Association Between P2RX7 Gene and Hepatocellular Carcinoma Susceptibility: A Case-Control Study in a Chinese Han Population

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Background: Hepatocellular carcinoma (HCC) is one of the most common types of liver cancer. It is hypothesized that P2RX7 genetic polymorphisms have strong association with HCC susceptibility. Therefore, a case-control study was designed and performed to verify the association between P2RX7 gene polymorphisms and HCC susceptibility.

Material/Methods: A total of 646 subjects were recruited in our study, including 323 HCC patients and 323 healthy controls. Five gene polymorphisms, –762C>T (rs2393799), 946G>A (rs28360457), 1513A>C (rs3751143), 1068G>A (rs1718119), and 1096C>G (rs2230911), were selected. Odds ratio (ORs) and 95% confidence interval (CI) were used to quantify the association between P2RX7 gene polymorphisms and the susceptibility to HCC. All tests were performed using SPSS 20 and a 2-sided P value of less than 0.05 was considered to be statistically significant.

Results: Our results suggest that allelic frequencies of these 5 SNPs all conformed to Hardy-Weinberg equilibrium (HWE). There was no significant difference in genotype and allele distributions of –762C>T and 1096C>G between the case group and the control group. However, an increased risk of HCC was associated with 946G>A (A vs. G: OR=1.48, 95%CI=1.09–2.01, P=0.013; GA+AA vs. GG: OR=1.46, 95%CI=1.03–2.07, P=0.033). A similar increased risk was associated with 1513A>C polymorphism (C vs. A: OR=1.37, 95%CI=1.05–1.79, P=0.021; AC+CC vs. AA: OR=1.40, 95%CI=1.01–1.93, P=0.041). On the other hand, a decreased risk of HCC was associated with gene polymorphism of 1068G>A (A vs. G: OR=0.68, 95%CI=0.51–0.91, P=0.010; GA+AA vs. GG: OR=0.68, 95%CI=0.49–0.96, P=0.027; AA vs. GG: OR=0.42, 95%CI=0.18–0.99, P=0.048).

Conclusions: Our results suggest that 3 of the 5 polymorphisms of P2RX7 described above (1513A>C, 946G>A, and 1068G>A) are significantly associated with HCC susceptibility in a Chinese Han population. Studies with larger sample sizes are recommended to confirm whether our results will be applicable to different ethnic populations in China.

MeSH Keywords: Carcinoma, Hepatocellular • Polymorphism, Genetic • Receptors, Purinergic P2X1

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Background

Hepatocellular carcinoma (HCC, also called malignant hepato-
toma) is one of the most common types of liver cancer and it
ranks as the third leading cause of cancer-related deaths
in the world [1]. There are more than one million new cases
of HCC every year [2]. Some epidemiological research indicat-
ated that more than 80% of HCC cases occurred in Asian and
African countries, and 40% to 90% of them were caused by
chronic hepatitis B virus (HBV) infections [3,4]. The HBV in-
fec tions have been demonstrated to interfere with viral activ-
ations and replications that are involved in signal pathways
such as NF-κB, PI-3K, and Hedgehog [5]. Besides HBV, there
are other risk factors which might be related with HCC tumori-
genesis, including HCV, cirrhosis, alcohol intake, aflatoxin,
nonalcoholic steatohepatitis, and diabetes [6,7]. Any environ-
mental agent that could cause chronic liver injury and even-
tually lead to cirrhosis is considered as an oncogenic agent.
However, the risk of HCC development varies from individu-
al to individual due to genetic factors even though people are
exposed to the same level of risk.

