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Effect of interleukin-10 gene promoter polymorphisms -1082 G/A and -592 C/A on response to therapy in children and adolescents with chronic hepatitis C virus infection

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ABSTRACT

Background and aim: Studying predictors of response to therapy for hepatitis C virus (HCV) infection in children may help avoid the inappropriate use of currently available costly therapy associated with numerous adverse effects. We tested the hypothesis that inheritance of single nucleotide polymorphisms (SNPs) of the interleukin-10 (IL-10) promoter gene might influence response to HCV treatment.
Patients and methods: The impact of SNPs, -1082 G/A and -592 C/A, in the promoter region of IL-10 gene, on response to HCV therapy was assessed in a cohort of 40 children treated with a combination of pegylated interferon (Peg-IFN) α2b and ribavirin.
Results: Sustained virological response was achieved in 48.7%. High viral load was associated with non-response to therapy. There was no association between histopathological degree of inflammation or fibrosis and response to therapy. There was no direct statistically significant association between polymorphisms in the IL-10 gene (-1082G/A and -592 C/A) as regards inflammation or response to therapy in children. As for the SNP -592 C/A; there was a statistically significant association with the score of fibrosis ($P < 0.004$), concluding that the A allele was protective from moderate and severe fibrosis. Meanwhile the SNP -1082G/A did not show any association with the fibrosis score.
Conclusion: We could not associate response to therapy for HCV with IL-10 polymorphisms -1082 G/A and -592 C/A. For the SNP -592 C/A, the A allele protected from moderate and severe fibrosis.

1. Introduction

According to 2014 World Health Organization report, at least 180 million people are chronically infected with hepatitis C virus (HCV) [1]. Egypt has the highest HCV prevalence in the world. It is estimated to be 8% in urban and 25% in rural areas [2,3].

Many cytokines secreted by Type 1 helper T cells (Th1) and Type 2 helper T cells (Th2) cells are involved in the immune response to HCV infection and progression of HCV-related liver disease [4]. Interleukin-10 (IL-10), secreted by Th2 cells, modulates hepatic injury by suppressing the Th1 response and counteracting fibrogenic effects of other cytokines [5] and serves to dampen inflammation that could be deleterious to the host and could limit potential tissue damage [6,7].

Regulatory mechanisms that control the production of IL-10 include genetic polymorphism particularly in the promoter region [8,9]. There is inter-individual variability in IL-10 production, which is associated with single nucleotide polymorphisms (SNPs) in the IL-10 promoter. This human genetic variation affects the innate immunity and adaptive responses against the virus and can play a significant role in the early control of viral infection. Studies found that SNPs have advantages over other genetic poly-

Abbreviations: HCV, hepatitis C virus; Th1, Type 1 helper T cells; Th2, Type 2 helper T cells; IL, interleukin; SNPs, single nucleotide polymorphisms; PEG-IFN, pegylated interferon; AST, aspartate amino transferase; ALT, alanine amino transferase; GGT, gamma-glutamyl transferase; AP, alkaline phosphatase; PCR, polymerase chain reaction; EVR, early virologic response; ETR, end of treatment response; SVR, sustained virological response; SDS, Sequence Detection System; IQR,, interquartile range; HAI, histological activity index.

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morphisms to characterize such genetic variation [10,11]. The majority of genetic association studies have focused on a series of 3 SNPs in the 5′ proximal region adjacent to IL10 consisting of −1082G/A (rs1800896), −819C/T (rs1800871), and −592C/A (rs1800872) [12].

There are conflicting results about the role of polymorphisms in IL-10 in clearance of HCV [13,14]. Several studies suggested that IL-10 polymorphisms might influence HCV outcome in the host [15–19], while others have not found association [20–23]. The current study aimed at testing the hypothesis that inheritance of IL-10 gene promoter polymorphisms (SNP -1082 G/A and -592 C/A) might influence response to antiviral treatment in HCV infected children.

