IL28B rs12980275 variant as a predictor of sustained virologic response to pegylated-interferon and ribavirin in chronic hepatitis C patients: A systematic review and meta-analysis

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Summary
Background and objective: The IL-28B rs12979860 CC and rs8099917 TT genotypes were proved to be predictor for pegylated-interferon (PEG-IFN)/ribavirin (RBV)-treated hepatitis C virus (HCV) patients. However, there were no identical conclusions on rs12980275 polymorphism. Our aim is to perform a meta-analysis in order to determine the association between rs12980275 polymorphism of IL28B and the sustain viral response (SVR) of HCV patients with PEG-IFN/RBV therapy.
Methods: Studies were retrieved from PubMed and Chinese China National Knowledge Infrastructure (CNKI). Data were extracted by two investigators and analyzed using Stata 11.0 software.
Results: Sixteen articles, containing 19 independent studies were included in the analysis. The results showed that patients with AA genotype of rs12980275 achieved higher SVR than patients with AG/GG genotypes. The overall OR (95% CI) was 3.118 (2.146, 4.529). In subgroup analysis by ethnicity, the ORs (95% CIs) were 3.084 (1.454, 6.542) and 2.736 (1.863, 4.018) in Asian and Caucasian population, respectively. Another subgroup analysis by HCV genotype, the ORs (95% CIs) were 3.976 (2.568, 6.158), 1.462 (0.504, 4.240) and 1.489 (0.916, 2.421) in patients with HCV genotype 1/4, mix genotype, and HCV genotype 2/3, respectively.

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Conclusion: AA genotype of rs12980275 was a predictive factor for SVR in HCV patients with PEG-IFN/RBV treatment, especially in HCV genotype 1/4.

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Introduction

The infection with hepatitis C virus (HCV) is a global health problem. About 60–80% of patients fail to clear the virus acutely and then develop chronic infection, with the long-term risks of cirrhosis, liver failure and liver-related death worldwide [1]. Death related to the complications of cirrhosis occurs at an incidence of approximately 4% per year, whereas hepatocellular carcinoma (HCC) occurs in this population at an estimated incidence of 1–5% per year [2].

Currently, a combination therapy with pegylated-interferon (PEG-IFN) and ribavirin (RBV) for 24 or 48 weeks was considered as the best treatment method [3]. Treatment success was evaluated based on a sustained virologic response (SVR), defined as undetectable HCV RNA levels 6 months after cessation of treatment [4,5]. However, this approach is not effective in all patients. The response of patients infected with chronic hepatitis C (CHC) to PEG-IFN plus RBV therapy is heterogeneous and is influenced by a wide range of factors. Clinical studies have been carried out in order to determine these factors, including viral factors, such as genotype, baseline viral load and nucleotide sequence in the viral genome, and host factors, including age, gender, race, liver fibrosis, and obesity et al. Recently, single nucleotide polymorphism (SNP) localized near the IL28B gene, encoding IFN-α3, (for example, rs12979860, rs8099917) have been shown to be associated with antiviral treatment response, such as SVR [6–8]. The prevalence rates of CC genotype of rs12979860 paralleled with the SVR in each population [9]. Because of its significant impact on the treatment outcome, a genetic testing for the genotype of SNP of IL28B before deciding on-treatment strategies has been proposed [9]. These polymorphisms might therefore serve as important factors to personalize antiviral therapy of patients with CHC [10].

At the same time, other SNPs of IL28B were also demonstrated to be highly associated with SVR, such as rs12980275 [11], and others [12,13], although some studies with adverse results were also reported [14]. Nevertheless, whether these SNPs would be influential on SVR was still undetermined. Therefore, we performed a meta-analysis to clarify the impact of rs12980275 in CHC patients treated with PEG-IFN plus RBV from available data.

