Research Article

**CD44, IL-33, and ST2 Gene Polymorphisms on Hepatocellular Carcinoma Susceptibility in the Chinese Population**

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1. Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide. The annual global incidence of primary liver cancer is 841,000; the death rate is 782,000; and it is the second highest mortality rate among males [1]. The mortality rate of liver cancer in China is the second highest among malignant tumors (new liver cancer accounts for more than 50% of the world’s total) and is following a trend of annual increase [2]. Surgical resection, liver transplantation, and tumor ablation are the potential curative therapies; however, these treatment options are only applicable to patients in the early stage of disease [3, 4]. Due to the highly malignant potential of HCC, diagnosis is not usually made until an advanced stage, at which point there are no effective therapies to be offered [5]. Therefore, it is of pressing importance to identify which genes are responsible for susceptibility to HCC. Single-nucleotide polymorphism (SNP), a well-defined molecular biomarker, has been widely applied in HCC susceptibility evaluation [6, 7].

Various factors, including viral infection and cirrhosis, may be involved in the development of HCC [8, 9]. Tumor stem cells (CSCs), a small number of stem cell-like cancer cell subsets in tumor tissues, are characterized by both cancer cells and stem cells and are decisive in the initiation of HCC formation, growth, and metastasis [10, 11]. The CSC marker CD44 is essential in several malignancies, including HCC, and is the primary adhesion molecule of the extracellular matrix, playing a pivotal role in tumor cell differentiation, invasion, and metastasis [12, 13]. Recent studies have
suggested that in the development of HCC, CD44+ cells were entwined with the genetic processes involved in cancer invasion and metastasis [14, 15]. HCC was often detected following the onset of cirrhosis, and the majority of patients worldwide with HCC have underlying cirrhosis [16]. The IL-33 gene, located on chromosome 9 (9p24.1), binds to ST2 and forms a trimer with IL-1R accessory protein (IL-1RACp), which recruits downstream signaling molecules through the Toll/IL-1R (TIR) domains of ST2. The signaling pathways of nuclear factor kappaB (NF-kB), activator protein 1 (AP-1), and mitogen-activated protein kinase (MAPK) are then activated, and the subsequent upregulation of the gene expression of proinflammatory cytokines leads to hepatic fibrosis [17–19]. Although CD44 and IL-33/ST2 have been well documented in human cancer metastasis or prognosis, respectively, evidence of the CD44 gene SNPs combined with IL-33/ST2 pathway functional polymorphisms in HCC susceptibility and clinical characteristics is scarce. We aimed to investigate in this study the association of these 3 polymorphisms with demographics, etiology, clinical features, and susceptibility to HCC.

2. Materials and Methods

2.1. Subjects and Clinical Data. The participant cohort was continuously recruited from September 2016 to December 2018 at the Affiliated Tumor Hospital of Guangxi Medical University. All patients were newly diagnosed and pathologically confirmed as having HCC, according to the American Association for the Study of Liver Diseases guidelines [20]. Ultimately, the study included 565 participants in the case group, 487 males and 78 females, aged 10–89 years. The control group was matched for sex and age and included a total of 561 cases (male = 488, female = 73), aged 22–78 years. The healthy control group patients were free of hypertension, diabetes, dyslipidemia, liver diseases, and cancer. Demographic data included medical record number, gender, age, drinking history, smoking history, tumor stage, and related biochemical indicators. All participants signed a written consent form after being informed of the study details. The Judging Committee approved the study of the Affiliated Tumor Hospital of Guangxi Medical University.

2.2. DNA Extraction and Genotyping Assays. We collected 2 ml of fasting peripheral blood from each participant and placed it in ethylenediaminetetraacetic acid- (EDTA-) K2 anticoagulant tube. After thorough mixing, it was stored in a refrigerator at -80°C for DNA extraction from the genome. Genomic DNA was extracted from the 2 ml of peripheral blood using a commercial kit according to the manufacturer’s instructions (Adelaide, Beijing, China) and stored at -80°C before genotyping. The genotyping of CD44 rs187115, IL-33 rs1929992, and ST2 rs3821204 was performed using the SNPShot method as previously described [21], and negative controls were used in each test to ensure accuracy of the genotype evaluation. The primers are listed in Table 1.

