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ORIGINAL ARTICLE

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Association of human platelet antigens polymorphisms with susceptibility to hepatitis C virus infection in Chinese population

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Summary

Hepatitis C virus (HCV) is a major cause of chronic hepatitis. Previous studies have identified a number of single nucleotide polymorphisms that are associated with HCV infection. Human platelet antigens (HPAs) polymorphisms play an important role in several diseases. Here, we demonstrated the association of the HPA-2, HPA-3, HPA-5 and HPA-15 polymorphisms with susceptibility to HCV infection in Chinese population. Overall, 118 patients with HCV and 167 controls were genotyped for HPAs. There were no significant differences in the allele and genotype frequency distribution for the HPA-3, HPA-5 and HPA-15 systems between the patients with chronic HCV infection and the healthy controls ($p > .05$). However, the genotype frequency of HPA-2aa was significantly lower, while HPA-2ab/bb was significantly higher in patients than that in the controls ($p = .006$). The allele frequency of HPA-2a in patients was significantly lower than that in the control group ($p = .005$). In contrast, HPA-2b in patients was significantly higher than that in the control group ($p = .005$). We conclude that HPA-2 polymorphism is associated with susceptibility to HCV infection, and individuals carrying the HPA-2b allele may have a higher risk of HCV infection compared with individuals carrying HPA-2a.

KEYWORDS
allele, genotype, hepatitis C virus, human platelet antigens, single nucleotide polymorphism

1 | INTRODUCTION

According to the recent estimates from World Health Organization, more than 185 million people around the world are currently affected by hepatitis C virus (HCV) infection (World Health Organization, 2014). The number of deaths per year attributed to HCV-related diseases, which include cirrhosis, hepatocellular carcinoma (HCC) and liver failure, continues to increase. For instance, the number of deaths due to HCV-related complications was 333,000 in 1990, 499,000 in 2010 and 704,000 in 2013(GBD Mortality and Causes of Death Collaborators, 2015; Lozano et al., 2012).

The predominant site of HCV replication and infection is human liver cells. However, HCV may also infect lymphocytes, monocytes and dendritic cells (Goutagny et al., 2003; Müller et al., 1993; Pavio & Lai, 2003). In addition, HCV can interact with platelets (Pugliese et al., 2004).

It is most well known that platelets play a key role in hemostasis. Furthermore, platelets also play an important role in cardiovascular disease, infectious diseases, parasitosis and even cancer through ligand–receptor interactions involving many glycoproteins (GPs) expressed on the cell membranes (Hamzeh-Cognasse et al., 2015; Honn, Tang, & Crissman, 1992; McMorran et al., 2012; Pain et al., 2001; Willoughby, Holmes, & Loscalzo, 2002). Platelet membrane GPs are expressed in polymorphic forms caused by single nucleotide polymorphisms (SNPs)

Zhou and Liang are equally contributed to this study.

in the genes that encode human platelet antigens (HPAs) (Curtis & McFarland, 2014).

Host genetic influences on HCV infection have been reported previously, particularly those with SNPs, such as SNPs in human leucocyte antigen (HLA) class II and some genetic factors located in the 19q13 region, including IL28B, KIR2DL3, TGFb1, LDLR and APOE (Romero-Gomez, Eslam, Ruiz, & Maraver, 2011). SNPs are the most common type of genetic variation in genomes among people. To date, over 10 million human reference SNPs have been deposited into the NCBI's public database dbSNP (Miller et al., 2005).

In a recent report, the HPA-1 seems to influence the interaction of platelets with HCV in vitro (Grotto et al., 2016). Verdichio-Moraes, Toralles-Pereira, Grotto, Silva, and Pardini (2009) indicated a possible association between HCV infection and HPA-5 in Brazil population. However, little is known regarding the association of HPA polymorphisms with susceptibility to HCV infection in Chinese population.

The goal of this study was thus to determine the association of the HPA-2, HPA-3, HPA-5 and HPA-15 polymorphisms (the HPA-1b and HPA-4b alleles are rare in Chinese population [Feng et al., 2006].) with susceptibility to HCV infection in Chinese population.

2 | MATERIALS AND METHODS

2.1 | Study cohorts

Overall, 118 patients with chronic hepatitis C infection and 167 control subjects were genotyped in this study. Patients were recruited at the Dalian sixth people's hospital Affiliated of Dalian Medical University between April 2015 and December 2015. The control subjects were randomly recruited from unrelated healthy blood donors at Dalian Blood Center. Inclusion criteria were as follows: the presence of HCV confirmed by the serological and nucleic acid testing. Exclusion criteria were as follows: patients with HBV or HIV positive, and the presence of other chronic liver diseases. Aliquots of EDTA-anticoagulated whole blood samples were collected from all study subjects.

