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Original Article

Genetic association between *CD44* polymorphisms and chronic hepatitis B virus infection in a Chinese Han population

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Abstract: Aims: This article aimed at discussing the association of chronic hepatitis B virus (HBV) infection with *CD44* polymorphisms in Chinese Han population; meanwhile, the interaction of polymorphisms was also analyzed based on chronic HBV infection. *Methods*: The genotyping of *CD44* polymorphisms was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 108 HBV infected and 130 healthy persons. The genotype distributions of *CD44* rs187115, rs13347 in the control group were checked by Hardy-Weinberg equilibrium (HWE). The strength of the relevance between polymorphism and disease was measured by odds ratio (OR) with corresponding 95% confidence interval (CI) calculated by χ^2 test. The 2×4 crossover analysis method was used to conduct the interaction analysis of polymorphisms. Results: The genotype distributions in controls conformed to HWE. GG genotype and G allele frequencies in rs187115 were obviously higher in cases than the controls (P=0.02, 0.04). Compared with the common genotype CC, individual who carried mutant genotypes (CT and TT) of rs13347 had a significantly high risk to suffer from HBV infection (OR=1.99, P=0.02 for CT; OR=3.56, P=3.00×10^{-3} for TT), furthermore, CT+TT genotype also showed a high susceptibility (OR=2.27, P=2.00×10^{-3}). Similarly, T allele of rs13347 increased 0.98 times risk in cases compared with controls (OR=1.98, 95% CI=1.34-2.92). The two polymorphisms in CD44 presented a positive interaction. Conclusion: *CD44* polymorphisms are associated with chronic HBV infection as the risk factors, and the synergistic action is also found between the two polymorphisms.

Keywords: *CD44*, polymorphism, chronic hepatitis B virus infection

Introduction

In China, about 93 million people infect hepatitis B virus (HBV) and among of them, nearly 22% develop the chronic HBV infection [1]. So far, it has become public health issues with high morbidity and lethality due to causing chronic liver disease and hepatocellular carcinoma (HCC) easily [2-4]. In Asian, 40% of chronic HBV-infected patients die from liver cirrhosis and HCC [5]. It is reported that HBV infection can lead to different clinical results [6], for example, 90%-95% patients who are HBV-infected first can eliminate virus to become self-limited hepatitis through immune system of the body in adults, the other 5%-10% advance chronic HBV carriers. Therefore, it is difficult to cure this disease only in means of drug therapy. Recently, a mass of researchers pay attention to the role of gene, especially gene polymorphism. *IL-18*, *TNF*, *IFN-γ*, *IL10*, *CCR5*, cytokine gene, vitamin D receptor gene has been proved to be associated with chronic HBV infection [7-10].

The CD44 antigen is a kind of glycoprotein attached to the surface of cells which participates in intercellular interactions, cell adhesion and migration and encoded by *CD44* gene located in human chromosome 11p13 [11, 12]. *CD44*, as the target gene of Wnt signaling pathway plays an important role in multiple cellular physiological and pathological processes through combining the ligands of cell surface owing to a mass of isoforms generated by alternative splicing and its abnormal expression can result in the malignant tumors and immune system diseases development [13, 14]. However, in previous studies, scholars focused their eyes on the effect of *CD44* on various cancers [15-

Table 1. The genotypes distribution of *CD44* gene polymorphisms between the cases and controls

Genotype/allele		Controls, n=130 (%)	Cases, n=108 (%)	OR (95% CI)	*P* value
rs187115	AA	81 (62.31)	59 (54.63)	1.00 (Ref.)	-
	AG	43 (33.08)	35 (32.41)	1.12 (0.64-1.95)	0.70
	GG	6 (4.61)	14 (12.96)	3.20 (1.16-8.83)	0.02
	AG+GG	49 (37.69)	49 (45.37)	1.37 (0.82-2.31)	0.23
	A	205 (78.85)	153 (70.83)	1.00 (Ref.)	-
	G	55 (21.15)	63 (29.17)	1.54 (1.01-2.33)	0.04
rs13347	CC	73 (56.15)	39 (36.11)	1.00 (Ref.)	-
	CT	47 (36.16)	50 (46.30)	1.99 (1.14-3.47)	0.02
	TT	10 (7.69)	19 (17.59)	3.56 (1.51-8.39)	3.00×10^{-3}
	CT+TT	57 (43.85)	69 (63.89)	2.27 (1.34-3.83)	2.00×10^{-3}
	C	193 (74.23)	128 (59.26)	1.00 (Ref.)	-
	T	67 (25.77)	88 (40.74)	1.98 (1.34-2.92)	1.00×10^{-3}

17]. The association of chronic HBV infection which is related to oneself immune system with *CD44* polymorphism is been concerned hardly.