2.3. Statistical Analysis. First, we assessed whether genotype frequencies were in the Hardy-Weinberg equilibrium (HWE). The Pearson two-sided chi-squared ($\chi^2$) test was used to analyze the HWE law to confirm whether the population of the study samples was representative of the populace [22]. When a $p > 0.05$ value was observed, the samples were representative of the population. Then, the $\chi^2$ test was used to examine the difference in clinicopathological features between the case group and the control group. The distribution data of alleles and genotypes were compared using the $\chi^2$ test and logistic regression analysis, and the relative risk was expressed by odd ratios (OR) and their 95% confidence intervals (CI). Additionally, unconditional logistic regression was used to correct the effects of confounding factors such as gender, age, drinking history, and smoking history on OR values and 95% CI. The haplotype model analysis of the gene interaction was performed using the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) [23]. eQTL are regions of the genome containing DNA sequence variants that influence the expression level of one or more genes. We further explored the effects of the three significant SNPs (CD44 rs187115, IL-33 rs1929992, and ST2 rs3821204) on their gene expression by investigating a public database, GTEx portal (https://gtexportal.org) [24]. Statistical analysis of the data was performed using the statistical software package SPSS 24.0 (SPSS Inc., IBM, Chicago, IL, USA), and the test was performed using a two-sided test with a test level of $\alpha = 0.05$. We used the false positive reporting probability (FPRP) to evaluate meaningful findings. We set 0.2 as the FPRP threshold, and 0.1 as the prior probability to detect the preponderance ratio (OR) associated with genotypes and haplotypes in the study to be 0.67/1.50 (protection/risk effect). Only significant results with an FPRP value of < 0.2 would be considered a noteworthy finding.

3. Results

3.1. Demographic and Clinical Characteristics of Participants. Initially, 593 subjects were enrolled in the HCC group, 21 cases had no pathological reports, and 7 cases had other cancers, and thus 28 cases were excluded. Ultimately, the study included 565 patients in the HCC group (males = 487, females = 78, age 10–89 years) and 561 in the healthy control group (males = 488, females = 73, age 22–78 years). Briefly, male participants showed a higher prevalence of HCC than females (487 vs. 78). Metastasis was found in 85 patients (15.0%). Stage of HCC was staged A or B in 259 patients (45.8%) and stage C or D in 306 patients (54.2%). Moreover,
Table 2: General characteristics of HCC patients and the normal controls.

| Characteristics | Cases (n = 565) | Controls (n = 561) | p value |
|-----------------|----------------|-------------------|---------|
| Age (year)      |                |                   | 0.546   |
| Range           | 10-89          | 22-78             |         |
| Mean            | 53.62          | 52.15             |         |
| <40             | 95             | 102               |         |
| 41-50           | 133            | 152               |         |
| 51-60           | 186            | 176               |         |
| >60             | 151            | 131               |         |
| Gender          |                |                   | 0.453   |
| Male            | 487            | 488               |         |
| Female          | 78             | 73                |         |
| BMI (kg/m²)     |                |                   | 0.406   |
| <18.5           | 62             | 51                |         |
| 18.5-23.9       | 366            | 359               |         |
| ≥24             | 137            | 151               |         |
| Smoking status  |                |                   | 0.734   |
| No              | 344            | 336               |         |
| Yes             | 221            | 225               |         |
| Alcohol drinker |                |                   | 0.113   |
| No              | 374            | 396               |         |
| Yes             | 191            | 165               |         |
| HBV infection   |                |                   |         |
| HbsAg (−)       | 47             | 479               |         |
| HbsAg (+)       | 497            | 75                | ≤0.001  |
| Liver cirrhosis |                |                   |         |
| Absent          | 487            | 491               |         |
| Present         | 78             | 70                | 0.51    |
| BCLC stage      |                |                   |         |
| A+B stage       | 259            |                   |         |
| C+D stage       | 306            |                   |         |
| Metastasis      |                |                   |         |
| No              | 480            |                   |         |
| Yes             | 85             |                   |         |

There were no statistical differences in the distributions of age, gender, smoking status, and alcohol consumption between the 2 groups. Detailed demographic and clinical characteristics of the study participants, including age, gender, smoking, and drinking status, Barcelona clinic liver cancer (BCLC) stage, and metastasis status, are provided in Table 2.