2. Patients and methods

This study was approved by the Ethical Committee of Kasr AlAiny School of Medicine, Cairo University. It was carried out between 2010 to 2012, on 40 children chronically infected with HCV and attending the Pediatric Hepatology Unit in Cairo University Pediatric Hospital. All children received treatment for chronic HCV in the form of pegylated interferon (Peg-IFN) α 2b (1.5 μg/kg weekly subcutaneously) and ribavirin (15 mg/kg/day orally) after taking a written consent from parents. According to the Helsinki Declaration [24], the purpose of the study was properly explained to all the subjects included in the study and their guardians.

Chronically infected HCV children above 3 years of age, of both sexes were included. Diagnosis was based on serological, virological and histological testing. Patients were not treated previously with IFN. Exclusion criteria were as follows: (1) decompensated liver disease (2) hemoglobin <10 g/dL, leukopenia (<3000/mm^3), neutropenia (<1500/mm^3), or thrombocytopenia (<100,000/mm^3) (3) high serum creatinine (4) Existence of autoimmunity, Wilson's disease, α-1-antitrypsin deficiency, hepatitis B infection, uncontrolled thyroid disorder, poorly controlled diabetes mellitus, or psychiatric diseases.

2.1. The following laboratory work up was done for all patients

2.1.1. Routine Tests

Complete blood count (on CELL-Dyn 3700, USA), liver function tests including determination of total and direct serum bilirubin, serum albumin, aspartate amino transferase (AST), alanine amino transferase (ALT), gamma-glutamyl transferase (GGT) and alkaline phosphatase (AP) (Hitachi 911*; Roche, GmbH Mannheim Germany).

2.1.2. HCV-RNA titer

HCV-RNA titer was done using quantitative real time polymerase chain reaction (PCR) at baseline, 12, 24, 48 weeks after start of therapy and 24 weeks after end of therapy on Applied Biosystems 7500 Real time PCR System using kits supplied by Qiagen (Qiagen GmbH (Hoffmann-La Roche AG) Max-Volmer-Strabe 4-40724-Hilden-Germany). The detection limit was 15 IU/ml. HCV genotype was not determined in this study, putting in consideration that the prevalent HCV genotype among Egyptians is genotype 4 (>90%) [25].

Children whose HCV RNA titer became negative or achieved a 2 log decrease in their viral load at week 12 i.e. early virologic response (EVR) continued the antiviral therapy. If not, the child was considered to be a non-responder for whom therapy was discontinued. For responders, HCV RNA was repeated after 24 weeks of therapy and if positive the child was considered to be a non-responder and therapy was discontinued. For responders, therapy was continued till 48 weeks. At end of therapy the HCV RNA was repeated to assess the end of treatment response (ETR). For those who achieved ETR, HCV RNA titer was repeated after 24 weeks to assess the sustained virological response (SVR). Only those who achieved SVR were considered as responders.

2.1.3. Genotyping of IL-10 gene -1082 G/A and -592 C/A polymorphisms by real time PCR

DNA extraction from whole blood was done using QIAamp DNA blood Mini kit- Qiagen and was then amplified using TaqMan SNP Genotyping Assays to define the IL-10 promoter SNPs at the -1082 and -592 positions according to the protocol proposed by Kusumoto et al. [26]. Genotyping was done on Applied Biosystem step one™ Real-Time PCR System. Allelic discrimination assays were designed using TaqMan SNP Genotyping Assays (Applied Biosystems)*. Assays perform genotyping of the G → A1082 (dbSNP ID: rs1800896, TaqMan SNP Genotyping Assays ID: C_1747360_10) and C → A 592 (dbSNP ID: rs1800872, TaqMan SNP Genotyping Assays ID: C_1747363_10).

PCR reaction mix consisted of the following: Taqman universal PCR master mix (2×) 12.5 μL, 20× working stock of SNP genotyping assay 1.25 μL, patient DNA 5 μL and that was completed to 25 μL with 5 DNase-free water. Sample denaturation and enzyme activation were done at 95 °C for 10 min, cycling: 50 cycles of PCR amplification of target DNA, at 92 °C for 15 s then at 62 °C for 60 s and finally allelic discrimination plate reading and analysis using the Sequence Detection System (SDS) Software. VIC dye and FAM-dye were used for allele discrimination (Figs. 1 and 2).

