). Especially for *ATG10* rs10514231, allele C of that was significantly correlated with higher expression of *ATG10* gene in both HCC tissues and normal liver tissues (Fig. 3).

3.4. Functional relevance of the ATG10 rs10514231

For the promising SNP rs10514231, HaploRegv4.1 was used to annotate the functional elements. We found that rs10514231 was located at a locus with transcription factor (GR, Hand1, Nanog, and TCF4) binding site and eQTL in many cell types, which suggests its allele-specific regulatory effect. Thus, we conducted the dual-luciferase reporter assay to validate the bioinformatics analysis findings. As shown in Fig. 4, both the Hep3B and SK-Hep1 cell lines transfected with vectors containing the risk C allele of rs10514231 showed higher relative luciferase activity compared to that containing the T allele ($P < 0.001$), which further confirmed its allele-specific regulatory effect.

4. Discussion

Genetic variants of ATGs have been has been widely explored in human cancers, and some of them have been identified to be associated with cancer risk or prognosis [22–26]. In current study, we explored associations between 14 functional variants of five ATGs (*ATG3, ATG5*, *ATG10*, *ATG12* and *ATG16L1*) and the development of HCC in a case-control study with 986 HCC cases and 1000 healthy controls among a Chinese population. We found five variants, including *ATG5* rs17067724, *ATG10* rs1864183, *ATG10* rs10514231, *ATG12* rs26537, and *ATG16L1* rs4663402, were significantly associated with risk of HCC. Most strikingly, *ATG10* rs10514231 kept significant association even adjusted for Bonferroni correction. Both bioinformatics analyses and dual-luciferase reporter assay showed that allele C of *ATG10* rs10514231 was significantly correlated with higher luciferase activity and expression of *ATG10* gene in both HCC tissues and normal liver tissues. These findings above suggested that *ATG10* rs10514231 might modulate the expression of its host gene *ATG10* by changing the regulation of transcriptional activity.

ATG10 gene, an E2-like enzyme, was located at 5q14.1 by Ensembl, and necessary for the initiation of autophagy [27,28]. It could mediate the formation of the autophagy essential Atg12-Atg5 conjugate without a specific E3 enzyme [29]. Jo et al. [30] first reported that increased