3.2. Association between CD44 rs187115 and Susceptibility and Clinicopathological Parameters of HCC. In this study, the distribution of CD44 gene rs187115 in the HCC group (p = 0.58) and the control group (p = 0.74) was consistent with the HWE. The frequency distribution differences of the GG, GA, and AA genotypes in the 2 groups were statistically significant (p = 0.010). We used the AA genotype and A allele as a reference to analyze the risk of HCC. Logistic regression was used to adjust the impact of confounding factors such as gender and age and for calculating the OR value of rs187115 on the risk of HCC and 95% CI. Ultimately, it was found that compared with other genotype individuals, people carrying the homozygous GG genotype were 2.469 times more likely to develop HCC than those carrying the AA gene (p = 0.027, adjusted OR (ORadj) = 2.469, 95% CI: 1.110–5.492). Furthermore, individuals who carried the heterozygous AG genotype were 1.359 times more likely to develop HCC than individuals carrying the GG gene (p = 0.026, ORadj = 1.359, 95% CI: 1.039–1.778). Moreover, compared with the A allele, individuals carrying the G allele had a 1.423 times higher risk of HCC (p = 0.003, OR = 1.423, 95% CI: 1.131–1.792), suggesting that the G allele mutation was associated with an increased risk of HCC.

The CD44 rs187115 polymorphism and allele frequencies for the HCC and control groups are listed in Table 3. Also, we further studied the clinical status of CD44 rs187115 gene polymorphism in HCC to assess whether there was a difference in the distribution of the rs187115 genotype among clinical subgroups. The results showed that the distribution of AG and GG genotypes of rs187115 in the hepatic fibrosis subgroup was significantly different (p = 0.038) (Table 4).

3.3. Association between IL-33 rs1929992 and Susceptibility and Clinicopathological Parameters of HCC. The distribution of IL-33 rs1929992 locus genotype in the HCC group (p = 0.084) and the control group (p = 0.750) was consistent with HWE, which indicates a good representation of the population. There was no significant difference in the frequency distribution of the GG, GA, and AA genotypes between the 2 groups (p = 0.597). Additionally, the results showed that rs1929992 genotyping of GA, G vector, and G allele was not associated with the risk of HCC (Table 3). Furthermore, there were no significant associations between IL-33 rs1929992 and any of the clinicopathological parameters (Table 5).

3.4. Association between ST2 rs3821204 and Susceptibility and Clinicopathological Parameters of HCC. The observed genotype frequency of ST2 rs3821204 was consistent with the expected distribution of HWE in both the HCC group (p = 0.071) and the control group (p = 0.125). The frequency distribution of the GG, CG, and CC genotypes in the 2 groups was statistically significant (p ≤ 0.001). The risk of HCC development was analyzed using the AA genotype and the A allele as a reference. After adjusting for gender and age using logistic regression, we calculated the OR value and 95% CI of rs187115 locus for the risk of HCC. Finally, the results showed that individuals with rs3821204 GG+CC genotype were 1.647 times more at risk of developing HCC than those carrying the GG gene (p ≤ 0.001, ORadj = 1.647, 95% CI: 1.296–2.083) (Table 3). Similarly, individuals carrying the homozygous CC genotype were 2.208 times more likely to develop HCC than individuals carrying the GG gene (p ≤ 0.001, ORadj = 2.208, 95% CI: 1.548–3.149). Furthermore, individuals who carried the heterozygous CG genotype were 1.483 times more likely to develop HCC than individuals...
carrying the GG gene ($p = 0.003, \text{OR}_{\text{adj}} = 1.483, \text{95\% CI:} 1.147\text{–}1.916$). Moreover, compared with the G allele, individuals carrying the C allele had a 1.532 times higher risk of HCC ($p \leq 0.001, \text{OR} = 1.527, \text{95\% CI:} 1.285\text{–}1.813$), suggesting that the C allele mutation was associated with an increased risk of HCC. Also, we further studied the clinical status of ST2 rs3821204 gene polymorphism in HCC; however, there was no significant difference in the distribution of the ST2 rs3821204 genotype in the clinical subgroup analysis ($p > 0.05$) (Table 6).

3.5. Haplotype Frequencies. Results obtained from SHEsis software suggested that the 3 SNPs (CD44, IL-33, and ST2) were in a strong linkage disequilibrium. The risk of HCC was affected when cooccurrence of these polymorphisms was examined using logistic regression analysis. We found 3 haplotypes to be associated with the risk of HCC. Compared to participants with other haplotypes, carrying the rs187115-rs1929992-rs3821204 G-G-C haplotype increased the risk of HCC by 3.181 times ($p \leq 0.001, \text{OR} = 3.181, \text{95\%CI:} 1.664\text{–}6.082$). The results of haplotype frequencies are summarized in Table 7, and Table 8 shows the FPRP values of significant results at different prior probability levels because their probability of being a false positive result was <20%.

