Cytokine 118 (2019) 124–129

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Genetic variants in *IL-33/ST2* pathway with the susceptibility to hepatocellular carcinoma in a Chinese population

Zhong-Heng Wei*, Yue-Yong Li, Shi-Qing Huang, Zhong-Qiu Tan

Department of Oncology, The Affiliated Hospital of Youjiang Medical University for Nationalities, Baise 533000, Guangxi, China

ARTICLE INFO

Keywords:
Interleukin-33
ST2
Polymorphism
Plasma
Hepatocellular carcinoma

ABSTRACT

Interleukin (IL)-33/ST2 pathway plays a pivotal role in tumorigenesis through influencing cancer stemness, tumor growth, metastasis, angiogenesis, and accumulation of regulatory T cells in tumor microenvironments. The aim of this study was to investigate the association of *IL-33* rs7025417 and *ST2* rs3821204 with the risk of hepatocellular carcinoma (HCC). Genotyping of *IL-33* rs7025417 and *ST2* rs3821204 was carried out using a Taqman assay. *IL-33* and *ST2* mRNA was examined using real-time PCR and plasma IL-33 and sST2 levels were measured using enzyme-linked immunosorbent assay. The *ST2* rs3821204 CC genotype was associated with a significantly increased risk of HCC (CC vs. GG: adjusted OR = 2.29, 95% CI, 1.39–3.78; dominant model: adjusted OR = 1.58, 95% CI, 1.12–2.23; recessive model: adjusted OR = 1.88, 95% CI, 1.21–2.93; C vs. G: adjusted OR = 1.53, 95% CI, 1.20–1.95). Gene-environment interaction analysis showed that the risk effect of rs3821204 CG/CC genotypes was more evident in smokers (adjusted OR = 1.70, 95% CI, 1.13–2.55) and drinkers (adjusted OR = 1.57, 95% CI, 1.04–2.37). The increased risk was also observed in combined analysis. Moreover, HCC patients with *ST2* rs3821204 CC genotype had higher levels of mRNA and protein expression ($P < 0.05$). These findings suggest that *ST2* rs3821204 CC genotype may contribute to hepatocarcinogenesis by enhancing *ST2* production at the transcriptional and translational level.

1. Introduction

Hepatocellular carcinoma (HCC) is most common type of primary liver malignancy and the sixth leading cause of cancer death worldwide [1]. The geographical disparities are pronounced throughout the world, with an estimated 82% of cases (and deaths) in less developed countries and 50% in China alone [1,2]. In 2015, an estimated 466,100 new cases and 422,100 mortalities occurred in China, making it the fifth most common cause of cancer incidence and cancer death [3]. It is well known that multiple risk factors are involved in the initiation and progression of HCC, including cigarette smoking, alcohol drinking, and viral hepatitis [4]. In addition to these factors, growing evidence suggests the attribution of genetic factors to HCC carcinogenesis. Nischalke et al. reported that an rs2228603 T allele in neurocan gene was associated a significantly increased risk of HCC [5]. Liu et al. reported a functional polymorphism 2578C/A in vascular endothelial growth factor A was significantly associated with a decreased risk of HCC, a reduced postoperative recurrence, and a longer overall survival time [6]. Although some susceptibility genes of HCC have been demonstrated in recent years [5–7], the exact etiology of HCC remains unclear.

Interleukin (IL)-33, belonging to the IL-1 superfamily, is widely expressed in endothelial cells, epithelial cells [8], and several organs, such as liver tissues [9] and central nervous system [10]. IL-33 is the ligand of interleukin 1 receptor-like 1 (ST2) that has 3 isoforms: a transmembrane form (ST2L), a soluble form (sST2), and a variant form [11]. By binding to ST2, IL-33 activates NF-kappaB, extracellular signal-regulated kinase (ERK), p38, serine/threonine protein kinase Akt, and c-Jun N-terminal kinase, and induces the expression of IL-4, IL-5, and IL-13, leading to a series of pathological changes [12]. Regarding the expression of IL-33 in HCC patients, Zhang et al. reported higher levels of protein IL-33 [9c], whereas Yang et al. reported lower levels of *IL-33* mRNA and reduced cytoplasmic staining [9a], indicating a dual role of IL-33 in HCC pathogenesis.

In the human genome, *IL-33* locates on chromosome 9p24.1 and *ST2* located on chromosome 2q12.1. Previously, a functional polymorphism rs7025417 in the promoter of *IL-33* was discovered, with the C allele yielding a reduced luciferase activity and lower levels of plasma IL-33 [13]. For genetic variants in *ST2*, a functional polymorphism rs3821204 was identified which located within the 3′ untranslated region of *ST2* [14]. The rs3821204 C allele disrupts its binding site to miR-202-3p, resulting in higher levels of plasma sST2 [14]. In this study, we aimed

* Corresponding author.
E-mail address: weizhonghengbs@sina.com (Z.-H. Wei).

https://doi.org/10.1016/j.cyto.2018.03.036
Received 4 February 2018; Received in revised form 26 March 2018; Accepted 26 March 2018
Available online 12 April 2018

to investigate the association between the 2 polymorphisms and HCC risk in a Chinese population. Gene-environment interaction and genotype-phenotype analyses were also examined.

2. Materials and methods

2.1. Ethics, consent and permissions

This study was approved by the Review Boards of Affiliated Hospital of Youjiang Medical College for Nationalities. All subjects agreed to participate in the study and provided informed consent.

