

## **Interleukin 28B Polymorphism Predicted Treatment Response in Egyptian Children with Chronic Hepatitis C Virus Infection**

**Yasser K Rashed<sup>1\*</sup>, Mohamed A El Guindi<sup>1</sup>, Hala H El said<sup>2</sup> and Ola Fouad<sup>1</sup>**

<sup>1</sup>*Department of Pediatric Hepatology, Egypt*

<sup>2</sup>*Department of Clinical Biochemistry, National Liver Institute, Menoufiya University, Egypt*

**\*Corresponding Author:** Yasser K Rashed<sup>1</sup>, Department of Pediatric Hepatology, Egypt.

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### **Abstract**

**Background:** Host genetic factors is the most important factor responsible for spontaneous and treatment-induced clearance of HCV infection from infected patients. Sustained virological response was associated with single nucleotide polymorphisms (SNPs) near the interleukin 28B (IL28B) Purpose: The aim of this study to evaluate the role of a single nucleotide polymorphism rs12979860 near the Interleukin 28B gene in predicting the response to Pegylated interferon- $\alpha$  and ribavirin treatment in chronic hepatitis C virus infected children.

**Methods:** Sixty nine patients were included in this study with chronic HCV infection, from Pediatric Hepatology Department, National Liver Institute, Menoufia University. They were 40 males and 29 females. The mean age of our patients was 11.1 $\pm$  3.6 years, Detection of the IL28B rs12979860 C/T polymorphism done in 67 patients.

**Results:** The CC genotype of rs12979860 had a higher SVR (78.9%) as compared to the non-CC genotypes (25.0%) with statistically significant difference (P < 0.0001).

**Conclusion:** IL28B rs12979860 genotyping could be a useful tool for predicting treatment outcome in children with chronic HCV.

**Keywords:** HCV; IL28B; Polymorphism; Interferon; Genotype

### **Abbreviations**

HCV: Hepatitis C virus; SNP: Single Nucleotide Polymorphism; IFN- $\lambda$ : Interferon Gamma I; L28B: Interleukin B28; PCR: Polymerase Chain Reaction

### **Introduction**

One of the major causes of chronic liver disease globally is hepatitis C virus infection. The long-term complications of HCV infection are extensive fibrosis, cirrhosis and hepatocellular carcinoma. About 170 million are chronically infected [1].

Chronic hepatitis C infection remains an important health care problem in different communities. Effective treatment of chronic hepatitis C can prevent the long-term complications of chronic infection and improve quality of life. Until recently Peg-IFN  $\alpha$ -2b in combination with ribavirin are the only approved for treatment of HCV in children since approved Food and Drug Administration (FDA) and European Medicines Agency (EMA) in December 2008 and September 2009 [2].

Host genetic factors are the most important spontaneous and treatment-induced clearance of HCV infection from infected. Single nucleotide polymorphism (SNP) near the IL28B gene on chromosomes 19 that encodes IFN- $\lambda$ 3, is associated with treatment-induced and spontaneous clearance of HCV from infected patients predicting the response to the antiviral therapy [3].

Direct-acting antivirals (DAAs) showed great success in the treatment of chronic hepatitis C in adults [4]. The use of Ledipasvir/Sofosbuvir in the treatment of adolescents with chronic hepatitis C virus was approved by the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) in 2017. (HCV) Genotypes 1, 4, 5 and 6, and infections with genotypes 2 and 3, respectively. Until now, DDA only approved in the form of combination of Ledipasvir/ Sofosbuvir for the treatment of children and young adults aged 12 - 17 years weighing more than 35 kg.

Advanced liver diseases were seen in 4% of children with chronic HCV infection. the progress to advanced liver disease in such children could be prevented by curing HCV infection [5].

To date, PEG IFN alpha-2a or -2b and ribavirin are the only drugs currently approved for the treatment of chronic hepatitis C in children under 12 years of age [6]. There is a great deal of toxicity from combined treatment with Interferon plus ribavirin. Approval for direct-acting antiviral (DAA) regimens for children aged 3 to 11 is expected in the near future.