Materials and methods

Search strategy

Studies on the associations between rs12980275 of IL28B gene and the treatment response (SVR) with PEG-IFN and RBV in HCV infected patients were retrieved from PubMed and CNKI through May 2014. The search terms were used as follows: (**"PEG-IFN"** OR **"interferon-lambda 3"** OR **"IFN lambda 3"**) AND (**"Hepatitis C"** OR **"HCV"**) AND (**"sustained virologic response"** OR **"SVR"**) AND (IL-28B polymorphisms). References of retrieved publications and review were also screened manually to search potential articles fitted the criteria.

The abstract of those papers were reviewed by two reviewers (Li and Zheng) independently. Studies were included if they met the following inclusion criteria:

- chronic HCV infected patients with the combined therapy of PEG-IFN and RBV;
- SVR was clearly defined as HCV RNA is undetectable 24 weeks or 6 months after the cessation of treatment;
- sufficient and accurate data could be extracted and calculated for estimating an odd ratio (OR) with 95% confidence interval (CI).

Studies would be excluded if patients had human immune-deficiency virus (HIV) infection or other complicated liver diseases such as chronic hepatitis B (CHB), hepatocellular carcinoma or underwent liver transplantation.

Data extraction

Data extraction was performed independently by two investigators according to the including or excluding criteria described previously. The following information was extracted from each study: the first author, date of publication, source of the subject, sample size, infection type, and therapy period, positive association, genotype method and Newcastle-Ottawa Scale score (NOS score). The data were then divided into two subgroups either by ethnicity or by HCV genotype.

The quality of the included articles was evaluated using NOS score. It is a risk of bias assessment tool for observational studies that is recommended by the Cochrane Collaboration. The NOS assigns up to a maximum of nine stars for the least risk of bias in three domains:

- selection of study groups (four stars);
- comparability of groups (two stars);
- ascertainment of exposure (three stars) for case–control studies [15].

Statistical analysis

Statistical analysis Stata11.0 software was used to perform meta-analysis. The overall strength of the association between potential factors and SVR was assessed by calculating the odd ratio (OR) and 95% confidence interval (CI).
OR of each study with 95% CI was calculated and displayed in a forest plot. Heterogeneity among studies was tested by Cochran’s Q-statistic and quantified by I² statistic, which was interpreted as the proportion of total variation contributed by between-study variation [16]. Then, the Mantel-Haenszel fixed-effect model [17] or the Dersimonian Laird random-effect model [18] was used to calculate the pooled OR and 95% CI. If there was a statistical difference in terms of heterogeneity (P < 0.1), a random-effect model was selected to pool the data. A fixed-effect model, otherwise, was employed. The method of Begg and Mazumdar was used to assess publication bias with a P < 0.05 considering statistical significance.