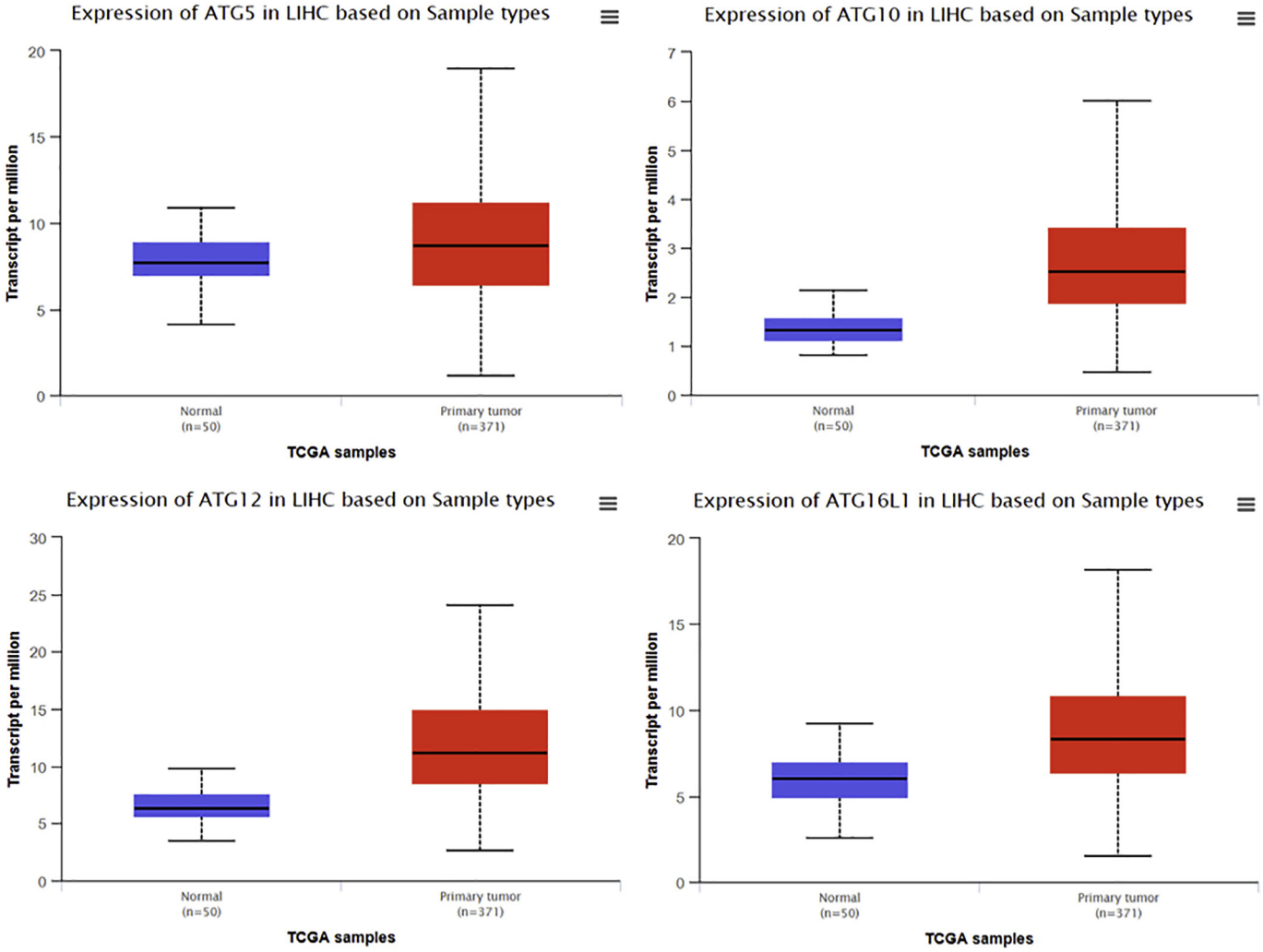

Fig. 1. Bioinformatics analysis of *the ATG5, ATG10, ATG12,* and *ATG16L1* gene in TCGA samples.

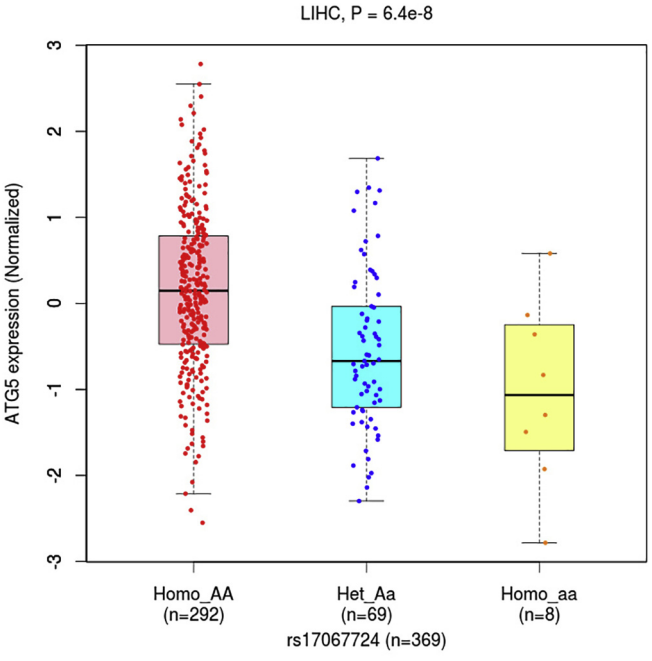

Fig. 2. Bioinformatics analysis of *ATG5* rs17067724 and expression of *ATG5* gene.