3.6. Expression Quantitative Trait Loci. We further explored biological effects of the three significant SNPs (CD44 rs187115, IL-33 rs1929992, and ST2 rs3821204) on their gene expression by investigating a public database (GTEx portal). We found that genotypes of these SNPs were not associated with their gene expression in liver tissue and whole blood cells.

4. Discussion

In this study, the results showed that the distribution frequency of the CD44 rs187115 allele and genotype in the HCC case group and the control group was statistically significant ($p < 0.05$), which was consistent with the results of Liu et al. in lung cancer and Winder et al. in gastric adenocarcinoma [25, 26]. Also, we further showed that there was a significant difference in the distribution of the rs187115 and GG genotypes in the liver fibrosis subgroup. CD44 belongs to the family of adhesion molecules and is also a hyaluronic acid receptor, which means it is mainly involved in the adhesion
between cells and the interstitial matrix [27]. It was reported that CD44 promoted HCC CSC stemness by regulating natural killer (NK) sensitivity or the tyrosine-protein kinase-Met-class I phosphoinositide 3-kinase-protein kinase B (c-Met-PI3K-AKT) signaling cascade, leading to poor prognoses of cancer and chemoresistance [28, 29]. Additionally, CD44 is involved in the maintenance of CSCs in HCC, possibly through the PI3K/AKT/mTOR pathway and the NOTCH3 signaling pathway [30]. In some genes, an SNP located in a coding, promoter, or regulatory region, may have a certain

| Characteristics | rs187115 | p value | rs187115 | p value | rs187115 | p value |
|-----------------|----------|---------|----------|---------|----------|---------|
| Age (year)      |          |         |          |         |          |         |
| <40             | 147      | 0.589   | 106      | 0.659   | 147      | 0.351   |
| ≥40             | 235      | 0.800   | 143      | 0.728   | 327      | 0.563   |
| Gender          |          |         |          |         |          |         |
| Male            | 55       |         | 20       |         | 55       |         |
| Female          | 327      |         | 143      |         | 327      |         |
| BMI (kg/m²)     |          |         |          |         |          |         |
| <18.5           | 43       |         | 16       |         | 43       |         |
| 18.5-23.9       | 242      |         | 109      |         | 242      |         |
| ≥24             | 97       |         | 38       |         | 97       |         |
| BCLC stage      |          |         |          |         |          |         |
| A+B stage       | 211      | 0.634   | 83       | 0.730   | 211      | 0.373   |
| C+D stage       | 171      |         | 80       |         | 171      |         |
| Smoking status  |          |         |          |         |          |         |
| No              | 230      |         | 100      |         | 230      |         |
| Yes             | 152      |         | 63       |         | 152      |         |
| Alcohol drinker |          |         |          |         |          |         |
| No              | 251      |         | 107      |         | 251      |         |
| Yes             | 131      |         | 56       |         | 131      |         |
| Metastasis      |          |         |          |         |          |         |
| No              | 272      |         | 181      |         | 272      |         |
| Yes             | 71       |         | 21       |         | 71       |         |
| Liver cirrhosis |          |         |          |         |          |         |
| Absent          | 116      |         | 53       |         | 116      |         |
| Present         | 266      |         | 110      |         | 266      |         |
| HBV infection   |          |         |          |         |          |         |
| HbsAg (−)       | 37       |         | 18       |         | 37       |         |
| HbsAg (+)       | 339      |         | 141      |         | 339      |         |
| HCV infection   | 6        |         | 4        |         | 6        |         |
| AST             |          |         |          |         |          |         |
| Negative        | 204      |         | 79       |         | 204      |         |
| Positive        | 178      |         | 84       |         | 178      |         |
| ALT             |          |         |          |         |          |         |
| Negative        | 235      |         | 163      |         | 235      |         |
| Positive        | 147      |         | 67       |         | 147      |         |
| GGT             |          |         |          |         |          |         |
| Negative        | 130      |         | 66       |         | 130      |         |
| Positive        | 252      |         | 97       |         | 252      |         |
| AFP             |          |         |          |         |          |         |
| Negative        | 154      |         | 62       |         | 154      |         |
| Positive        | 228      |         | 101      |         | 228      |         |

ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: γ-glutamyl transpeptidase; AFP: alpha fetoprotein.
Table 5: Association of IL-33 rs1929992 genotype with clinical characteristics in HCC patients.

