Analysis of Interleukin-28B Polymorphisms and Pegylated-Interferon/Ribavirin Response of Indonesian Chronic Hepatitis C

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Running Head: IL-28B SNPs in Indonesian Chronic Hepatitis C

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ABSTRACT

This study demonstrated that the Indonesian chronic hepatitis C patients (mostly ethnic Java people) mostly were infected with HCV genotype 1, however they carried mainly the major genotypes of IL-28B SNPs (rs12979860 CC, rs11881222 TT, rs8103142 AA, rs8099917 TT) and mostly achieved sustained virological response to Pegylated-Interferon/Ribavirin treatment.

Keywords: chronic hepatitis C, IL-28B SNPs, Peg-IFN/RBV, virological response.

Word count: 1,369 words

Plasma and PBMC were collected from chronic hepatitis C (CHC) patients in Dr. Saiful Anwar General Hospital, Malang, East Java Province, and a private clinic in Sanglah, Denpasar, Bali Province, Indonesia. All patients were treated with Pegylated-Interferon (PEG-IFN)-α-2a (Pegasys) and Ribavirin. Patients with HCV genotype 1 were treated for 48 weeks and patients with HCV genotype 2 or 3 were treated for 24 weeks. The ethnicity of each patient was carefully documented for three previous generations, both maternally and paternally. Plasma and PBMC were examined in the Institute of Tropical Disease, Airlangga University, Surabaya, East Java Province. Data on the pre-treatment HCV viral loads and HCV genotypes of patients were obtained from their medical records. Ethical clearance for this study was obtained from the Ethics Committee of Dr. Saiful Anwar General Hospital, Malang.

HCV RNA was extracted from 140 µL plasma using a commercially available kit (QIAamp viral RNA kit; QIAGEN, Tokyo, Japan). To amplify the NS5B region of the HCV genome, the extracted RNA was reverse transcribed and amplified using SuperScript One-step RT-PCR (Invitrogen, Tokyo, Japan) and a set of primers. PCR amplifications using outer primers (nt 7999-8825) and inner primers (8159-8630) were performed as previously described (1) using
Hot Star Taq Master Mix (Qiagen, Tokyo, Japan). The amplified fragments were sequenced by a
direct sequencing method with the Big Dye Terminator Cycle Sequencing v.1.1. kit and an ABI
Prism 310 Sequencer (Applied Biosystems, Foster City, CA, USA). Based on the sequence
similarity to the reported sequences from International DNA databases (DDBJ/EMBL/GenBank)
using the program Genetyx-Win version 9.0 (Genetyx Corporation, Tokyo, Japan), each HCV
isolate was assigned an HCV subtype (2). The HCV genotypes/subtypes were re-examined to
confirm the HCV-genotype/subtype data obtained from the medical records.

The quantification of plasma HCV RNA titers was performed with the Taqman® Gene
Expression Master Mix using the Applied Biosystems 7300 real time PCR machine. HCV 5’-
NCR was amplified with a primer and probe set, as described previously (3). The lowest
detectable titer with this kit was 3.0 log10 RNA copies/ml. This assay was used to measure the
HCV viral load post treatment or during treatment (more than 12 weeks of the treatment) for
determining the virological response to PEG-IFN/RBV based on the pretreatment HCV viral
load data obtained from the medical records.

Host DNA was extracted from each PBMC sample using a QIAmp DNA kit (Qiagen,
Tokyo, Japan) following the manufacturer’s guidelines. To determine IL-28B single nucleotide
polymorphisms (SNPs), PCRs amplified a short fragment containing rs12979860, rs11881222,
rs8103142, and rs8099917 using specific primer pairs (4-5). PCR amplification was performed
as previously described (4-5) using Hot Star Taq Master Mix (Qiagen, Tokyo, Japan). The
amplified fragments were sequenced with Big Dye on an ABI Prism 310 Sequencer (Applied
Biosystems, Foster City, CA, USA).

