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The –94Ins/DelATTG polymorphism in NFKB1 promoter modulates chronic hepatitis C and liver disease progression

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Abstract

Infection with Hepatitis C Virus (HCV) is one of the most important risk factor of hepatocellular carcinoma (HCC). HCV is suspected to induce HCC primarily through chronic inflammation and promotion of cirrhosis, a well-known pre-neoplastic condition. The NF-κB pathway is a key regulator of immune and inflammatory processes and plays a pivotal role in oncogenesis. Genetic variations affecting the pathway may alter NF-κB activity in response to HCV infection and contribute to liver tumorigenesis. The present study aims to evaluate the association between -94Ins/DelATTG (rs28362491) polymorphism in NF-κB1 gene promoter region and 2758G>A (rs696) single nucleotide polymorphism in the 3'UTR region of NFκBIA and the outcomes of HCV infection.

In this case-control study, 559 subjects (343 patients with HCV infection including 237 mild chronic hepatitis patients and 106 patients with Advanced Liver Disease (AdLD), 78 individuals who naturally cleared HCV and 138 healthy subjects) were genotyped for the NFκB1 and NFκBIA SNPs using PCR-RFLP. Logistic regression was used to assess the association between polymorphisms and the outcome and progression of the infection. Variation at rs696 was not associated with HCV resolution or progression (P>0.05). By contrast, the Ins/Ins genotype was associated with a 4-fold increase of AdLD risk when compared to mild chronic hepatitis C (OR= 4.69; 95% CI, 2.15-10.19; P= 0.0001) and the risk was more pronounced when compared to healthy controls (OR= 5.02; 95% CI, 2.30-10.98; P= 0.00005). Furthermore, carriage of Ins allele at rs28362491 was significantly associated with higher viral loads (P= 0.003).

Our results suggest that variation in NFKB1 gene promoter modulates the progression of chronic hepatitis C toward advanced liver disease.

Highlights

- Hepatitis C virus may induce liver cancer through chronic inflammation and promotion of cirrhosis,
- NF-κB pathway is a key regulator of inflammatory process and may contribute to liver tumorigenesis,
- The NF-κB polymorphism is not associated with naturel clearance of HCV infection,
- The NF-κB polymorphism may modulate the progression of HCV infection to advanced liver disease.

Keywords: Hepatitis C Virus; Inflammation; Hepatocellular Carcinoma; NF-κB; IκB.

Abbreviations

HCV: Hepatitis C Virus
PRR: Pattern Recognition Receptor
NF-κB:Nuclear Factor-kappa B
TNFR1 : Tumor Necrosis Factor Receptor 1
NS5B:non-structural protein 5A
IκB: Inhibitor of kappa B
TNFA: Tumor Necrosis Factor Alpha
IL8: Interleukin 8
IL1B: Interleukin 1B

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1. Introduction

Hepatitis C virus (HCV) is a positive strand RNA virusmember of the Hepacivirus genus within the Flaviviridae family (Alter, 1997). The WHO estimates that about 3% of the world's population is infected with HCV, with three to four million people newly infected each year (Liang et al., 2000). Only15 to 30% of individuals acutely infected with HCV spontaneously clear the virus. In the remaining 70 to 85% of cases the chronic hepatitis that develops due to viral persistence was proven to play a decisive role in hepatocarcinogenesis (Hoofnagle, 2002). However, the molecular and cellular mechanisms linking inflammation and liver cancer remain unclear (Freeman, 2002).Overall, the clinical outcome of HCV infection is highly variable, and multiple factors, including host genes variations, influence disease progression following infection (Ezzikouri et al., 2013; Thomas et al., 2000; Thomas et al., 2009).

