Polymorphisms of a Disintegrin and Metalloproteinase with Thrombospondin Motifs 5 and Aflatoxin B1–Related Hepatocellular Carcinoma

Xiao-Ying Huang1, Jin-Guang Yao1, Bing-Chen Huang1, Yun Ma2, Qiang Xia3, and Xi-Dai Long1,3

Abstract

Background: Altered expression of a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) is observed in hepatocellular carcinoma. The genetic polymorphisms of this gene in aflatoxin B1 (AFB1)–related hepatocellular carcinoma have not yet been elucidated.

Methods: We conducted a hospital-based case–control study, including 1,706 hepatocellular carcinoma cases and 2,270 controls without any liver diseases or tumors, to assess the association between 74 polymorphisms in ADAMTS5 and AFB1-related hepatocellular carcinoma risk and prognosis. Genotype, mRNA levels, and TP53 gene mutation (TP53M) related to AFB1 exposure were tested using TaqMan-PCR or sequencing technique. ADAMTS5 protein level and microvessel density were analyzed by IHC.

Results: Among these 74 polymorphisms, only rs2830581 affected hepatocellular carcinoma risk. Compared with the homozygote of rs2830581 G alleles (rs2830581-GG), the genotypes of rs2830581 A alleles (rs2830581-GA or -AA) increased hepatocellular carcinoma risk (OR: 1.85 and 4.40; 95% CI: 1.57–2.19 and 3.43–5.64, respectively). Significant interactive effects between risk genotypes and AFB1 exposure status were also observed in the joint effects analysis. Furthermore, the rs2830581 polymorphism modified the tumor recurrence-free survival and overall survival of patients. This polymorphism not only affected pathologic features of hepatocellular carcinoma such as tumor dedifferentiation and microvessel density, but also modified ADAMTS5 expression and the effects of transarterial chemoembolization treatment on hepatocellular carcinoma.

Conclusions: These results suggest ADAMTS5 polymorphisms may be risk and prognostic biomarkers of AFB1-related hepatocellular carcinoma, and rs2830581 is a potential candidate.

Impact: Our findings support the hypothesis that ADAMTS5 rs2830581 polymorphism modifies AFB1-related hepatocellular carcinoma risk and prognosis. Cancer Epidemiol Biomarkers Prev; 25(2): 334–43. ©2015 AACR.

Introduction

Hepatocellular carcinoma, in addition to being the most common cancer worldwide in 2008, is the most common histopathologic type of liver cancer (1). Approximately 50% all hepatocellular carcinoma cases and deaths were estimated to occur in the People's Republic of China (especially in the southeast areas such as Guangxi), mainly because of high aflatoxin B1 (AFB1) exposure and/or chronic infection of hepatitis B and C viruses (HBV and HCV, respectively; ref. 2). Therefore, a better understanding of the molecular characteristics and underlying mechanism are of the utmost importance and may prove useful in developing novel strategies for the prevention, early detection, treatment, and prognosis of hepatocellular carcinoma. The hotspot mutation of the TP53 gene (TP53M) has been shown to be positively associated with AFB1 exposure levels and hepatocellular carcinoma risk factor (2, 3). Until now, it has been regarded as an important biomarker of AFB1-related hepatocellular carcinoma as well as the serum AFB1 albumin adducts (AAA; refs. 2, 3). Recently, increasing evidence has shown potential associations of common genetic variants with AFB1-related hepatocellular carcinoma risk in biologically plausible pathways including DNA repair and detoxification pathways (4). These genetic factors could feasibly be utilized as biomarkers of hepatocellular carcinoma induced by AFB1 exposure and prognostic indicators.