| Characteristics                  | rs1929992 | rs1929992 | rs1929992 |
|----------------------------------|-----------|-----------|-----------|
|                                  | AA        | GA        | GG        | AA        | GA        | GG        | AA        | GA        | GG        |
| Age (year)                       |           |           |           |           |           |           |           |           |           |
| Range                            | 13-83     | 10-89     | 24-87     | 10-89     | 24-87     | 13-83     | 10-89     |           |           |
| Mean                             | 52.6      | 52.1      | 54.3      | 52.1      | 54.3      | 52.6      | 53.1      |           |           |
| <40                              | 23        | 51        | 15        | 51        | 15        | 23        | 663       |           |           |
| ≥40                              | 146       | 210       | 120       | 210       | 120       | 0.056     | 146       | 330       | 0.057     |
| Gender                           |           |           |           |           |           |           |           |           |           |
| Male                             | 138       | 233       | 116       | 233       | 116       | 138       | 349       |           |           |
| Female                           | 31        | 28        | 19        | 28        | 19        | 31        | 47        | 31        | 47        |
| BMI (kg/m²)                      |           |           |           |           |           |           |           |           |           |
| <18.5                            | 15        | 32        | 14        | 32        | 14        | 15        | 46        |           |           |
| 18.5-23.9                        | 112       | 164       | 90        | 164       | 90        | 112       | 254       |           |           |
| ≥24.0                            | 42        | 65        | 31        | 65        | 31        | 0.735     | 42        | 96        | 0.182     |
| BCLC stage                       |           |           |           |           |           |           |           |           |           |
| A+B stage                        | 90        | 145       | 71        | 145       | 71        | 90        | 216       |           |           |
| C+D stage                        | 79        | 116       | 64        | 116       | 64        | 0.575     | 79        | 180       | 0.778     |
| Smoking status                   |           |           |           |           |           |           |           |           |           |
| No                               | 98        | 166       | 80        | 166       | 80        | 98        | 246       |           |           |
| Yes                              | 71        | 95        | 55        | 95        | 55        | 453       | 71        | 150       | 0.357     |
| Alcohol drinker                  |           |           |           |           |           |           |           |           |           |
| No                               | 114       | 178       | 88        | 178       | 88        | 114       | 266       |           |           |
| Yes                              | 55        | 83        | 47        | 83        | 47        | 0.398     | 55        | 130       | 0.948     |
| Metastasis                       |           |           |           |           |           |           |           |           |           |
| No                               | 143       | 218       | 119       | 218       | 119       | 143       | 337       |           |           |
| Yes                              | 26        | 43        | 16        | 43        | 16        | 0.221     | 26        | 59        | 0.882     |
| Family history of cancer         |           |           |           |           |           |           |           |           |           |
| No                               | 145       | 219       | 121       | 219       | 121       | 145       | 340       |           |           |
| Yes                              | 24        | 42        | 14        | 42        | 14        | 0.121     | 24        | 56        | 0.985     |
| Liver cirrhosis                  |           |           |           |           |           |           |           |           |           |
| Absent                           | 47        | 89        | 35        | 89        | 35        | 47        | 124       |           |           |
| Present                          | 122       | 172       | 100       | 172       | 100       | 0.096     | 122       | 272       | 0.404     |
| HBV infection                    |           |           |           |           |           |           |           |           |           |
| HbsAg (−)                        | 18        | 23        | 14        | 23        | 14        | 18        | 37        |           |           |
| HbsAg (+)                        | 146       | 225       | 128       | 225       | 128       | 146       | 353       |           |           |
| HCV infection                    | 5         | 3         | 3         | 0.684     | 3         | 3         | 0.792     | 5         | 6         | 0.379     |
| AST                              |           |           |           |           |           |           |           |           |           |
| Negative                         | 82        | 135       | 76        | 135       | 76        | 82        | 211       |           |           |
| Positive                         | 87        | 126       | 59        | 126       | 59        | 87        | 185       | 0.300     |           |
| ALT                              |           |           |           |           |           |           |           |           |           |
| Negative                         | 97        | 155       | 94        | 155       | 94        | 97        | 249       |           |           |
| Positive                         | 72        | 106       | 41        | 106       | 41        | 0.056     | 72        | 147       | 0.221     |
| αGT                              |           |           |           |           |           |           |           |           |           |
| Negative                         | 65        | 106       | 56        | 106       | 56        | 65        | 162       |           |           |
| Positive                         | 104       | 155       | 79        | 155       | 79        | 0.868     | 104       | 234       | 0.587     |
| AFP                              |           |           |           |           |           |           |           |           |           |
| Negative                         | 67        | 108       | 53        | 108       | 53        | 67        | 161       |           |           |
| Positive                         | 102       | 153       | 82        | 153       | 82        | 0.684     | 102       | 235       | 0.822     |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; AFP, alpha fetoprotein.
### Table 6: Association of ST2 rs3821204 genotype with clinical characteristics in HCC patients.