In this article, two polymorphisms of *CD44* gene (rs187115, rs13347) were selected to discuss the relevance with chronic HBV infection risk. The genotying was conducted in 108 patients infected chronic HBV and 130 healthy people from Chinese Han population. Through this study, we hope to provide a guide for exploring the etiology of chronic HBV infection.

Materials and methods

Study subjects

In this case-control study, 108 chronic HBV infected persons clinical diagnosed by pathogenic serologic method according to epidemiological data in 302 Military Hospital from March, 2012 to March 2015 was enrolled as the case group, including 50 males and 58 females without relationship by blood. Their age rang was 21-53 years old. The control group was consisted of healthy persons who experienced the physical examination in the same hospital at the same period. They were also not the family history of liver disease. There was no obvious difference between the two groups in gender and age. All subjects were not related by blood and our research was supported by the Ethics Committee of 302 Military Hospital. Written consents were obtained from every participant before collecting blood samples.

DNA extraction

Every individual enrolled in our study population offered 2 ml peripheral venous blood and they were put into the EDTA anticoagulative tube. Genome DNA was extracted using the conditional chloroform-isopentanol extraction method and finally stored at -20°C.

The genotyping of CD44 polymorphisms in the case and control groups

The genotyping was conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR primers were designed by primer 5.0 software and synthesized by Shanghai Sangon Company. The primers sequences were listed as follows: rs187115, the forward primer: 5'-CCTTCAGATGCAAGTACA-3', the reverse primer: 5'-CTGCCCAATAAAGCCAAT-3'; rs13347: the forward primer: 5'-ACGATAGAAATAAGGGAGG-3', the reverse primer: 5'-GCAAGGGTTTGTGAAGAC-3'. The PCR reaction solution was a volume of 25 μl and the program was followed: initial denaturation at 95°C for 5 min, denaturation at 94°C for 30 s, annealing at 58°C for 45 s and extension at 72°C for 45s with 34 cycles, and finally extension at 72°C for 8 min. The products were digested by restriction enzymes and separated through 3% agarose gel electrophoresis and the EB staining.

Statistical strategies

The genotype distributions of *CD44* rs187115, rs13347 polymorphisms in the control group

Table 2. The interaction analysis of *CD44* rs187115, rs13347 polymorphisms

Polymorphism	Polymorphism	Controls, n (%)	Cases, n (%)	OR	S	AP	EREL
rs187115	rs13347				2.87	0.51	2.28
-	-	67 (51.54)	36 (33.33)	1.00			
-	+	23 (17.69)	25 (23.15)	2.02			
+	-	28 (21.54)	18 (16.67)	1.20			
+	+	12 (9.23)	29 (26.85)	4.50			

Note: ''-'' represents a wild genotype, ''+'' represents a mutant genotype.

were checked whether were consistent with Hardy-Weinberg equilibrium (HWE). Odds ratio (OR) and 95% confidence interval (95% CI) were used to represent the strength of the association between gene polymorphisms and chronic HBV infection risk which was calculated by the chi-square test. Above-mentioned data analysis was performed by SPSS18.0 software. The interaction of polymorphisms was calculated by the 2×4 crossover analysis method. The results were presented by the synergy index (S), attributable proportion of interaction (AP) and relative excess risk of interaction (EREI).

Results

HWE test

In study population, genotype frequencies of *CD44* polymorphisms conformed to the HWE requirement in the control group, so it had the representativeness and our results were relative reliable.