2.2. Study population

A hospital-based case-control study was conducted to assess *IL-33* rs7025417 and *ST2* rs3821204 polymorphisms in relation to HCC risk. Cases were consecutively recruited from the Affiliated Hospital of Youjiang Medical College for Nationalities between January 2010 and February 2017. All cases were newly diagnosed and histologically confirmed HCC. During the study period, a total of 511 HCC patients agreed to participate in the study. Patients with alcoholic liver diseases, metabolic liver diseases, or a history of familial cancer were excluded from the study. Controls were healthy volunteers who came to the same hospital for routine physical examination during the same period. To avoid selection bias, the inclusion criteria for controls were as follows: (a) frequency matched to the cases on age, gender, and residential area; (b) individuals with normal B-mode ultrasound images. Controls were excluded if they had a history of cancer, or a family history of cancer. Finally, 592 controls were included in this study. All the cases and controls were genetically unrelated Han Chinese. Clinical data including age, gender, and HBV surface antigen (HBsAg) was acquired from medical records. After signing written informed consent, each participant was interviewed using a defined questionnaire to collect information of environmental exposure history. Individuals smoking ≥ 1 cigarette/day for over 1 year were categorized as smokers, and those consuming ≥ 3 alcohol drinks a week for over 6 months were defined as alcohol drinkers. After interview, about 3 ml venous blood was taken from each participant. All the samples were centrifuged at 1000*g* for 10 min, plasma was aliquoted and stored at $-80\,^{\circ}C$ until analysis.

2.3. DNA extraction and genotyping assays

Genomic DNA was isolated using a commercial kit (BioTeke, Beijing, China). Genotyping of *IL-33* rs7025417 and *ST2* rs3821204 was carried out using a Taqman assay. TaqMan probes were purchased form Applied Biosystems (Foster City, CA, USA). The assays ID of probes are C_31940410_20 for rs7025417 and C_1226153_10 for rs3821204. Genotyping analysis was performed using the ABI PRISM_7500HT Sequence Detection System (Applied Biosystems) according to manufacturer's instructions. The genotyping results were verified by DNA sequencing and the concordance between the 2 methods was identical.

2.4. RNA isolation and real-time quantitative PCR

Total RNA was extracted from peripheral blood cells using TRIpure LS Reagent (BioTeke, Beijing, China) and reverse-transcribed to cDNA using a PrimeScript® 1st Strand cDNA Synthesis Kit (Takara Bio Inc., Nojihigashi, Kusatsu, Shiga, Japan) following the manufacturer's instructions. Real-time quantitative PCR was performed using cDNA as the template and SYBR® Premix Ex Taq™ II (Takara Bio Inc.) in an ABI PRISM_7500HT Sequence Detection System (Applied Biosystems). The forward and reverse primers for *IL-33* amplification were 5′-ATCCCA ACAGAAGGCCAAAG-3′ and 5′-CCAAAGGCAAAGCA CTCCAC-3′, respectively. Primers for *ST2* amplification were 5′-GGATTGAGGCCACTC TGCTC-3′ (forward) and 5′-CCGCCTGCTCTTTCGTATGT-3′ (reverse), and primers for *β-actin* amplification (internal control) were 5′-TTGC CGACAGGATGCAGAA-3′ (forward) and 5′-GCCGATCCACACGGAGT ACT-3′ (reverse) [15]. A comparative $2^{-\Delta Ct}$ method was used to calculate the relative *IL-33* and *ST2* mRNA expression.

2.5. Plasma levels of IL-33 and sST2 in HCC patients and controls

Plasma concentrations of IL-33 and sST2 were measured in duplicate by enzyme-linked immunosorbent assay (ELISA) using a commercial kit purchased from R&D Systems (Minneapolis, MN, USA). The assay range was 6.3–400 pg/mL for IL-33 and 31.3–2000 pg/mL for sST2.

2.6. Statistical analysis

Differences of demographic characteristics between cases and controls were compared using the Student's *t* test for continuous variables and χ^2 test for categorical variables. Each polymorphism was tested using the chi-square 'goodnessof-fit' test to ensure the fitting with Hardy–Weinberg equilibrium (HWE). Unconditional logistic regression analysis was used to examine the association between *IL* and *33/ST2* polymorphisms and HCC risk by computing the odds ratios (ORs) and 95% confidence intervals (CIs), adjusted for age, gender, smoking status, drinking status, and HBsAg status. The statistical *P* level of significance was set as 0.0125 (0.05/4) after Bonferroni's correction for multiple testing. Plasma levels of IL-33 and sST2 in HCC patients and controls were analyzed using Mann-Whitney *U* test. *P* values < 0.05 were considered statistically significant. Data analyses were performed using the statistical software package SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Characteristics of the study subjects

The general characteristics of the 511 HCC patients and 592 controls are summarized in Table 1. There was no significant difference between the cases and controls in terms of the distribution of age, gender, tobacco smoking, and alcohol consumption status ($P > 0.05$). Approximately, 80.2% of the HCC cases had positive HBsAg, which was significantly higher than that of the controls (10.6%) ($P < 0.001$).

Table 1
Characteristics of HCC patients and controls.

Characteristics	Controls (n = 592)	HCC patients (n = 511)	*P* value
Age (years, mean ± SD)	56.6 ± 10.1	57.5 ± 10.7	0.13
Gender, n (%)			
Male	460 (77.7)	376 (73.6)	0.11
Female	132 (22.3)	135 (26.4)	
Smoking status, n (%)			
Never	191 (32.3)	143 (28.0)	0.12
Ever	401 (67.6)	368 (72.0)	
Drinking status, n (%)			
Never	168 (28.4)	127 (24.9)	0.19
Ever	424 (71.6)	384 (75.1)	
HBsAg, n (%)			
Positive	63 (10.6)	410 (80.2)	< 0.001
Negative	529 (89.4)	101 (19.8)	

HCC, hepatocellular carcinoma; SD, standard deviation; HBsAg, HBV surface antigen.

Table 2
Association between polymorphisms in *IL-33/ST2* and HCC risk.

