

## Interleukin-12 P40 (IL-12 B) *Taq-1* Polymorphism association in pharmacogenomics of HCV genotype 3a infection from Pakistani population

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**Abstract.** Interleukin-12 (IL-12) is a heterodimeric cytokine consisting of P35 (IL-12A) and P40 (IL-12B). Along with multiple functions, this cytokine has been shown to have potent role as an antiviral and antitumor cytokine. HCV clearance has also been associated with this cytokine. Currently very limited therapeutic options are available for HCV treatment and their outcome is also not encouraging. Fifteen percent of the HCV infected patients clear HCV without any treatment due to presence of efficient immune system. Among individuals receiving anti-HCV therapy (interferon, PEG-interferon or ribavirin), only 55 percent respond to the therapy and attain sustained virological response (SVR), while others are non responders or face relapse after treatment. The fate of anti-HCV treatment is determined by viral and host factors. The aim of this study was to access IL-12 gene polymorphism at 3'-UTR (1188-A/C) in pharmacogenomics of HCV genotype 3a among the Pakistani population as a genetic factor. No allele or genotype pattern was associated in pharmacogenomics of HCV. Some virological parameters were associated with therapy success as reported previously in other studies.

**Key word:** HCV, Pharmacogenomics, Interleukin-12 P40, SVR

### 1. Introduction

Hepatitis C virus is a serious health issue globally and particularly for developing countries including Pakistan<sup>[1]</sup>. In HCV pathogenesis 15 percent individual clear the virus from the body without any treatment due to presence of efficient immune system<sup>[2]</sup> and 85 percent face chronicity of infection and later other associated complications of the disease like Hepatocellular carcinoma<sup>[3-5]</sup>. It is known that Th1-type cellular immune responses are important in the resolution of HCV infection, both treatment-related and spontaneously occurring<sup>[6-8]</sup>. An imbalanced T- helper (Th) lymphocytes response plays important role in fate of HCV infection as Th2 cytokines IL4 and IL10 have elevated levels in chronic HCV infection<sup>[9]</sup>. Current HCV therapies include IFN and antiviral nucleoside/nucleotide analogues such as lamivudine, adefovir, dipivoxil and ribavirin<sup>[10]</sup>. These limited HCV therapeutic options have not very much encouraging outcome, with only 55 percent individuals responding efficiently to Interferon, PEGylated Interferon and ribavirin therapy<sup>[11,12]</sup>.

Regarding pharmacogenomics different Single Nucleotide Polymorphism (SNPs) in coding and non coding (promoter/regulatory)<sup>[13]</sup> reveal the influence of human leukocyte antigens, killer immunoglobulin like receptors, chemokines, interleukins and interferon stimulated genes on spontaneous hepatitis C clearance<sup>[14]</sup>. Many HCV patients can not respond or tolerate the typical full-dose combination therapy (FCT) or modified FCT<sup>[15,16]</sup>. Merely a small number of patients can have therapeutic success with normalization of alanine aminotransferase (ALT) and improved histological grading of liver biopsy. HCV genotype, duration of HCV infection, pretreatment viral load, Age, body mass index (BMI), ethnicity<sup>[17,18]</sup>, expression of IFN receptor genes IFNAR1 and IFNAR2 and Early virological response (EVR)<sup>[19]</sup> are major predictor of Sustained Virological response (SVR)<sup>[20]</sup>.