Actually, factors within cellular microenvironment conditions
(e.g., cytokines, enzymes, autacoids, reactive oxygen, and nu-
cleotides) are associated with tumor formation and develop-
ment. Among those factors, purine metabolites are particular-
ly important [8]. Functioning as the key energy currency, ATP
can trigger various cell responses, ranging from cell duplica-
tion to cell apoptosis [9,10]. P2RX7 is one of the most critical
receptors among all purinergic receptors and it is highly ex-
pressed in tumor cells. Purinergic receptor P2X7 (P2RX7) is an
ATP-gated ion channel which is encoded by the P2RX7 gene
in humans [11,12]. Previous studies have confirmed that P2X7
receptor plays an important role in cytotoxic activity and could
affect cell proliferation. Elena et al. found that P2RX7 exhib-
it significant tumor growth-promoting effects in vivo [8].
Fu et al. also suggested that the activation of P2RX7-mediated
apoptosis inhibits DMBA/TPA-induced formation of skin papil-
lomas and cancer in mice [13]. Moreover, Emmett et al. provided
evidence that P2X7 receptors play a functional role in he-
patocyte Na+ and Ca2+ transport, and it may contribute to the
mechanism for autocrine regulation of hepatic glycoprotein me-
tabolism [14]. Therefore, we hypothesized that P2RX7 might
be associated with hepatocellular carcinoma.

The P2X7 gene (also called P2RX7 gene) is located on chro-
some 12 (12q24), which is a highly polymorphic gene with more
than 11 single-nucleotide polymorphisms (SNPs); these SNPs
have specific effects on P2RX7 function, including loss-of-function
or gain-of-function [15]. Furthermore, P2RX7/polymorphisms
have been discovered to have clinical significance in many dif-
f erent diseases and in tumorigenesis. Some studies provided
evidence that P2RX7 polymorphisms are clinically significant in
tuberculosis and mood disorders [16–21]. Wessellius et al. sug-
gested that 2 genetic polymorphisms of P2RX7 gene (Glu496Ala
and Gly150Arg) are associated with increased risk of osteopo-
rosis [15]. Gadeock et al. suggested an association between
P2RX7 SNPs and organic cation uptake in human myeloid
leukemic KG-1 cells, and they also discovered that functional
SNPs could contribute to alterations in survival and inva-
siveness of myeloid leukemic cells [15]. In conclusion, empiri-
cal evidence has suggested that P2RX7 SNPs are significantly
associated with diseases, cell regulation, and tumorigenesis.
Based on the genotype data obtained from the HapMap da-
tabase for Han Chinese in Beijing (dbSNP b126), we chose 5
tag-SNPs (rs2393799, rs28360457, rs3751143, rs1718119, and
rs2230911) situated on P2RX7 that have not been extensively
studied in Chinese populations to determine if they play an im-
portant role in HCC development in a Han Chinese population.

Material and Methods

Ethics statement

This study protocol was approved by the Ethics Committee of
the Chinese PLA General Hospital. A consent form approved by
the local ethics committee of the Chinese PLA General Hospital
was signed by every volunteer in advance.

Subjects

A total of 646 subjects were selected in this study, including
323 HCC patients and 323 healthy controls. All HCC patients
were recruited from the Department of Oncology at the Chinese
PLA General Hospital, and the healthy controls were volunteers
without prior history of HCC, other cancer, or family history of
HCC. All selected subjects were of Chinese Han ethnicity. The
diagnosis of every HCC patient was confirmed with pathologic
evidence, and the relevant medical information was obtained
by questionnaires and laboratory examinations. The compari-
son of relevant characteristics between HCC patients and con-
trols are shown in Table 1, including age, sex, smoking status,
drinking habits, and HBsAg expression status. Characteristics
of HCC patients, such as BCLC state, tumor size, alanine trans-
aminase (ALT) concentration, alpha fetal protein (AFP) con-
centration, and Child-Pugh score, are also included in Table 1.
For every HCC patient, we classified the disease based on the
criteria used by the WHO and disease was staged based on
the American Joint Committee on Cancer TNM classification.