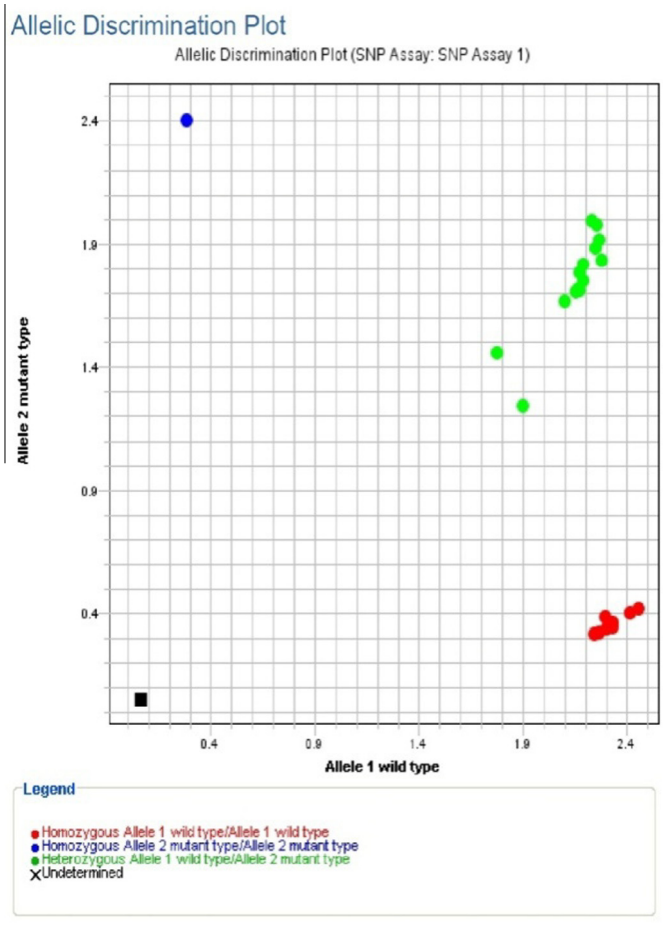

Fig. 1. Allelic discrimination plot SNP assay1 G/A-1082 done on the Applied Biosystem Step One™ Real-Time PCR System.

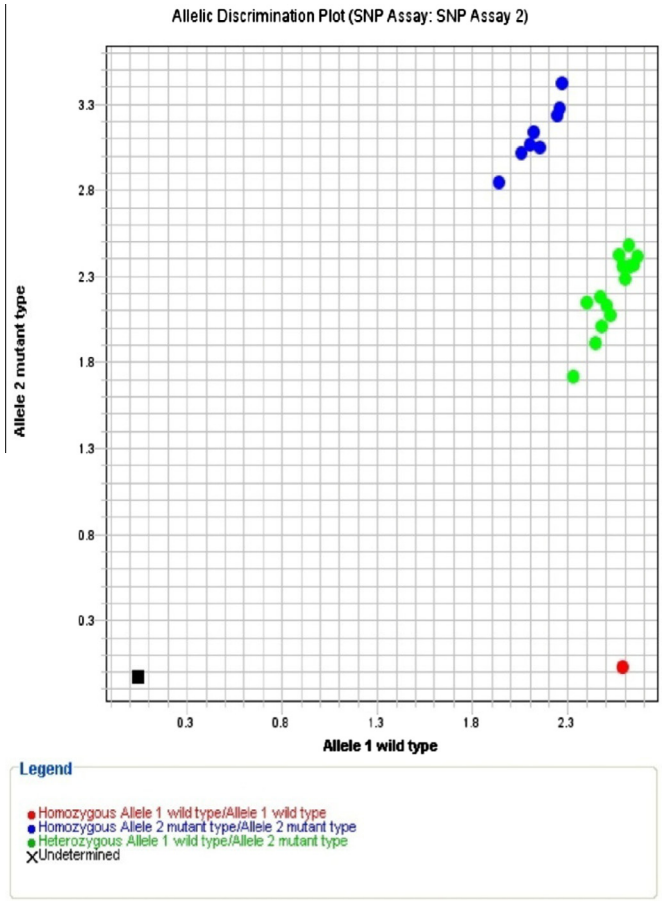

Fig. 2. Allelic discrimination plot SNP assay2 C/A-592 done on the Applied Biosystem Step One™ Real-Time PCR System.