Polymorphisms	Controls, n = 592 (%)	HCC, n = 511 (%)	Adjusted OR (95% CI)[a]	Adjusted *P* value[a]
rs7025417				
TT	171 (28.9)	163 (31.9)	1.00 (Ref)	
CT	287 (48.5)	235 (46.0)	0.79 (0.53–1.16)	0.23
CC	134 (22.6)	113 (22.1)	0.90 (0.58–1.40)	0.64
Dominant	421 (71.1)	348 (68.1)	0.82 (0.57–1.18)	0.28
Recessive	458 (77.4)	398 (77.9)	1.01 (0.68–1.51)	0.96
T allele	629 (53.1)	561 (54.9)	1.00 (Ref)	
C allele	555 (46.9)	461 (45.1)	0.92 (0.73–1.17)	0.51
rs3821204				
GG	262 (44.3)	189 (37.0)	1.00 (Ref)	
CG	250 (42.2)	212 (41.5)	1.36 (0.94–1.96)	0.10
CC	80 (13.5)	110 (21.5)	2.29 (1.39–3.78)	0.001
Dominant	330 (55.7)	322 (63.0)	1.58 (1.12–2.23)	0.009
Recessive	512 (86.5)	401 (78.5)	1.88 (1.21–2.93)	0.005
G allele	774 (65.4)	590 (57.7)	1.00 (Ref)	
C allele	410 (34.6)	432 (42.3)	1.53 (1.20–1.95)	< 0.001

HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval.
[a] Adjusted based on age, gender, smoking status, drinking status, and HBsAg status.

3.2. Association of IL-33 rs7025417 and ST2 rs3821204 polymorphisms with HCC risk

The genotype and allelic frequencies of *IL-33* rs7025417 and *ST2* rs3821204 among HCC cases and controls are presented in Table 2. The observed genotype frequencies of the 2 SNPs were in agreement with that expected distributions according to HWE in controls ($P = 0.52$ for rs7025417 and $P = 0.10$ for rs3821204, respectively). In multivariate logistic regression models, the *ST2* rs3821204 CC genotype was associated with a significantly increased risk of HCC (CC vs. GG: adjusted OR = 2.29, 95% CI, 1.39–3.78, $P = 0.001$; dominant model: adjusted OR = 1.58, 95% CI, 1.12–2.23, $P = 0.009$; recessive model: adjusted OR = 1.88, 95% CI, 1.21–2.93, $P = 0.005$). The rs3821204 C allele was also observed to be associated with a significantly increased risk of HCC compared to the G allele (adjusted OR = 1.53, 95% CI, 1.20–1.95, $P < 0.001$). However, no significant association was observed between *IL* and *33* rs7025417 and HCC risk.

We then performed stratification analyses and the results are shown in Tables 3 and 4. Although null results were found between *IL* and *33* rs7025417 and HCC risk in overall analysis, stratification analysis showed that *IL-33* rs7025417 CT/CC genotypes were associated with a reduced risk of HCC risk in subjects with negative HBsAg (adjusted OR = 0.56, 95% CI, 0.36–0.87, $P = 0.01$) (Table 3). Additionally, the risk effect of rs3821204 CG/CC genotypes was more evident in male subjects (adjusted OR = 1.71, 95% CI, 1.15–2.55, $P = 0.007$), ever smokers (adjusted OR = 1.70, 95% CI, 1.13–2.55, $P = 0.009$), and ever drinkers (adjusted OR = 1.57, 95% CI, 1.04–2.37, $P = 0.03$) (Table 4).

Further combined analysis was conducted to evaluate if the 2 SNPs had combined effect on the susceptibility to HCC. As shown in Table 5, the combined genotypes of rs7025417TT-rs3821204CG/CC were associated with a significantly increased risk of HCC compared to the combined genotypes of rs7025417TT-rs3821204GG (OR = 1.86, 95% CI, 1.19–2.90, $P = 0.006$).

Table 3
Stratified analyses of association between *IL* and *33* rs7025417 polymorphism and HCC risk.

Variables	Genotypes	Controls (n = 592)	HCC (n = 511)	Adjusted OR (95% CI)[a]	Adjusted *P* value[a]
Gender					
Male	TT	129 (28.0)	114 (30.3)	1.00 (Ref)	
	CT/CC	331 (72.0)	262 (69.7)	0.94 (0.62–1.44)	0.79
Female	TT	42 (31.8)	49 (36.3)	1.00 (Ref)	
	CT/CC	90 (68.2)	86 (63.7)	0.52 (0.24–1.12)	0.09
Smoking status					
Never	TT	67 (35.1)	52 (36.4)	1.00 (Ref)	
	CT/CC	124 (64.9)	91 (63.6)	0.77 (0.38–1.55)	0.46
Ever	TT	104 (25.9)	111 (30.2)	1.00 (Ref)	
	CT/CC	297 (74.1)	257 (69.8)	0.83 (0.54–1.27)	0.39
Drinking status					
Never	TT	46 (27.4)	36 (28.4)	1.00 (Ref)	
	CT/CC	122 (72.6)	91 (71.7)	0.73 (0.37–1.45)	0.37
Ever	TT	125 (29.5)	127 (33.1)	1.00 (Ref)	
	CT/CC	299 (70.5)	257 (66.9)	0.87 (0.57–1.35)	0.54
HBsAg					
Positive	TT	24 (38.1)	123 (30.0)	1.00 (Ref)	
	CT/CC	39 (61.9)	287 (70.0)	0.67 (0.38–1.17)	0.17
Negative	TT	147 (27.8)	40 (39.6)	1.00 (Ref)	
	CT/CC	382 (72.2)	61 (60.4)	0.56 (0.36–0.87)	0.01

HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval; HBsAg, HBV surface antigen.
[a] Adjusted based on age, gender, smoking status, drinking status, and HBsAg status (excluded the stratified factor in each stratum).