Sample collection

We collected 5-ml venous blood samples from the HCC case
group and the control group using Vacutainer tubes, and then
blood samples were transferred and tested using test tubes
Table 1. Comparison of HCC patients and controls by clinical characteristics.

| Clinical characteristics | HCC patients (n=323) | Relative frequency | Healthy controls (n=323) | Relative frequency | P value |
|--------------------------|----------------------|--------------------|--------------------------|--------------------|---------|
| Gender                   |                      |                    |                          |                    |         |
| Male                     | 235                  | 72.8%              | 222                      | 68.7%              | 0.261   |
| Female                   | 88                   | 27.2%              | 101                      | 31.3%              |         |
| Age group (year)         |                      |                    |                          |                    |         |
| ≤50                      | 131                  | 40.6%              | 137                      | 42.4%              | 0.391   |
| 50–60                    | 95                   | 29.4%              | 95                       | 29.4%              |         |
| 60–70                    | 66                   | 20.4%              | 55                       | 17.0%              |         |
| >70                      | 31                   | 9.6%               | 43                       | 11.2%              |         |
| Smoking                  |                      |                    |                          |                    |         |
| Ever                     | 137                  | 42.4%              | 121                      | 37.5%              | 0.199   |
| Never                    | 186                  | 57.6%              | 202                      | 62.5%              |         |
| Drinking                 |                      |                    |                          |                    |         |
| Ever                     | 189                  | 58.5%              | 113                      | 35%                | <0.001  |
| Never                    | 134                  | 41.5%              | 210                      | 65%                |         |
| HBsAg                    |                      |                    |                          |                    |         |
| Positive                 | 221                  | 68.4%              | 29                       | 9.0%               | <0.001  |
| Negative                 | 102                  | 31.6%              | 294                      | 91.0%              |         |
| BCLC                     |                      |                    |                          |                    |         |
| 0+A                      | 156                  | 48.3%              | --                       | --                 |         |
| B+C                      | 167                  | 51.7%              | --                       | --                 |         |
| Tumor size               |                      |                    |                          |                    |         |
| <5 cm                    | 138                  | 42.7%              | --                       | --                 |         |
| ≥5 cm                    | 185                  | 57.3%              | --                       | --                 |         |
| ALT (U/l)                |                      |                    |                          |                    |         |
| ≤40                      | 136                  | 42.1%              | --                       | --                 |         |
| >40                      | 187                  | 57.9%              | --                       | --                 |         |
| AFP (ng/ml)              |                      |                    |                          |                    |         |
| ≤20                      | 83                   | 25.7%              | --                       | --                 |         |
| >20                      | 240                  | 74.3%              | --                       | --                 |         |
| Child-Pugh               |                      |                    |                          |                    |         |
| A                        | 301                  | 93.2%              | --                       | --                 |         |
| B                        | 22                   | 6.8%               | --                       | --                 |         |

HCC – hepatocellular carcinoma; HBsAg – hepatitis B surface antigen; ALT – alanine transaminase; AFP – alpha fetal protein.
with ethylenediaminetetraacetic acid (EDTA). The rest of the blood samples were stored at -4ºC for further use.

Tag SNPs selection and primer design

The \( P2RX7 \) SNPs were selected using the genotype data obtained from the HapMap database for Han Chinese in Beijing (HapMap Data Rel 24/phase II Nov08, on NCBI B36 assembly, dbSNP b126). The pairwise option of HaploView 4.2 (Broad Institute, Cambridge, MA) software was used to identify the tag-SNPs and an \( r^2 \) of 0.8 was selected as the threshold for the analysis. Mass ARRAY Assay Designer 3.1 (Sequenom, Inc., San Diego, CA) was selected to design proper primer for PCR amplification of tag SNPs. Five tag-SNPs (rs2393799, rs28360457, rs3751143, rs1718119, and rs2230911) were selected at a resolution of 1 SNP per 7 kb, which captured all variant alleles with a mean \( r^2 \) of 0.979. The identification number and relative position of the 5 tag-SNPs, together with the LD plot of the tag-SNPs (rs2393799, rs28360457, rs3751143, rs1718119, and rs2230911).

![Figure 1](image-url). The identification number and relative position of the 5 tag-SNPs together with the LD plot of the tag-SNPs (rs2393799, rs28360457, rs3751143, rs1718119, and rs2230911).

