The Distribution of Genotype and Allelic Frequency of *IL28B* Gene Polymorphism in Andhra Pradesh, India

Siddapuram Sivaprasad*, Padaki Nagaraja Rao**, Rajesh Gupta**, Kaitha Ashwini*, Duvvuru Nageshwar Reddy**

*Department of Medical Genetics, Institute of Basic Sciences and Translational Research, **Asian Institute of Gastroenterology, Hyderabad, India

Background: The single nucleotide polymorphism (SNP) of *IL28B* gene on chromosome 19, encoding for the interferon (IFN)- λ -3 is strongly associated with treatment response to pegylated-IFN and ribavirin in patients infected with different genotypes of hepatitis C virus (HCV). Difference between ethnicity and treatment response rates suggesting a key role of host genetics. The *IL28B* polymorphism (rs12979860C/T) shows a marked differential distribution between racial groups. **Aim:** The present study is aimed to evaluate genotype and allelic frequency of *IL28B* gene polymorphism (rs12979860C/T) in Andhra Pradesh, India. **Methods:** A total of 220 healthy controls were recruited for the study. The genotyping of SNP rs12979860C/T on *IL28B* gene was performed by polymerase chain reaction-direct sequencing method. **Results:** The frequency of CC genotype was found to be significantly (59.09%) higher compared to CT (34.09%) and TT (6.81%) genotypes, respectively. The frequency of major allele C is 0.762 whereas minor allele T is 0.238. **Conclusion:** The higher distribution of genotype 'CC' of SNP, rs12979860C/T of *IL28B* gene in study subjects is suggestive of better response of HCV patients to standard anti-HCV therapy. (J CLIN EXP HEPATOL 2012;2:112–115)

Chronic hepatitis C virus (CHC) infection is a major cause for developing cirrhosis and hepatocellular carcinoma (HCC) which often results in liver failure and require transplantation. According to World Health Organization (WHO), 180 million people are infected worldwide and annually 3–4 million new infections were estimated.¹ The current standard therapy for CHC consists of polyethylene glycol pegylated-interferon- α and ribavirin (PEG-IFN- α /ribavirin [RBV]).² Interferon- α acts through a single receptor and signals through the well-characterized janus kinase-signal transducer and activator of transcription protein (JAK-STAT) pathway to up- or down-regulate hundreds of genes that function in immune response pathways.³ Attainment of sustained virological response (SVR) in patients with hepatitis C virus (HCV) infection depends on virus-specific characteristics, disease and host genetic factors.⁴ The human genome is composed of 3.3 billion base pairs and about 10 million of these may vary in nucleotide sequence between individuals (single nucleotide polymorphism or SNP). Some of these variations

may result in altered expression of the gene or altered processing of the gene product (post-translational modification) or altered functional activity (e.g., receptor binding). Identifying polymorphisms that result in altered clinical expression (phenotype) is a challenge to unravel the disease process as well as the therapeutic response.⁵

However, in the past 2 years, genome-wide association studies (GWAS) have confirmed that polymorphisms near the *IL28B* gene on chromosome 19, which encodes the type-III IFN, predict SVR upon treatment with PEG-IFN- α /RBV in HCV-monoinfected patients bearing genotype-1.^{6–9} Specifically, SNP rs12979860C/T, located 3 kilobases upstream of the *IL28B* gene, is strongly associated with more than 2-fold difference in the rate of SVR.⁶ *IL28B* encodes IFN- λ 3, a cytokine, distantly related to type I IFNs and the interleukin (IL)-10 family. Together with *IL28A* (IFN- λ 2) and *IL29* (IFN- λ 1), *IL28B* forms a cytokine gene cluster on a chromosomal region mapped to 19q13. Expression of the cytokines encoded by these three genes can be induced by ribonucleic acid (RNA) virus infection.¹⁰

The genetic marker *IL28B* may help us to select patients who are more or less prone to respond to PEG-IFN- α /RBV. While the investigations of *IL28B* genotype have been restricted to HCV genotype 1-infected patients, recent studies extended to patients infected with HCV genotype 2 and 3 also demonstrated the striking influence of the *IL28B* genotype.¹¹ The difference between ethnicity and treatment response rate is poorly explained by host clinical factors, suggesting a key role of host genetics.^{12,13} The polymorphism (rs12979860C/T) of *IL28B* gene shows a marked differential distribution between racial groups and strongly associated with SVR in HCV-infected patients.^{6,14} This

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Address for correspondence: Siddapuram Sivaprasad, Department of Genetics, Institute of Basic Sciences and Translational Research, Asian Institute of Gastroenterology, Hyderabad, India

E-mail: shiv.sidda@gmail.com

Abbreviations: CHC: chronic hepatitis C virus; GWAS: genome-wide association studies; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; IL: interleukin; PEG-IFN- α : pegylated-interferon- α ; RBC: red blood cells; RBV: ribavirin; SNP: single nucleotide polymorphism; SVR: sustained virological response; WBC: white blood cells

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study is aimed to establish the distribution of genotype and allelic frequency of *IL28B* polymorphism (rs12979860C/T) in Andhra Pradesh, India.