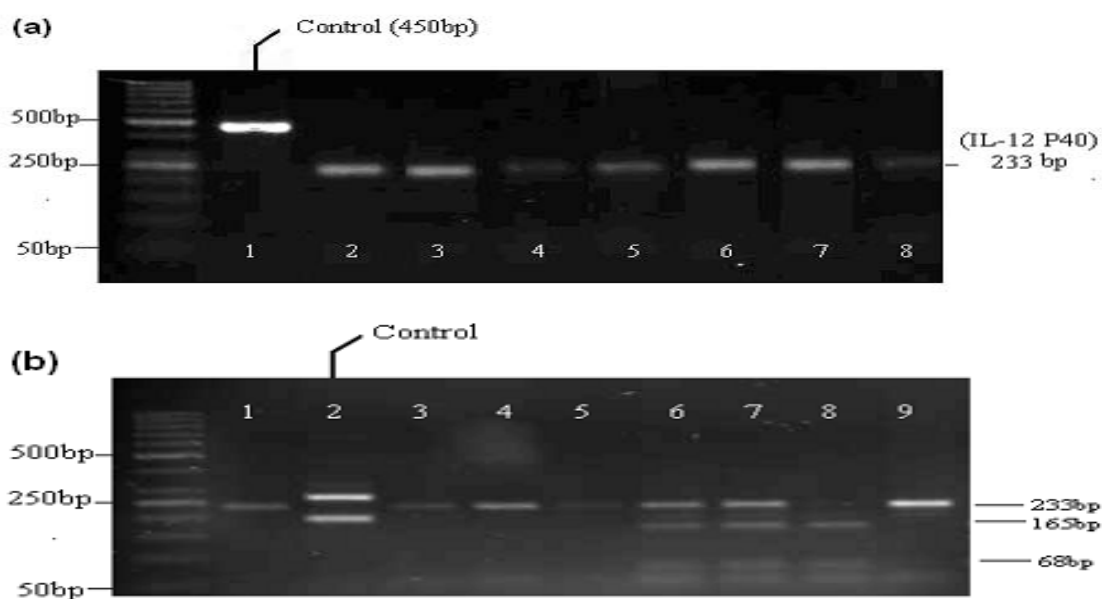
The genes for the IL-12 subunits p35 and p40 reside at independent loci in the human genome on -chromosomes 3p12-3q13.2 and 5q31-33, respectively [21]. IL12B (3'-UTR 1188-A/C), has been associated with outcome of HCV infection in Chinese and British population [22]. IL12B 3'-UTR 1188-C allele carriers population is more likely to respond efficiently to antiviral combination therapy and ultimately reduce relapse rate [23]. Interleukin-12 (IL-12) is a heterodimeric protein of 70 KD, comprises of two disulfide bonds between P35 and P40 subunits. In this study we reported a possible involvement of IL-12 P40 *TaqI* polymorphism in course of pharmacogenomics among the Pakistani subjects bearing 3a genotype of HCV.

## 2. Material and Methods

A total 122 HCV subjects, having any final fate of their therapy (SVR or relapse/none responders) with genotype 3a, were included in this study. The amplified fragment covers position 1188 at 3'-UTR of the p40 gene which encodes either the nucleotide A (allele A), or C (allele B) which results in the creation of a *TaqI* restriction enzyme site. All PCR products were digested with the restriction enzyme *TaqI*, which generated two products if allele B (1188-C) was present of size 68 and 165 base pairs, and remains uncut (233 bp) if allele A is present 1188-A) [24]. The frequency of this polymorphism was studied in HCV therapeutic outcome of populations from the Pakistan. Data was expressed as mean  $\pm$  SD and Chi-square test was performed by using *GraphPad Online Statistical Software* and 1x1 contingency tests was done by *StudyResults Trial online* (Sweden) statistical software.

## 3. Results

The electropherograms of PCR product (332 bp) and *TaqI* digestion of these products are shown fig-1(a,b). The selected 80 subjects were those who attained the SVR at the end of treatment and rest of 44 individuals were none responding or attained the relapse. IL-12 P40 (A/C) in SVR allelic frequencies were A allele (n= 114) 71 % and C allele (n=46) 29 % and NR allelic frequency A (n= 58) and C (n = 26) allele were 69 and 30 % respectively with P=0.83 as observations are shown in fig.2 (a) and graphical distribution in fig. 2(b). In SVR IL-12 P40 3'-UTR genotyping were CC = 45 %, CA= 52.5 % and AA = 2.5 %, while in IFN-NR (n= 21) group IL-12 P40 3'-UTR genotyping were AA = 55.2 %, AC= 33.3 % and CC = 14.3 %, *P values* in all genotypes were 0.58, 0.15, and 0.08 respectively. The above mentioned results show that no genotypes or allelic frequencies has any significance with SVR or none responders.

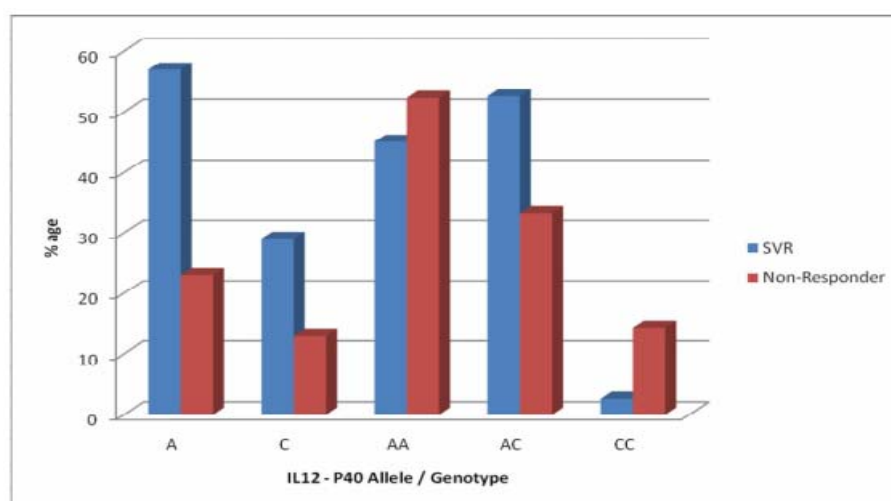


**Fig-1:** Electropherogram (a) of amplified PCR product of IL-12 P40 (3'-UTR). Lane 1 has a 450 bp *TaqI* control which is amplified from exon 4 of NF-kB gene. Lane 2 – 8, 233 bp amplified product of IL-12 P40 (3'-UTR) is shown.