expression of *ATG10* was associated with lymphovascular invasion and lymph node metastasis of colorectal cancer. Cao et al. [31] found ATG proteins, including *ATG10,* might serve as novel prognostic biomarkers in gastric cancer. Interestingly, Xie et al. [14] identified that *ATG10* rs10514231 could influence lung cancer survival through regulating *ATG10* expression. These results validated our findings that *ATG10* rs10514231 might modulate the expression of *ATG10* by changing the regulation of transcriptional activity, and further influence the development and progression of carcinogenesis. Although Qin et al. [32] found rs1864182 and rs10514231 in *ATG10* were significantly associated with a decreased risk of breast cancer. Guo et al. [33] reported *ATG10* rs73134739 could significantly change the promoter activities. Wang et al. [34] found rs1864183 was significantly associated with the overall survival of non-small cell lung cancer patients treated with platinum-based chemotherapy. Fernandez-Mateos [35] reported that *ATG10* rs1864183 was associated with a higher susceptibility to develop laryngeal cancer. *ATG10* rs10036653 and rs1864182 were also associated with primary or acquired resistance to gefitinib in advanced lung adenocarcinoma [36].

ATG12 and *ATG5* genes could form a conjugate, and interact with *Atg16L1* to form a complex, which is necessary for autophagosome formation [37]. *ATG5* rs2245214 have a higher probability to develop thyroid carcinoma [38]. A allele of *ATG5* rs473543 had an increased risk of recurrence and shorter DFS compared genotype GG [25]. Among EGFR-mutant patients, *ATG5* rs688810 and rs510432, *ATG12* rs26538, *ATG16L1* rs2241880 significantly contributed to prognosis of lung

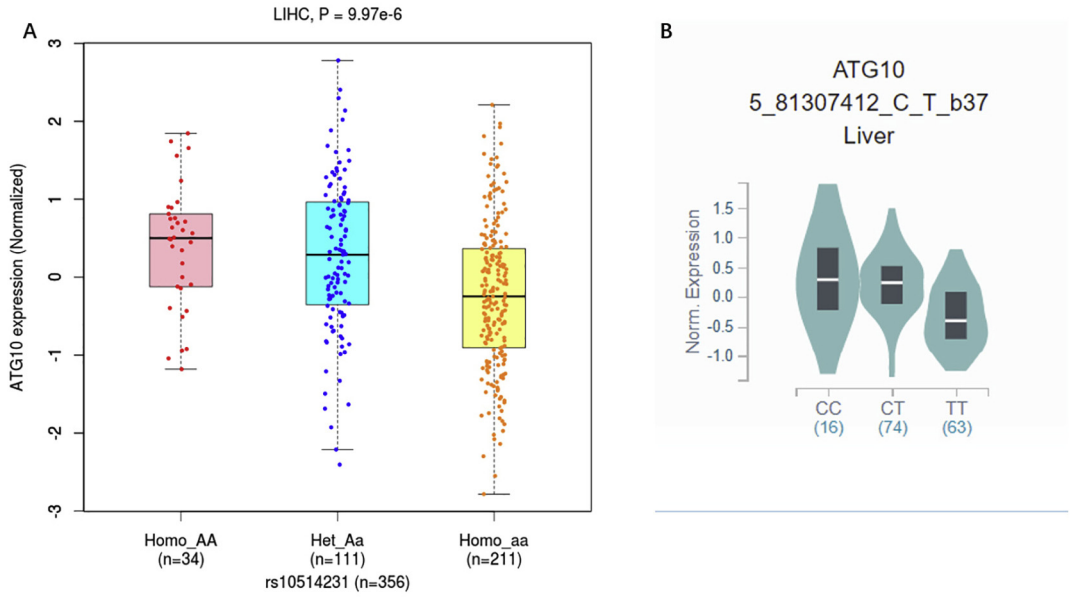

Fig. 3. Bioinformatics analysis of *ATG10* rs10514231 and expression of *ATG10* gene.

adenocarcinoma [36]. Song et al. [15] found *ATG12* eQTL SNP rs26537 might contribute to an allele-specific effect on the expression of host gene *ATG12* and explain a fraction of head and neck squamous cell carcinoma (HNSCC) genetic susceptibility. In our results, *ATG12* rs26537 was also identified to be associated with increased risk of HCC. *ATG12* rs26532 was significantly associated with an increased risk of brain metastases in patients with non-small cell lung cancer [39]. *ATG12* rs26538 were also significantly associated with survival of lung adenocarcinoma patients [36]. Besides, *ATG16L1* rs2241880 was associated with improved survival in human colorectal cancer and increased risk of brain metastases in patients with non-small cell lung cancer [39,40]. *ATG16L1* rs4663402 was significantly associated with the increased risk of HNSCC [15]. Fernandez-Mateos [35] reported that *ATG16L1* rs2241880 was associated with a higher susceptibility to develop oral carcinoma. However, in current study, we only replicated the promising finding for rs4663402, while got null result for rs2241880.