The disintegrin-like and metalloproteinase (ADAMTS) family member ADAMTS5 acts as a proteoglycanase and has been shown to be a major aggrecanase in cartilage destruction (5, 6). Recently, a preponderance of evidence has implicated aberrant ADAMTS5 expression in the development and progression of some tumors including breast cancer, colorectal cancer, glioblastoma, hepatocellular carcinoma, and T-cell acute lymphoblastic
leukemia (7–18). Despite the collected data, the association of altered ADAMTS5 expression and genetic variants of AFB1-related hepatocellular carcinoma remains to be elucidated. Here, we evaluated whether 74 SNPs in this gene modify AFB1-related hepatocellular carcinoma risk and prognosis.

**Materials and Methods**

**Study subjects**

We utilized a hospital-based case-control study of AFB1-related hepatocellular carcinoma in the Guangxi area according to a previously described protocol (19, 20). Briefly, patients diagnosed with histopathologically confirmed hepatocellular carcinoma in hospitals affiliated with Guangxi Medical University and Youjiang Medical College for Nationalities in the Southwestern Guangxi from January 2004 to July 2014 were utilized. Individuals without clinical evidence of hepatic disease or tumors were recruited from the general health check-up center at the same hospitals during the same period for comparison. To control the effects of confounders, cases were individually matched (1:1 or 1:2) to controls based on gender, ethnicity (Han, Zhuang), age (<5 years), and HBV and HCV infection. All controls were surveyed to ascertain their willingness to participate in the study and to provide preliminary demographic data. In this study, a total of 1,706 cases and 2,076 controls, representing 95% of eligible cases and 98% of eligible controls were interviewed and included the final analysis. From these subjects, 200 matched cases and controls were randomly selected for inclusion in the screening set, whereas the remainder was utilized for the validation group. Once informed, written consent was obtained for all participants and 4 mL of peripheral blood was collected along with demographic data. Surgically removed tumor samples and 207 fresh cancer tissue specimens were collected for ADAMTS5 expression analysis. All protocols were approved by the ethic committees of the participating hospitals and more detailed data collection can be found in the Supplementary Materials and Methods.

**AFB1 exposure analysis**

In this study, subject AFB1 exposure level was evaluated via the serum AAA levels using a comparative ELISA (21). For statistical analysis, AAA values were logarithmically transformed and then were divided into three subgroups: low (<2.18 ln fmol/mg), medium (2.18–2.98 ln fmol/mg), and high (>2.98 ln fmol/mg), according to the mean logit value of serum AAA among controls and cases. These three subgroups represented low, medium, and high AFB1 exposure, respectively.

Mutation of TP53 gene was examined using the TaqMan-PCR methods (21) to assess the differences between groups, demographic characteristics, AFB1-exposure information, and SNP genotypes. From the individually matched design, conditional logistical regression was conducted (with multivariate factors including known causes of hepatocellular carcinoma among Guangxi population) to estimate ORs for hepatocellular carcinoma along with the 95% confidence intervals (CI). Screening the main effects of the 74 SNPs utilized was based on the additive model with the genotypes treated as ordinal variables. For the correction of multiple testing in the screen stage, the Bonferroni correction treats each SNP test as an independent test. This correction is overly conservative for SNPs that are in the linkage disequilibrium (LD), because it ignores the correlation among SNPs. To address this limitation, the correlation matrix-based method was used to take into account LD between SNPs (22). On the basis of this method, two-sided P values smaller than 6.74 × 10^{-4} were considered significant for the main effects of SNPs in the screen stage. In the joint analysis stage of SNPs and AFB1 exposure, genotype frequencies in these groups were further adjusted for multiple comparisons using Bonferroni method, and the significance threshold was lowered to \( \alpha_{\text{correct}} = 3.37 \times 10^{-4} \).

Kaplan-Meier survival analysis with the log-rank test was used to evaluate the effects of rs2830581 polymorphism on hepatocellular carcinoma prognosis. Risk factors for hepatocellular carcinoma prognosis were selected using the Cox multivariate regression model (including all possible multiplicatively interactive variables) with stepwise forward selection based on a likelihood ratio test. HRs and 95% CIs for risk factors were then calculated from a multivariate Cox regression model. All statistical analyses were carried out utilizing the SPSS version 18 (SPSS Institute).