After the sensing of HCV by pattern recognition receptors (PRRs), the first-line antiviral host defense response involves the activation of transcription factors and leads to induction and release of interferon, interleukin and other antiviral cytokines (Waris et al., 2003). In fact, during HCV infection, the nuclear factor-kappa B (NF-κB) signaling pathway is maintained in an activated state by two different mechanisms. The first trigger involves HCV core protein which can bind to the death domain of TNF receptor 1 (TNFR1) and activates NF-κB (Bonizzi and Karin, 2004). The second pathway depends on HCV non-structural protein 5A (NS5A) that induces endoplasmic reticulum stress leading to NF-κB activation via reactive oxygen species or IkB pathways (Waris et al., 2003).

NF-κB has been shown to regulate the transcription of more than 200 genes involved in innate and adaptive immunity, inflammation, and developmental processes. It is considered as a central positive regulator of the inflammatory response by the induction of genes such as those encoding for tumor necrosis factor alpha (TNFA), interleukin 8 (*IL8*), and 1β (IL1β)

(Bonizzi and Karin, 2004; Wagoner et al., 2007). NF-κB is also detected in tumors where it is suspected to connect inflammation with tumor progression (Bonizzi and Karin, 2004).

The NF-κB family consists of five members, NF-κB1 (p105 processed to p50), NF-κB2 (p100 processed to p52), RelA (p65), RelB, and cRel. All members of this transcription factors family form homo- or hetero-dimers and share some structural features, including a Rel homology domain (RHD), essential for dimerization as well as binding to cognate DNA elements and regulation of targeted genes expression (Baeuerle, 1998; Del Prete et al., 2011)[9, 10]. The most abundant form is the p50/p65 heterodimer. The observed over-expression of p50 in various malignancies including brain cancer, prostate cancer, gastric cancer, breast cancer, colorectal cancer, colon cancer, melanoma and bone cancer, suggests the potential involvement of p50 in tumorigenesis (Brown et al., 2007; Miyamoto and Verma, 1995).

In non-stimulated hepatocytes, NF-κB is sequestered in the cytoplasm by IκB. The IκB family includes the classical IκB proteins such as IκBα, NF-κB precursor proteins (p100 and p105), and the nuclear IκBs. These inhibitors are characterized by ankyrin repeats, which associate with the DNA-binding domains of the transcription factors thereby making them transcriptionally inactive (May and Ghosh, 1997). IκBα has been recognized to be involved in the regulation of NF-κB activity during oncogenic transformation of liver cells (Arsura et al., 2000).

The NF-κB1 (p105/p50) protein is encoded by the NFKB1 gene, which is located on chromosome 4q23-24 and consists of 24 exons. p105, an inactive precursor, is activated, by proteasome-mediated processing, to p50, a DNA binding protein. In most cancer cell lines and tissues, NF-κB dimers are activated, entailing their involvement in oncogenesis. Previous studies reported that a functional polymorphism in the promoter region of NFKB1 gene, namely the -94in/DelATTG (rs28362491) polymorphism, has a regulatory effect on the

NFKB1 gene (Karban et al., 2004) and could potentially influence the transcription of the gene and the level and function of NF-κB protein. This variation is suspected to play a role in the susceptibility of individuals to inflammatory diseases and various malignancies (Sun and Zhang, 2007).

NFKBIA gene, which encodes IκBα, consists of 6 exons and is located on chromosome 14q13. Studies on a 3'-untranslated region (3'-UTR) polymorphism 2758G>A (rs696) in NFKBIA gene were relatively rare and findings were contradictory (Curran et al., 2002; Gao et al., 2007). Recent studies associated *NFKB1* and *NFKBIA* polymorphisms with increased risk and severity in sporadic colorectal and oral cancers (Curran et al., 2002; Tan et al., 2013).

The rich and unique migration history of Morocco due to its particular geographical position, in addition to its dual Arabic-Berberic predominant ethnicity confer to Moroccan population commonalities with neighboring countries of North Africa. Consequently, Moroccan population can be considered to be representative of Maghreb population. No data assessing the relationship between the *NFKB1* and *NFKBIA* genetic variants and spontaneous clearance or progression of HCV infection are available so far.