To the best of our knowledge, the present study is the first focusing on associations of the potentially functional variants of ATGs with HCC risk with an adequate sample size. Using Quanto (version 1.2.4), we have 93.4% statistical power to detect such an association for *ATG10* rs10514231 (C vs T: OR = 1.41) with our sample size. Some limitations of this study also should be considered when interpreting the results. First, potential selection bias was inevitable for hospital-based case-control study. Second, great difference of HBV infection rate between HCC cases and controls might confound our results, although we have adjusted them by HBV infection rate. Therefore, larger studies with more functional investigations are warranted.

5. Conclusion

Conclusively, our study identified that genetic variants of ATGs could influence the corresponding biological functions and consequently contribute to development of HCC. Specifically, *ATG10* rs10514231 might contribute to an allele-specific effect on the expression of host gene *ATG10* and explain a fraction of HCC genetic susceptibility. However, further larger studies in different populations and mechanical studies are required to validate our findings.

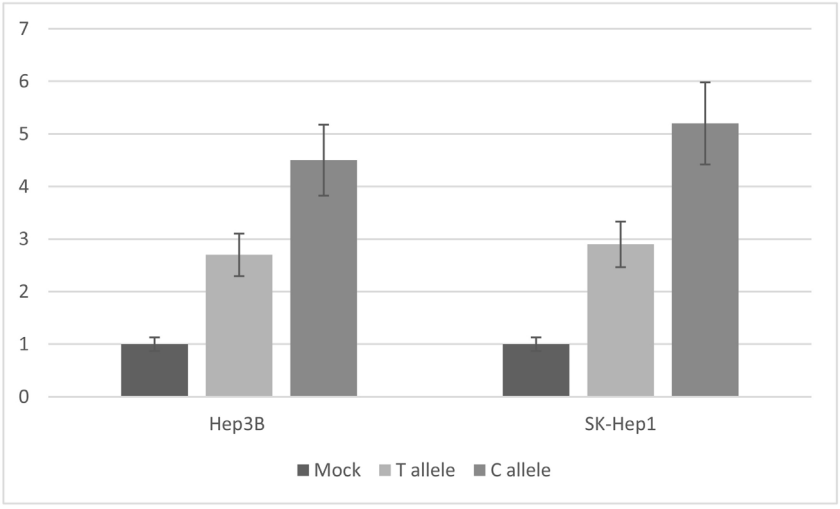

Fig. 4. *ATG10* rs10514231 affect luciferase activities in both Hep3B and SK-Hep1 cell lines.

Author contributions

Lin Lin conceived and designed the research; Mingyang Shen performed the experiments; Lin Lin and Mingyang Shen contributed reagents/materials/analysis tools; Lin Lin and Mingyang Shen wrote the paper. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116675.

References

[1] G.B.D.C.o.D. Collaborators, Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the global burden of disease study 2017, Lancet 392 (10159) (2018) 1736–1788.

[2] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, F. Bray, A. Jemal, X.Q. Yu, J. He, Cancer statistics in China, 2015, CA Cancer J. Clin. 66 (2) (2016) 115–132.

[3] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, CA Cancer J. Clin. 69 (1) (2019) 7–34.

[4] K.A. McGlynn, J.L. Petrick, W.T. London, Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability, Clinics in Liver Disease 19 (2) (2015) 223–238.

[5] L.Y. Mak, V. Cruz-Ramon, P. Chinchilla-Lopez, H.A. Torres, N.K. LoConte, J.P. Rice, L.E. Foxhall, E.M. Sturgis, J.K. Merrill, H.H. Bailey, N. Mendez-Sanchez, M.F. Yuen, J.P. Hwang, Global Epidemiology, Prevention, and Management of Hepatocellular Carcinoma, American Society of Clinical Oncology Educational Book, 38 ASCO. American Society of Clinical Oncology, 2018, pp. 262–279 Meeting.