3.3. Association between ST2 rs3821204 and mRNA and protein levels

IL-33 and *ST2* mRNA and protein levels in HCC patients and controls were examined using real-time PCR and ELISA. As shown in Fig. 1A and B, there was no significant difference of *IL-33* mRNA and protein levels between HCC patients and controls. The *ST2* mRNA and protein levels, however, were significantly higher in HCC patients than those in controls ($P < 0.05$). Genotype-phenotype analysis was then performed to assess whether rs3821204 influences *ST2* mRNA and protein expression. As shown in Fig. 2A and B, in controls, no significant association was observed between rs3821204 and *ST2* mRNA and protein levels. Nevertheless, HCC patients carrying rs3821204 CC genotype yielded increased levels of *ST2* mRNA (Fig. 2C) and protein expression (Fig. 2D) compared to rs3821204 GG genotype ($P < 0.05$).

4. Discussion

In this report, we analyzed the association of *IL-33* rs7025417 and *ST2* rs3821204 with HCC risk and found that the *ST2* rs3821204 was associated with a significantly increased risk of HCC in homozygote comparison, dominant genetic comparison, recessive genetic comparison, and allele comparison. Combined analysis showed that the combined genotypes of rs7025417TT-rs3821204CG/CC were associated with a significantly increased risk of HCC. Gene-environment interaction analysis showed that the risk effect of rs3821204 CG/CC genotypes

Table 4
Stratified analyses of association between *ST2* rs3821204 polymorphism and HCC risk.

Variables	Genotypes	Controls (n = 592)	HCC (n = 511)	Adjusted OR (95% CI)[a]	Adjusted *P* value[a]
Gender					
Male	GG	201 (43.7)	136 (36.2)	1.00 (Ref)	
	CG/CC	259 (56.3)	240 (63.8)	1.71 (1.15–2.55)	0.007
Female	GG	61 (46.2)	53 (39.3)	1.00 (Ref)	
	CG/CC	71 (53.8)	82 (60.7)	1.19 (0.58–2.45)	0.63
Smoking status					
Never	GG	87 (45.5)	55 (38.5)	1.00 (Ref)	
	CG/CC	104 (54.5)	88 (61.5)	1.26 (0.64–2.48)	0.51
Ever	GG	175 (43.6)	134 (36.4)	1.00 (Ref)	
	CG/CC	226 (56.4)	234 (63.6)	1.70 (1.13–2.55)	0.009
Drinking status					
Never	GG	72 (42.9)	45 (35.4)	1.00 (Ref)	
	CG/CC	96 (57.1)	82 (64.6)	1.55 (0.82–2.96)	0.18
Ever	GG	190 (44.8)	144 (37.5)	1.00 (Ref)	
	CG/CC	234 (55.2)	240 (62.5)	1.57 (1.04–2.37)	0.03
HBsAg					
Positive	GG	32 (50.8)	154 (37.6)	1.00 (Ref)	
	CG/CC	31 (49.2)	256 (62.4)	0.58 (0.34–1.00)	0.05
Negative	GG	230 (43.5)	35 (34.7)	1.00 (Ref)	
	CG/CC	299 (56.5)	66 (65.3)	1.48 (0.94–2.32)	0.08

HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval; HBsAg, HBV surface antigen.

[a] Adjusted based on age, gender, smoking status, drinking status, and HBsAg status (excluded the stratified factor in each stratum).

Table 5
Combined analyses of *IL-33* rs7025417 and *ST2* rs3821204 with HCC risk.

Polymorphisms	Controls (%)	Cases (%)	OR (95% CI)	*P* value
rs7025417TT-rs3821204GG	82 (13.9)	54 (10.6)	1.00 (Ref)	
rs7025417CC/CT-rs3821204GG	180 (30.4)	135 (26.4)	1.14 (0.76–1.72)	0.53
rs7025417TT-rs3821204CG/CC	89 (15.0)	109 (21.3)	1.86 (1.19–2.90)	0.006
rs7025417CC/CT-rs3821204CG/CC	241 (21.8)	213 (19.3)	1.34 (0.91–1.98)	0.14

HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval.

was more evident in smokers and drinkers. Genotype-phenotype analysis showed that HCC patients carrying rs3821204 CC genotype had higher levels of *ST2* mRNA and protein expression. Taken together, our findings suggest that rs3821204 CC genotype may contribute to hepatocarcinogenesis by enhancing *ST2* production.

Accumulating data has revealed that IL-33 plays a pivotal role in tumorigenesis through influencing cancer stemness, tumor growth, metastasis, angiogenesis, polarization of M2 tumor-associated macrophages, and accumulation of regulatory T cells in tumor microenvironments [16]. IL-33 is a dual function cytokine with conflicting data of expression in different cancer types. Some research groups reported an up-regulation of IL-33 in glioma [17], gastric cancer [18], colorectal cancer [19], ovarian cancer [20], and squamous cell carcinoma of tongue [21] while some research groups reported a down-regulation of IL-33 in breast cancer [22], lung cancer [23], and multiple myeloma [24]. Even in the same cancer type of HCC, inconsistent results were observed. Zhang et al. reported increased IL-33 protein levels in serum and liver tissue from HCC patients [9c], whereas Bergis et al. did not find significant difference of IL-33 serum levels between HCC patients and healthy controls [25]. In agreement with the negative results, we failed to find any significant difference of *IL-33* mRNA and protein levels among HCC patients and controls. Previously, a polymorphism rs7025417 in the promoter of *IL-33* was discovered, and the T allele increased the risk of coronary artery disease by enhancing reporter gene expression and IL-33 protein levels [13]. Since it is functional, we selected and investigated whether it is related to the risk of HCC. Even though negative results were found in overall analysis, stratification analysis showed that *IL-33* rs7025417 CT/CC genotypes were associated with a reduced risk of HCC risk in subjects with negative HBsAg, indicating that rs7025417 CT/CC genotypes were protective factors for HCC patients without hepatitis B vaccination.