In the present study, we attempted to determine the association between *NFKB1*-94in/DelATTG (rs28362491) and *NFKBIA*2758G>A (rs696) variants and the outcomes of HCV infection in a Moroccan population.

2. Material and Methods

2.1. Study subjects

The subjects were enrolled at the Medical Center of Biology at the Pasteur Institute of Morocco and Service of Medicine B CHU Ibn Rochd Hospital, Casablanca, from January 2010 to April 2013. In-person interview was conducted using a structured questionnaire to get information about demographic data, medical history, lifestyle and other characteristics. One hundred-thirty eight controls were selected from healthy individuals who visited the Pasteur Institute, for regular health examination. They had normal alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, and were apparently free from any form of cancer. In addition, 343 patients were positive for anti-hepatitis C virus (anti-HCV) antibodies and HCV RNA by a quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) for at least six months defined as HCV persistent group. Among them, 237 had mild chronic hepatitis C (mCHC, patients with minimal fibrosis score F0 and F1) and 106with HCV-related-AdLD (patients with advanced fibrosis F3-F4 and HCC). The group with spontaneous viral clearance comprised 78 subjects who were positive for anti-HCV and negative for HCV RNA by qRT-PCR from at least two measurements more than 6 months apart. HCV genotyping was performed by sequencing as described previously (Brahim et al., 2012). All patients were HBV and HIV negatives. The study was conformed to the ethical guidelines of the 1975 Declaration of Helsinki and approved by the ethics committee of the Faculty of Medicine of Casablanca. All participants of this study gave their informed consent.

2.2. DNA isolation

Peripheral blood was collected from the study subjects into EDTA-containing vacutainers. Genomic DNA was isolated from PBMC using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. The concentration and the purity of

the genomic DNA were assessed using a NanoVue plus spectrophotometer (GE Healthcare, US). DNA samples were stored at -20°C until use for genotyping.

2.3. Genotyping of *NFKB1* and *NFKBIA* SNPs

Genotyping of each subject for NFKB1 -94 Ins/DelATTG (rs28362491) and *NFKBIA* 3'UTR 2758G>A (rs696) polymorphisms was performed by restriction fragment length polymorphism (RFLP) as described previously (Senol Tuncay et al., 2010). Briefly, regions of 285bp in *NFKB1* and 425bp in *NFKBIA* were amplified using the PCR primer sets NFKB1 forward: 5'-TGGGCACAAGTCGTTTATGA-3' and reverse:5'-CTGGAGCCGGTAGGGAAG-3', and NFKBIA forward:5'-GGCTGAAAGAACATGGACTTG-3' and reverse: 5'-GTACACCATTTACAGGAGGG-3'. PCR was performed in a final volume of 25µl, containing 25 ng of genomic DNA, 20 pmol/µl of each primer, 1.5 mM $MgCl_2$, 200µM of each dNTP, and 0.5 unit of Taq DNA polymerase (Invitrogen, France). The PCR cycle consisted of an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30s, annealing at 62°C and 55°C for 45s, for rs28362491 and rs696, respectively, extension at 72°C for 30s and final extension step at 72°C for 7min in a 2720 thermal cycler Applied Biosystems. Ten microliters of PCR products were digested with PfIMI (10U/µl, Fermentas) for rs28362491 and HaeIII (10U/µl, Sigma) for rs696 at 37°C overnight followed by 2% agarose gel electrophoresis. For rs28362491 polymorphism, the Insertion allele (Ins), which has an ATTG Insertion, has a unique recognition site taking the form 5'...CCANNNN$^{\downarrow}$NTGG...3' and was cleaved by PfIMI, into two fragments of 45 bp and 240 bp after restriction digestion. Deletion allele (Del) presents only one ATTG and is consequently undigested. The G allele, in the rs696 polymorphism, was recognised by HaeIII (5'...GG$^{\downarrow}$CC...3') and cleaved into two fragments of 108 bp and 316 bp. Whereas, the A allele has no HaeIII recognition site.