**Results**

**Characteristics of subjects**

The distributions of demographic characteristics (including sex, age, race, and HBV and HCV status) between hepatocellular carcinoma cases and controls were not significantly different. However, hepatocellular carcinoma cases exhibited a higher level of AFB1-album adducts in the peripheral serum than controls (2.98 vs. 2.18 ln fmol/mg, \( P = 1.81 \times 10^{-17} \)). Logistic regression
analysis revealed that hepatocellular carcinoma risk increased with an increasing exposure level (OR, 1.89–5.88; P < 0.01; Supplementary Table S2). However, there was not a significant difference of AFB1 levels between the early stage and the advanced stage patients (2.99 ± 0.98 vs. 2.97 ± 0.75 in fmol/mg, P = 0.61).

Effects of ADAMTS5 polymorphisms on hepatocellular carcinoma risk

Analysis of the selected SNPs revealed the genotype distribution of control individuals was consistent with predictions from the Hardy–Weinberg equilibrium (Supplementary Table S3; P > 0.05). Logistic regression analysis indicated that four SNPs (rs162499, rs233895, rs62215997, and rs2830581) were significant upon initial screening (Table 1, Supplementary Table S4). From the validation group, it was found that only rs2830581 (located on the 3′ UTR of the ADAMTS5 gene) was significantly associated with hepatocellular carcinoma risk (OR = 2.35; 95% CI, 2.03–2.72; P = 3.38 × 10−3). In the combined analysis utilizing all subjects (Merged set, Table 1), the adjusted OR for hepatocellular carcinoma among those heterozygous for rs2830581 G and A alleles (rs2830581-GA) versus those homozygous for rs2830581 G alleles (rs2830581-GG) was 1.85 (1.57–2.19). The corresponding OR for those homozygous for rs2830581 A alleles (rs2830581-AA) was 4.40 (3.43–5.64). Thus, hepatocellular carcinoma risk was associated with the number of rs2830581 A alleles.

To evaluate possible interactive effects of individually matching variables (including age, race, gender, HBV infection, and HCV infection) and ADAMTS5 rs2830581 polymorphism on hepatocellular carcinoma risk, the polymorphism analysis was stratified by matching factors. Results showed that these factors did not substantially modulate the effect of ADAMTS5 rs2830581 on cancer risk (Pinteraction > 0.05; Supplementary Table S5).

Joint effects of ADAMTS5 rs2830581 polymorphism and AFB1 exposure on hepatocellular carcinoma risk

The joint effects of ADAMTS5 rs2830581 polymorphism and AFB1 exposure levels on hepatocellular carcinoma risk was carried out with the lowest risk group (those with rs2830581-GG and low level of AFB1 exposure) as a reference (Table 2). It was found that higher levels of AFB1 exposure consistently increased hepatocellular carcinoma risk; the risk was more pronounced among subjects with ADAMTS5 risk genotypes. There was evidence of multiplicatively interactive effects of genotypes and exposure levels on hepatocellular carcinoma risk [16.67 > (1.88 × 3.97)] according to the previously published formula [ORgen × ORexp; ref. 23]. To determine more detailed interactive effects, we conducted multiplicative interactive analysis. In this analysis model, risk factors for hepatocellular carcinoma risk were selected using a multivariate logistic regression model (including all possible multiplicatively interactive variables) with stepwise forward selection based on a likelihood ratio test. Next, the ORinteraction and 95% CIs for AFB1–genotype interactive variables were calculated in the same multivariate model (simultaneously including all risk variables and multiplicatively interactive variables). We found significantly interactive values [ORinteraction (95% CI/P), 1.63 (1.08–2.46/0.02) for high AFB1 exposure level × rs2830581-GA; 4.37 (1.76–10.86/1.48 × 10−3) for high AFB1 exposure level × rs2830581-AA, respectively]. Together, the data indicated that AFB1 exposure multiplicatively interacted with ADAMTS5 rs2830581 polymorphism.
ADAMTS5 rs2830581 polymorphism modified ADAMTS5 expression