ST2 was originally considered as an orphan receptor till the specific ligand (i.e., IL-33) was demonstrated [26]. Subsequent studies identified the importance of ST2 in anti-tumor immunity [27]. Previous reports and current work have demonstrated higher levels of sST2 in several cancers including HCC [25,28]. *In vitro* and *in vivo* studies revealed that ST2-deficiency protects from tumor development [29]. Recently, a polymorphism rs3821204 in the 3′ untranslated region of *ST2* was found to be functional, with the rs3821204 C allele disrupting the binding site to miR-202-3p and resulting in higher levels of plasma sST2 [14]. In this study, we hypothesized that the rs3821204 may be associated with the risk of HCC. Our findings confirmed this hypothesis. We found an increased effect of *ST2* rs3821204 CC genotype on HCC risk and the risk effect was more evident in smokers and drinkers, indicating that gene-environment interaction may contribute to the etiology of HCC in addition to genetic factor of *ST2*. We then explored the possible mechanism and found rs3821204 CC genotype carriers exhibiting higher levels of *ST2* mRNA and protein expression in HCC patients. Taken together, we may conclude that *ST2* rs3821204 modifies HCC susceptibility by regulating its expression.

Given the association of *ST2* rs3821204 CC genotype with smokers, we speculated a potential impact of the *ST2* polymorphism on other

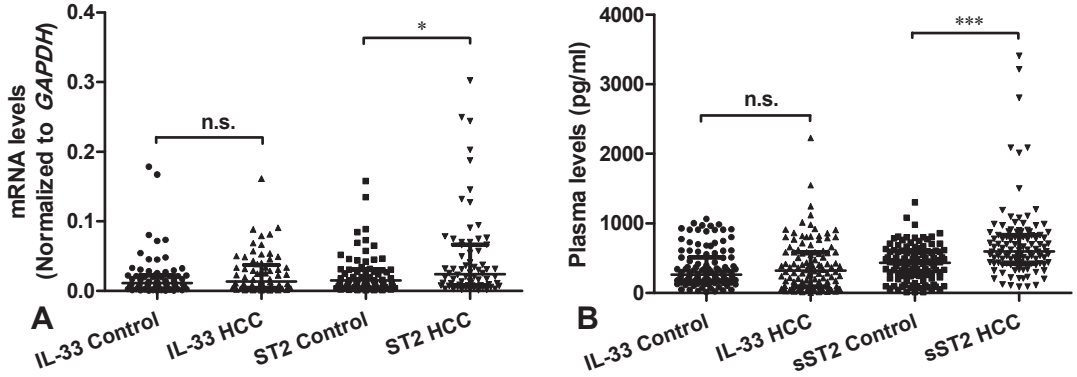

Fig. 1. *IL-33/ST2* mRNA and protein levels in HCC patients and controls. Data was shown as median with interquatile range (n.s., not significant; * $P < 0.05$; *** $P < 0.001$). A, *IL-33* and *ST2* mRNA levels in HCC tissues and adjacent normal tissues (controls); B, Plasma levels of IL-33 and sST2 in patients with HCC and controls.