To further validate the genotyping results, 20 samples were chosen at random and subjected to DNA sequencing as described previously (Ezzikouri et al., 2014). The results of sequencing were 100% concordant with those of digestion.

2.4. Statistical analysis

Hardy-Weinberg equilibrium was assessed by a goodness-of-fit χ^2 test with 1 degree of freedom to compare observed and expected genotype frequencies. Continuous and categorical variables were compared by Mann-Whitney U test and Fisher's exact test. Differences between groups were considered significant for P values of <0.05. The association between the different groups and allelic and genotypic frequencies, measured by the odds ratio (OR) and its corresponding 95% confidence interval (CI), was estimated using multiple logistic regression models with and without adjustment for age, sex and viral load. Statistical analyses were carried out by using SPSS version 20.0 (SPSS, Chicago, Illinois, USA).

3. Results

3.1. Clinico-demographical features

In the present study, 559 volunteers were enrolled, among them 343 patients with persistent HCV infection, 78 resolvers and 138 healthy controls. Selected baseline characteristics of study subjects are shown in Table 1.The majority of the patients were women (59.9%) and distribution of gender and age between all groups were not statistically significant (P>0.05). As expected, mean AST and ALT levels were significantly higher in persistently infected patients than in resolvers and controls (P<0.05). Most of the patients were infected with HCV genotype 1 followed by genotype 2 common in West Africa. HCV subtype 1b was predominant, a situation conform to the Moroccan epidemiology (Brahim et al., 2012).

3.2. Allele and genotype frequencies in Moroccan population

Genotyping *NFKB1*-94Ins/DelATTG and *NFKBIA* 2758G>A polymorphisms in the healthy controls group enabled, for the first time, the estimation of genotype frequencies in a population of the Middle East and North Africa (MENA) region (see Table 3**)**. The genotype distribution at the rs28362491-94Ins/DelATTG loci was identified as follows: Del/Del homozygous in 48 (34.8%) individuals, heterozygosity in 60 (43.5%) and homozygote Ins/Ins in 30 (21.7%) and at the rs696 G/A, the genotype distribution was G/G 34.1%, G/A 20%, A/A 23.9%. These data result in allele frequencies of 56.5% for Del allele (± 3.15% IC 95%) atrs28362491 and 55.1% for G allele (± 3.21% IC 95%) at rs696 (Table 2). The genotype distributions at both variants were in Hardy-Weinberg equilibrium in healthy controls (P= 0.175 for rs28362491 and P= 0.077 for rs696).

3.3. Association between the -94Ins/DelATTG polymorphism in *NFKB1* promoter region and HCV infection outcomes

The outcomes of HCV infection, including spontaneous clearance of the virus and progression of infection to advanced stage (AdLD), were analyzed according to genotypic distributions (Table 2 and Table 3**)**. Regarding the NFKB1-94Ins/DelATTG polymorphism, the frequency of Ins allele was not significantly different between individuals who spontaneously cleared HCV (49.4%) and those with persistent infection (52.6%) (P=0.461, Table 2).

To test the association between the rs28362491-94Ins/DelATTG and the progression of HCV infection, we genotyped this polymorphism in 106 patients with HCV related-AdLD and compared them with 237 patients with mCHC (Table 3). The frequency of Ins/Ins genotype was 40.6% in AdLD group, 26.6% in mCHC and 21.7% in healthy controls, while that of heterozygous Del/Ins was 47.1% in AdLD, 41.8% in mCHC and 43.5% in healthy controls. The Ins/Ins genotype was, thus, significantly overrepresented in AdLD patients compared to mCHC (p= 0.0001). This difference was even more pronounced when we compared patients with AdLD and healthy controls (p= 0.00005) (Table 3). Moreover, the frequency of the Ins allele in AdLD group (64.2%) was significantly higher than in patients with mCHC (47.5%, p= 0.00005). In multivariate logistic regression analysis using the Del/Del genotype as reference, we found that the Ins/Ins genotype was significantly associated with an increased risk of AdLD development when compared to mCHC (P= 0.0001, OR=4.69, 95% CI= 2.15-10.19) and the risk was pronounced when compared to healthy controls (P= 0.00005, OR= 5.02, 95% CI= 2.3-10.98) (Table 3).