### Results

The literature review identified 92 articles in PubMed and other database using search terms. Forty-three articles included after first assessing. The remaining studies underwent further evaluation with the inclusion criteria. Studies on other liver disease such as CHB, hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Authors (Year) [Ref.]</th>
<th>Source of subject</th>
<th>Sample size</th>
<th>Infection genotype</th>
<th>Therapy period</th>
<th>Significant association</th>
<th>NOS</th>
<th>Genotyping method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdo et al. (2013) [19]</td>
<td>Kingdom of Saudi Arabia</td>
<td>129</td>
<td>HCV4</td>
<td>48 w</td>
<td>Yes</td>
<td>6</td>
<td>Sequencing</td>
</tr>
<tr>
<td>Bochud et al. (2011) [14]</td>
<td>Multicenter</td>
<td>241</td>
<td>HCV1.2.3</td>
<td>48 w</td>
<td>No</td>
<td>7</td>
<td>Taqman</td>
</tr>
<tr>
<td>Covolo et al. (2014) [20]</td>
<td>Multicenter</td>
<td>121</td>
<td>HCV1,4</td>
<td>48 w</td>
<td>Yes</td>
<td>7</td>
<td>Taqman</td>
</tr>
<tr>
<td>Domagalski et al. (2013) [21]</td>
<td>Poland</td>
<td>82</td>
<td>HCV1/4</td>
<td>48 w</td>
<td>No</td>
<td>7</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>Fattovich et al. (2011) [22]</td>
<td>Italy</td>
<td>280</td>
<td>HCV1,2,3,4</td>
<td>24 w or 48 w</td>
<td>Yes</td>
<td>7</td>
<td>Taqman</td>
</tr>
<tr>
<td>Fischer et al. (2012) [23]</td>
<td>Multicenter</td>
<td>931</td>
<td>HCV1</td>
<td>48 w</td>
<td>Yes</td>
<td>8</td>
<td>SSP-PCR</td>
</tr>
<tr>
<td>Gao et al. (2012) [24]</td>
<td>China</td>
<td>97</td>
<td>HCV1</td>
<td>48 w</td>
<td>Yes</td>
<td>6</td>
<td>Sequencing</td>
</tr>
<tr>
<td>Gouda et al. (2014) [25]</td>
<td>Egyptian</td>
<td>111</td>
<td>HCV4</td>
<td>48 w</td>
<td>Yes</td>
<td>7</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>Grebely et al. (2010) [26]</td>
<td>Australia</td>
<td>58</td>
<td>HCV1,2,3,4</td>
<td>24 w</td>
<td>No</td>
<td>8</td>
<td>Sequenom MassARRAY iPLEX</td>
</tr>
<tr>
<td>Heo et al. (2014) [27]</td>
<td>Korean</td>
<td>156</td>
<td>HCV1,2,3,6</td>
<td>24 w or 48 w</td>
<td>No</td>
<td>7</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>Lagging et al. (2011) [28]</td>
<td>Multicenter</td>
<td>168</td>
<td>HCV1</td>
<td>48 w</td>
<td>Yes</td>
<td>7</td>
<td>Taqman</td>
</tr>
<tr>
<td>Lazarevic et al. (2013) [29]</td>
<td>Serbia</td>
<td>106</td>
<td>HCV1</td>
<td>48 w</td>
<td>Yes</td>
<td>7</td>
<td>SSP-PCR</td>
</tr>
<tr>
<td>Lin et al. (2011) [13]</td>
<td>China</td>
<td>191</td>
<td>HCV1</td>
<td>24 w</td>
<td>Yes</td>
<td>8</td>
<td>SSP-PCR</td>
</tr>
<tr>
<td>Sarrazin et al. (2011) [30]</td>
<td>Germany</td>
<td>267</td>
<td>HCV2,3</td>
<td>24 w–48 w</td>
<td>No</td>
<td>8</td>
<td>Sequencing</td>
</tr>
<tr>
<td>Venagas et al. (2011) [31]</td>
<td>Chile</td>
<td>99</td>
<td>HCV1</td>
<td>24 w</td>
<td>Yes</td>
<td>6</td>
<td>PCR-RFLP</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus; w: week; Ref.: reference.

a Refer to the association between polymorphism of rs12980275 and SVR.
b Represent Newcastle-Ottawa Scale.
c Refer to genotyping method for IL28B rs12980275.

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and liver transplantation were also excluded. Finally, 16 articles (19 studies) that investigated the association between rs12980275 AA genotype and SVR of HCV patients with PEG-IFN plus RBV treatment meet the criterion, and they were included in this meta-analysis [11,13,14,19–31] (Fig. 1). Table 1 shows the characteristics of the study included in our analysis. As shown in the Table 1, 16 articles (19 studies) were eligible for examining the association between the IL28B polymorphism rs12980275 and treatment response of hepatitis C, including 1952 cases with SVR and 1271 cases with non-SVR.

Meta-analysis

In the 16 included articles, most of them compared the favorable genotype AA with unfavorable genotype AG+GG. Therefore, in this meta-analysis, the SVR of AA genotype carriers was compared with that of AG and GG carriers. The higher SVR rate was found in patients with HCV2/3 genotype (486/577) than HCV1/4 genotype patients (1432/2588) (P<0.001). For the association between AA genotype of rs12980275 and SVR, the overall OR (95% CI) were 3.118 (2.146, 4.529). In the subgroup analysis by ethnicity, the OR value and 95% CI were 3.084 (1.454, 6.542) for Asian and 2.736 (1.863, 4.018) for Caucasian (Fig. 2). In addition, the subgroup analysis by HCV genotype was also performed.