2.1.4. Percutaneous liver Biopsy

Percutaneous liver biopsy was done using suction technique with core aspiration needle, Menghini needle (secure cut biopsy needle 16 G, HS Hospital Service S.p.A.Via A. Vacchi 23/25 Aprilia [LT], Italy). Hepatic necroinflammatory activity and liver fibrosis were assessed using Ishak staging and grading scores [27]. Histological activity index (HAI) was classified into mild (grades 1–5), moderate (grades 6–8), and severe (grades 9–18). Fibrosis was classified into mild (stage 1), moderate (stages 2–3), and severe fibrosis or cirrhosis (stages 4–6).

2.2. Statistical methods

Results were analyzed by SPSS computer software package, version 15.0. Qualitative data were expressed as frequencies. Quantitative data were expressed as mean and SD and quantitative data as median and IQR. Differences between groups were compared by Student *t* and Mann Whitney test for normally and non-normally distributed data respectively. Multivariate logistic regression analysis was done to detect independent factors predicting response to IFN therapy in chronic hepatitis C patients [28]. Differences were considered significant at P value $\leqslant 0.05$.

3. Results

Patients were divided into two groups according to their response to therapy:

Group I: Responders included patients who achieved SVR with normalization of aminotransferases (ALT and AST) levels and clearance of the virus denoted by negative HCV RNA by PCR 24 weeks after completing treatment course. This group included 19 patients, 12 males and 7 females, whose ages ranged between 6 and 17 years. One patient dropped out at end of treatment.

Group II: Non-responders included 20 patients, 14 males and 6 females, whose age ranged between 5 and 16 years.

Demographic and laboratory data of the 2 groups are presented in Table 1. Quantitative HCV RNA and GGT were significantly higher in non-responders (P = 0.001, P = 0.001 respectively) (Table 1). Multivariate logistic regression to assess the influence of different factors on response to therapy showed that only pre-treatment levels of HCV-RNA were significantly associated with treatment outcome (P = 0.001), where a lower count was observed in responders. Histological activity index (HAI) and fibrosis score in the liver biopsy done for the tested subjects showed no differences between the 2 groups. There was no statistically significant difference in the genotype and haplotype distribution in both SNPs between responders and non-responders (Tables 2 and 3). As for the second SNP -592 C/A; there was a statistically significant association with stage of fibrosis (P = 0.004), concluding that the A allele was protective from development of moderate and severe fibrosis (Table 4). When classifying the genotypes as risky genotypes (GG, GA) and (CC, CA) and non-risky genotype (AA) for SNP -1082 and SNP -592 respectively to predict response to IFN therapy in HCV infected patients; no statistically significant difference was found (Table 5).

4. Discussion

The currently available therapy for HCV infected children (Peg IFNα-2b and ribavirin), is expensive, associated with significant side effects, with only 50–70% SVR. Thus, predicting the likelihood of response to treatment before initiating therapy is very useful [29].

The main target in this study was to assess the role of the SNPs in the promoter region of IL-10 gene (-1082 G/A and -592 C/A) in the response to HCV therapy and viral clearance. We found no statistically significant difference between responders and non-responders regarding the frequency of distribution of different

Table 1
Baseline data in responders and non-responders to combined interferon/ribavirin therapy.