### Aim of the study

The aim of this study to evaluate the role of a single nucleotide polymorphism rs12979860 near the Interleukin 28B gene in predicting the response to pegylated interferon- $\alpha$  and ribavirin treatment in hepatitis C virus infected children.

### Methods

Patients selected in this study were sixty seven patients with chronic HCV infection, from Pediatric Hepatology Department, National Liver Institute, Menoufia University. the study extended from September 2011 till August 2016. Selected patients were 40 males and 29 females. The mean age of our patients was  $11.1 \pm 3.6$  years, they underwent full history taking and thorough clinical examination and laboratory investigations including HCV antibody, Real time PCR for HCV-RNA (The detection limit is 15 IU/mL) and hepatitis B viral markers. Sixty Seven (67) patients received antiviral therapy consisting of Peg-IFN- $\alpha$  2b with a dose of 60 ug / 1.73 m plus ribavirin 15 mg/kg/day orally for 24 weeks. According to the response, patients were classified into two groups, responders; they were 27 patients, 18 males and 9 females and non-responders; they were 40 patients, 20 males and 20 females while the virus spontaneously cleared in only 2 patients. Patients were divided into two categories, responders and nonresponders, according to sustained virological response (SVR) (undetectable HCV RNA at 24 weeks after completion of treatment).

Viral markers were done using manual ELISA technique. HCV antibodies were done by kit from inngentics, Ghent- Belgium (Pawlotsky, 2002). Using the ELISA procedure, HBV surface antigen (HBsAg) and HBV core antibody [(HBcIgM) and (HBcIgG)] were performed using a kit from Sorin Biomedica Co, Spain [7]. HCV-RNA real-time PCR: This was conducted using COBAS Ampliprep /COBAS TaqMan, Roche Molecular Systems, Inc., Branchburg, NJ, USA [8]. It is a procedure for the quantification of HCV-RNA for nucleic acid amplification.

Specimen preparation is automated using the COBAS Ampliprep instrument and using the COBAS TaqMan analyzer for automated amplification and detection. The detection limit is 15 IU/mL [9].

### Liver biopsy

Ultrasound guided liver biopsies were done, after sedation using midazolam (0.3 mg/kg/ dose), for all patients by true cut needle. Biopsy specimens were fixed in formalin-buffered saline, embedded in paraffin followed by a histological examination using hematoxylin and eosin stains, orcién stain and Periodic acid Schiff (PAS) stain for routine histopathological evaluation. Hepatic necroinflammatory activity and liver fibrosis were evaluated according to Ishak staging and grading scores [10]. Necroinflammatory activity was classified into no necroinflammatory activity (score 0) minimal (score 1-2) mild (score 3-5), moderate (score 6-8) and severe (score 9-18). Fibrosis was classified into no fibrosis (stage 0) mild (stage 1), moderate (stages 2-3), and severe fibrosis or cirrhosis (stages 4-6) [11].

### Detection of the IL28B rs12979860 C/T polymorphism

For genotyping of the IL28B rs12979860 C/T polymorphism using a PCR-based constraint fragment duration polymorphism test, 5ml of whole blood was obtained in vacutainer tubes containing EDTA (RFLP).

### Principle

#### Restriction fragment length polymorphism (RFLP)

Restriction endonucleases cut double-stranded DNA into fragments of reproducible size; the same enzyme produces the same fragments in different specimens if the specimens contain the same DNA sequence. If an alternation in the DNA abolishes or creates a cleavage site recognized by the enzyme (or changes the spacing between two cleavage sites), then electrophoresis of digested fragments will reveal those changes (or polymorphisms) in fragment length: hence the name restriction fragment length polymorphism (RFLP).