Table 2. Primers and probe sequences of five SNP in \( P2RX7 \) gene.

| SNP       | Primers for PCR amplification | Product size (bp) | Probes          | Product size (bp) |
|-----------|------------------------------|-------------------|-----------------|-------------------|
| rs2393799 | Forward: 5'- GTAGCTCTTCTGGTGTTG-3' Reverse: 3'-CAGTATCTAGCTGGACGG-5' | 120 | SP: 5'- AGCAGCCTCAATTTACAAC-3' | 330 |
| rs28360457| Forward: 5'- CACTTTCAAGGGATCTTA-3' Reverse: 3'- ATTACAGAGACAGGGTGGGTCG-5' | 156 | SP: 5'- TTTCTCTCTACAGATAGCGCAAG-3' | 171 |
| rs3751143 | Forward: 5'- TAGGAAAATGGTTTGGAG-3' Reverse: 3'- GACAGGGGACGATGAAAT-5' | 124 | SP: 5'- CTTGGCCCTCCATGACACA-3' | 247 |
| rs1718119 | Forward: 5'- TGGCTTCTCTCTATCTT-3' Reverse: 3'- GTTGGCTTGGTCATGCTT-5' | 461 | SP: 5'- ATAAATAGGGAGGCGACA-3' | 297 |
| rs2230911 | Forward: 5'- ATAAATAGGGAGGCGACA-3' Reverse: 3'- ACTCTCTTAGCTACTTGT-5' | 115 | SP: 5'- ATAAATAGGGAGGCGACA-3' | 85 |

SNP – single-nucleotide polymorphism; SP – specific probe; F – forward; R – reverse.

DNA extraction and genotyping

Genomic DNA was isolated from whole blood with the TIANamp Blood DNA kit (Tiangen Biotech, Beijing). Quantitation was performed by spectrophotometric (260 nm) and all samples were normalized to 10 ng/µl. For PCR reactions, 2 µl of extracted genomic DNA (40 µl) was used. In this study, SNaPshot assay was used to detect genotype DNA samples for \( P2RX7–762T>C \), \( 946G>A \), \( 1513A>C \), \( 1068G>A \), and \( 1096C>G \) according to the manufacturer’s instructions (Applied Biosystems). The specific reaction was performed in 50 µl reaction volume containing 3 µl extracted DNA, 1.5 mM MgCl2, 1 µl of 15 mM dNTP, 5 µl PCR buffer (10×), and 2U TaqDNA polymerase. The amplification of the tag-SNPs, are presented by the HaploView 4.2 software in Figure 1. The positions of the 5 tag-SNPs were on intron5, exon10, exon12, and exon14 regions, respectively. Primer designing and PCR application are shown in Table 2.
process started with 94°C for 5 min, followed by 40 cycles of 94°C for 20 s, 30 s at 56°C, and then 45 s at 72°C for 6 min. After amplification, the unincorporated PCR products were purified using Shrimp Alkaline Phosphatase (SAP) methods. Then 0.2 U of SAP and 0.2 U of Exonuclease III were put in the PCR product purifying system, at a condition of 37°C for 22 min followed by 75°C for 6 min. Finally, the extension products were analyzed in a 96-well PRISM 3730 DNA Sequencer (Applied Biosystems). All genotyping procedures were carried out using a double-blind control approach and 5% of the samples were randomly selected for confirming; the results were 100% repeatable.

Statistical analysis

Pearson’s χ² test and the Student’s t-test were used to compare the difference in clinical characteristics between the HCC patient group and the control group. Hardy-Weinberg equilibrium (HWE) test was also performed to analyze genotype frequencies of 2 alleles in the 5 SNPs. Furthermore, a multivariate logistic regression model was used to quantify the association between P2RX7 gene polymorphisms and the susceptibility to HCC. For every single-nucleotide polymorphism, 4 genetic models (dominant model, homozygous model, recessive model, and allelic model) were used to evaluate the association between each SNP and HCC susceptibility. Odds ratios (ORs) and their 95% confidence intervals (CIs) were used to investigate how different genotype distributions affect the susceptibility to HCC. All statistical tests were performed using SPSS 20 and a 2-sided P value of less than 0.05 was considered to be statistically significant.