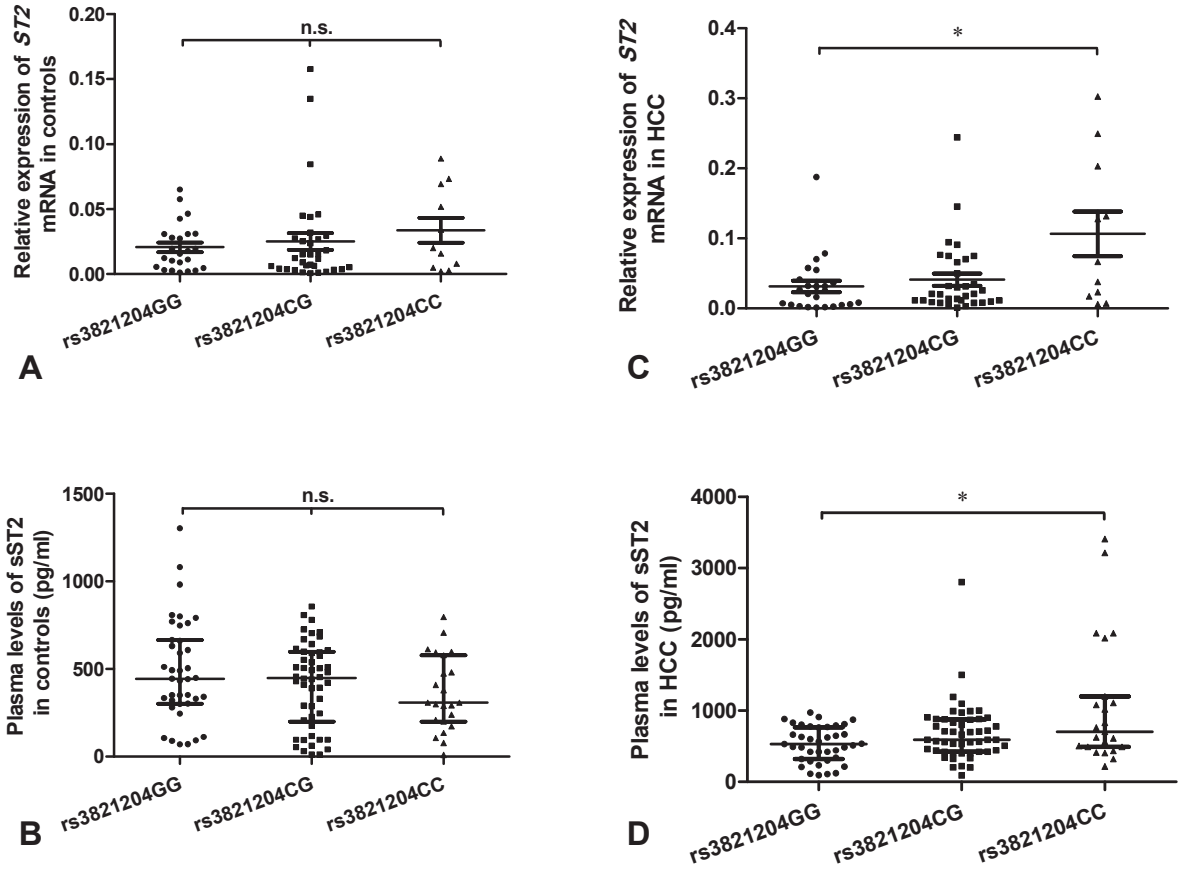

Fig. 2. Comparison of *ST2* rs3821204 with mRNA and protein levels in HCC patients and controls. Data was shown as median with interquatile range (n.s., not significant; * $P < 0.05$). In controls, no significant association of rs3821204 with mRNA (A) and protein levels (B). In HCC patients, rs3821204 CC genotype was associated with an increased level of mRNA (C) and protein expression (D).

smoking-related cancers, such as hepatocellular carcinoma [30], lung cancer, and pancreatic cancer [31]. It is demonstrated that serum levels of ST2 were elevated in patients with hepatocellular carcinoma [25] and chronic obstructive pulmonary disease (COPD) [32]. Interleukin-33/ST2 signaling can promote production of IL-6 and IL-8 in systemic inflammation in cigarette smoke- induced COPD mice [33]. COPD is a well established risk factor for lung cancer [34]. Additionally, IL-33/ST2 axis might act as a crucial mediator in inflammation-associated pancreatic carcinogenesis [35]. It is likely, therefore, that *ST2* rs3821204 CC genotype may be a risk factor for the development of hepatocellular carcinoma, lung cancer, and pancreatic cancer. Further studies are warranted to verify the hypothesis.

As HCC is a complex disease involving in multiple genes, combined analysis was performed to assess whether *IL-33* and *ST2* have combined effect on the susceptibility to HCC. Not surprising, we found that the combined genotypes of rs7025417TT-rs3821204 CG/CC had a 1.86-fold increased risk of HCC. This finding seems to be biological plausible because interaction of *IL-33* and *ST2* in tumorigenesis has been previously reported [36]. By binding to ST2, IL-33 promotes cancer-associated inflammation, tumor progression and metastasis [27b,29b,36,37]. Deletion of IL-33/ST2 axis attenuates tumor growth through inducing the secretion of some inflammation-related cytokines, such as interferon-gamma, IL-17 and tumor necrosis factor-α [37a].

This study has limitations with respect to study design and statistical power. The study design is hospital-based and the controls were selected from the same hospital as the cases, which might not be representative of the general population. Our sample size is limited to 511 cases and 592 controls that are not sufficiently large. The control groups with the rs3821204 CC genotype do not display the same increase in ST2 levels as observed in HCC patients with the same CC genotype (Fig. 2). It is difficult to exactly explain the discrepancy. Although no significant difference was observed, there is a trend of increasing ST2 mRNA levels in control subjects carrying rs3821204 CC genotype (Fig. 2A), and thus the limited sample size may be responsible for the discrepancy in mRNA levels (n = 68). For the discrepancy in protein levels (Fig. 2C and D), we cannot rule out the possibility for selection bias of controls tested in ELISA assay. Confirmation studies are required in larger prospective studies.

Despite these limitations, our study provides initial evidence that *ST2* rs3821204 CC genotype was a new genetic risk factor for HCC in the Chinese population, highlighting it as a promising biomarker for personalized diagnosis and treatment for HCC. Association between *ST2* rs3821204 and HCC risk in diverse populations needs further validation. Moreover, further investigation of the mechanism of *ST2* rs3821204 in HCC development and progression will help to improve prevention and therapeutic strategies.

Author contribution

Zhong-Heng Wei designed and wrote the manuscript. Yue-Yong Li performed experiments. Shi-Qing Huang collected samples and performed genotyping. Zhong-Qiu Tan performed statistical analysis.

Compliance with ethical standards

Conflict of Interest: Zhong-Heng Wei declares that he has no conflict of interest. Yue-Yong Li declares that he has no conflict of interest. Shi-Qing Huang declares that he has no conflict of interest. Zhong-Qiu Tan declares that he has no conflict of interest.

Ethical approval: All procedures performed in studies involving

human participants were in accordance with the ethical standards of the Affiliated Hospital of Youjiang Medical College for Nationalities and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

References

[1] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2015) 87–108.

[2] D.M. Parkin, F. Bray, J. Ferlay, P. Pisani, Global cancer statistics, 2002, CA Cancer J. Clin. 55 (2005) 74–108.

[3] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, F. Bray, et al., Cancer statistics in China, 2015, CA Cancer J. Clin. 66 (2016) 115–132.

[4] (a) C.J. Chen, D.S. Chen, Interaction of hepatitis B virus, chemical carcinogen, and genetic susceptibility: multistage hepatocarcinogenesis with multifactorial etiology, Hepatology 36 (2002) 1046–1049;
(b) C.J. Chen, M.W. Yu, Y.F. Liaw, Epidemiological characteristics and risk factors of hepatocellular carcinoma, J. Gastroenterol. Hepatol. 12 (1997) S294–S308.

[5] H.D. Nischalke, P. Lutz, B. Kramer, J. Sohne, T. Muller, J. Rosendahl, et al., A common polymorphism in the NCAN gene is associated with hepatocellular carcinoma in alcoholic liver disease, J. Hepatol. 61 (2014) 1073–1079.

[6] F. Liu, L. Luo, Y. Wei, W. Wang, T. Wen, J. Yang, et al., Association of VEGFA polymorphisms with susceptibility and clinical outcome of hepatocellular carcinoma in a Chinese Han population, Oncotarget 8 (2017) 16488–16497.