### Procedure

Genomic DNA was collected from entire blood samples using the manufacturer's instructions for the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The 139-bp product was amplified with the following primers using purified genomic DNA: forward primer 5' - C C A G G G C C C C T A A C C T C T G C A - 3' and reverse primer 5' - GGGAGCGGAGTGCAATTCA-3'. Amplification was carried out with a total volume of 50 µL containing 10 mmol/L of Tris HCl (pH 8.3), 50 mmol/L of KCl, 0.01% of Tween-20, 0.2 mmol/L of deoxyribonucleotides, 2-4 pmol of each primer, 2.0 mmol/L of MgCl<sub>2</sub>, 0.5 units of hot-start Taq DNA polymerase (Thermo Taq, Thermo Scientific, Pittsburgh, PA, USA) and approximately 10 ng of genomic DNA. The thermal protocol for amplification included 35 cycles of denaturation at 95 °C for 60 s, annealing at 62 °C for 60 s, and elongation at 72 °C for 60 s. Ten amplicon microliters were digested at a total volume of 20 µL at 37 °C overnight with 1 unit BstUI (New England Biolabs, Hitchin, UK). After staining with ethidium bromide, the fragments were resolved by 4 percent agarose electrophoresis. The TT genotype indicated a band of 139 bp, the CC genotype indicated 109 bp, and the CT genotype indicated 139 + 109 bp.

### Exclusion criteria

Patients with combined viral infection, other coexistent liver disease such as Wilson' disease, autoimmune hepatitis etc., decompensated liver disease, or impaired renal functions were all excluded from the study.

### Inclusion criteria

HCV RNA positive by PCR, Liver biopsy in the past 18 months with METAVIR score over A2 and over or equal to F1, or over or equal A1 and over F2, ALT over 1.5 time the normal range in the 24 weeks prior to inclusion (Week-28; W-2);, Patients never treated with ribavirin, IFNalpha or PEG-IFNalpha, Normal albumin, HBs antigen negative, Hemoglobin over or equal 11g/dl,, Leucocytes over or equal 3000/mm<sup>3</sup>, neutrophils over or equal 1500/mm<sup>3</sup>, Platelets over or equal 100 000/mm<sup>3</sup>, normal creatinine, normal TSH, No features of other causes of chronic liver diseases.

### Statistical analysis

Data were collected analyzed using the SPSS package for Windows, version 18.0, SPSS Inc., Chicago, Illinois, USA. The frequency and percentage were expressed as qualitative data. The mean ± standard deviation of quantitative results was seen as (SD). Tables and graphs were placed with the results.

The following tests were conducted to test for significance:

- Chi square tests were done to compare qualitative variables.
- Student t-test or Mann-Whitney test were done to compare of 2 sets of quantitative data as appropriate.
- Kruskal-Wallis test or ANOVA test were used to compare of multiple sets of quantitative variables as appropriate.
- Correlation coefficients were calculated by Spearman’s test.
- Probability (P) value was considered to be significant if it was ≤ 0.05.

**Ethical points**

The study followed the ethical standards of national liver institute- Menofiya university- Egypt, committee (IRB00003413). During the interview, the respondents (parents) of the children were simply informed about the aims of this study. Written consent was taken from the Parents who accompanied the child during attending the mentioned hospitals before participating in the research.

**Results**

The present study included 67 children with proved chronic HCV infection, 38 (56.7%) patients were males and 29 (43.3%) were female.

According to the response, the remaining 67 patients were classified into two groups:

Responders: Patients who achieved SVR. They were 27 patients, 18 males and 9 females with age range 9.1±3.2 years.

Non responders: Patients who didn’t achieve SVR. They were 40 patients, 20 males and 20 females with age range 9.4 ± 3.8 years.

Pretreatment serum HCV- RNA (IU/ml) 888103.18 ± 1768081.42 in responders while was 1548752.7 ± 4093936.28 in non –responders.