[6] K. Zhang, J. Chen, H. Zhou, Y. Chen, Y. Zhi, B. Zhang, L. Chen, X. Chu, R. Wang, C. Zhang, PU.1/microRNA-142-3p targets ATG5/ATG16L1 to inactivate autophagy and sensitize hepatocellular carcinoma cells to sorafenib, Cell Death Dis. 9 (3) (2018) 312.

[7] Z. Zhao, J. Wu, X. Liu, M. Liang, X. Zhou, S. Ouyang, J. Yao, J. Wang, B. Luo, Insufficient radiofrequency ablation promotes proliferation of residual hepatocellular carcinoma via autophagy, Cancer Lett. 421 (2018) 73–81.

[8] J. Li, P.W. Wu, Y. Zhou, B. Dai, P.F. Zhang, Y.H. Zhang, Y. Liu, X.L. Shi, Rage induces hepatocellular carcinoma proliferation and sorafenib resistance by modulating autophagy, Cell Death Dis. 9 (2) (2018) 225.

[9] Q. Fan, L. Yang, X. Zhang, Y. Ma, Y. Li, L. Dong, Z. Zong, X. Hua, D. Su, H. Li, J. Liu, Autophagy promotes metastasis and glycolysis by upregulating MCT1 expression and Wnt/beta-catenin signaling pathway activation in hepatocellular carcinoma cells, J. Exp. Clin. Cancer Res. 37 (1) (2018) 9.

[10] S.Y. Wu, S.H. Lan, S.R. Wu, Y.C. Chiu, X.Z. Lin, I.J. Su, T.F. Tsai, C.J. Yen, T.H. Lu, F.W. Liang, C.Y. Li, H.J. Su, C.L. Su, H.S. Liu, Hepatocellular carcinoma-related cyclin D1 is selectively regulated by autophagy degradation system, Hepatology 68 (1) (2018) 141–154.

[11] S.B. Hong, B.W. Kim, J.H. Kim, H.K. Song, Structure of the autophagic E2 enzyme Atg10, Acta Crystallogr D Biol Crystallogr 68 (Pt 10) (2012) 1409–1417.

[12] A. Kunanopparat, I. Kimkong, T. Palaga, P. Tangkijvanich, B. Sirichindakul, N. Hirankarn, Increased ATG5-ATG12 in hepatitis B virus-associated hepatocellular carcinoma and their role in apoptosis, World J. Gastroenterol. 22 (37) (2016) 8361–8374.

[13] H. Wei, J. Hu, J. Pu, Q. Tang, W. Li, R. Ma, Z. Xu, C. Tan, T. Yao, X. Wu, X. Long, J. Wang, Long noncoding RNA HAGLROS promotes cell proliferation, inhibits apoptosis and enhances autophagy via regulating miR-5095/ATG12 axis in hepatocellular carcinoma cells, Int. Immunopharmacol. 73 (2019) 72–80.

[14] K. Xie, C. Liang, Q. Li, C. Yan, C. Wang, Y. Gu, M. Zhu, F. Du, H. Wang, J. Dai, X. Liu, G. Jin, H. Shen, H. Ma, Z. Hu, Role of ATG10 expression quantitative trait loci in non-small cell lung cancer survival, Int. J. Cancer 139 (7) (2016) 1564–1573.

[15] X. Song, Z. Yuan, H. Yuan, L. Wang, P. Ji, G. Jin, J. Dai, H. Ma, ATG12 expression quantitative trait loci associated with head and neck squamous cell carcinoma risk in a Chinese Han population, Mol. Carcinog. 57 (8) (2018) 1030–1037.

[16] J. Tan, L. Fu, H. Chen, J. Guan, Y. Chen, J. Fang, Association study of genetic variation in the autophagy lysosome pathway genes and risk of eight kinds of cancers, Int. J. Cancer 143 (1) (2018) 80–87.

[17] N. Li, X. Fan, X. Wang, H. Deng, K. Zhang, X. Zhang, Q. Han, Y. Lv, Z. Liu, Autophagy-related 5 gene rs510432 polymorphism is associated with hepatocellular carcinoma in patients with chronic hepatitis B virus infection, Immunol. Investig. 48 (4) (2019) 378–391.