	Responder (n = 19)	Non responder (n = 20)	p-value
Age (years): (mean ± SD)	11.5 ± 3.2	9.98 ± 3.7	0.17
Gender			
Males (n,%)	12 (63.2%)	14 (70%)	0.74
Females (n,%)	7 (36.8%)	6 (30%)	
Serum bilirubin (mg/dl) [median (IQR)]	0.6 (0.4)	0.7 (0.5)	0.6
ALT (U/L) [median (IQR)]	57 (52)	78 (76)	0.58
AST (U/L) [median (IQR)]	47.3 (30.7)	53 (44.2)	0.6
AP (U/L) [median (IQ range)]	207 (69.2)	198.5 (125)	0.8
GGT (U/L) [median (IQ range)]	20 (16)	53 (57)	0.001
Serum albumin (g/dl) [mean + SD]	4.6 ± 0.6	4.6 ± 0.5	0.6
WBCs ($\times 1000 mm^3$) [median(IQ range)]	6.9 (3.89)	6 (3.25)	0.06
Quantitative HCV RNA level (IU/ml) [median (IQ range)]	55765 (225660)	439935 (3482665)	0.001
HAI; N (%)			
Mild	18 (94.7)	16 (80)	
Moderate	1 (5.26)	4 (20)	0.76
Fibrosis Score; N (%)			
Absent to mild	16 (84.2)	16 (80)	
Moderate to severe	3 (15.8)	4 (20)	0.54

p value was considered significant at $\leqslant 0.05$.

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Table 2
Genotypes of the two SNPs in responders and non-responders to combined interferon/ribavirin therapy.

	Responders (n = 19)	Non responders (n = 20)	p- value
SNP -1082			
GG	9 (47.4%)	6 (30%)	0.48
GA	7 (36.8%)	11 (55%)	
AA	3 (15.8%)	3 (15%)	
SNP -592			
CC	1 (5.3%)	2 (10%)	0.85
CA	10 (52.6%)	10 (50%)	
AA	8 (42.1%)	8 (40%)	

Results were presented as numbers and percentage. p value was considered significant at ⩽0.05.

Table 3
Haplotypes of the two SNPs in responders and non-responders to combined interferon/ribavirin therapy.

	Responders (n = 19)	Non responders (n = 20)	p value
GC/GC	2 (10%)	1 (5.3%)	0.761
GC/GA	3 (15%)	6 (31.6%)	
GC/AA	7 (35%)	4 (21.1%)	
GA/GA	1 (5%)	2 (10.5%)	
GA/AA	4 (20%)	3 (15.8%)	
AA/AA	3 (15%)	3(15.8%)	

Results were presented as numbers and percentage. p value was considered significant at ⩽0.05.

genotypes of the two studied SNPs, and percentage distribution of different haplotypes. Literature reports are contradictory [21,30–34] (Table 6).

In the current study, factors affecting response to therapy, other than IL-10 genotype, were also analyzed. Similar to other studies, we found that patients with SVR had significantly lower pretreatment HCV RNA levels [35,36] and multivariate logistic regression identified that pretreatment HCV RNA level is the most significant predictor of response (p = 0.001).

As regards fibrosis stages and its relation to response to treatment, contrary to other studies [37–39], we found that fibrosis staging was not associated with response to IFN treatment which could be attributed to the low prevalence of significant fibrosis among our cases.

The present study failed to demonstrate any relation between SNP -1082 G/A in IL-10 gene and the degree of fibrosis or HAI. These findings were in concordance with other studies [15,18,40]. As for the SNP -592 C/A, there was a statistically significant association between the stage of fibrosis and different genotypes (P < 0.004), concluding that the A allele was protective from moderate to severe fibrosis. Meanwhile, another meta-analysis study reported that the haplotype GCC is more likely to be associated with less severe liver fibrosis in chronic HCV patients [41].

The limitation of the present study is the small number of subjects included. However, in the literature there is scarcity of data on chronic HCV in children as compared to adults. Further studies

Table 4
Relation between gender, HAI and fibrosis, and the genotype in chronic hepatitis C infected children.