Electropherogram (b) of an agrose gel electrophoresis of IL-12 P40 (3'-UTR)

Characters	SVR		None-Responder		P-value
	= n	Frequency (%)	= n	Frequency (%)	
A allele	114	71.25	58	69.04	0.83
C Allele	46	28.75	26	30.95	
Genotyping					
AA	36	45	22	52.23	0.58
AC	42	52.5	14	33.33	0.15
CC	2	2.5	6	14.28	0.08

**Table-1:** It describes the age ( $p = 0.0057$ ), ALT ( $p = <0.0001$ ), and Viral Load ( $P = <0.0001$ ) have significantly relationship in individuals who attain the SVR as compared to those who were none responders. But other non-contiguous parameter like sex has no relation. Total Leucocytes count (TLC)  $P = 0.61$ , Hemoglobin (Hb)  $P = 0.87$  and Platelets Counts (PLT count)  $P = 0.064$  have no relation (data not shown)



**Fig.2:** Tabular and graphical representation of Genotypic and Allelic frequencies of IL-12 P40

#### 4. Discussion

IL-12 is mainly responsible for the development of TH1 side of immunity, cell mediated immunity and production of interferon gamma. In the present study, we found the 3'-UTR (A>C) SNPs in IL-12B association in the pharmacogenomics of HCV infection and role in anti-HCV therapy the probability of treatment out come. In our study for SVR and Non responder groups, A or C allele did not show any significance in case of IL-12 P40 (IL-12 B) A/C-1188 but C allele were none significantly high as also reported by Hegazy *et al* (2008). IFN responder (R-IFN) group had high value of C allele as compared to A but contradictory results were obtained in case of PEGylated Interferon group where A allele was high in case of responders. C allele is high IL-12 producer. Non significant results in case of all groups whether broader or small can be due to small number of sample population. PEGylated IFN is second choice of therapy and these individuals are usually IFN non responders so this can also be reason for this deviation from unusual trend.

Our study indicates high level AA genotype in Non responders, AC was high in SVR patients while CC was observed in relapse but none of the genotypes of IL-12B found any significance association with these groups. Same observations were noted in case of IFN therapy group that frequency of AA genotype is high in NR. IL-12B 3'-UTR 1188-C-allele carriers appear to be capable of responding more efficiently to antiviral combination therapy as a consequence of a reduced relapse rate<sup>[23]</sup>. But contradictory results were also shown by some others which are supporting to our data.

Second phase of our study was analysis of biochemical and physiological parameters which include Age, Gender, ALT, Viral load, TLC, Hb and Platelets count. Age, ALT and viral load were highly associated in SVR and Non responder groups. Our study shows that age ( $P=0.005$ ) of the patient has association in outcome of HCV therapy in broader group of SVR and NR. Patient in early age respond the therapy efficiently as compared to later one. HCV chronicity outcome has previously been shown to be influenced by age<sup>[26]</sup>. Other studies show that patients infected at a higher age have more severe histological lesions and a more rapid evolution to cirrhosis<sup>[26,27]</sup>. Age and gender found no significant relation in therapy outcome group or IL-12 genotype based associations. In case of SVR and responder this can be low immunity with increasing age. Chronic patients of HCV infection have high level of IL-12, which correlated with intrahepatic HCV-specific CTL activity, due this cytotoxicity serum ALT also elevates. Such data suggests the involvement of IL-12 in the immunopathogenesis of chronic HCV infection, especially in cell-mediated immunity, which might be important in spontaneous or interferon, mediated viral clearance<sup>[28, 29]</sup>. This finding also correlate with our study which indicates high level of ALT in case of CC genotype as C is the high level of IL-12 producer allele. Platelet count has not shown any significant change in therapeutic outcome of HCV patients in general SVR group and NR group or in patients with treatment specific (IFN or PEG-IFN) out come or in case of IL-12 (both IL-12A and IL-12B) genotypic based distribution of Platelets count. But in case of viremia a general trend of low platelets count is observed in HCV patients in Taiwani population<sup>[30]</sup>. However, data distribution indicates the potential of IL-12 P40 (3'-UTR) involvement pharmacogenomics of HCV. This study can be repeated on large population to focus the importance of IL-12 polymorphisms in HCV therapy success.

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