| Characteristics          | rs3821204 |       |       | p value | rs3821204 |       |       | p value |
|--------------------------|-----------|-------|-------|---------|-----------|-------|-------|---------|
|                          | GG        | CG    | CC    |         | GG        | CG    | CC    |         |
| Age (year)               |           |       |       |         |           |       |       |         |
| Range                    | 10-78     | 19-87 | 24-89 | 19-87   | 24-89     | 10-78 | 19-89 |         |
| Mean                     | 52.8      | 52.2  | 52.9  | 52.2    | 52.9      | 52.8  | 52.6  |         |
| <40                      | 28        | 36    | 15    | 36      | 15        | 28    | 51    |         |
| >40                      | 170       | 220   | 97    | 0.981   | 220       | 97    | 0.864 | 170     | 317     | 0.926  |
| Gender                   |           |       |       |         |           |       |       |         |
| Male                     | 176       | 219   | 92    | 219     | 92        | 176   | 311   |         |
| Female                   | 22        | 36    | 20    | 0.250   | 36        | 20    | 0.359 | 22      | 56      | 0.173  |
| BMI (kg/m²)              |           |       |       |         |           |       |       |         |
| <18.5                    | 21        | 30    | 11    | 30      | 11        | 21    | 41    |         |
| 18.5-23.9                | 125       | 168   | 74    | 168     | 74        | 125   | 242   |         |
| ≥24.0                    | 52        | 57    | 27    | 0.883   | 57        | 27    | 0.832 | 52      | 84      | 0.670  |
| BCLC stage               |           |       |       |         |           |       |       |         |
| A+B stage                | 110       | 131   | 64    | 131     | 64        | 110   | 195   |         |
| C+D stage                | 88        | 124   | 48    | 0.501   | 124       | 48    | 0.308 | 88      | 172     | 0.582  |
| Smoking status           |           |       |       |         |           |       |       |         |
| No                       | 123       | 152   | 68    | 152     | 68        | 123   | 220   |         |
| Yes                      | 75        | 103   | 44    | 0.863   | 103       | 44    | 0.842 | 75      | 147     | 0.613  |
| Alcohol drinker          |           |       |       |         |           |       |       |         |
| No                       | 131       | 165   | 77    | 165     | 77        | 131   | 242   |         |
| Yes                      | 67        | 90    | 35    | 0.752   | 90        | 35    | 0.452 | 67      | 125     | 0.958  |
| Metastasis               |           |       |       |         |           |       |       |         |
| No                       | 122       | 211   | 95    | 211     | 95        | 122   | 306   |         |
| Yes                      | 17        | 44    | 17    | 0.417   | 44        | 17    | 0.623 | 17      | 61      | 0.222  |
| Family history of cancer |           |       |       |         |           |       |       |         |
| No                       | 167       | 221   | 96    | 221     | 96        | 167   | 317   |         |
| Yes                      | 31        | 34    | 16    | 0.783   | 34        | 16    | 0.807 | 31      | 50      | 0.511  |
| Liver cirrhosis          |           |       |       |         |           |       |       |         |
| Absent                   | 62        | 77    | 31    | 77      | 31        | 62    | 108   |         |
| Present                  | 136       | 178   | 81    | 0.798   | 178       | 81    | 0.626 | 136     | 259     | 0.641  |
| HBV infection            |           |       |       |         |           |       |       |         |
| HbsAg (−)                | 16        | 11    | 11    | 11      | 11        | 16    | 22    |         |
| HbsAg (+)                | 179       | 239   | 99    | 239     | 99        | 179   | 338   |         |
| HCV infection            | 3         | 5     | 2     | 0.304   | 5         | 2     | 0.144 | 3       | 7       | 0.617  |
| AST                      |           |       |       |         |           |       |       |         |
| Negative                 | 108       | 127   | 60    | 127     | 60        | 108   | 187   |         |
| Positive                 | 90        | 128   | 52    | 0.575   | 128       | 52    | 0.506 | 90      | 180     | 0.415  |
| ALT                      |           |       |       |         |           |       |       |         |
| Negative                 | 121       | 148   | 75    | 148     | 75        | 121   | 223   |         |
| Positive                 | 77        | 107   | 37    | 0.271   | 107       | 37    | 0.107 | 77      | 144     | 0.936  |
| GGT                      |           |       |       |         |           |       |       |         |
| Negative                 | 90        | 95    | 42    | 95      | 42        | 90    | 137   |         |
| Positive                 | 108       | 160   | 70    | 0.821   | 160       | 70    | 0.964 | 108     | 230     | 0.060  |
| AFP                      |           |       |       |         |           |       |       |         |
| Negative                 | 83        | 99    | 44    | 99      | 44        | 83    | 143   |         |
| Positive                 | 115       | 156   | 68    | 0.789   | 156       | 68    | 0.933 | 115     | 224     | 0.494  |

ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: γ-glutamyl transpeptidase; AFP: alpha fetoprotein.
function, whereas CD44 SNP rs187115 is located in the first intron of CD44 [31, 32]. Until this point, the regulatory mechanism of CD44 intron 1 has not been reported, but it has been found that the polymorphism of the CD44 rs187115 gene may act on chemical resistance and cellular stress response in a p53-dependent manner in HCC [33].

As a bifunctional factor, IL-33 has both transcription factors and cytokine activity [34]. There is a strong correlation between the level of IL-33 and the progression of disease in a variety of malignant epithelial tumors [19, 35]. Previous studies have shown that rs1929992 was associated with the risk of several autoimmune diseases. Since it is functional and has not reported its relationship to HCC, we studied whether it is associated with HCC risk [36–38]. However, the results showed that the difference in distribution frequency of rs1929992 locus alleles and genotypes in the HCC case group and control group was not statistically significant (p > 0.05), which was similar to the findings of Jafarzadeh et al. in breast cancer [39]. Some studies have reported overexpression of IL-33 in colorectal cancer [19, 40], while in

| Haplotype | Case (%) N = 565 | Control (%) N = 561 | p   | OR (95% CI) |
|-----------|-----------------|---------------------|-----|------------|
| rs187115-rs1929992-rs3821204 | 227.91 (0.202) | 168.09 (0.150) | 0.001 | 1.434 (1.152, 1.785) |
| rs187115-rs1929992-rs3821204 | 264.26 (0.234) | 343.55 (0.306) | ≤0.001 | 0.692 (0.574, 0.834) |
| rs187115-rs1929992-rs3821204 | 168.53 (0.149) | 149.51 (0.133) | 0.279 | 1.140 (0.899, 1.446) |
| rs187115-rs1929992-rs3821204 | 267.30 (0.237) | 310.86 (0.277) | 0.027 | 0.808 (0.669, 0.977) |
| rs187115-rs1929992-rs3821204 | 44.16 (0.039) | 34.13 (0.030) | 0.261 | 1.296 (0.823, 2.042) |
| rs187115-rs1929992-rs3821204 | 62.66 (0.055) | 44.23 (0.039) | 0.073 | 1.431 (0.965, 2.122) |
| rs187115-rs1929992-rs3821204 | 38.40 (0.034) | 12.27 (0.011) | ≤0.001 | 3.181 (1.664, 6.082) |
| rs187115-rs1929992-rs3821204 | 56.77 (0.050) | 59.36 (0.053) | 0.774 | 0.947 (0.652, 1.376) |

Table 7: The association of haplotype of each gene with hepatocellular cancer risk.

| Genotype/haplotype | OR (95% CI) | p value | Statistical powera | Prior probability |
|--------------------|------------|---------|--------------------|------------------|
| CD44 rs187115      |            |         |                   |                  |
| AG/AA              | 1.359 (1.039-1.778) | 0.025 | 0.764 | 0.919 | 0.091 | 0.971 | 0.097 |
| GG/AA              | 2.443 (1.099-5.430) | 0.028 | 0.116 | 0.429 | 0.961 | 0.996 | 1.000 |
| AG+GG/AA           | 1.429 (1.101-1.855) | 0.007 | 0.642 | 0.095 | 0.563 | 0.921 | 0.991 |
| ST2 rs3821204      |            |         |                   |                  |
| CG/GG              | 1.478 (1.144-1.910) | 0.003 | 0.545 | 0.016 | 0.345 | 0.842 | 0.982 |
| CC/GG              | 2.229 (1.564-3.177) | ≤0.001 | 0.014 | 0.002 | 0.065 | 0.411 | 0.875 |
| CG+CC/GG           | 1.648 (1.297-2.093) | ≤0.001 | 0.220 | 0.002 | 0.020 | 0.168 | 0.668 |