SNP -1082	GG	GA	AA	p value
Gender				
Male	8 (20%)	16 (40%)	4 (10%)	0.09
Female	7 (17.5%)	3 (7.5%)	2 (5%)	
HAI				
Mild	13 (32.5%)	13 (32.5%)	6 (15%)	0.58
Moderate	1 (2.5%)	3 (7.5%)	0 (0%)	
Fibrosis				
No fibrosis	3 (7.5%)	3 (7.5%)	1 (2.5%)	
Mild	8 (20%)	10 (25%)	5 (12.5%)	0.8
Moderate	3 (7.5%)	2 (5%)	0 (0%)	
Severe	0 (0%)	1 (2.5%)	0 (0%)	
SNP -592	CC	CA	AA	p value
Gender				
Male	3 (7.5%)	14 (35%)	11 (27.5%)	1
Female	1 (2.5%)	6 (15%)	5 (12.5%)	
HAI				
Mild	3 (7.5%)	13 (32.5%)	16 (40%)	0.07
Moderate	0 (0%)	4 (10%)	0 (0%)	
Fibrosis				
No fibrosis	0 (0%)	3 (7.5%)	4 (10%)	
Mild	0 (0%)	11 (27.5%)	12 (30%)	0.004
Moderate	3 (7.5%)	2 (5%)	0 (0%)	
Severe	0 (0%)	1 (2.5%)	0 (0%)	

Results were presented as numbers and percentage. p value was considered significant at ⩽0.05.

Table 5
Association between response to combined interferon/ribavirin therapy in HCV infected children as regards genotype: genotypes (GG, GA) and (CC, CA) and protective genotype AA.

	Responder (n = 19)	Non responder (n = 20)	OR	95% CI interval	p value
SNP -1082					
GG and GA	16 (84.2%)	17 (85%)	0.94	0.16–5.36	1
AA	3 (15.8%)	3 (15%)			
SNP -592					
CC and CA	11 (57.9%)	12 (60%)	0.92	0.26–3.29	1
AA	8 (42.1%)	8 (40%)			

Results were presented as numbers and percentage. p value was considered significant at ⩽0.05. OR = Odds ratio. CI interval = Confidence interval.

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Table 6
Supporting and conflicting studies of the major conclusions of the present study.

Conclusion of the present study	Supporting studies	Conflicting studies	Points of differences in conflicting studies
No impact of genotype distribution on response to therapy	[21,30,31]	[32]	Although the contradicting study regarding the SNP -1082 was performed on Egyptian patients as well as our study, but it was performed on adults, unlike our study, which was conducted on children only
No difference between responders and non responders regarding haplotype distribution		[33,34]	The protective factor in the conflicting studies seems to be due to inheritance of the extended haplotype rather than to inheritance of a single polymorphism of the IL-10 gene promoter and the synergistic effect of the three SNPs in the promoter region affecting IL-10 expression and consequently its role in HCV eradication as well as response to HCV therapy
Lower pretreatment HCV RNA level is a good predictor to response to therapy	[35,36]		
No association between fibrosis staging and response to therapy		[37–39]	This could be attributable to the low prevalence of significant fibrosis among our cases
No association between SNP -1082 and SNP -592 and HAI	[15,18,40]		
No association between SNP -1082 and the degree of fibrosis	[15,18,40]	[41]	The conflicting study was conducted on Asian population and concluded that a significantly decreased risk of moderate/severe fibrosis was associated with the GCC haplotype (IL10-1082G, -819C and -592C) in the overall chronic hepatitis B/C patients (OR: 0.547, 95% CI: 0.317–0.946, P = 0.031)
In SNP -592 C/A, A allele was protective from moderate to severe fibrosis			

are still needed on a larger pediatric population to conclude predictors of response to therapy in order to avoid using an expensive therapy that having numerous adverse effects. This is of particular importance, in the era of newer antiviral therapies for HCV that are not yet licensed in children. Till then, a combination of Peg-IFN and ribavirin is the accepted and available therapy for HCV infected children at the present time.

In conclusion, there was no direct association between polymorphisms in the IL-10 gene (-1082 G/A and -592 C/A) as regards response to therapy in HCV infected children. There was a significant relation between SNP -592 and degree of fibrosis in HCV infected children; allele A being protective from moderate and severe fibrosis. Higher baseline HCV viral load predicted non-response to IFN based therapy in HCV infected children.

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