[18] D.S. Chandrashekar, B. Bashel, S.A.H. Balasubramanya, C.J. Creighton, I. Ponce-Rodriguez, B. Chakravarthi, S. Varambally, UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses, Neoplasia 19 (8) (2017) 649–658.

[19] G.T. Consortium, The genotype-tissue expression (GTEx) project, Nat. Genet. 45 (6) (2013) 580–585.

[20] J. Gong, S. Mei, C. Liu, Y. Xiang, Y. Ye, Z. Zhang, J. Feng, R. Liu, L. Diao, A.Y. Guo, X. Miao, L. Han, PancanQTL: systematic identification of cis-eQTLs and trans-eQTLs in 33 cancer types, Nucleic Acids Res. 46 (D1) (2018) D971–D976.

[21] L.D. Ward, M. Kellis, HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease, Nucleic Acids Res. 44 (D1) (2016) D877–D881.

[22] B. Liu, T. An, M. Li, Z. Yi, C. Li, X. Sun, X. Guan, L. Li, Y. Wang, Y. Zhang, B. Xu, F. Ma, Y. Zeng, The association between early-onset cardiac events caused by neoadjuvant or adjuvant chemotherapy in triple-negative breast cancer patients and some novel autophagy-related polymorphisms in their genomic DNA: a real-world study, Cancer Commun (Lond) 38 (1) (2018) 71.

[23] M. Nikseresht, M. Shahverdi, M. Dehghani, H. Abidi, R. Mahmoudi, G. Ghalamfarsa, L. Manzouri, S. Ghavami, Association of single nucleotide autophagy-related protein 5 gene polymorphism rs2245214 with susceptibility to non-small cell lung cancer, J. Cell. Biochem. (2018).

[24] M.D. Berger, S. Yamauchi, S. Cao, D.L. Hanna, Y. Sunakawa, M. Schirripa, S. Matsusaka, D. Yang, S. Groshen, W. Zhang, Y. Ning, S. Okazaki, Y. Miyamoto, M. Suenaga, S. Lonardi, C. Cremolini, A. Falcone, V. Heinemann, F. Loupakis, S. Stintzing, H.J. Lenz, Autophagy-related polymorphisms predict hypertension in patients with metastatic colorectal cancer treated with FOLFIRI and bevacizumab: results from TRIBE and FIRE-3 trials, Eur. J. Cancer 77 (2017) 13–20.

[25] M. Li, F. Ma, J. Wang, Q. Li, P. Zhang, P. Yuan, Y. Luo, R. Cai, Y. Fan, S. Chen, Q. Li, B. Xu, Genetic polymorphisms of autophagy-related gene 5 (ATG5) rs473543 predict different disease-free survivals of triple-negative breast cancer patients receiving anthracycline- and/or taxane-based adjuvant chemotherapy, Chin J Cancer 37 (1) (2018) 4.

[26] H.M. Kim, E.S. Kim, J.S. Koo, Expression of autophagy-related proteins in different types of thyroid cancer, Int. J. Mol. Sci. 18 (3) (2017).

[27] M. Shams Nooraei, A. Noori-Zadeh, S. Darabi, F. Rajaei, Z. Golmohammadi, H.A. Abbaszadeh, Low level of autophagy-related gene 10 (ATG10) expression in the 6-hydroxydopamine rat model of Parkinson's disease, Iran. Biomed. J. 22 (1) (2018) 15–21.

[28] T. Hanada, Y. Ohsumi, Structure-function relationship of Atg12, a ubiquitin-like modifier essential for autophagy, Autophagy 1 (2) (2005) 110–118.

[29] M. Yamaguchi, N.N. Noda, H. Yamamoto, T. Shima, H. Kumeta, Y. Kobashigawa, R. Akada, Y. Ohsumi, F. Inagaki, Structural insights into Atg10-mediated formation of the autophagy-essential Atg12-Atg5 conjugate, Structure 20 (7) (2012) 1244–1254.