To characterize the functional relevance of the ADAMTS5 rs2830581 polymorphism, we conducted a correlation analysis between the rs2830581 genotypes and the expression of ADAMTS5 protein assessed using IHC on the cancerous tissues collected in the study. The results showed that the genotypes with rs2830581 A alleles were significantly related to decreased ADAMTS5 expression in hepatocellular tumor tissues, compared with rs2830581-GG (Fig. 1A; P < 0.01). To analyze this correlation further, subjects were divided into three groups based on ADAMTS5 expression scores in the tumors, representing low, medium, and high expression of ADAMTS5. ADAMTS5 expression was found to be negatively related to the expression of the polymorphism (r = −0.313; Supplementary Table S6; Fig. 1B). Moreover, mRNA levels of ADAMTS5 in cancerous tissues with rs2830581-GA or-AA were significantly lower than in those with rs2830581-GG (P < 0.01; Fig. 1C). Together, these results suggest that this polymorphism modulates the expression of ADAMTS5.

ADAMTS5 rs2830581 polymorphism correlated with microvessel density

From previous reports, it is known that ADAMTS5 expression is capable of regulating hepatocellular carcinoma angiogenesis (14). Thus, we explored how expression of ADAMTS5 rs2830581 polymorphism affected MVD (Fig. 1D). The data showed that rs2830581-GA or-AA had a higher frequency of MVD, compared with rs2830581-GG [OR (95% CI): 2.50 (1.98–3.16)/1.65 × 10−14] for rs2830581-GA and 7.29 (5.09–10.43)/2.33 × 10−27 for rs2830581-AA, respectively. Moreover, these risk-associated genotypes increased the risk of portal vein tumor [PVT; OR = 2.14 (1.65–2.79) and 5.74 (3.64–9.06) for rs2830581-GA and-AA, respectively; Fig. 1E].

ADAMTS5 rs2830581 polymorphism modified hepatocellular carcinoma prognosis

To study the effects of ADAMTS5 rs2830581 polymorphism on the outcome of hepatocellular carcinoma patients, survival follow-up information of all hepatocellular carcinoma patients was analyzed. The association analysis between high-risk genotypes (namely, genotypes with rs2830581 A alleles; rs2830581-GA/AA) or low/no-risk genotypes (rs2830581-GG) and the pathologic characteristics of hepatocellular carcinoma were performed separately (Supplementary Table S7). A significant difference between genotypes among different AFB1 exposure levels, tumor size, tumor grade and TNM stage, and PVT and TP53M status, but not in age, gender, race, HBsAg, anti-HCV, or cirrhosis was observed. Results of the Kaplan–Meier survival analysis showed that ADAMTS5 polymorphism significantly correlated with shorter overall survival (OS) and recurrence-free survival (RFS) of hepatocellular carcinoma cases (Fig. 2A and B), particularly under conditions of high aflatoxin exposure (Fig. 2C–H). From Cox regression analysis (Table 3) we showed that the ADAMTS5 polymorphism is capable of affecting hepatocellular carcinoma prognosis [rs2830581-AA risk value, HR = 3.57 for RFS and 2.18 for OS, respectively]. Furthermore, some evidence of multiplicative interaction was found for rs2830581 polymorphism and AFB1 exposure (Pinteraction < 0.05). Taken together, these results indicated that this polymorphism is independent of other clinical covariates and suggested its potential as an independent prognostic factor for hepatocellular carcinoma.