The results showed significant association in HCV 1/4 subgroup. The OR (95% CI) were 3.976 (2.568, 6.158), 1.462 (0.504, 4.240) and 1.489 (0.916, 2.421) in HCV1/4 subgroup, mixed subgroup and HCV2/3 subgroup, respectively (Fig. 3). Four of five studies included in Asian subgroup were of HCV genotype 1 or 4. So, the subgroup analysis by HCV genotype was performed in Caucasian population (Fig. 4). The results showed that association exist in HCV genotype 1/4 subgroup of Caucasian population with the OR (95% CI) was 3.714 (2.270, 6.077). No association was found in HCV genotype 2/3 subgroup among Caucasian population.

Heterogeneity

The heterogeneity among all studies was calculated using the Q-statistic and the I² test. The overall between-study heterogeneity was significant (I² = 72.9%, P<0.001). As shown in Table 2, in the subgroup analysis by ethnicity, the between-study heterogeneity was also significant either in Asian subgroup (I² = 72%, P = 0.003) or in Caucasian subgroup (I² = 64.1%, P = 0.001). In the subgroup analysis by HCV genotype subgroup, the between-study heterogeneity was not in HCV2/3 subgroup (I² = 0%, P = 0.525). However, the moderate heterogeneity was found in HCV1/4 genotype subgroup (I² = 76.5%, P<0.001).

Figure 1 Flow diagram of literature search.
Publication bias

Egger’s test was performed to assess the publication bias of the literature in this meta-analysis. All the P-values of Egger’s tests are shown in Table 2. The P-value with more than 0.05 was considered as statistical significance. The results showed that the P-value of publication bias for overall included studies was 0.334. As shown in Table 2, there were no publication bias either in HCV1/4 genotype analysis or in HCV2/3 genotype analysis (PEgger’s=0.074 and PEgger’s=0.493, respectively). In the subgroup analysis by ethnicity, the P-values for publication bias were 0.129 in Asian population subgroup and 0.291 in Caucasian population subgroup, respectively. The Egger’s test results suggested that publication bias in our meta-analysis was not significant in two subgroups meta-analysis.

### Table 2  Association between rs12980275 AA genotype and SVR in CHC patients with PEG-IFN/RBV treatment.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Studies number</th>
<th>Heterogeneity</th>
<th>M²</th>
<th>OR (95% CI)</th>
<th>Publication bias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F² (%)</td>
<td>P-value</td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>6</td>
<td>72.0</td>
<td>0.003</td>
<td>3.084 (1.454, 6.542)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caucasian</td>
<td>12</td>
<td>64.1</td>
<td>0.001</td>
<td>2.736 (1.863, 4.018)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV1/4</td>
<td>14</td>
<td>76.5</td>
<td>&lt;0.001</td>
<td>3.976 (2.568, 6.158)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV2/3</td>
<td>4</td>
<td>0</td>
<td>0.525</td>
<td>1.489 (0.916, 2.421)</td>
<td>0.108</td>
</tr>
</tbody>
</table>

SVR: sustain viral response; CHC: chronic hepatitis C; PEG-IFN: pegylated-interferon; RBV: ribavirin; OR: odd ratio; 95% CI: 95% confidence interval; HCV: hepatitis C virus. The study number of HCV2/3 was four because one study did not specify the data source and was not included in this subgroup.

- M: model; R: random-effect model; F: fix effect model.
- P-value for Egger’s test.
IL28B was identified as a key factor of the immune response to HCV. The protein product of IL28B is IFN-λ-3, one of the three members of type III IFN family (IFN-λ-1/2/3 = IL29, IL28A, and IL28B) [32]. They have been studied previously in the context of HBV and HCV infection and shown to suppress both hepatitis B virus (HBV) and HCV replication. Recently, several genome-wide association studies have shown that genetic polymorphisms at or near the IL28B (IFN-λ) gene, including rs12979860, rs12980275 and rs8099917, are associated with higher rate of SVR in CHC patients undergoing PEG-IFN alpha and RBV therapy [11,33]. However, no meta-analysis about rs12980275 and SVR was published yet. Moreover, these studies on rs12980275 did not come into the conclusion.