The genotype frequencies comparison of CD44 gene polymorphisms in two groups

As was shown in **Table 1**, in *CD44* gene, rs187115 GG genotype had a significantly high frequency in the case than control group (P=0.02), similarly, its G allele increased the risk of infecting chronic HBV (OR=1.54, 95% CI=1.01-2.33). Parallel results, variants genotypes CT and TT carriers were more inclined to suffering from chronic HBV infection than CC genotype (CT vs. CC: OR=1.99, 95% CI=1.14-3.47; TT vs. CC: OR=3.56, 95% CI=1.51-8.39), rs13347 CT+TT genotype also supported the above opinion (OR=2.27, 95% CI=1.34-3.83). In addition, T allele of rs13347 was also associated with the remarkably increased risk of chronic HBV infection (OR=1.98, 95% CI=1.34-2.92).

The interaction analysis of CD44 gene polymorphisms

There was a positive interaction between rs187115 and rs13347 polymorphisms of *CD44* gene based on the additive effect model (S=2.87) (**Table 2**). When people carried the variant genotypes of rs187115 (AG, GG) and rs13347 (CT, TT) simultaneously, 51% of chronic HBV infected persons were resulted from the interaction of this two polymorphisms (AP=0.51). What's more, this interaction factor was 2.28 times risk compared with other factors (EREL=2.28).

Discussion

The publications report that the outcome of HBV infection mainly depends on the age infected HBV first. Most adults can clean virus through their immune system of the body when they infect HBV, only 5%-10% of people develop the chronic HBV infection, but not in 90% of children infected HBV [18, 19]. What's more, the same HBV subtype infect people who come from the same region with similar age, the outcomes may be different [20]. Therefore, the genetic factors play a vital role in chronic HBV infection. This opinion has been confirmed by multiple studies.

Kim et al. find that A allele of *TNF-α* promoter polymorphisms are significantly associated with spontaneously cleaning HBV and generating the protective antibody, furthermore, there is the linkage disequilibrium between the -308A allele of *TNF-α* and *HLA-DRB1**13 allele which has been involved in chronic HBV infection [21]. Ren et al. summarize the studies about the effect of *IL10* A-819 C/T on chronic HBV infection from various counties and populations, the conclusion shows that this polymorphism presence increased the risk of infecting chronic

HBV and exists the linkage disequilibrium with the other polymophsims in *IL10* gene [22]. According to the study of Thio et al. *CCR5* Delta32 is found to have an influence on recovery from the HBV infection and provide a potential therapeutic for patients suffering from chronic HBV infection [23]. In addition, *MBL*, *VDR*, *IFN-γ* and *eNOS* genes are also considered to play roles in chronic HBV infection.

CD44 is a target gene in Wnt signaling pathway downstream. In normal cells, the Wnt signaling pathway is in inactivated and β-catenin is degraded, but in diseases cells, Wnt signal is activated to inhibit the synthesis of β-catenin complex and β-catenin phosphorylation [24, 25]. And then accumulated β-catenin transfers to cell nucleus and forms the complex with transcription factor TCF/LEF, which motivates the Wnt target genes transcription, such as *CD44*, *MMP7* [26]. Furthermore, HBV can lead to the change of the Wnt signal transduction. The *CD44* haplotypes (rs353644-rs353630-rs7937602) are analyzed in the study of Ryckman et al. but not the single polymorphism [27].

In present study, the effect of *CD44* single polymorphism on chronic HBV infection was analyzed in Han population of Shandong region, China, in addition the interaction analysis of polymorphisms (rs187115, rs13347) was displayed. The conclusion demonstrated that GG genotype and G allele of rs187115 were associated with chronic HBV infection obviously. People carried the CT, TT genotypes of rs133477 increased 0.99 and 2.56 times risk infected chronic HBV, compared with CC genotype carriers. What's more, rs13347 CT+TT genotype also obtained above result, in addition, its T allele had a significantly different frequency in two study groups as well. Both of *CD44* rs187115, rs13347 polymorphisms were strongly risk factors to induce chronic HBV infection. Afterwards, the interaction analysis showed that these two polymorphisms were positively related and half of patients infected chronic HBV were the outcome of this interaction.

The genetic research of chronic HBV infection can directly show its molecular basis and the disease diagnosis, and provide more reasonable prevention and treatment methods, which cure chronic HBV infection early. But chronic HBV infection is a complicated polygenes disease and gene polymorphism is different in different regions and races. So, further studies with various races in different districts and large sample size are required, considering all kinds of factors in the future.

Disclosure of conflict of interest

None.

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