Human Immunology 77 (2016) 1248–1253

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

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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ARTICLE INFO

Article history:
Received 19 October 2014
Revised 18 September 2016
Accepted 18 September 2016
Available online 19 September 2016

Keywords:
HCV
Interleukin-10
Peg-IFN
SNP -1082 G/A
SNP -592 C/A

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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http://dx.doi.org/10.1016/j.humimm.2016.09.005
0198-8859/

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).

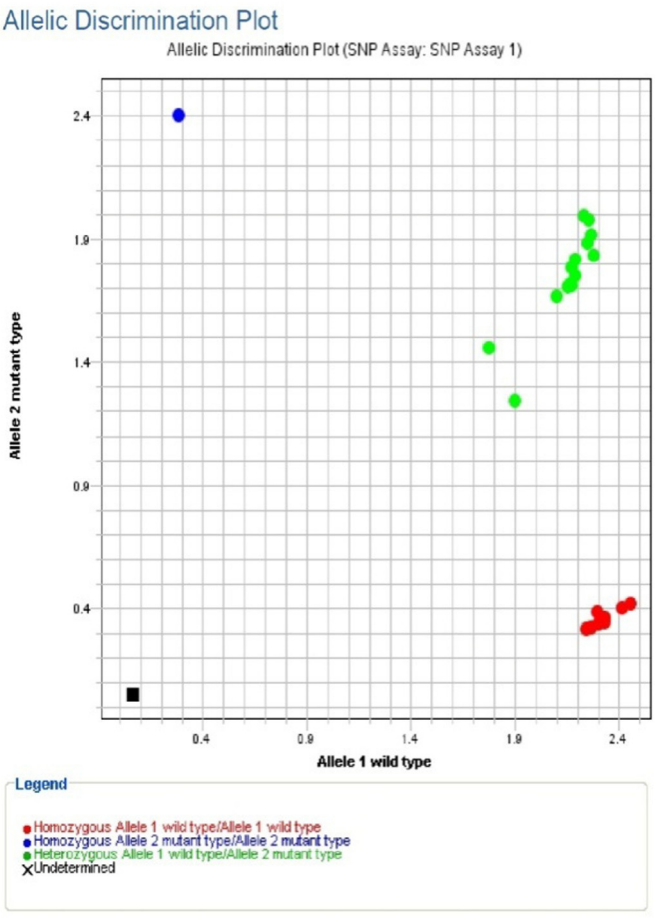

Fig. 1. Allelic discrimination plot SNP assay1 G/A-1082 done on the Applied Biosystem Step One™ Real-Time PCR System.