The three rs12979860 genotypes in HCV-infected patients are CC, CT and TT. We found a higher frequency of CT genotype (50.7%) than CC (27.5%) and TT (21.7 patients%).

There was a statistically significant difference between responders and non-responders regarding IL28B genotype rs12979860 (P-value < 0.0001). Sustained virological response to antiviral therapy was obtained from 55.6% of the CC genotype, 40.7% of the CT genotype, and 3.7% of the TT genotype.

The CC genotype of rs12979860 had a higher SVR (78.9%) as compared to the non-CC genotypes (25.0%) with statistically significant difference (P < 0.0001).

Studied variables TT N=13 (19.4%)		IL28B			P-value	
		CT N=35 (52.2%)	CC N=19 (28.4%)			
Sex	Male	8 (61.5%)	17 48.6%	13 68.4%	0.34	
	female	5 (38.5%)	18 51.4%	6 31.6%		
Age(years)	Mean	9.80	8.32	10.07		0.14
	SD	(4.31)	(3.54)	(3.35)		

**Table 1:** Correlation of IL28B genotype with the age and sex of studied patients.

IL28B genotype	Responders n = 27		Non-responders n = 40		P-value
	n	%	N	%	
TT	1	(3.7)	12	(30)	<0.0001
CT	11	(40.7)	24	(60.0)	
CC	15	(55.6)	4	(10.0)	

Table 2: Comparison between responders and non-responders regarding IL28B genotype.

IL28B genotype	Responders n = 27		Non-responders n = 40		P-value
	N	%	N	%	
CC N=19	15	(78.9%)	4	(21.1%)	<0.0001
Non-CC N=48	12	(25.0%)	36	(75.0%)	

Table 3: Comparison Between Responders and Non-Responders Regarding IL28B Genotype (CC and Non CC).

Studied variable TT N=13		IL28B			P-value
		CT N=35	CC N=19		
HCV level of viremia	Mean	823102.9231	1801595.485	640669.1579	0.417
	SD	1525524.89	4428907.055	1227481.698	

Table 4: Correlation of IL28B genotype with pretreatment serum level of HCV RNA.

Studied variables TT N=9		IL28B genotype			P-value
		CT N=24	CC N=13		
Grade of activity	no activity	1	0	0	0.029
		11.1%	0.0%	0.0%	
	minimal	3	16	7	
		33.3%	66.7%	53.8%	
	mild	3	8	6	
		33.3%	33.3%	46.2%	
moderate	2	0	0		
	22.2%	0.0%	0.0%		
Stage of fibrosis	no fibrosis	1	2	0	0.800
		11.1%	8.3%	0.0%	
	mild fibrosis	6	18	11	
		66.7%	75.0%	84.6%	
	moderate fibrosis	2	4	2	
		22.2%	16.7%	15.4%	

Table 5: Correlation of IL28B genotype with histopathological parameters in liver biopsy.

## Discussion

There is no doubt that chronic hepatitis C infection remains an important health care problem in children and adolescents. Chronically HCV infected children may be at risk for social problems and impaired quality of life. Effective treatment of chronic hepatitis C virus at an early age may help to prevent the long-term complications of chronic infection [2].

Grade of activity no activity 11.1% 0.0% 0.0% 0.029 minimal 3 16 7 33.3% 66.7% 53.8% mild 3 8 6 33.3% 33.3% 46.2% moderate 2 0 0 22.2% 0.0% 0.0% Stage of fibrosis no fibrosis 1 2 0 0.800 11.1% 8.3% 0.0% mild fibrosis 6 18 11 66.7% 75.0% 84.6% moderate fibrosis 2 4 2 22.2% 16.7% 15.4% In 1 percent to 2 percent of children with CHC, cirrhosis was reported, and liver transplantation was performed in advanced disease for end-stage liver disease. Medical conditions associated with increased risk of more severe disease include obesity, cancer, congenital anemias requiring chronic transfusions, social factors as alcohol and coinfection with HIV or hepatitis B virus (HBV) [12].