ADAMTS5 rs2830581 polymorphism modified the effects of transcatheter arterial chemoembolization (TACE) treatment on hepatocellular carcinoma

The effect on patient survival in ADAMTS5 rs2830581 polymorphism was investigated to determine whether there was a correlation with effectiveness of a chosen therapy. Information from TACE-treated hepatocellular carcinoma patients who received curative resection treatment (Supplementary Table S8) was collected in accordance with the Chinese Manage Criteria of hepatocellular carcinoma (24). Although TACE treatment improved hepatocellular carcinoma prognosis as a whole (Fig. 3A and B, Supplementary Fig. S1A and S1B), stratified analysis based on the different genotypes of ADAMTS5 rs2830581 revealed that TACE treatment had no effect on the RFS and OS of hepatocellular carcinoma patients with rs2830581-AA genotype (Fig. 3C and D, Supplementary Fig. S1C and S1D). Interestingly, patients with rs2830581-GA or-GG polymorphisms that received TACE treatment had a prolonged OS and RFS (Fig. 3E–H, Supplementary Fig. S1E–S1H). By using these with rs2830581-AA but not receiving TACE treatment as a reference, a joint statistical analysis between ADAMTS5 genotypes and TACE treatment on hepatocellular carcinoma prognosis was performed (Supplementary Fig. S2A–S2D). The results indicated that hepatocellular carcinoma cases with rs2830581-GG would face a noticeably decreased risk of death and tumor reoccurrence under the conditions of TACE treatment (OS: HR = 0.28, 95% CI, 0.22–0.36; RFS: HR = 0.17, 95% CI, 0.12–0.24). Taken together, the data

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<th>ADAMTS5 genotype</th>
<th>Controls (n = 2,270) n (%)</th>
<th>Cases (n = 1,706) n (%)</th>
<th>OR (95% CI)</th>
<th>Pvalue</th>
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<tr>
<td>Low/GG</td>
<td>908 (40.0)</td>
<td>269 (15.8)</td>
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<td>216 (9.5)</td>
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<td>72 (3.2)</td>
<td>63 (3.7)</td>
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<td>GA/AA</td>
<td>288 (12.7)</td>
<td>161 (9.4)</td>
<td>1.88 (1.49–2.35)</td>
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<td>Medium/GG</td>
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<td>258 (15.3)</td>
<td>1.72 (1.40–2.10)</td>
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<td>137 (6.0)</td>
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<td>3.01 (2.28–3.99)</td>
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<td>AA</td>
<td>35 (1.5)</td>
<td>82 (4.8)</td>
<td>7.91 (5.20–12.02)</td>
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<td>GA/AA</td>
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<td>203 (11.9)</td>
<td>4.01 (3.14–5.12)</td>
<td>1.04 × 10−28</td>
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<tr>
<td>High/GG</td>
<td>378 (16.4)</td>
<td>463 (27.1)</td>
<td>4.97 (4.07–6.05)</td>
<td>1.27 × 10−56</td>
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<tr>
<td>GA</td>
<td>66 (2.9)</td>
<td>239 (14.0)</td>
<td>12.35 (9.08–16.73)</td>
<td>1.75 × 10−58</td>
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<tr>
<td>AA</td>
<td>6 (0.3)</td>
<td>113 (6.6)</td>
<td>64.56 (28.07–148.49)</td>
<td>1.05 × 10−22</td>
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<tr>
<td>GA/AA</td>
<td>72 (3.2)</td>
<td>352 (20.6)</td>
<td>16.67 (12.50–22.25)</td>
<td>1.86 × 10−31</td>
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suggest that ADAMTS5 rs2830581 polymorphism could potentially modify the effects of TACE treatment and improve the prognosis of hepatocellular carcinoma patients.