Results

Clinical characteristics of the case and the control groups

A total of 646 subjects (323 HCC and 323 healthy controls) were included. The frequency distributions by different clinical characteristics of the cases and controls are demonstrated in Table 1. As shown by Table 1, the relative frequency of sex, age group, and smoking status were not significantly different between the control and the case group, implying that the associations between these clinical characteristics and HCC were not significant (all P > 0.05). However, the relative frequency of drinking status and HBsAg between the 2 groups was significantly different, which indicates a significant association between the 2 clinical characteristics and the susceptibility to HCC. There were more non-drinkers in the control group (65%) than in the HCC case group (41.5%) (P < 0.001), and the percentage of positive HBsAg in the case group (68.4%) was significantly higher than in the control group (9.0%) (P < 0.001). In our study, the percentage of tumor grade for I and II, and III and IV were 11.8% and 88.2%, respectively. HCC patients were classified by tumor size; 42.7% of the HCC patients had tumors <5 cm and 57.3% of them had tumors ≥5 cm.

Associations between P2RX7 polymorphisms and the risk of HCC

The characteristics of the 5 selected SNPs in P2RX7 gene are shown in Table 2. The −762C>T polymorphism was located on intron 5, 946G>A on exon 10, 513A>C on exon 14, and 1068G>A and 1096C>G on exon 12. All the allele frequencies of the 5 tag SNPs were compliant with the HWE test. The genotype and allele distributions of the 5 tag SNPs in the case and control groups are presented in Table 3. No significant difference in genotype and allele distributions of −762C>T (rs2393799) and 1096C>G (rs2230911) was observed between the case and the control group (all P > 0.05). However, we had sufficient evidence to suggest that 946G>A, 1513A>C, and 1068G>A are significantly associated with the susceptibility to HCC.

For 946G>A (rs28360457) polymorphism, there was no statistically significant association between SNP and HCC in the homozygous (OR = 2.58, 95% CI: 0.98–6.84, P = 0.048) and recessive (OR = 2.39, 95% CI: 0.91–6.31, P = 0.069) models. However, the allelic model (A vs. G: OR = 1.48, 95% CI: 1.09–2.01, P = 0.013) indicated that AA variant genotype was associated with an increased risk of HCC. The dominant (GA + AA vs. GG) model also showed an increased HCC risk accompanied by A variant genotype (OR = 1.46, 95% CI: 1.03–2.07, P value = 0.033). All the above evidence suggests that 946G>A polymorphism is associated with an increased risk of HCC.

For 1513A>C (rs3751143) polymorphism, taking AA wild genotype as a reference, recessive (AA vs. AA+ AC) and homozygous (CC vs. AA) models showed no significant associations between 1513A>C and HCC risk (P > 0.05). The allelic model (C vs. A: OR = 1.37, 95% CI: 1.05–1.79, P = 0.021) indicated that CC variant genotype was associated with an increased risk of HCC. A similar association was presented in the dominant (AC + CC vs. AA) model (OR = 1.40, 95% CI: 1.01–1.93, P = 0.041). Therefore, 1513A>C is also likely to be a risk factor for HCC susceptibility.

For 1068G>A (rs1718119) polymorphism, the recessive model (GG vs. GG + GA: OR = 0.46, 95% CI: 0.46–1.08, P = 0.066) revealed no significant association between 1068G>A and HCC. However, significant associations were found in the allelic, dominant, and homozygous models. Considering GG wild genotype as a reference, the allelic (A vs. G: OR = 0.68, 95% CI: 0.51–0.91, P = 0.010), dominant (GA + AA vs. GG: OR = 0.68, 95% CI: 0.49–0.96, P = 0.027) and homozygous (AA vs. GG: OR = 0.42, 95% CI: 0.18–0.99, P = 0.048) models suggested that AA variant genotype is significantly associated with decreased risk of HCC. Therefore, 1068G>A polymorphism could be considered as a protective factor for HCC.
Table 3. Allele and genotype frequencies of five SNPs in \( P2RX7 \) gene in HCC patients and controls.