Next, the biochemical and virological data were also analyzed according to the rs28362491-94Ins/DelATTG polymorphism (Figure 1). Patients with Ins allele showed significantly higher viral loads compared to Del allele (P= 0.003).However, we did not observe any

significant difference regarding liver enzyme ALT. Moreover, no significant correlation was found between NFKB1-94Ins/DelATTG genotypes and AST levels (data not shown).

3.4. Impact of *NFKB1*-94Ins/DelATTG polymorphism on responsiveness to peginterferon-alpha (PegIFNα) and Ribavirin (RBV) treatment

To examine whether the -94Ins/DelATTG polymorphism affects responsiveness to treatment, 51 patients with chronic HCV infection were stratified according to their response to PegIFNα plus RBV treatment. No significant correlation was found between NFKB1-94Ins/DelATTG genotypes and/or alleles and sustained virological response (data not shown).

3.5. Association between *NFKBIA* 2758G>A polymorphism in the 3'UTR region and HCV infection outcomes

Next, we decided to check the association between the NFKBIA 2758 G>A SNP and natural HCV clearance or progression to AdLD (Table 2 and 3). Genotype and allele frequencies distributions of NFKBIA 2758 G>A SNP in resolvers and persistent infected patients did not show any significant differences (P>0.05, Table 2). Furthermore, the polymorphism was not associated with progression to AdLD status (Table 3).

5. Discussion

Many regulators are involved in the inflammation-fibrosis-liver cancer sequence(Ribeiro et al., 2004), and the NF-κB signaling pathway appears to have an important role in liver function and pathophysiology (Robinson and Mann, 2010). In fact, in chronic hepatitis C, NF-κB activation is markedly modulated by viral proteins including both structural (core) and non-structural (NS5A) proteins (Sato et al., 2006; Waris et al., 2003). This virus-dependant NF-κB activation leads to persistently elevated expression of proinflammatory cytokines, chemokines and matrix metalloproteinases, which can eventually lead to fibrosis and cirrhosis, increasing the risk of progression to HCC(Sato et al., 2006). In fact, it seems that HCV developed the ability to hijack NF-kB function in his own advantage to establish a persistent infection (Ribeiro et al., 2004).

In the present study, we found that NFKB1-94Del/InsATTG is not associated with HCV spontaneous clearance. However, the -94 (Ins/+Del/Ins) genotypes were significantly associated with an increased risk of AdLD (P= 0.0004, OR= 3.27, 95% CI= 1.69-6.34) when compared to those with mCHC or with healthy controls (P= 0.0001, OR 3.7, 95% CI= 1.87-7.31). Previous works reported that the -94Ins/DelATTG polymorphism is associated with chronic inflammatory diseases, prostate, colorectal, nasopharyngeal carcinoma, breast and gastric cancers (Nakshatri et al., 1997; Yang et al., 2014; Zou et al., 2011). To the best of our knowledge, this is the first report on rs28362491-94Ins/DelATTG polymorphism and AdLD in patients with HCV infection. Our data seems to be line with recently published reports showing that -94 Ins allele is associated with hepatitis B virus-induced hepatocarcinogenesis. Indeed, *in vitro* functional assay has shown that the -94Ins allele could result in significantly higher promoter activity and increased the production of p105/p50 protein and increased nuclear protein binding ability (Karban et al., 2004). Moreover, it has been shown that alterations of *NFkB1* expression play an important role in the protection from apoptosis,

conducting to carcinogenesis (Sonenshein, 1997). Previous studies postulated that the observed correlation between -94Ins allele and the increasing risk of HCV progression could be due to the expression and activity of p50. Findings reported that p50 expression in cancer tissues is higher in individuals with the Ins/Ins genotype than in those carrying the Del allele (Riemann et al., 2007).