The data were analyzed by the chi-square test or Fisher’s exact test for categorical
variables. A P value <0.05 was considered significant.
A total of 34 samples were collected from 19 (55.9%) women and 15 (44.1%) men (aged 32-76 years, mean 58.8±10.90 years) with CHC. The majority of these patients were ethnic Java people (82.4%), and the other 6 patients were Batak-Lampung (Sumatera) (2.9%), Java-Madura (2.9%), Gorontalo (Sulawesi) (2.9%), Japan-Toraja (Sulawesi) (2.9%) and Bali (5.9%) people. Among the 34 patients, 28 (82.4%) completed the entire course of the PEG-IFN/RBV treatment and were followed-up for 24 weeks. The other 6 patients were treated for more than 12 weeks.

In this study, virological responses to PEG-IFN/RBV were classified into two groups: (i) non-virological response (NVR)/ transient virological response (TVR) (poor response) and (ii) early virological response (EVR)/sustained virological response (SVR) (good response). Overall, 25 (73.5%) patients achieved EVR/SVR, while 9 (26.5%) had NVR/TVR. No significant differences were observed in age, gender, race, pre-treatment ALT, severe fibrosis (F3-F4), or HCV genotype among the virological responses (all \( P > 0.05 \)) (Table 1). All these factors may not have influenced the virological response; however, this has been attributed to the small number of patients with poorly distributed factors, i.e. a large number of HCV-genotype 1 patients together with a small number of TVR or NVR. Most patients (24/34, 70.6%) were infected with HCV genotype 1. The confirmation assay showed that HCV subtype 1b (56%, 5/9) was predominant among all positive PCR products obtained, followed by HCV subtypes 1a (22%, 2/9), and 1c and 2a (11%, 1/9 each). These results were consistent with the findings of previous studies in Indonesia, in which HCV subtype 1b was predominant in HCV-associated liver disease patients (6). Patients infected with HCV genotype 1, as documented, were mostly slow responders and required longer treatment duration than patients infected with genotypes 2 and 3 (7). In the United States and Europe, 42%-52% of patients with HCV genotype 1 achieved SVR (8). However, the response rate was markedly higher in China when patients were treated
with the corresponding regimen (9). Our study on the Indonesian population showed that the proportion of patients with HCV genotype 1 was higher (64.0%) in the EVR/SVR group. As has long been suspected, host genetic factors may be the key determinants for CHC treatment.

The results showed that most patients (94%) carried the major genotypes (rs12979860 CC, rs11881222 TT, rs8103142 AA, rs8099917 TT). The frequencies of the major genotypes of the four SNPs were higher in the EVR/SVR group (75.0%-75.8%) than in the NVR/TVR group (24.2%-25.0%) (Table 2); however, these differences were not statistically significant ($P >0.05$ for each SNP), which may have been due to the rare event of the heterozygous/minor genotype of IL-28B SNPs. The majority of patients (64.0%) who achieved EVR/SVR were infected with HCV genotype 1, and most of them (93.8%) carried the major genotypes of the four SNPs of IL-28B. Homozygosity for the major allele of SNPs associated with IL28B was correlated with a better response to PEG-IFN/RBV treatment, and minor allele positive patients were found to be poor responders (10). Most of the limited number of patients with HCV genotype 2 or 3 infection (90.0%, 9/10) achieved EVR/SVR, and all carried the major genotypes of the four SNPs. In contrast to the data on HCV genotype 1 infection, several studies have not demonstrated any clear association between IL-28B polymorphisms and SVR in patients with HCV genotype 2 or 3 infection (11-12). The role of predictive factors such as IL-28B polymorphisms in patients with HCV genotype 2 or 3 infection may not be as important as those in the former group (12).

One patient with the heterozygous genotypes of the four SNPs showed NVR, while another patient with the major genotypes of rs12979860, rs11881222, rs8099917 and the heterozygous genotype of rs8103142 achieved SVR. Akkarathamrongsin et al. (2010) reported that most patients with heterozygous and minor homozygous genotypes of rs8103142 and rs11881222 (70% and 100%, respectively) were non-responders (4). However, another study
found NVR in a patient with the heterozygous genotypes of rs8099917 and rs12979860 and with
the major genotypes of rs11881222 and rs8103142 (10). Therefore, both rs8099917 and
rs12979860 may have a stronger influence on the treatment stronger than rs11881222 and
rs8103142.

