LETTER

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KIR2DL2/C1 is a Risk Factor for Chronic Infection and Associated with Non-response to PEG-IFN and RBV Combination Therapy in Hepatitis C Virus Genotype 1b Patients in China

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Dear Editor,

Natural killer (NK) cells are lymphocytes that play important roles in the host defense against hepatitis C virus (HCV) infection. Killer cell immunoglobulin-like receptors (KIRs) are a group of regulatory molecules expressed on NK cells and a subset of T cells (Parham 2005). Ligands for KIRs are human leukocyte antigen (HLA) class I molecules, and HLA-C1 is a ligand for the inhibitory receptors KIR2DL2, KIR2DL3 and the activating receptor KIR2DS2 (Robinson et al. 2003; Du et al. 2007). In 2002, the National Institutes of Health Consensus Development Conference concluded that a combination therapy of pegylated alpha interferon (PEG-IFN) with ribavirin (RBV) manages HCV infections effectively (Gebo and Bartlett 2002). Before the direct-acting antiviral agent treatment was approved, PEG-IFN and RBV were the main antiviral treatments for chronic HCV in China (Chinese Society of Hepatology et al. 2015). Patients that receive the same standard combination therapies are classified as non-responders (NR) and sustained virological responders (SVR) according to their responses to the treatment by detecting HCV RNA 24 weeks after treatment (Asselah 2012). The NR do not mount a sufficient anti-HCV response, which is defined by a consistent positive viral

load during treatment, at its end, or at 24-weeks post-treatment. The SVR are defined by consistent undetectable HCV RNA levels in serum at 24-weeks post-treatment (Fried et al. 2002).

To investigate the association of the KIR frequencies with HCV infection and therapy responses in Chinese Han population, we recruited 333 patients infected with HCV-1b and 320 healthy individuals in Hubei Province between October 2010 and August 2012. In 333 HCV patients, 98 treated by PEG-IFN and RBV were successfully tracked and divided into NR and SVR groups, which were detected with Real-Time Quantitative PCR Detection System and ELISA (Kehua Bio-Engineering Co, Shanghai, China). All subjects were negative from other disorders, such as infection with hepatitis B virus, hepatitis D virus, and HIV, which were detected by quantitative PCR and ELISA, as well as diabetes, malignant tumor, or any autoimmune diseases.

We extracted the genomic DNA from the blood of donors with a SE Blood DNA kit (Omega Bio-Tek Inc, Norcross, GA, USA), and the KIR alleles were determined by the sequence-specific PCR primers (Bunce et al. 1995; Hsu et al. 2002). The results were analyzed with a Chi square test based on P values and odds ratios (OR). The gene frequencies of *KIR2DL2* and *KIR2DS3* were significantly higher in HCV patients (Supplementary Table S1, Table 1) ($P < 0.01$), and only the frequency of *KIR2DL2* was significantly higher in the SVR group (Supplementary Table S2). The gene frequency of full-length *KIR2DS4* was significantly lower in HCV patients and the SVR group. HLA-C1, as a ligand, is necessary for the function of KIR2DL2 (Bashirova et al. 2006; Du et al. 2007).

Therefore, we analyzed the association among KIR2DL2, its ligand HLA-C1, HCV-1b infection, and treatment response. As shown in Table 1, although the frequency of HLA-C1 was not different compared with healthy controls, both KIR2DL2 and HLA-C1 positive (KIR2DL2+/C1+ pairs) showed a risk association with

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Table 1 Clinical characteristics and occurrence of KIR2DL2 and HLA-C1 in HCV and healthy patients.

Characteristic	HCV patients (n = 333)	Healthy controls (n = 320)	P value[a]	Odds ratio[a] (95% CI)
Sex (M:F)	1.41:1	1.22:1		
Mean age (span; median)	(24–55); 40	(27–55); 41		
Mean ALT ± SD (IU/L)	101.11 ± 28.22	25.2 ± 12.11		
HCV RNA (copies/mL)	$(5.13 \pm 1.56) \times 10^5$	–		
Anti-HCV (detected by ELISA)	+	–		
HLA-C1	307 (92.19%)	289 (90.31%)	0.40	1.26 (0.73–2.18)
KIR2DL2	186 (55.86%)	94 (29.38%)	< 0.01	3.04 (2.20–4.20)
KIR2DL2+/HLA-C1−	6 (1.80%)	10 (3.12%)	0.27	0.57 (0.20–1.58)
KIR2DL2+/HLA-C1+	180 (54.05%)	84 (26.25%)	< 0.01	3.31 (2.38–4.60)

[a]P value of Z-test for pooled odds ratio.

HCV-1b infection. As shown in Table 2, in contrast to the SVR group, the gene frequency of *KIR2DL2* was significantly higher in the NR group, whereas the frequency of *HLA-C1* was not different. The joint analysis revealed that the frequency of *KIR2DL2+/C1+* pairs in the NR group was significantly higher than the SVR group (*P* < 0.05), and the *KIR2DL2+/C1−* pairs showed no difference. This suggests that the patients carrying *KIR2DL2/C1* genes have a high risk of being infected by HCV-1b and they are not beneficial for HCV treatment. But 186 of the 333 HCV patients had the *KIR2DL2* gene, of which 180 had *HLA-C1* group genes and 6 did not. Ninety-four of the 320 healthy control participants had the *KIR2DL2* gene, of which 84 had the *HLA-C1* gene and 10 did not. There was no statistical difference in the *P* values and OR values of *KIR2DL2+/C1−* between HCV patients and the healthy controls.