[7] (a) K. Matsuura, H. Sawai, K. Ikeo, S. Ogawa, E. Iio, M. Isogawa, et al., Genome-wide association study identifies TLL1 variant associated with development of hepatocellular carcinoma after eradication of hepatitis C virus infection, Gastroenterology 152 (2017) 1383–1394;
(b) Y. Shi, W. Zhai, B. Wang, D. Zhao, H. Jin, Y. Wang, et al., Genetic susceptibility of eight nonsynonymous polymorphisms in HLA-DRB1 gene to hepatocellular carcinoma in Han Chinese, Oncotarget 7 (2016) 80935–80942.

[8] C. Moussion, N. Ortega, J.P. Girard, The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel 'alarmin'? PloS One 3 (2008) e3331.

[9] (a) Y. Yang, J.B. Wang, Y.M. Li, Y.U. Zhao, R. Wang, Q. Wu, et al., Role of IL-33 expression in oncogenesis and development of human hepatocellular carcinoma, Oncol. Lett. 12 (2016) 429–436;
(b) P. Marvie, M. Lisbonne, A. L'Helgoualc'h, M. Rauch, B. Turlin, L. Preisser, et al., Interleukin-33 overexpression is associated with liver fibrosis in mice and humans, J. Cell. Mol. Med. 14 (2010) 1726–1739;
(c) P. Zhang, X.K. Liu, Z. Chu, J.C. Ye, K.L. Li, W.L. Zhuang, et al., Detection of interleukin-33 in serum and carcinoma tissue from patients with hepatocellular carcinoma and its clinical implications, J. Int. Med. Res. 40 (2012) 1654–1661.

[10] C.A. Hudson, G.P. Christophi, R.C. Gruber, J.R. Wilmore, D.A. Lawrence, P.T. Massa, Induction of IL-33 expression and activity in central nervous system glia, J. Leukocyte Biol. 84 (2008) 631–643.

[11] K. Tago, T. Noda, M. Hayakawa, H. Iwahana, K. Yanagisawa, T. Yashiro, et al., Tissue distribution and subcellular localization of a variant form of the human ST2 gene product, ST2V, Biochem. Biophys. Res. Commun. 285 (2001) 1377–1383.

[12] (a) C. Bouffi, M. Rochman, C.B. Zust, E.M. Stucke, A. Kartashov, P.C. Fulkerson, et al., IL-33 markedly activates murine eosinophils by an NF-kappaB-dependent mechanism differentially dependent upon an IL-4-driven autoinflammatory loop, J. Immunol. 191 (2013) 4317–4325;
(b) V. Carriere, L. Roussel, N. Ortega, D.A. Lacorre, L. Americh, L. Aguilar, et al., IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo, PNAS 104 (2007) 282–287.

[13] X. Tu, S. Nie, Y. Liao, H. Zhang, Q. Fan, C. Xu, et al., The IL-33-ST2L pathway is associated with coronary artery disease in a Chinese Han population, Am. J. Hum. Genetics 93 (2013) 652–660.

[14] F. Wu, L. Li, Q. Wen, J. Yang, Z. Chen, P. Wu, et al., A functional variant in ST2 gene is associated with risk of hypertension via interfering MiR-202-3p, J. Cell. Mol. Med. 21 (2017) 1292–1299.

[15] P.P. Li, X.M. Zhang, D. Yuan, X. Liu, Y. Li, N.N. Shan, Decreased expression of IL-33 in immune thrombocytopenia, Int. Immunopharmacol. 28 (2015) 420–424.

[16] (a) M. Fang, Y. Li, K. Huang, S. Qi, J. Zhang, W. Zgodzinski, et al., IL33 promotes colon cancer cell stemness via JNK activation and macrophage recruitment, Cancer Res. 77 (2017) 2735–2745;
(b) K. Wang, S. Shan, Z. Yang, X. Gu, Y. Wang, C. Wang, et al., IL-33 blockade suppresses tumor growth of human lung cancer through direct and indirect pathways in a preclinical model, Oncotarget 8 (2017) 68571–68582;
(c) Y. Zhang, C. Davis, S. Shah, D. Hughes, J.C. Ryan, D. Altomare, et al., IL-33 promotes growth and liver metastasis of colorectal cancer in mice by remodeling the tumor microenvironment and inducing angiogenesis, Mol. Carcinogenesis 56 (2017) 272–287;
(d) M. Akimoto, R. Maruyama, H. Takamaru, T. Ochiya, K. Takenaga, Soluble IL-33 receptor sST2 inhibits colorectal cancer malignant growth by modifying the tumour microenvironment, Nat. Commun. 7 (2016) 13589.

[17] J. Zhang, P. Wang, W. Ji, Y. Ding, X. Lu, Overexpression of interleukin-33 is associated with poor prognosis of patients with glioma, Int. J. Neurosci. 127 (2017) 210–217.

[18] P. Sun, Q. Ben, S. Tu, W. Dong, X. Qi, Y. Wu, Serum interleukin-33 levels in patients with gastric cancer, Dig. Dis. Sci. 56 (2011) 3596–3601.

[19] X. Liu, L. Zhu, X. Lu, H. Bian, X. Wu, W. Yang, et al., IL-33/ST2 pathway contributes to metastasis of human colorectal cancer, Biochem. Biophys. Res. Commun. 453 (2014) 486–492.

[20] X. Tong, M. Barbour, K. Hou, C. Gao, S. Cao, J. Zheng, et al., Interleukin-33 predicts poor prognosis and promotes ovarian cancer cell growth and metastasis through regulating ERK and JNK signaling pathways, Mol. Oncol. 10 (2016) 113–125.

[21] K. Ishikawa, S. Yagi-Nakanishi, Y. Nakanishi, S. Kondo, A. Tsuji, K. Endo, et al., Expression of interleukin-33 is correlated with poor prognosis of patients with squamous cell carcinoma of the tongue, Auris Nasus Larynx 41 (2014) 552–557.