2.2 | Extraction of DNA

Genomic DNA was extracted from whole blood samples using a commercially available DNA isolation kit, on MagCore® Automated Nucleic Acid Extractor (RBCBioscience, Taipei, Taiwan) according to the manufacturer's instructions.

2.3 | 5´-Nuclease assay (NA) with TaqMan minor groove-binding (MGB) probes for HPA-2, HAP-3, HPA-5 and HPA-15 genotyping

Sequences of the primers and probes for the 5´-NA with TaqMan-MGB Probes were obtained from a previous study (Higgins, Hughes, Buzzacott, & Lown, 2004). The primers and probes were synthesized by Takara Biotechnology and Thermo Fisher Scientific Inc., respectively.

The polymerase chain reaction (PCR) mixtures included 1 μl of purified genomic DNA, 10 μl of 2 × TaqMan Universal PCR Master Mix II (Thermo Fisher Scientific Inc), 0.9 μl of each primer (20 μM), 0.2 μl of each probe (20 μM) and 6.8 μl of distilled water in a final reaction volume of 20 μl. The 5′-NA was performed on the ABI Prism7,300 sequence detection system (Applied Biosystems) under the following conditions: pre-PCR heat step at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min. The genotyping results were analysed with SDS software v1.4 (Applied Biosystems), using the allele discrimination function to detect the end-point fluorescent intensity of FAM and VIC in each well. The genotyping results were sorted into three distinct groups, corresponding to the three genotypes, homozygous aa, bb and heterozygous ab.

2.4 | Statistical analysis

Statistical analyses were performed using SPSS 13.0 for windows package (SPSS Inc., Chicago, IL, USA). The Hardy–Weinberg equilibrium test was carried out to evaluate the distribution of gene frequencies of HPA-2, HPA-3, HPA-5 and HPA-15 using Chi square test. The association between HPA polymorphisms and patients with chronic HCV infection was performed using Chi square test. A two-tailed p value of less than .05 ($p < .05$) was considered statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) were determined by logistic regression analysis.

3 | RESULTS

In total, 285 cases of human samples from healthy controls or patients with chronic hepatitis C infection were involved in this study. All included subjects were Chinese Han. Among those, there were 118 cases of patient samples with chronic hepatitis C infection, including 60 women and 58 men, with an average age of 53.7 ± 13.6 years. In addition, there were 167 cases of healthy donors, including 73 women and 94 men, with an average age of 40.2 ± 10.7 years. Figure 1 shows the representative HPA genotyping by the 5´-NA with TaqMan-MGB Probes. The HPA-2, HPA-3, HPA-5 and HPA-15 allele and genotype frequencies in patients with chronic HCV infection and the healthy controls are summarized in Table 1. Genotyping was performed in all patients and control subjects for HPA. Genotypes did not deviate from the Hardy–Weinberg equilibrium among patients and healthy controls and showed no sign of linkage disequilibrium (Table 2). There were no significant differences in the allele and genotype frequency distribution for the HPA-3, HPA-5 and HPA-15 between patients with chronic HCV infection and the healthy controls ($p > .05$). However, distributions of the allele and genotype frequency for HPA-2 were significantly different between patients and the control group. The genotype frequency of HPA-2aa was significantly lower in patients than that in the control group, and the genotype frequency of HPA-2ab/bb was significantly higher in patients than that in the control group ($p = .006$). The allele frequency of HPA-2a in patients was found to be significantly lower than that in the control group, and HPA-2b