| SNP     | Genotype frequencies | HWE test | Allele frequency 1 | Allele frequency 2 | MAF | Allelic model P value OR 95%CI | Dominant model P value OR 95%CI | Recessive model P value OR 95%CI | Homozygous model P value OR 95%CI |
|---------|----------------------|----------|-------------------|-------------------|-----|--------------------------------|---------------------------------|----------------------------------|----------------------------------|
| –762C>T | Control              | 323      | 120 140 63 0.059  | 380               | 266 | 0.442                          | 1.10                            | 0.398 [0.88 – 1.06]               | 1.23 [0.84 – 1.79]               | 0.290 [1.22 – 1.85]               |
|         | Case                 | 323      | 116 133 74       | 365               | 281 |                                |                                  |                                  |                                  |                                  |
|         | 946G>A               | Control  | 323 248 69 6     | 0.640             | 565 | 81                            | 1.48                            | 0.013 [1.09 – 1.16]               | 0.033 [1.03 – 2.07]               | 0.069 [2.58 – 6.84]               |
|         | Case                 | 323 224 85 14 | 533              | 113               |     |                                |                                  |                                  |                                  |                                  |
| 1196G>A  | Control              | 323 211 95 17 0.151 | 517 | 129 | 0.323 | 0.68                            | 0.010 [0.51 – 1.05]               | 0.027 [0.49 – 0.96]               | 0.066 [0.46 – 1.08]               | 0.42 [0.18 – 0.99]               |
|         | Case                 | 323 237 78 8 | 552 | 94 |                                  |                                  |                                  |                                  |                                  |                                  |
| 1313C>G  | Control              | 323 201 107 15 0.875 | 509 | 137 | 0.165 | 0.77                            | 0.058 [0.58 – 1.02]               | 0.082 [0.66 – 0.75]               | 0.308 [0.29 – 1.48]               | 0.60 [0.27 – 1.37]               |
|         | Case                 | 323 222 91 10 | 535 | 111 |                                  |                                  |                                  |                                  |                                  |                                  |

MAF – minor allele frequency; \( P \) value – \( P \) value of heterogeneity test; HCC – hepatocellular carcinoma; OR – odds ratio; CI – confidence interval.

Discussion

HCC is an epithelial carcinoma which could be caused by environmental and genetic factors. \( P2RX7 \) (Purinergic receptor P2X) is an ATP-gated ion channel encoded by the \( P2RX7 \) gene. The genetic polymorphisms of \( P2RX7 \) gene could affect purine metabolism, which plays an important role in HCC tumorigenesis. We performed this hospital-based case-control study of 323 HCC patients and 323 controls to investigate the association between SNPs in \( P2RX7 \) gene and HCC susceptibility in the Chinese Han population. Our results suggest that genotype and allele distributions of –762C>T (rs2393799) and 1096C>G (rs2230911) are not significantly associated with HCC. However, we discovered that other polymorphisms – 1196G>A (rs3751143) and 946G>A (rs28360457) – are associated with an increased risk of HCC development, while 1068G>A (rs1718119) is associated with a decreased risk of HCC development.

To the best of our knowledge, this is the first report showing a significant association of SNP 946G>A variant with increased risk of HCC. In previous studies, Ursu et al. have revealed that 946G>A variant was one of several SNPs responsible for gain and loss of function of the P2X7 receptor [22]. Moreover, 946G>A variant was shown to be associated with decreased LS-BMD (lumbar spine bone mineral density) [23], increased risk of bone loss [24], and increased risk of cumulative hazard after hip arthroplasty revision [25]. Genetic polymorphisms could contribute to a series of cell changes, which can functionally affect our cells and organs. Thus, the effect of \( P2RX7 \) polymorphisms on HCC susceptibility might be associated with the changes in cellular microenvironment conditions. In addition, the 946G>A variants, located on the exon region of \( P2RX7 \), might give rise to the occurrence of mis-sense Arg307Gln, affecting purine metabolites and cell apoptosis, which eventually might contribute to HCC tumorigenesis. Therefore, we suspect that 946G>A variant is involved in the functional dysregulation of the P2X7 receptor. Further
studies on 946G>A variant are recommended to confirm the role of this SNP in HCC.