MATERIALS AND METHODS

A total of 220 healthy controls were recruited in the study. The participants were patients' attendants and voluntary blood donors. All participants were confirmed to be healthy on the basis of their past medical history and routine clinical and laboratory investigations. The study is conducted at Institute of Basic Sciences and Translational Research, Asian Institute of Gastroenterology, Hyderabad, Andhra Pradesh, India. An informed consent was obtained from all the study participants and the study protocol was approved by the Institutional Ethics Committee. About 3 mL of peripheral blood sample was collected from all the study participants and processed for deoxyribonucleic acid (DNA) isolation. The DNA was isolated from white blood cells (WBC) using standard method.¹⁵ Whole blood was subjected to erythrocyte lyses buffer, and red blood cells (RBC) were removed. Total WBC pellet was subjected to proteinase-K for protein digestion and further DNA was purified. The genomic DNA was used to genotype *IL28B* polymorphism (rs12979860C/T).

Genotyping of *IL28B* Polymorphism (rs12979860C/T)

The genomic region associated with HCV treatment response lies on chromosome 19 and contains multiple SNPs in linkage disequilibrium around the *IL28B* gene.^{6,8} We genotyped the most strongly associated SNP rs12979860C/T, located 3kB upstream of the *IL28B* gene by using polymerase chain reaction (PCR)-direct sequencing method. PCR was used to amplify the polymorphic region by using a set of specific forward (5'-GGCCTGAG-GATGCAGAGAAG-3') and reverse (5'-GAGGGACCGCT-ACGTAAGTCA-3') primers. The primers flanking the

polymorphic region were designed using online software (http://cedar.genetics.soton.ac.uk/public_html/primer3.html). The PCR was carried out in a total volume of 25 μ L, containing 100–150 ng of template DNA, 5 pmol of each forward and reverse primers, 1 $\times$ buffer (100 mM Tris-HCl, [pH 8.8 at 25°C]), 500 mM KCl, and 0.8% Nonidet P40), a significant amount of MgCl₂, 100 mM dNTP, and 1 U/ μ L *Taq* DNA polymerase (Fermentas Life Sciences, Burlington, ON, Canada). Amplification was performed using thermal cycler, Eppendorf Mastercycler pro S (Eppendorf AG, Hamburg, Germany). The initial denaturation was allowed at 94°C for 4 minutes; then 34 cycles were performed with denaturation at 94°C for 1 minute, annealing at 62°C for 1 minute, and extension at 72°C for 1 minute. The final extension was carried out at 72°C for 7 minutes. A 10 μ L of amplified product was mixed with 2 μ L loading buffer and subjected to agarose gel (2%) electrophoresis to visualize 795 bp fragment (Figure 1). The PCR products were purified and sequenced individually on both strands (Figure 2) using Big Dye Terminator Cycle Sequencing

Figure 1 Electrophoresis of PCR-amplified products of single nucleotide polymorphism rs12979860(C/T) of *IL28B* gene. Lane 1: 100 bp molecular marker; lanes 2 and 3: participants PCR product.

Figure 2 Chromatogram showing sequence of single nucleotide polymorphism rs12979860 (C/T) of *IL28B* gene. Lanes 1–3 show CC, CT, and TT genotypes orderly.

Table 1 Distribution of genotype, allelic frequency, and genotype proportions of *IL28B* gene polymorphism.

Single nucleotide polymorphism of <i>IL28B</i> gene	Frequency of genotypes			Frequency of alleles		Proportion of genotypes			P value between proportions of genotypes		
	CC	CT	TT	C	T	CC	CT	TT	CC/CT	CC/TT	CC/(CC+TT)
rs12979860(C/T)	59.09	34.09	6.81	0.762	0.238	0.591	0.341	0.067	* $P < 0.0001$	* $P < 0.0001$	* $P < 0.0002$

* P value < 0.05 is statistical significant.

Ready kit (Bioserve Biotechnologies, Genome Valley, Hyderabad, Andhra Pradesh, India).

Statistical Analysis

The distribution of genotype and allele frequencies was analyzed using the principles of Hardy-Weinberg equilibrium. The frequency of genotypes was analyzed and compared by using 'proportion test'. SPSS software was used for all statistical analysis (version 11.5; SPSS, Chicago, IL, USA).