[22] C. Kretschmer, A. Sterner-Kock, F. Siedentopf, W. Schoenegg, P.M. Schlag, W. Kemmner, Identification of early molecular markers for breast cancer, Mol. Cancer 10 (2011) 15.

[23] M.S. Kim, E. Kim, J.S. Heo, D.J. Bae, J.U. Lee, T.H. Lee, et al., Circulating IL-33 level is associated with the progression of lung cancer, Lung Cancer 90 (2015) 346–351.

[24] C. Musolino, A. Allegra, M. Profita, A. Alonci, S. Saitta, S. Russo, et al., Reduced IL-33 plasma levels in multiple myeloma patients are associated with more advanced stage of disease, Br. J. Haematol. 160 (2013) 709–710.

[25] D. Bergis, V. Kassis, A. Ranglack, V. Koeberle, A. Piiper, B. Kronenberger, et al., High serum levels of the interleukin-33 receptor soluble ST2 as a negative prognostic factor in hepatocellular carcinoma, Translational Oncol. 6 (2013) 311–318.

[26] J. Schmitz, A. Owyang, E. Oldham, Y. Song, E. Murphy, T.K. McClanahan, et al., IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines, Immunity 23 (2005) 479–490.

[27] (a) I. Jovanovic, G. Radosavljevic, M. Mitrovic, V.L. Juranic, A.N. McKenzie, N. Arsenijevic, et al., ST2 deletion enhances innate and acquired immunity to murine mammary carcinoma, Eur. J. Immunol. 41 (2011) 1902–1912;
(b) I.P. Jovanovic, N.N. Pejnovic, G.D. Radosavljevic, J.M. Pantic, M.Z. Milovanovic, N.N. Arsenijevic, et al., Interleukin-33/ST2 axis promotes breast cancer growth and metastases by facilitating intratumoral accumulation of immunosuppressive and innate lymphoid cells, Int. J. Cancer 134 (2014) 1669–1682.

[28] (a) D.P. Lu, X.Y. Zhou, L.T. Yao, C.G. Liu, W. Ma, F. Jin, et al., Serum soluble ST2 is associated with ER-positive breast cancer, BMC Cancer 14 (2014) 198;
(b) D. Bergis, V. Kassis, H.H. Radeke, High plasma sST2 levels in gastric cancer and their association with metastatic disease, Cancerbiomarkers: Section A Disease Markers 16 (2016) 117–125.

[29] (a) K.D. Mertz, L.F. Mager, M.H. Wasmer, T. Thiesler, V.H. Koelzer, G. Ruzzante, et al., The IL-33/ST2 pathway contributes to intestinal tumorigenesis in humans and mice, Oncoimmunology 5 (2016) e1062966;
(b) J.Y. Kim, S.C. Lim, G. Kim, H.J. Yun, S.G. Ahn, H.S. Choi, Interleukin-33/ST2 axis promotes epithelial cell transformation and breast tumorigenesis via upregulation of COT activity, Oncogene 34 (2015) 4928–4938.

[30] O. Abdel-Rahman, D. Helbling, O. Schob, M. Eltobgy, H. Mohamed, J. Schmidt, et al., Cigarette smoking as a risk factor for the development of and mortality from hepatocellular carcinoma: An updated systematic review of 81 epidemiological studies, J. Evidence-based Med. 10 (2017) 245–254.

[31] M. Inoue-Choi, P. Hartge, L.M. Liao, N. Caporaso, N.D. Freedman, Association between long-term low-intensity cigarette smoking and incidence of smoking-related cancer in the national institutes of health-AARP cohort, Int. J. Cancer 142 (2018) 271–280.

[32] J. Xia, J. Zhao, J. Shang, M. Li, Z. Zeng, J. Zhao, et al., Increased IL-33 expression in chronic obstructive pulmonary disease, Am. J. Physiol. Lung Cell. Mol. Physiol. 308 (2015) L619–L627.

[33] H. Wu, S. Yang, X. Wu, J. Zhao, J. Zhao, Q. Ning, et al., Interleukin-33/ST2 signaling promotes production of interleukin-6 and interleukin-8 in systemic inflammation in cigarette smoke-induced chronic obstructive pulmonary disease mice, Biochem. Biophys. Res. Commun. 450 (2014) 110–116.

[34] (a) J.P. de-Torres, J.M. Marin, C. Casanova, V. Pinto-Plata, M. Divo, C. Cote, et al., Identification of COPD patients at high risk for lung cancer mortality using the COPD-LUCSS-DLCO, Chest 149 (2016) 936–942;
(b) Y. Takiguchi, I. Sekine, S. Iwasawa, R. Kurimoto, K. Tatsumi, Chronic obstructive pulmonary disease as a risk factor for lung cancer, World J. Clin. Oncol. 5 (2014) 660–666.

[35] A. Schmieder, G. Multhoff, J. Radons, Interleukin-33 acts as a pro-inflammatory cytokine and modulates its receptor gene expression in highly metastatic human pancreatic carcinoma cells, Cytokine 60 (2012) 514–521.

[36] Y. Yang, P. Andersson, K. Hosaka, Y. Zhang, R. Cao, H. Iwamoto, et al., The PDGF-BB-SOX7 axis-modulated IL-33 in pericytes and stromal cells promotes metastasis through tumour-associated macrophages, Nat. Commun. 7 (2016) 11385.

[37] (a) M. Milovanovic, V. Volarevic, G. Radosavljevic, I. Jovanovic, N. Pejnovic, N. Arsenijevic, et al., IL-33/ST2 axis in inflammation and immunopathology, Immunologic Res. 52 (2012) 89–99;
(b) G. Cui, H. Qi, M.D. Gundersen, H. Yang, I. Christiansen, S.W. Sorbye, et al., Dynamics of the IL-33/ST2 network in the progression of human colorectal adenoma to sporadic colorectal cancer, Cancer Immunol., Immunotherapy : CII 64 (2015) 181–190.