Single nucleotide polymorphisms in INFL3-INFL4 region are associated with fibrosis and cirrhosis in Egyptian patients with chronic hepatitis C

Khaled Thabet¹, Amany A. Bekhit¹, Elham Ahmed Hassan², Abeer Shara³, Heba M. Eltahir¹, Meky M. Abouzied², Abo Bakr F. Ahmed*  

¹ Department of Biochemistry, Faculty of Pharmacy, Minia University, Minia, 61519, Egypt  
² Department of Gastroenterology and Tropical Medicine, Faculty of Medicine, Assiut University, Assiut, Egypt  
³ Department of Pharmacology and Toxicology, College of Pharmacy, Taibah University, Al-Madinah Almunawwarah, Kingdom of Saudi Arabia  
* Correspondence: Abo Bakr F. Ahmed  
Tel.: (+20) +2013443561  
Fax: (+20) 086-236-9075  
Email Address: abobakr_fathe@mu.edu.eg

Abstract

Genetic variables have a notable and polygenic impact on the incidence and development of hepatic fibrosis and cirrhosis in individuals with chronic hepatitis C (CHC). Genetic polymorphisms in interferon-λ3-interferon- λ4 (INFL3-INFL4) region, rs12979860 and its highly linked disequilibrium polymorphisms (rs368234815 and rs4803217) are linked to liver inflammation and fibrosis progression in CHC individuals from different ethnic groups. For Egyptian patients, no data available about the degree of linkage disequilibrium between these variants and their effect on liver fibrosis. In this study, 99 healthy controls and 176 CHC patients were genotyped for 3 variants in (INFL3-INFL4) region rs12979860, rs4803217, and rs368234815. We found that these variants are highly linked disequilibrium in Egyptian CHC cohort. Moreover, we found that the major alleles of these 3 polymorphisms (rs12979860, rs4803217, and rs368234815) significantly increase the risk of liver fibrosis ($P = 0.015, 0.026$, and $0.007$ respectively). Additionally, these variants were linked to stage of hepatic fibrosis. This report shows that the 3 linked disequilibrium variants in INFL3-INFL4 region may be valuable foretellers of liver fibrosis and cirrhosis in CHC Egyptian patients.

Keywords: INFL3-INFL4 variants, liver fibrosis, cirrhosis, CHC, Egyptian patients

1. Introduction

Hepatitis C virus (HCV) causes an inflammatory liver illness (hepatitis C) that ranges from acute mild condition to most likely serious chronic disease (in 70-80% of patients) which can cause serious liver damage, usually hepatic fibrosis which develops into liver cirrhosis and then hepatocellular carcinoma (HCC) [1,2]. Globally, about 58 million individuals with chronic hepatitis C (CHC) virus infection, and 1.5 million new cases are reported each year [3]. According to the WHO, 290,000 deaths from hepatitis C in 2019, mainly from cirrhosis and HCC [3]. The outcomes of HCV infection are greatly affected by complicated interactions between viral, host genetic and environmental factors. All these factors control the inter-individual variability in the hazard and development of liver inflammation and consequential fibrosis [4-7]. With approximately 14.7 percent in 2009 and 10 percent in 2015 which is greater than the global average [8]. Despite improvements in the country's national HCV treatment programme and HCV management plan, current data indicate that HCV is still transmitted in Egypt, with higher prevalence rates than in other countries [8]. Recently, direct-acting antiviral drugs (DAADs) increase the eradication rate of HCV; however, some genetic variants increase the susceptibility of hepatic fibrosis and subsequent cirrhosis due to CHC infection [9,10].

Till now, there are no approved anti-fibrotic drugs and there are many reports elaborated the core role of studying human genetics in identifying novel therapeutic targets; therefore, CHC candidates are considered excellent paradigm for exploring the genetic origin of fibrosis [7,11-13].

HCV does not have direct effect on hepatocytes, therefore; the caused liver damage, fibrosis and cirrhosis are mainly due to inflammation and mediated by immune response [14]. Several reports confirm that genetic factors have a main role in controlling the response of the immune system and interactions between host and virus; moreover, studies explain that several genetic factors collectively influence the probability of developing hepatic fibrosis and cirrhosis due to their mild separated effects [11,12,15].