Discussion

On the basis of a large-scale case–control study, we investigated the association between ADAMTS5 polymorphisms and the risk of hepatocellular carcinoma in a high AFB1 exposure area of Guangxi province. It was found that the genotypes of ADAMTS5 with rs2830581 A alleles had a substantial association with the increasing risk of hepatocellular carcinoma (OR, 2.37; 95% CI, 2.05–2.74). Interestingly, an interaction between genotype and environment was found for populations having the rs2830581 A alleles, and exposed to different levels of AFB1. This study represents the first report indicating the potential of ADAMTS5 polymorphisms as biomarkers and prognostic indicators of hepatocellular carcinoma. In addition, we report that these polymorphisms alter the effects of TACE treatment as well.
The association between hepatocellular carcinoma (HCC) prognosis and both AFB1 exposure and ADAMTS5 rs2830581 polymorphism in 1,706 hepatocellular carcinoma patients. A and B, the genotypes with rs2830581 A alleles (rs2830581-GA and -AA) were found to have a poorer overall survival (OS) or tumor recurrence-free survival (RFS) than rs2830581-GG. C–H, the joint effects of AFB1 exposure and rs2830581 polymorphism further modified hepatocellular carcinoma prognosis. Cumulative hazard function was plotted by Kaplan–Meier’s methodology, and P value was calculated with two-sided log-rank tests. MST, the median overall survival time; MRT, the median tumor recurrence-free survival time.

**Figure 2.**
ADAMTS5 is well known for its role in cartilage biology (1, 25), and that it is the major aggrecanase in mouse cartilage in an inflammation model of arthritis (26). Previously, studies have suggested that ADAMTS5 may be involved in the development and progression of cancers affecting the breast, prostate, brain, colorectal tissue, and liver (10, 14, 16, 27–29). Porter and colleagues (27) found that ADAMTS5 expression in breast cancer tissues was downregulated in cancerous tissues with respect to nonneoplastic mammary tissue, irrespective of the heterogeneity of the samples and the tumor type or grade. Kim and Roman-Gomez (10, 16) observed that ADAMTS5 gene exhibited hypermethylation in colorectal cancer and T-cell acute lymphoblastic leukemia. Additional studies have indicated that it may be possible for this gene to suppress tumor growth and progress (9, 14, 16). In contrast, other reports indicated that ADAMTS5 expression was upregulated in human glioblastoma compared with normal brain tissues and promoted glioma cell invasiveness in culture (28). Together, the collected data indicate that ADAMTS5 may serve different functions in different cancer types. Thus, the role of ADAMTS5 in cancer, including hepatocellular carcinoma, needs to be further evaluated.

More than 1,000 SNPs have been reported in the ADAMTS5 gene, some of which were found in correlation with human disease (11, 13, 17, 18). In the current study, 74 known SNPs were analyzed because of their indicated involvement with the structure and expression of ADAMTS5, and potential involvement in hepatocellular carcinoma carcinogenesis (13). Of the polymorphisms analyzed, only rs2830581 polymorphism (located in the 3’ UTR of ADAMTS5) was found to be associated with increased hepatocellular carcinoma risk from first stage screening and second stage validation. Furthermore, this polymorphism was found in correlation with AFB1 exposure status in hepatocellular carcinoma patients. These data suggest this polymorphism may be an important genetic susceptibility factor in cancer risk. Coincidentally, a previous case–control study from Chongqing Region, a non-AFB1 exposure area in China, has shown that rs2830585 modifies hepatocellular carcinoma risk. Supplementary the aforementioned modifying role, the data indicated that the polymorphism afforded hepato-cellular carcinoma risk in the first-stage screening analysis, despite a lack of statistical significance. Therefore, the possibility that the genetic polymorphisms in ADAMTS5 influence hepatocellular carcinoma risk through an alternative pathway should not be ignored.

To explore the effect of ADAMTS5 rs2830581 polymorphism on AFB1-related hepatocellular carcinoma risk, expression of ADAMTS5 and the pathologic features of hepatocellular carcinoma were examined. It was found that this polymorphism was associated with the downregulation of ADAMTS5 expression, increasing MVD, tumor progression, dedifferentiation, and metastasis. A recent in vivo and in vitro study has shown that downregulation of this gene accelerates hepatocellular carcinoma angiogenesis and carcinogenesis by modifying VEGF expression (14). The survival analysis further indicated that the rs2830581 polymorphism was associated with poor outcome of hepatocellular carcinoma patients, highlighting the potential roles of this polymorphism in predicting the prognosis of hepatocellular carcinoma induced by AFB1 exposure. Collectively, these results suggest that ADAMTS5 rs2830581 polymorphism could modulate the antitumorigenesis role of ADAMTS5, increasing AFB1-related hepatocellular carcinoma risk and shortening prognosis of this tumor.