RESULTS

In this study, all 220 individuals were genotyped for SNP, rs12979860C/T of *IL28B* gene. The mean age standard deviation of the study participants was 38.75 years (± 11.70). Among study cohort, males were 170 (77.27%) and females 50 (22.73%). The distribution of genotype and allelic frequencies of SNP, rs12979860C/T, is summarized in Table 1. The distribution of allelic frequencies was in accordance to the Hardy-Weinberg equilibrium. In this study, the frequency of C/C genotype (reported to be associated with HCV clearance) was considerably high (59.09%) compared with C/T and T/T genotypes. The distribution of genotype T/T (reported to be poor in HCV clearance) was found to be the lowest. Further, allelic distribution of this polymorphism was analyzed (Table 1). The frequency of allele 'C' was found to be substantially higher and it would be denoted as 'major allele' in the study group while 'T' as 'minor allele'. A comparative analysis of proportions of different genotypes is represented in Table 1. The proportion of 'C/C' genotype of SNP, rs12979860C/T of *IL28B* gene was significantly higher compared with the proportions of genotypes 'C/T' ($P < 0.0001$) and 'T/T' ($P < 0.0001$). Furthermore, the proportional distribution of 'C/C' genotype was significantly higher when compared with C/T and T/T genotypes together ($P < 0.0002$) in study cohort.

DISCUSSION

The prediction of SVR in patients with CHC before initiation of antiviral therapy is important in order to estimate the potential for treatment success. These predictive markers can help clinicians in the decision of better patient management. This information can also prompt patients who might have a high chance for virological response. Previous studies showed that HCV genotype,

RNA concentration of HCV, age, gender, body mass index (BMI), fibrosis stage, alanine aminotransferase (ALT), gamma-glutamyl-transpeptidase (GGT) levels, insulin resistance, and host genetic polymorphism of several genes (human leukocyte antigen, chemokines, ILs, and IFN-stimulated genes) are associated with SVR.^{16,17} Presently, in clinical practice guidelines, only the HCV genotype and concentration of HCV-RNA at baseline are recommended to be used in determining treatment duration but still the therapy is frequently complicated by treatment-limiting side effects.¹⁸⁻²¹ The most recent genome-wide association studies reported association of different SNPs of *IL28B* gene (IFN- λ gene region) with response to antiviral therapy, where SNP, rs12979860(C/T) of *IL28B* gene, was reported to be strongly associated.⁶⁻⁸ Hence, SNP rs12979860(C/T) of *IL28B* gene is considered to be a significant host genetic marker in prediction of SVR by standard therapy. In this study, we genotyped SNP rs12979860(C/T) of *IL28B* gene for the first time in India. Our study showed that the frequency of the allele C is significantly higher in Andhra Pradesh population. A recent GWAS study analyzed a random multi-ethnic population samples with unknown hepatitis C status and reported a substantially higher frequency of the C allele in East Asian population followed by European-American, Hispanic, African-American populations and also reported the C allele association with spontaneous clearance.⁶ Hence, this study is in concordance with earlier studies and may be predicted that the study population has superior chance of spontaneous clearance and therapeutic response for HCV infection.

The previous studies estimated that approximately half of the difference in SVR can be accounted due to the difference in frequency of the C allele between African-Americans and individuals of European ancestry. Interestingly, it has also been well documented that East Asians have higher SVR rates than patients of European ancestry.^{22,23} The SVR rates across different population groups displayed a striking concordance with C allele frequency which emphasizes the greater importance of individual genotype than ethnicity in predicting treatment response.²⁴ A previous study from Germany, extended to HCV genotype 2 and 3 infected patients, also analyzed 200 healthy controls for comparison and reported the frequency of CC, CT, and TT genotypes with 49%, 42.5%, and 8.5%, respectively.¹¹ Another study conducted on a group of 154 patients co-infected with HIV and HCV from European

ancestry analyzed rs12979860 genotype and reported the genotype CC in 68 patients (44%), CT in 66 patients (43%), and TT in 20 patients (13%). Although, the frequency of C allele was significantly higher among patients achieved SVR, the genotype CC was not significantly deviated from genotype CT.²⁵ A recent study from East Asian patients of CHC with genotypes 2a and 2b reported a substantially higher frequency (78.58%) of CC genotype of SNP rs12979860.²⁶ The frequency of CC genotype reported from East Asia is higher compared with the frequency of CC genotype of this study. However, all previous studies reported the gradual reduction in frequency of rs12979860C allele of *IL28B* gene from East Asian population to African-American population.

In conclusion, we genotyped SNP, rs12979860C/T of *IL28B* gene, and report that the frequency of CC genotype is significantly higher (59.09%) than that of CT (34.09%) and TT (6.81%) genotypes. The frequency of major allele C is 0.762 while that of minor allele T is 0.238. Since the CC genotype is reported to be a strong predictor for therapeutic clearance of HCV, extension of present findings using larger study cohort might provide information for better management of HCV-mediated disease in Indian subjects.

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CONFLICTS OF INTEREST

All authors have none to declare.

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