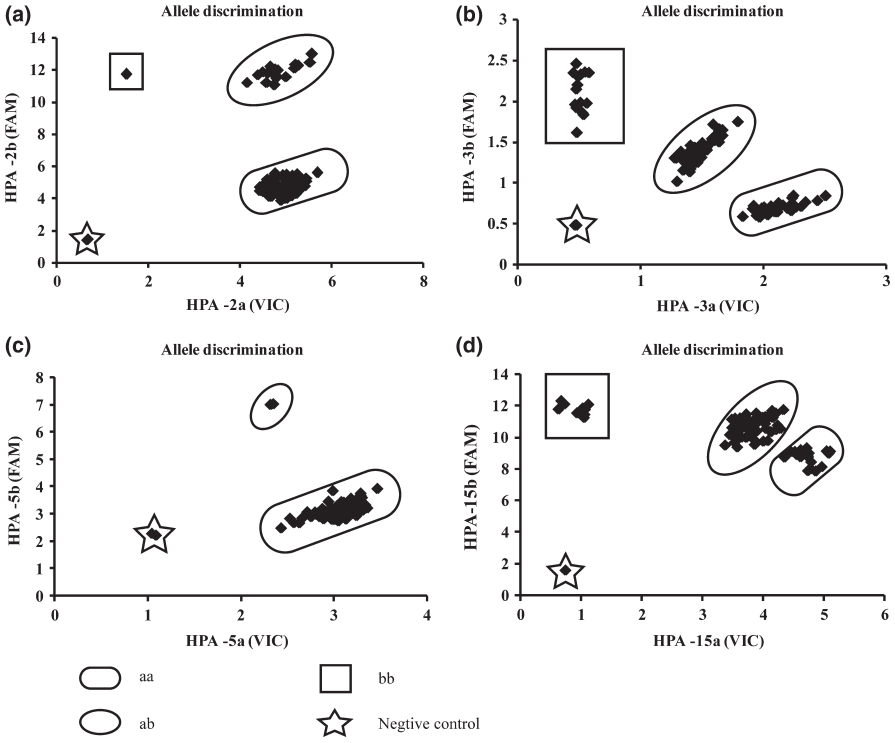

FIGURE 1 Representative genotyping results of human platelet antigens using 5´-nuclease assay with TaqMan minor groove-binding probes. End-point fluorescent signals were from representative samples. Homozygous aa showed an increased fluorescence along the *X*-axis, homozygous bb along the *Y*-axis, whereas heterozygous ab showed an increase in fluorescence intensity along both the *X*-axis and *Y*-axis. Genotyping of Human platelet antigens (HPA)-2, HPA-3, HPA-5 and HPA-15 were shown in a, b, c and d, respectively

TABLE 1 Allelic and genotypic frequencies for Human platelet antigens (HPA)-2, HPA-3, HPA-5 and HPA-15 in patients with chronic hepatitis C virus infection compared with control group

	Patients with chronic hepatitis C virus infection (%)	Control group (%)	*p*-Value[b]	Odds ratios(95%CI)[c]
Genotypes				
2aa	95 (80.51)	153 (91.62)	**.006**	1
2ab	22 (18.64)	14 (8.38)		2.598 (1.235-5.186)
2bb	1 (0.85)	0		N/A
2ab/bb	23 (19.49)	14 (8.38)		2.646 (1.298-5.392)
3aa	42 (35.59)	56 (33.53)	.683	1
3ab	55 (46.61)	86 (51.50)		0.893 (0.441-1.807)
3bb	21 (17.80)	25 (13.59)		0.761 (0.389-1.490)
5aa	116 (98.31)	159 (95.21)	.284	1
5ab	2 (1.69)	8 (4.79)		0.343 (0.071-1.644)
5bb	0	0		
15aa	33 (27.97)	56 (33.53)	.101	1
15ab	65 (55.08)	71 (42.51)		1.179 (0.592-2.345)
15bb	20 (16.95)	40 (23.95)		1.831 (0.972-3.450)
Alleles[a]				
2a	212 (89.83)	320 (95.81)	**.005**	1
2b	24 (10.17)	14 (4.19)		2.588 (1.309-5.116)
3a	139 (58.90)	198 (59.28)	.927	1
3b	97 (41.10)	136 (40.72)		1.016 (0.724-1.426)
5a	234 (99.15)	326 (97.60)	.288	1
5b	2 (0.85)	8 (2.40)		0.348 (0.073-1.655)
15a	131 (55.51)	183 (54.79)	.865	1
15b	105 (44.49)	151 (45.21)		0.971 (0.695-1.358)

Bold indicates $p < .05$.

[a]Numbers of alleles are counting chromosomes not patients.

[b]*p*-Values were determined by Chi square test.

[c]Odds ratios(95% CI)were determined by logistic regression analysis.

TABLE 2 The evaluation of the distribution of gene frequencies of Human platelet antigens (HPA)-2, HPA-3, HPA-5 and HPA-15 by Hardy–Weinberg equilibrium test

	Patients with chronic hepatitis C virus infection			Control group		
	Observed number	Expected number	*p*-Value	Observed number	Expected number	*p*-Value
Genotypes						
2aa	95	95.2	.824	153	153.3	.572
2ab	22	21.6		14	13.4	
2bb	1	1.2		0	0.3	
3aa	42	40.9	.685	56	58.7	.389
3ab	55	57.1		86	80.6	
3bb	21	19.9		25	27.7	
5aa	116	116.0	.926	159	159.1	.751
5ab	2	2.0		8	7.8	
5bb	0	0.0		0	0.1	
15aa	33	36.4	.211	56	50.1	.067
15ab	65	58.3		71	82.7	
15bb	20	23.4		40	34.1	

The Chi square test was used to test Hardy–Weinberg equilibrium.

in patients was significantly higher than that in the control group (p = .005). The OR of the HPA-2ab/bb for HCV infection was 2.646 (95% CI 1.298-5.392), and the OR of the HPA- 2b was 2.588 (95% CI 1.309-5.116).