aStatistical power was calculated using the number of observations in the subgroup and the OR and p values in this table.
HCC, inconsistent results were observed. Zhang et al. found that increased IL-33 protein levels were present in HCC patients’ serum and liver tissue [41], whereas Bergis et al. did not find a significant difference in IL-33 serum levels between HCC patients and healthy controls [42]. Thus, further replication studies with larger sample sizes are needed to identify the relationship of rs1929992 to the risk of HCC.

Recent studies have also found that soluble ST2 levels were highly expressed in the liver, lung, and breast cancer; malignant glioma; and other tumor tissues; this was associated with the occurrence, invasion, and metastasis of tumors [43–45]. In the present study, we found that the frequency of the ST2 rs3821204 C allele was higher in the HCC group than in the healthy control group; our results were consistent with the findings of Wei et al. on Chinese HCC patients [46]. The ST2 rs3821204 is located in the three prime untranslated regions (3’UTR) of sST2 mRNA, and the CC genotype of rs3821204 is highly correlated with elevated plasma sST2 levels in vivo [47]. The ST2 rs3821204 CC genotype may contribute to hepatocarcinogenesis by enhancing ST2 production at the transcriptional and translational levels [46]. The physiological effect of ST2 gene rs3821204 polymorphism on HCC patients is mainly exerted by enhancing the synthesis of ST2 protein [46]. Additionally, studies have shown that ST2 deficiency could prevent tumor progression in a mouse model [48]. Based on the above mechanisms, individuals with rs3821204 are prone to develop HCC.

In this study, by performing a haplotype analysis, we found that CD44 rs187115, IL-33 rs1929992, and ST2 rs3821204 have a combined effect on HCC susceptibility. By analyzing the reasons, the development of HCC was related to various genetic polymorphisms, and changes in multiple genes could cause genetic and molecular abnormalities [49, 50]. There are several studies related to the IL-33/ST2 axis and CSCs [51, 52]. Our previous research clarified that IL-33 binds to its receptor ST2 and induces phosphorylation of c-Jun N-terminal kinase activation (JNK), which leads to the expansion of colon cancer cell stemness [19]. Also, some researchers have found that IL-33 can promote the activation of p38, increasing the levels of liver CSC markers expression, and epithelial to mesenchymal transition- (EMT-) like changes [53]. We suppose that IL-33/ST2 may enhance CD44 expression to initiate HCC, but further experimentation is required to elucidate the mechanism.

In summary, our genetic results suggest an association between SNPs (CD44 rs187115, ST2 rs3821204) and the risk of HCC. The combination of CD44 rs187115, IL-33 rs1929992, and ST2 rs3821204 might be used as a marker to identify a subgroup at higher risk of HCC among the Chinese population. Unfortunately, the signaling pathway for HCC development from CD44 to IL-33/ST2 axis has not been reported, and the small sample size of this study may limit the applicability of these results. Therefore, future research will be required, recruiting larger sample sizes, careful design, and more clinical information to identify risk factors for HCC development from CD44 rs187115, IL-33 rs1929992, and ST2 rs3821204 polymorphisms and the underlying biological mechanisms leading to HCC.

Data Availability

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

Ethical Approval

The Ethics Committee approved this study of the Affiliated Tumor Hospital of Guangxi Medical University.

Consent

Participants provided written informed consent before the commencement of this study. Participant consent was provided for the publishing of this article.

Conflicts of Interest

The authors have no competing interests to declare.

Authors’ Contributions

Min Fang designed the experiment and analyzed and interpreted the data. Xiaolan Pan, Meiqin Li, and Lei Huang carried out the experiments and analyzed and interpreted the data. Xiaolan Pan and Min Fang wrote the manuscript. Xiaolan Pan, Yihua Liang, Zhaodong Huang, and Bo Zhu collected peripheral blood samples. Meiqin Li and Dan Mo performed the data analysis of demographic and clinical characteristics of research participants. All authors drafted, reviewed, edited, read, and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work is appropriately investigated and resolved as required. Xiaolan Pan, Meiqin Li, Lei Huang Xiaolan Pan, Meiqin Li and Lei Huang contributed equally to this work.

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