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Interleukin-28B Polymorphisms and Response of Chronic Hepatitis C Patients from Indonesia to Pegylated Interferon/Ribavirin Treatment

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This study demonstrated that Indonesian patients with chronic hepatitis C (mostly ethnic Java people) mostly were infected with hepatitis C virus (HCV) genotype 1; however, they carried mainly the major genotypes of interleukin 28B (IL-28B) single nucleotide polymorphisms (SNPs) (rs12979860 CC, rs11881222 TT, rs8103142 AA, and rs8099917 TT), and they mostly achieved sustained virological responses to pegylated interferon/ribavirin treatment.

Plasma and peripheral blood mononuclear cells (PBMC) were collected from chronic hepatitis C (CHC) patients in Dr. Saiful Anwar General Hospital, Malang, East Java Province, or a private clinic in Sanglah, Denpasar, Bali Province, Indonesia. All the patients were treated with pegylated interferon (PEG-IFN)-alfa-2a (Pegasys) and ribavirin (RBV). Patients with hepatitis C virus (HCV) genotype 1 were treated for 48 weeks, and patients with HCV genotype 2 or 3 were treated for 24 weeks. The maternal and paternal ethnicities of each patient were carefully documented for three previous generations. Plasma and PBMC were examined at the Institute of Tropical Disease, Airlangga University, Surabaya, East Java Province. Data on the pretreatment HCV viral loads and HCV genotypes of the patients were obtained from their medical records. Ethical clearance for this study was obtained from the Ethics Committee of Dr. Saiful Anwar General Hospital.

HCV RNA was extracted from 140 μl plasma using a commercially available kit (QIAmp 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 reverse transcription-PCR (RT-PCR) (Invitrogen, Tokyo, Japan) and a set of primers. PCR amplifications using outer primers (nucleotides [nt] 7999 to 8825) and inner primers (8159 to 8630) were performed as previously described (1) using Hot Star *Taq* master mix (Qiagen). The amplified fragments were sequenced by a direct sequencing method with the BigDye Terminator v1.1 cycle sequencing kit and an ABI Prism 310 sequencer (Applied Biosystems, Foster City, CA, USA). Based on the sequence similarity to the reported sequences from the international DNA databases (DDBJ, EMBL, and 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 reexamined 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. The HCV 5′ noncoding region (NCR) was amplified with a primer and probe set, as described previously (3). The lowest detectable titer with this kit was 3.0 $\log_{10}$ RNA copies/ml. This assay was used to measure the HCV viral load posttreatment or during treatment (after 12 weeks of treatment) to determine 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 the interleukin 28B (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). The amplified fragments were sequenced with a BigDye kit 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 of <0.05 was considered significant.

A total of 34 samples were collected from 19 (55.9%) women and 15 (44.1%) men (32 to 76 years of age; mean ± standard deviation, 58.8 ± 10.90 years) with CHC. The majority of these patients were ethnic Java people (82.4%), and the other 6 patients were of Batak-Lampung (Sumatera) (2.9%), Java-Madura (2.9%), Gorontalo (Sulawesi) (2.9%), Japan-Toraja (Sulawesi) (2.9%), or Bali (5.9%) ethnicity. Among the 34 patients, 28 (82.4%) completed the entire course of the PEG-IFN/RBV treat-

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TABLE 1 Demographic and clinical characteristics of HCV-infected patients according to their virological response

Factor	All patients	Patients with EVR/SVR	Patients with NVR/TVR	*P*
Age > 40 yr	31/34 (91.2)[a]	24/25 (96.0)	7/9 (77.7)	0.16
Gender, male	15/34 (44.1)	12/25 (48.0)	3/9 (33.3)	0.70
Race, Javanese	28/34 (82.4)	21/25 (84.0)	7/9 (77.7)	0.64
Pretreatment ALT level (IU/liter)	103.5 ± 90.50	110.50 ± 104.80	89.50 ± 55.14	0.53
HCV genotype 1	24/34 (70.6)	16/25 (64)	8/9 (88.9)	0.23
Pretreatment Metavir score, F3 to F4	3/24 (12.5)	1/15 (6.7)	2/9 (22.2)	0.25

[a] Data indicate either the number of patients with the indicated characteristic/total number of patients (%) or the mean ± standard deviation.

ment and were followed up for 24 weeks. The other 6 patients were treated for >12 weeks.