We further performed meta-analysis to assess the association between *KIR2DL2* and the response to therapy. Twelve studies were identified through databases searching

(e.g., PubMed, Science Direct) for all case-control studies evaluating KIRs and HCV-1b treatment in humans (up to April 2017). Studies were further selected if they fulfilled the following criteria: (1) have a NR-SVR design, used the same therapy strategy, reported the *KIR2DL2* genotype frequencies, and confirmed that the recruited HCV patients had no other disease; (2) supplied sufficient information to calculate the OR in a peer-reviewed journal. Finally, five articles covering populations in three countries (Brazil, Spain, Ireland) were eligible for meta-analysis (Carneiro *et al.* 2010; Vidal-Castineira *et al.* 2010; Dring *et al.* 2011; de Vasconcelos *et al.* 2013; Vidal-Castineira *et al.* 2014). A significant association of the *KIR2DL2* gene with the response to therapy was detected under the fixed effects model, including 934 NRs and 732 SVRs (shown in Supplementary Table S3 and Fig. 1). The aggregated OR was 1.41 (*P* < 0.01, 95% CI = 1.05–1.88), and the heterogeneity was moderate (*P* = 0.16, I^2 = 39%). The publication bias of the literature was estimated by funnel plots (shown in Supplementary Figure S1). The shapes of funnel

Table 2 Clinical characteristics and occurrence of KIR2DL2 and HLA-C1 in NR and SVR HCV patients.

Characteristic	NR patients (n = 36)	SVR patients (n = 62)[a]	P value[b]	Odds ratio[b] (95% CI)
Sex (M:F)	1.25:1	1.69:1		
Mean age (span; median)	(28–55); 41	(27–51); 41		
Mean ALT ± SD (IU/L)	72.5 ± 17.6	45.2 ± 11.3		
HCV RNA (copies/mL)	$(4.76 \pm 1.34) \times 10^4$	–		
Anti-HCV (detected by ELISA)	+	–		
HLA-C1	34 (94.44%)	57 (91.94%)	0.64	1.49 (0.27–8.11)
KIR2DL2	26 (72.22%)	32 (51.61%)	< 0.05	2.44 (1.01–5.90)
KIR2DL2+/HLA-C1−	2 (5.56%)	4 (6.45%)	0.86	0.83 (0.15–4.90)
KIR2DL2+/HLA-C1+	24 (66.67%)	28 (45.16%)	< 0.05	2.43 (1.03–5.71)

[a]There is a KIR2DL2-/C1- in SVR patients.
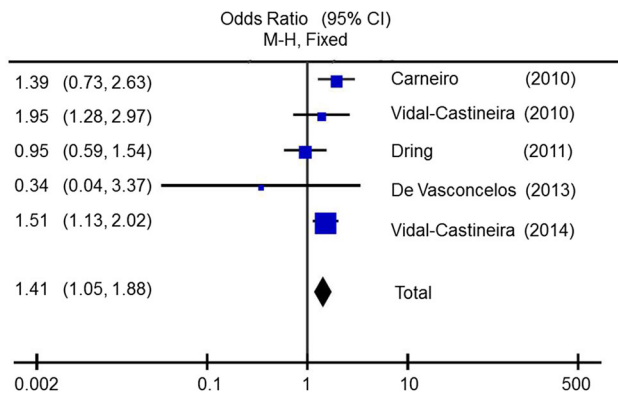
[b]P value of Z-test for pooled odds ratio.

Fig. 1 Forest plot of the meta-analysis for KIR2DL2 in NR and SVR HCV patients under the fixed effects model.

plots appeared symmetrical for NR versus SVR in the studies. Together with our data, *KIR2DL2* is not beneficial for HCV therapy in multiple populations. However, none of the above papers discussed the relationship between the *KIR2DL2/C1* pair and HCV patients.

This study displayed a significantly higher frequency of the *KIR2DL2* gene among patients infected with HCV-1b compared with healthy controls in a Chinese Han population. As previously mentioned, the frequency of the *KIR2DL2* gene in this Chinese Han population was 10%–30% and is present in 40%–60% of Caucasians (Single *et al.* 2007). In this study, the *KIR2DL2* gene frequency in Chinese Han HCV patients (55.86%) is similar to that in Caucasian patients, which also implies that KIR2DL2 is a risk factor for HCV-1b infection in the Chinese Han population.

KIR2DL2 is an inhibitory receptor on the surface of NK cells, which can transmit inhibitory signals to the cell, but all these require the involvement of HLA-C1. In the early stages of virus infection and development, the elimination of viruses in the body also depends on innate immunity, including NK cells. The killing ability of NK cells carrying KIR2DL2 may be impaired, which has also been verified in studies on KIR2DL2 and HIV (Zwolinska *et al.* 2016). In this study, one possibility is that the inhibition of virus infection mediated by KIR2DL2 can overcome the activation of NK cells by IFN-alpha and decrease the antiviral ability of NK cells.

In conclusion, KIR2DL2/C1 is a risk factor of HCV-1b infection and is associated with nonresponse to PEG-IFN and RBV combination therapy in Chinese Han patients. These findings might contribute to our understanding of the pathogenic mechanisms of HCV infection and to the development of more efficient therapeutic strategies for HCV that consider host genetic factors.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Animal and Human Rights Statement This study conformed to the 1975 Declaration of Helsinki guidelines and permission was obtained from the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. Prior to the study, informed consent was obtained from each individual.

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