Another important finding in this study was that ADAMTS5 rs2830581 polymorphism was found to modify the effects of TACE treatment on hepatocellular carcinoma patients. This is likely due to the downregulation of ADAMTS5 expression and increased MVD, an important control factor for TACE treatment.

In the current study, major strengths were its prospective two-stage design, large sample size, and the panel of a relatively large number of systematically identified SNPs in ADAMTS5. The effects of possible confounders (including age, gender, national, and HBV and HCV infection status) were controlled with an individually matched design. In addition, AAA and TP53M was used to test this exposure and correlation because AAA is a stable biomarker reflecting long-term AFB1 exposure status, whereas TP53M is the characteristic genetic change resulting from AFB1 exposure, and higher frequency of this mutation predicts higher
Figure 3.
The different therapeutic value of TACE on hepatocellular carcinoma (HCC) cases in three groups with different genotypes of ADAMTS5 rs2830581 from 1,299 subjects. A and B, TACE treatment was found to improve overall survival (OS) or tumor recurrence-free survival (RFS). C–H, the effects of TACE treatment on hepatocellular carcinomas’ OS and RFS within strata of ADAMTS5 rs2830581 genotypes. Cumulative hazard function was plotted by Kaplan–Meier’s methodology, and P value was calculated with two-sided log-rank tests. MST, the median overall survival time; MRT, the median tumor recurrence-free survival time.
AFB1 exposure and higher hepatocellular carcinoma risk. In the current study, we found hepatocellular carcinoma cases had a higher serum AAA and more than 70% of them featured TP53M, which was similar to the data from other countries of South East Asia (30).

However, there were several limitations to our study. First, because 74 SNPs were evaluated in the screening set, whereas only 4 were evaluated in the validation set, false-negative results were a considerable issue. In this study, the correction for multiple testing was done using the correlation matrix-based method, which takes into account the LD between SNPs (22). Second, potential selection bias might have occurred through the selection of hospital-based control subjects. Third, the increased risk with AFB1 exposure seen in this study was probably underestimated because liver disease itself may affect the metabolism of AFB1 and modify the levels of AFB1 adducts (2, 31). Finally, although we analyzed the effects of the ADAMTS5 rs2830581 polymorphisms on the ADAMTS5 expression and MVD, we failed to accomplish more functional analysis. Thus, more functional analyses deserve elucidation based on large samples and a combination of genes and AFB1 exposure.

This study represents the first report describing genetic polymorphisms of ADAMTS5 and their association with AFB1-related hepatocellular carcinoma risk and prognosis. The findings provide an additional insight into the ADAMTS5 rs2830581 polymorphism as biologic determinants of hepatocellular carcinoma risk and prognosis related to AFB1 exposure. Interestingly, the effects of ADAMTS5 polymorphism on TACE therapy for hepatocellular carcinoma treatment indicated a possible association between the two. Understanding the different value in individual patients will allow more information counseling with regard to screening, prevention, treatment options, follow-up plans, and approaches for secondary prevention. Therefore, these findings, in combination with clinicopathologic information of hepatocellular carcinoma, could improve the identification of high-risk populations, especially from high AFB1 exposure areas.

References

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: X.-D. Long
Development of methodology: X.-Y. Huang, X.-D. Long
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.-G. Yao, Y. Ma
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X.-Y. Huang, J.-G. Yao, B.-C. Huang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Q. Xia, X.-D. Long
Study supervision: Q. Xia, X.-D. Long

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