This study included 69 patients with chronic HCV. All patients were anti-HCV and HCV-RNA positive, 67 patients received antiviral therapy consisting of Peg-IFN  $\alpha$  2b (peg-intron, Schering-Plough Brinny, U.S.A) plus ribavirin adjusted for patient's weight while only 2 patients cured the virus spontaneously. The mean age of our patients was  $9.1 \pm 3.6$  years.

In the current study, polymorphisms of IL28B (rs12979860) were analyzed and their association with the virological response to Peg-IFN alpha treatment was determined in children with HCV infection.

The three rs12979860 genotypes in HCV-infected patients are CC, CT and TT. We found a higher frequency of CT (52.2%) than CC (28.4%) and TT (19.4 patients%) (Table 1) which in agreement with other studies [13] which reported predominance genotype CT in their population in 52.7% of their patients also Cieřla., *et al.*, 2012 [14] who detected a higher frequency of CT (53%) in their patients. Same findings documented by Domagalski., *et al.* 2013 [15] found that the CT genotype was detected in 54.9% of children with HCV.

Our study reported CC (28.4%) and TT (19.4 patients%) (Table1), Uruguayan study documented that the favorable genotypes rs12979860-CC and rs8099917-TT were present in 29.5% and 57.7% of the Uruguayan population infected with HCV, respectively [16].

It was observed that, the mean values of pretreatment level of viremia had not statistically significantly difference with IL28B genotypes (P value > 0.05).

In our study SVR to antiviral therapy was obtained from 55.6% of the CC genotype, 40.7% of the CT genotype, and 3.7% of the TT genotype (P < 0.0001) (Table 2). Our results are in agreement with other studies [17] who found that SVR was obtained from 66.7% of the CC genotype, 42.9% of the CT genotype, and 28.3% of the TT genotype (P = 0.001).

In our study the CC genotype of rs12979860 had a higher SVR (78.9%) as compared to the non-CC genotypes (25.0%) with statistically significant difference (P < 0.0001) (Table 3). Our results are in agreement with (Aziz *et al.*, 2015) who found that the CC genotype of rs12979860 had a higher SVR (84.5%) than the non-CC genotypes (48.9%) (p = 0.0001). Domagalski., *et al.* 2013 [15] in their study of the impact of IL-28B polymorphisms on pegylated interferon plus ribavirin treatment response in children, they found that the CC genotype of rs12979860 had a higher SVR (76.5%) as compared to the non-CC genotypes (32.3%) (P = 0.001).

Viral load, it was found that the pretreatment serum level of HCV-RNA were lower in the CC genotype than CT and TT genotypes with no statistically significant difference (P- value > 0.05). Our results are in agreement with the study done by Montes., *et al.* 2010 [18].

In our study, minimal activity in the liver biopsy was detected in 53.8% in CC group and in 66.7% and 45.5% of CT and TT groups respectively with no statistically significant difference (Table 5).

Regarding to the stage of fibrosis, higher stages of fibrosis (moderate fibrosis) was detected in TT group (18.2%) followed by CT group (16.7%), while it was detected in 15.4% of CC group again with no statistically significant difference. Other studies [18] and [13] detected higher stage of fibrosis in CT group than other groups (CC and TT) (Table 5). The different results in our study may be because the number of patients underwent liver biopsy was lower.

### Conclusion

Finally, we can conclude that IL28B rs12979860 genotyping could be a useful tool for predicting treatment outcome in children with chronic HCV.

### Conflict of Interest

The study has no conflict of interest.

### Funding

This study was funded by the National Liver Institute, Menofiya University, Egypt, without any particular role in the study design, recruitment of individuals, data analysis or the writing of the manuscript and the authors declare that they have no competing interests.

The study was approved by the Research Ethics Committee of the National Liver Institute, Menoufia University, and is in accordance with the Helsinki Declaration of 1975 and as revised in Seoul, Korea, October 2008

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