The second significant SNP in our study was 1513A>C variant, which was also associated with an increased risk of HCC. It has been reported that this missense variant may cause differences in protein expressions (Glu496Ala), which could result in functional changes in different subtypes of P2X7 receptor [26]. Liu et al. reported that 1513A>C might affect the morbidity of Parkinson’s disease (PD) and late-onset PD, based on a case-control study [27]. A meta-analysis conducted by Areeshi et al. has shown that 1513A>C may be associated with an increased risk of tuberculosis [28]. Ursu et al. also discovered that 1513A>C variant can cause loss-of-function of P2RX7, which contributes to a lower risk of IHD (ischemic heart disease) and IS (ischemic stroke) [29]. Moreover, studies have shown that 1513A>C polymorphism is related to cancers, including papillary thyroid cancer and leukemic lymphocytes [22,27,28,30]. However, the present study is the first to report the association between 1513A>C and HCC. Our results suggest that 1513A>C variant is associated with elevated risk of HCC. However, studies with large sample sizes should be performed to confirm the association between 1513A>C and HCC.

The third significant SNP was 1068G>A variant (rs1718119), which was associated with a decreased risk of HCC. This SNP may modify polar threonine to unipolar alanine at amino acid 348, which plays a critical role in P2RX7 receptor function [22,31]. It was reported that 1068G>A variant is associated with a gain-of-function of P2RX7, as well as enhanced IL-1β secretion from monocytes [32]. Furthermore, the SNP rs1718119 was also associated with susceptibility to cognitive symptoms of mania in a Swedish population [33] and to systemic lupus erythematosus (SLE) in a Chinese population [31]. Although the association between 1068G>A and decreased risk of HCC remains to be confirmed, it provides a novel target for down-regulating HCC risk at genomic levels. Understanding the role of 1068G>A variant in P2RX7 could contribute to the discovery of HCC pathogenesis and the development of alternative treatments.

Mutations of rs2230911 could also alter coding of amino acids (Ser357 to Thr), which was associated with a partial function loss of P2X7R through affecting channel and pore function [31]. It was reported that rs2230911 may impair mycobacterial killing induced by ATP through macrophages, but it was not associated with SLE in a Chinese population [34]. However, the rs2230911 variant investigated in the present study was not associated with risk of HCC. Generally, rs2393799, as a variant of P2X7R, was applied to study its correlations with pulmonary tuberculosis susceptibility in several previous studies [35–37], yet it was reported to be unassociated with pulmonary tuberculosis susceptibility in Asians or whites in a previous meta-analysis [37]. Our study showed that there was no significant difference in genotype or allele distributions of rs2393799 between the case and control groups.

This study suggests several potential associations between P2RX7 gene polymorphisms and HCC susceptibility. However, a few important limitations need to be considered. Firstly, our study is limited due to small patient samples, which might fail to detect the pathogenic roles of P2RX7 polymorphisms. Secondly, this case-control study included subjects from the hospital and therefore selection bias is another inevitable limitation. Moreover, the above results may not be applicable to other Chinese ethnicities, as all subjects were selected from the Chinese Han population. Although environmental factors, viral infections, and their interactions with genetic factors could have substantial impact on the susceptibility to HCC, no stratified analysis was conducted by distinct epigenetic factors. Large-scale studies are recommended to confirm our results in different ethnicities and improve the statistical power of the study.

Conclusions

Our results suggest that 3 polymorphisms of P2RX7 (946G>A, 1513A>C, and 1068G>A) are associated with HCC susceptibility in a Chinese Han population. The AA variant genotype of 946G>A polymorphism and the CC variant genotype of 1513A>C polymorphism were associated with an increased risk of HCC, whereas the AA variant genotype of 1068G>A polymorphism was associated with a decreased risk of HCC. Apart from that, the distinct effects of polymorphisms on HCC susceptibility might be attributed to the location of polymorphisms. Further studies are recommended to validate our findings in different ethnicities and to clarify the functional relationship between P2RX7 polymorphisms and the susceptibility to HCC.

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