Based on IL-28B polymorphisms, the frequency of HCV clearance varies markedly
across ethnic groups. The protective allele of rs12979860 was reported predominant in East
Asian population (13) and that of rs8099917 was predominant in European and Japanese
 ancestries (14-15). The several other SNPs within the IL-28B gene (including rs11881222 and
rs8103142) showed strong linkage disequilibrium with rs12979860 and rs8099917 (15). Among
the 25 patients achieved EVR/SVR, 84.0% (21/25) were Javanese, all of whom carried the major
genotypes of the four SNPs of IL-28B. The remaining 4 patients with EVR/SVR were Java-
Madura (1), Bali (1) and Batak-Lampung (1) people with the major genotypes of the four SNPs,
and the other Balinese (1) patient carried the major genotypes of rs12979860, rs11881222,
rs8099917 and the heterozygous genotype of rs8103142. Further studies using more samples
from patients from other ethnic groups are warranted because Indonesia has hundreds of ethnic
groups.

This study showed that although these patients (mostly Javanese) were mostly infected
with HCV genotype 1, most of them achieved good responses (EVR/SVR) to the PEG-IFN/RBV
treatment. The major types of IL-28B polymorphisms may have contributed to these results.
Further study in other ethnic groups of Indonesians is now underway in our laboratory.
The authors are grateful to all participants who donated their blood to this study. This study was supported by a grant from the Ministry of Research and Technology, Indonesia, the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), and the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. The authors would also like to thank Dr. Masashi Mizokami of the National Center for Global Health and Medicine, Ichikawa, Japan for his kindness in supporting this study.
REFERENCES


Table 1. Demographic and clinical characteristics of HCV-infected patients according to their virological responses

<table>
<thead>
<tr>
<th>Factor</th>
<th>Total</th>
<th>EVR/SVR</th>
<th>NVR/TVR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years): &gt;40 (%)</td>
<td>31/34 (91.2%)</td>
<td>24/25 (96.0%)</td>
<td>7/9 (77.7%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Gender: male (%)</td>
<td>15/34 (44.1%)</td>
<td>12/25 (48.0%)</td>
<td>3/9 (33.3%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Race: Javanese (%)</td>
<td>28/34 (82.4%)</td>
<td>21/25 (84.0%)</td>
<td>7/9 (77.7%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Pre-treatment ALT (IU/L)</td>
<td>103.5±90.50</td>
<td>110.5±104.80</td>
<td>89.5±55.14</td>
<td>0.53</td>
</tr>
<tr>
<td>HCV genotype 1 (%)</td>
<td>24/34 (70.6%)</td>
<td>16/25 (64%)</td>
<td>8/9 (88.9%)</td>
<td>0.23</td>
</tr>
<tr>
<td>Pre-treatment Metavir score: F3-F4 (%)</td>
<td>3/24 (12.5%)</td>
<td>1/15 (6.7%)</td>
<td>2/9 (22.2%)</td>
<td>0.25</td>
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</table>
Table 2. Virological responses according to IL-28B polymorphisms

<table>
<thead>
<tr>
<th>Virological Responses</th>
<th>No. of IL-28B Polymorphisms (%)</th>
<th>rs12979860</th>
<th>rs11881222</th>
<th>rs8103142</th>
<th>rs8099917</th>
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<tbody>
<tr>
<td></td>
<td>CC*</td>
<td>CT**</td>
<td>TT*</td>
<td>TC**</td>
<td>AA*</td>
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<tr>
<td>NVR/TVR (Σ = 9)</td>
<td>8</td>
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<td>8</td>
<td>1</td>
<td>8</td>
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<tr>
<td></td>
<td>(24.2)</td>
<td>(100)</td>
<td>(24.2)</td>
<td>(100)</td>
<td>(25.0)</td>
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<tr>
<td>SVR/EVR (Σ = 25)</td>
<td>25</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>24</td>
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<tr>
<td></td>
<td>(75.8)</td>
<td>(75.8)</td>
<td>(75.8)</td>
<td>(75.8)</td>
<td>(75.0)</td>
</tr>
<tr>
<td>Total</td>
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<td>33</td>
<td>1</td>
<td>32</td>
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<tr>
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<td>(100)</td>
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<tr>
<td>P value</td>
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<td>0.265</td>
<td>0.465</td>
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<tr>
<td>Odds Ratio (95% CI)</td>
<td>NA</td>
<td>NA</td>
<td>3.00</td>
<td>NA</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(0.17-53.71)</td>
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