Surprisingly, a nested case-control study conducted in Shanghai found a positive association between Ins/Del or Del/Del genotypes with higher risk of liver cancer (OR=1.54, 95% CI 1.04-2.28)(Gao et al., 2014). However, at variance with the present report, Chinese patients were HBV-infected. Taken together, this report and our data emphasize the intrinsic context-dependent duality of NF-κB pathway during liver tumorigenesis. The difference in the protein subunits level produced between carriers of -94Del and -94In alleles may contribute to the inter-individual variations and influence the susceptibility of HCV infection progression. All together these data suggest that in HCV-infected subjects-94 Ins allele and viral-dependent NF-κB activation exert synergistic effect and lead to persistently and constitutively elevated expression of pro-inflammatory cytokines, chemokines and matrix metalloproteinases, which can eventually lead to chronicity, fibrosis, cirrhosis and progression to liver cancer (Elsharkawy and Mann, 2007).

In non-stimulated cells, inactive NF-κB complexes are present in the cytoplasm and bind to a class of inhibitor proteins known as NFKBIA inhibitors (IκBα). The *NFKBIA* 3'UTR 2758G>A polymorphism might affect the mRNA stability, translational efficacy or nuclear RNA processing and export, influencing the expression of IκBα and thus the activation of NF-κB (Cheng et al., 2013). In the present study, we investigated the association between *NFKBIA* 3'UTRG>A polymorphism and the risk to develop chronic carrier state or to progress from moderate chronic disease to advanced stage. In this study, we did not found any association between rs696 and natural clearance or with the risk of HCV progression. Our

observation seems to be line with recently published reports showing a lack of association between many SNPs in *NFKBIA*, including rs696, and liver cancer in HBV-infected patients(Gao et al., 2014).In addition, previous association studies fail to report an association between *NFKBIA* 3'UTR - 2758A>G polymorphism and breast cancer (Zhao et al., 2014). However, a Chinese study found that frequency of GA heterozygous genotype was increased in patients ≥50 years of age with colorectal cancer (Gao et al., 2007). The role of this polymorphism in cancer is thus still controversial potentially variable between tissues.

In conclusion, we presented hereby the first study connecting*NFKB1* polymorphism with hepatitis C outcome as well as progression to chronic liver disease. Overall, we conclude to an absence of activity of NFKB1-94Ins on the naturel clearance of HCV infection. However, this polymorphism is strongly associated with progression of HCV infection to advanced stages including cirrhosis and liver cancer in Moroccan patients. This situation is most probably due to constitutive activation of genes modulating inflammation and subsequent cell damage and carcinogenesis. By contrast, no association was found with the *NFKBIA* 3'UTR -2758A>G polymorphism and HCV infection outcomes. This finding highlights the potentialrole of the *NFKB1*-94Ins/DelATTG polymorphism as a predisposing factor for liver tumorigenesis.

Conflict of interest statement

The authors declare no conflict of interest.

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Figures legends

Figure 1: Biochemical and virological data according tors28362491-94Ins/DelATTG and rs696 3'UTR -2758A>G polymorphisms. (A) and (B) represent ALT and HCV viral load according to rs696 polymorphism, respectively. (C) and (D) represent ALT and HCV viral load according to rs28362491 polymorphism, respectively.

Table 1. Demographic and clinical characteristics of the subjects included

	Persistently infected patients (n=343)	Spontaneousclearance subjects (n=78)	Healthy Controls (n=138)
Male (%)	131 (38.2)	27 (34.6)	66 (47.8)
Female (%)	212 (61.8)	51 (65.4)	72 (52.2)
Mean Age ± SD, years	62.47±13.53	58.58±11.94	56.26±10.53
Median viral load (IU/l) [range]	540000 [$1.1.10^3$-$2.1.10^9$]	na	na
Viral genotypes			
Genotype 1 (1b)	69.6% (55.6%)	na	na
Genotype 2	30.4%	na	na
Alanine aminotransferase (IU/l)	71.92	36.16	28.17
Aspartate aminotransferase (IU/l)	65.88	36.9	26.7
Bilirubin (µmol/l)	43.01	19.13	-
Creatinin (mmol/l)	104.08	77.87	-

na: non applicable

Table 2. Impact of *NFKB1* and *NFKBIA* polymorphisms on the spontaneous clearance of HCV infection