In this analysis, we pooled the studies on associations between rs12980275 near IL28B gene and the treatment effect of PEG-IFN/RBV in chronic HCV patients. Roughly, a 3-fold significant increase of possibility to clear virus (SVR) was observed for rs12980275 AA genotype, especially in HCV genotype 1 or 4, with the OR and 95% CI 3.65 (2.44–5.47). No relationship was found in patients infected with HCV genotype 2 or 3, which is agreement with the previous study on rs12979860 and rs8099917 [6]. However, when examining the data by ethnicity subgroup, a significant association was found in Asian subgroup, with OR and 95% CI 4.62 (2.56–8.36). It was worth mentioning that the patients of four studies included in Asian subgroup were of HCV genotype 1 or 4. At the same time, the subgroup analysis by HCV genotype was also performed in Caucasian population. The results again indicated association between AA genotype of rs12980275 and SVR in HCV1/4 genotype.

The main finding of this study is that rs12980275 polymorphism has a better performance as the previously investigated rs12979860 and rs8099917 for predicting SVR in patients with HCV genotype 1 or 4. This finding is not unexpected due to the strong linkage disequilibrium. Moreover, ss469415590, a newly discovered dinucleotide variant that creates a novel gene, designated IFNL4, was reported as a better predictor than rs12979860 of response to IFN-based therapy in HCV genotype 1 [26]. Both ss469415590 and rs2979860 are located within IFNL4 gene, separated by 367 pairs of bases and, therefore, in high LD. However, some interethnic variations exist in the correlation level between ss469415590 TT and rs2979860C alleles with stronger correlation in Caucasian than in Africans. This data might explain why we did not observe any differences in the performance of these markers to predict SVR in our Caucasian population, and why these differences are found in Africans. Similar conclusions were obtained by Stattermayr et al. [34] and Real et al. [35] recently. They studied a large sample of HCV, mainly Caucasians, and concluded that ss469415590 marker shows equivalent performance to predict SVR to
PEG-IFN/RBV than the rs12979860 variant in Caucasian and there is no benefit in additional testing for ss469415590 for treatment prediction in these patients.

Due to its high statistical efficiency and lower bias and uncertainty, meta-analysis is now increasingly used to help making clinical decision. However, conclusions arising from a meta-analysis might affected by a series of selection bias, which are caused by studies that are rapidly published with statistically significant results. In this meta-analysis, funnel plots (a graph of estimates of the effect of each trial versus sample size) are used to detect publication bias by asymmetric evaluation. Publication bias is considered to exist when funnel plots are asymmetrical. In addition, we also used other statistical methods such as Egger’s method to detect publication bias since it has stronger statistical power than Begg’s method or Mac skill’s method [36,37].

It is important to note that this meta-analysis has some limitations. First of all, in the present analysis, several studies were excluded during the calculation of OR value due to insufficient data. Inclusion of those studies might have had a slight change in OR values, but may not have affected the final conclusion. Second, although publication bias did not exist in our analysis, it is preferable for a meta-analysis to include all available data: published or unpublished. However, in our meta-analysis, only published studies were included. Finally, some of the subgroups such as race were relatively small in size, and to some extent might impact the application of our conclusions. Future studies of larger groups from different ethnicities should be conducted to validate the relationship.

In conclusion, AA genotype of rs12980275, which in strong linkage disequilibrium with rs12979860, plays an important role in the prediction of SVR in HCV patients with PEG-IFN/RBV treatment, especially in HCV genotype 1 or 4.

Author contributions

Design by Man Li, data analysis by Man Li and Hao Zheng, literature retrieval by Bing Chi and Xiao-xue Wu, drafting article by Man Li and Dian-wu Liu.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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