In this study, virological responses to PEG-IFN/RBV were classified as being in one of two groups, (i) nonvirological response (NVR)/transient virological response (TVR) (poor response) or (ii) early virological response (EVR)/sustained virological response (SVR) (good response). Overall, 25 (73.5%) patients achieved an EVR/SVR, while 9 (26.5%) had an NVR/TVR. No significant differences in age, gender, race, pretreatment alanine aminotransferase (ALT) level, severe fibrosis (stage F3 or F4), or HCV genotype were observed 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 patients with a TVR or NVR. Most of the 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 subtypes 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 durations than patients infected with genotype 2 or 3 (7). In a study performed in the United States and Europe, 42% to 52% of patients with HCV genotype 1 achieved an 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 success.

The results showed that most of the patients (94%) carried the major genotypes (rs12979860 CC, rs11881222 TT, rs8103142 AA, and rs8099917 TT). The frequencies of the major genotypes of the four SNPs were higher in the EVR/SVR group (75.0% to 75.8%) than in the NVR/TVR group (24.2% to 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 an 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 IL-28B was correlated with a better response to PEG-IFN/RBV treatment, and minor allele-positive patients were found to be poor responders (10). Of the limited number of patients with HCV genotype 2 or 3 infection, most (90.0%, 9/10) achieved an EVR/SVR, and all of them 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 that in the former group (12).

One patient with the heterozygous genotypes of the four SNPs showed an NVR, while another patient with the major genotypes of rs12979860, rs11881222, and rs8099917 and the heterozygous genotype of rs8103142 achieved an SVR. Akkarathamrongsin et al. (1) reported that most patients with heterozygous and minor homozygous genotypes of rs8103142 and rs11881222 (70% and

TABLE 2 Virological responses according to IL-28B polymorphisms

Virological response (*n*) or data type	No. (%) of IL-28B polymorphism[a]: rs12979860 CC[b]	rs12979860 CT[c]	rs11881222 TT[b]	rs11881222 TC[c]	rs8103142 AA[b]	rs8103142 AG[c]	rs8099917 TT[b]	rs8099917 TG[c]
NVR/TVR (9)	8 (24.2)	1 (100)	8 (24.2)	1 (100)	8 (25.0)	1 (50)	8 (24.2)	1 (100)
SVR/EVR (25)	25 (75.8)	0	25 (75.8)	0	24 (75.0)	1 (50)	25 (75.8)	0
Total	33 (100)	1 (100)	33 (100)	1 (100)	32 (100)	2 (100)	33 (100)	1 (100)
P value	0.265		0.265		0.465		0.265	
Odds ratio (95% CI[d])	NA[e]		NA		3.00 (0.17–53.71)		NA	

[a] Minor types of IL-28B polymorphisms are not indicated, since there was no sample obtained.
[b] Major types.
[c] Heterozygous types.
[d] CI, confidence interval.
[e] NA, not available.

100%, respectively) were nonresponders (4). However, another study found an NVR in a patient with the heterozygous genotypes of rs8099917 and rs12979860 and with the major genotypes of rs11881222 and rs8103142 (10). Therefore, rs8099917 and rs12979860 may have stronger influences on treatment than those of rs11881222 and rs8103142.

Based on IL-28B polymorphisms, the frequencies of HCV clearance vary markedly across ethnic groups. The protective allele of rs12979860 was reported to be predominant in an 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 who achieved an 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 an EVR/SVR were people of Java-Madura ($n = 1$), Bali ($n = 1$), or Batak-Lampung ($n = 1$) ethnicity with the major genotypes of the four SNPs, and the other 1 Balinese patient carried the major genotypes of rs12979860, rs11881222, and 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 infected mostly 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.

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