Genotypes	Persistent infection (n=343) (%)	Resolvers (n=78) (%)	P value	Crude OR (95% CI)	Ad P value	Ad OR (95% CI)
NF-κB1						
Del/Del	88 (25.7)	18 (23.1)	0.061	1.80 (0.97-3.33)	0.056	1.82 (0.99-3.37)
Del/Ins	149 (43.4)	43 (55.1)	0.508	1.27 (0.62-2.62)	0.450	1.32 (0.64-2.72)
Ins/Ins	106 (30.9)	17 (21.8)	-	1.00 (reference)	-	1.00 (reference)
Recessive model	88 (25.7)	18 (23.1)	0.509	0.78 (0.38-1.61)	0.508	0.78 (0.38-1.61)
Dominant model	237 (69.1)	61 (78.2)	0.636	1.15 (0.64-2.05)	0.627	1.15 (0.65-2.06)
Del allele	325 (47.4)	79 (50.6)	0.461	1.14 (0.81-1.61)	-	-
Ins allele	361 (52.6)	77 (49.4)	-	1.00 (reference)	-	-
NF-κBIA						
G/G	98 (28.6)	25 (32.1)	0.506	1.23 (0.67-2.24)	0.530	1.21 (0.66-2.22)
G/A	131 (38.2)	31 (39.7)	0.388	1.32 (0.70-2.49)	0.385	1.32 (0.70-2.50)
A/A	114 (33.2)	22 (28.2)	-	1.00 (reference)	-	1.00 (reference)
Recessivemodel	98 (28.6)	25 (32.1)	0.705	1.34 (0.81-2.20)	0.627	1.15 (0.65-2.06)
Dominantmodel	229 (66.8)	56 (71.8)	0.636	1.15 (0.64-2.05)	0.705	0.87 (0.43-1.77)
G allele	327 (47.7)	81 (51.9)	0.337	1.19 (0.84-1.68)	-	-
A allele	359 (52.3)	75 (48.1)	-	1.00 (reference)	-	-

*Ad: Adjusted P value and OR for age and gender.

Table 3. Association of the *NFKB1* and *NFKBIA* polymorphisms with the HCV infection progression