[30] Y.K. Jo, S.C. Kim, I.J. Park, S.J. Park, D.H. Jin, S.W. Hong, D.H. Cho, J.C. Kim, Increased expression of ATG10 in colorectal cancer is associated with lymphovascular invasion and lymph node metastasis, PLoS One 7 (12) (2012) e52705.

[31] Q.H. Cao, F. Liu, Z.L. Yang, X.H. Fu, Z.H. Yang, Q. Liu, L. Wang, X.B. Wan, X.J. Fan, Prognostic value of autophagy related proteins ULK1, Beclin 1, ATG3, ATG5, ATG7, ATG9, ATG10, ATG12, LC3B and p62/SQSTM1 in gastric cancer, Am. J. Transl. Res. 8 (9) (2016) 3831–3847.

[32] Z. Qin, J. Xue, Y. He, H. Ma, G. Jin, J. Chen, Z. Hu, X. Liu, H. Shen, Potentially functional polymorphisms in ATG10 are associated with risk of breast cancer in a Chinese population, Gene 527 (2) (2013) 491–495.

[33] X. Guo, W. Lin, J. Bao, Q. Cai, X. Pan, M. Bai, Y. Yuan, J. Shi, Y. Sun, M.R. Han, J. Wang, Q. Liu, W. Wen, B. Li, J. Long, J. Chen, W. Zheng, A comprehensive cis-eQTL analysis revealed target genes in breast cancer susceptibility loci identified in genome-wide association studies, Am. J. Hum. Genet. 102 (5) (2018) 890–903.

[34] S.M. Wang, X. Song, X.Y. Zhao, H.Y. Chen, J.C. Wang, J.J. Wu, Z.Q. Gao, J. Qian, C.X. Bai, Q. Li, B.H. Han, D.R. Lu, Association between polymorphisms of autophagy pathway and responses in non-small cell lung cancer patients treated with platinum-based chemotherapy, Yi Chuan 39 (3) (2017) 250–262.

[35] J. Fernandez-Mateos, R. Seijas-Tamayo, J.C.A. Klain, M.P. Borgonon, E. Perez-Ruiz, R. Mesia, E. Del Barco, C.S. Coloma, A.R. Dominguez, J.C. Daroqui, E. Fernandez Ruiz, J.J. Cruz-Hernandez, R. Gonzalez-Sarmiento, Analysis of autophagy gene polymorphisms in Spanish patients with head and neck squamous cell carcinoma, Sci. Rep. 7 (1) (2017) 6887.

[36] J. Yuan, N. Zhang, L. Yin, H. Zhu, L. Zhang, L. Zhou, M. Yang, Clinical implications of the autophagy core gene variations in advanced lung adenocarcinoma treated with Gefitinib, Sci. Rep. 7 (1) (2017) 17814.

[37] Y. Fujioka, N.N. Noda, H. Nakatogawa, Y. Ohsumi, F. Inagaki, Dimeric coiled-coil structure of Saccharomyces cerevisiae Atg16 and its functional significance in autophagy, J. Biol. Chem. 285 (2) (2010) 1508–1515.

[38] T.S. Plantinga, E. van de Vosse, A. Huijbers, M.G. Netea, L.A. Joosten, J.W. Smit, R.T. Netea-Maier, Role of genetic variants of autophagy genes in susceptibility for non-medullary thyroid cancer and patients outcome, PLoS One 9 (4) (2014) e94086.

[39] Q.X. Li, X. Zhou, T.T. Huang, Y. Tang, B. Liu, P. Peng, L. Sun, Y.H. Wang, X.L. Yuan, The Thr300Ala variant of ATG16L1 is associated with decreased risk of brain metastasis in patients with non-small cell lung cancer, Autophagy 13 (6) (2017) 1053–1063.

[40] W.A. Grimm, J.S. Messer, S.F. Murphy, T. Nero, J.P. Lodolce, C.R. Weber, M.F. Logsdon, S. Bartulis, B.E. Sylvester, A. Springer, U. Dougherty, T.B. Niewold, S.S. Kupfer, N. Ellis, D. Huo, M. Bissonnette, D.L. Boone, The Thr300Ala variant in ATG16L1 is associated with improved survival in human colorectal cancer and enhanced production of type I interferon, Gut 65 (3) (2016) 456–464.