4 | DISCUSSION

To date, 33 HPAs expressed on six different platelet glycoproteins, GPIIb, GPIIIa, GPIbα, GPIbβ, GPIa and CD109 have been defined by immune sera, of which, twelve antigens are grouped into six biallelic systems (HPA-1, HPA-2, HPA-3, HPA-4, HPA-5 and HPA-15) (Curtis & McFarland, 2014). Clinically, HPAs play an important role in several diseases such as neonatal alloimmune thrombocytopenia, post-transfusion purpura and platelet transfusion refractoriness (Hurd, Cavanagh, Schuh, Ouwehand, & Metcalfe, 2002). Furthermore, a recent study has shown that HPA-2b allele was associated with idiopathic thrombocytopenic purpura (Pavkovic, Stojanovic, Karanfilski, Cevreska, & Spiroski, 2012). In the present study, we focused on the association of the HPA-2, HPA-3, HPA-5 and HPA-15 polymorphisms with susceptibility to hepatitis C virus infection. To our knowledge, this may be the first report to evaluate the association of HPA polymorphisms with susceptibility to HCV infection in Chinese population.

Our study showed that HPA-2ab/bb genotype and HPA-2b allele were more frequent in patients infected with HCV compared to the control group. This result suggested that the HPA-2 polymorphisms rather than HPA-3, HPA-5 and HPA-15 are associated with susceptibility to HCV infection. Individuals carrying the HPA-2b allele had a 2.588-fold increased risk of HCV infection compared with those carrying HPA-2a. Verdichio-Moraes et al. (2009) determined the frequency of the HPA-1 to HPA-5 in patients infected with HCV in Brazil population. They found that the allelic and genotypic frequency of HPA-5a in patients infected with HCV was significantly lower and HPA-5b was significantly higher than that in the control group. However, our study is not consistent with this finding. The genetic background of ethnicity is one of the main factors involved in HCV spontaneous clearance (Romero-Gomez et al., 2011); therefore, the reason for the inconsistency may be due to differences in the genetic background of ethnicity.

The HPA-2 polymorphic antigenic determinants are located on human platelet GPIbα (Metcalfe et al., 2003). GPIbα is essential for membrane development and distribution in maturing megakaryocytes (Poujol, Ware, Nieswandt, Nurden, & Nurden, 2002). The main receptor for HCV in hepatocytes is cluster differentiation 81 (CD81) molecule, which is not expressed on platelets. Recently, a study showed that HCV interacts with platelets despite the absence of the receptor CD81 molecule (Padovani et al., 2013). Pugliese et al. (2004) concluded that the binding of HCV to PLTs seems to involve fibronectin (FN). Moreover, Grotto et al. (2016) evaluated HCV–platelet and HIV–platelet interactions in vitro as well as HPA-1, HPA-3 and HPA-5 polymorphisms. The authors concluded that platelet–HCV interaction seems to be influenced by the presence of the HPA-1b allele. However, the role of HPA-2 in the interaction of HCV with platelets remains unclear. Because HPA-2 polymorphism caused a single amino acid change (Thr145Met) in GPIbα (Metcalfe et al., 2003), we speculate that HPA-2 polymorphism likely affects interaction of HCV with platelets via changing GPIbα structure and function which affects the host susceptibility to HCV.

A limitation of the current study is not considering the differences between Chinese subpopulations. The Chinese Han population can be genetically divided into two distinct groups, North Chinese and South Chinese, on the basis of population genetics studies (Shao

et al., 2016; Zhao & Lee, 1989). However, in the present study, it is difficult to obtain the reliable information on Chinese subpopulation in patients infected with HCV and the control group. The role of HPA may be different between North Chinese Han and South Chinese Han. Another methodologic limitation is possible selection bias in this study, possibly due to difference in risk behaviour for HCV infection between patients infected with HCV and the control group. Unfortunately, we cannot estimate the impact of the risk behaviour for HCV infection on our study because we cannot obtain reliable information on risk behaviour for HCV infection from patients and the control group.

Silva et al. (2012) evaluated the association between the genotype frequencies of HPA-1, HPA-3, and HPA-5 and degree of fibrosis in HCV-infected patients in Brazil population. The authors demonstrated the association of the HPA-1a/1b with the development of fibrosis in HCV infection with time. Therefore, it would be of interest to further investigate the association between HPA polymorphisms and the development of fibrosis during the course of chronic infection by HCV.

5 | CONCLUSION

This study demonstrated that HPA-2 polymorphism is associated with susceptibility to HCV infection, and individuals carrying the HPA-2b allele had a higher risk of HCV infection compared with those carrying HPA-2a. This finding would be useful for further exploring the role of platelet in HCV infection.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Signed informed consents were obtained from all the blood donors and patients, and the approval was obtained from the Institute Research Ethics Committee.

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