Genotypes	Healthy (n=138) (%)	mCHC (n=237) (%)	AdLD (n=106) (%)	P value	mCHC vs AdLD Crude OR (95% CI)	Ad P value	mCHC vs AdLD Ad OR (95%CI)	P value	Crude OR (95% CI)	Ad P value	Ad O.R (95%CI)
NF-κB1											
Del/Del	48 (34.8)	75 (31.6)	13 (12.3)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)
Del/Ins	60 (43.5)	99 (41.8)	50 (47.1)	0.002	2.91 (1.48-5.75)	0.004	2.73 (1.38-5.43)	0.002	3.08 (1.50-6.31)	0.002	3.04 (1.48-6.25)
Ins/Ins	30 (21.7)	63 (26.6)	43 (40.6)	0.0001	3.94 (1.95-7.97)	0.0001	4.69 (2.15-10.19)	0.00002	5.29 (2.45-11.43)	0.00005	5.02 (2.30-10.98)
Del/Del	48 (34.8)	75 (31.6)	13 (12.3)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)
Del/Ins +	90 (65.2)			0.0002	3.31 (1.74-6.29)	0.0004	3.27 (1.69-6.34)	0.0001	3.81 (1.94-7.51)	0.0001	3.70 (1.87-7.31)
Ins/Ins		162 (68.4)	93 (87.7)								
Del/Del +	108 (78.3)			-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)
Del/Ins		174 (73.4)	63 (59.4)								
Ins/Ins	30 (21.7)	63 (26.6)	43 (40.6)	0.01	1.88 (1.16-3.06)	0.005	2.05 (1.23-3.40)	0.001	2.46 (1.40-4.30)	0.003	2.35 (1.33-4.14)
Del allele	156 (56.5)	249 (52.5)	76 (35.8)	-	1.00 (reference)	-	-	-	1.00 (reference)	-	-
Ins allele	120 (43.5)	225 (47.5)	136 (64.2)	0.00005	1.98 (1.42-2.76)	-	-	0.000006	2.33 (1.61-3.36)	-	-
NF-κBIA											
G/G	47 (34.1)	71 (30.0)	27 (25.5)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)
G/A	58 (42)	89 (37.5)	42 (39.6)	0.462	1.24 (0.70-2.21)	0.549	1.20 (0.66-2.19)	0.463	0.79 (0.43-1.47)	0.485	0.80 (0.43-1.49)
A/A	33 (23.9)	77 (32.5)	37 (34.9)	0.438	1.26 (0.70-2.28)	0.530	1.21 (0.66-2.22)	0.05	1.95 (1.00-3.80)	0.05	1.97 (1.01-3.85)
G/G	47 (34.1)	71 (30.0)	27 (25.5)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)
G/A+A/A	91 (65.9)	166 (70.0)	79 (74.5)	0.396	1.25 (0.75-2.1)	0.507	1.20 (0.70-2.05)	0.149	1.51 (0.86-2.65)	0.151	1.51 (0.86-2.66)
G/G+G/A	109 (76.1)	160 (67.5)	69 (65.1)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)
A/A	33 (23.9)	77 (32.5)	37 (34.9)	0.661	1.11 (0.69-1.81)	0.785	1.07 (0.65-1.77)	0.06	1.71 (0.98-2.98)	0.05	1.77 (1.01-3.11)
G allele	152 (55.1)	231 (48.7)	96 (45.3)	-	1.00 (reference)	-	-	-	1.00 (reference)	-	-
A allele	124 (44.9)	243 (51.3)	116 (54.7)	0.403	1.15 (0.83-1.59)	-	-	0.032	1.48 (1.03-2.12)	-	-

*Ad: Adjusted P value and OR for age, gender and viral genotype.

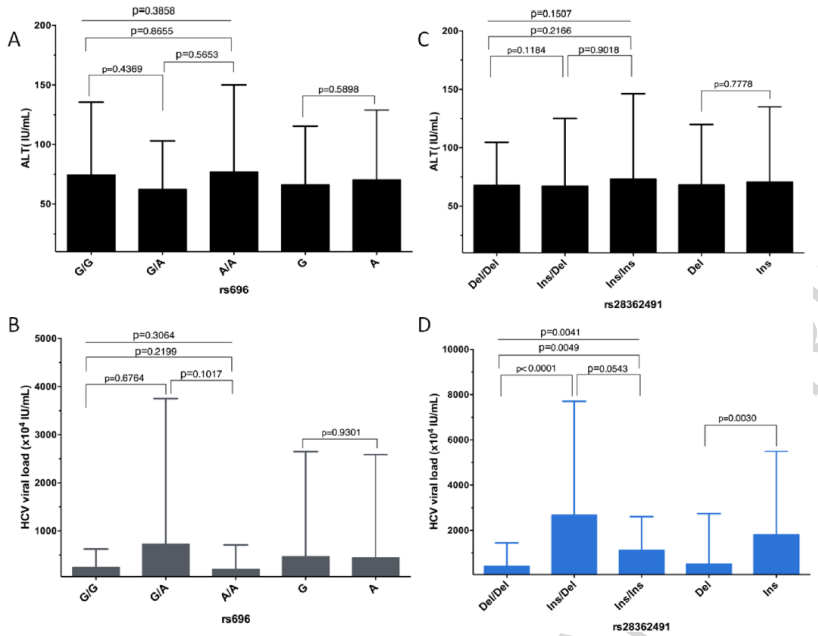

Figure 1