The interferon group plays a crucial role in fighting against a broad range of infectious diseases, and inflammation has a major effect in this role [16]. Type III interferons (IFN-λ) have antiviral effect in the liver, and it is the predominant interferon subtype synthesized during HCV infection [16,17]. At present, several reports show several genetic and functional proofs that IFN-λ has a fundamental effect on liver inflammation and fibrosis hazard, with many studies exploring that SNPs in the IFNL3–IFN4 region, mainly detected by genome-wide association studies...
GWAS as for the prediction of HCV clearance, are a robust predictor of hazard [18–20]. Concentrations of IFN-α are raised and associated with inflammation and fibrosis markers [21,22]. Moreover, in cell culture models of HCV, IFN-α directly stimulates a positive feedback inflammatory cascade [23]. Additionally, while production of type I and II interferons is reduced during HCV infection and in advanced hepatic disease, IFN-α receptor production is unaffected [24–26].

SNPs in the INFL3-INFL4 region, rs12979860, rs4803217 and rs368234815 were determined as a powerful forerunner of HCV clearance [27–29]. Several reports showed the genetic and functional association of these variants with liver fibrosis in different ethnicities [30–32]. This association was found with other hepatic infection, like HBV [33,34], other hepatic illnesses, like non-alcoholic fatty liver (NAFLD) [33,35], and even other organs fibrosis [36,37].

Till now, majority of the reports exploring the genotypic linkage of INFL3-INFL4 region single nucleotides polymorphisms (rs12979860, rs4803217 and rs368234815) and the hazard of liver fibrosis and cirrhosis in CHC patients have not focused on Egyptian population. It is well known that the influences of genotypic variations are greatly affected by ethnicity, because dissimilarity in allele/genotype ratio, distribution of haplotype [38–40]. Therefore, for the first time in this report, we explored the effect of INFL3-INFL4 region variants (rs12979860, rs4803217 and rs368234815) on the hazard and grade of liver fibrosis and cirrhosis in CHC Egyptian patients. This investigation is important in directing the progress in polygenic risk-score-based models with an advanced predictive importance which can recognize the hazard and development of hepatic fibrosis.

**Patients and Methods**

**Patient cohort**

In this study 275 participants (176 CHC patients and 99 healthy control) were recruited from Al-Rajhi Liver Center, Assiut University Hospital, Assiut, Egypt from 2021 to 2022. To be included in the study, patients must undergo a FibroScan® and FibroTest® with fibrosis stage scoring before beginning any antiviral treatment. Any subject that has a positive result for other organs fibrosis (e.g., HBV, NHLD, etc.) was excluded from this study. To be involved in the control group, the candidate should not have any history of chronic hepatic illnesses. Before starting this work, Ethical approval for this study was given by the research ethics committee, Faculty of Pharmacy, Minia University. Each subject provided a written informed assent and was obtained information about the nature of disease and the performed diagnostic tests.

**Hepatic fibrosis staging**

In order to stage fibrosis without using invasive methods like getting biopsies, FibroScan®, a non-invasive technique for assessment of hepatic fibrosis, and FibroTest®, a highly precise serum biomarker technique for assessment hepatic fibrosis [41]. Level of fibrosis was determined by hepatic stiffness evaluation by FibroScan®. Stage of fibrosis was on a scale: < 7 Kpa for stage F0 (no fibrosis)-F1 (low fibrosis), ≥ 7 Kpa for F2 (moderate fibrosis), ≥ 9.5 Kpa for F3 (severe fibrosis), and ≥ 12 Kpa for F4 (cirrhosis) [42].

**Genotyping**

As described previously [30], genotyping for 3 polymorphisms in INFL3-INFL4 region (rs12979860, rs4803217 and rs368234815) was undertaken by TaqMan® SNP genotyping allelic discrimination tool (Cat. No.: 4351379, Assay ID: C-7820464-10 for rs12979860, Cat. No.: 4351379, Assay ID: C-27922735-10 for rs4803217, and Cat. No.: 4351379, Assay ID: C-203097338-10 for rs368234815, ThermoFisher Scientific, USA). All genotyping was blinded to any clinical data of the patients.

**Data analysis**

Statistics was done by Prism- GraphPad version 8 (GraphPad software, USA) and SPSS version 16 for Windows (IBM Corp., USA). The Shapiro-Wilk Test for Normality was used to determine data normality. Comparison of continuous data was done by Student’s t-test or Mann-Whitney U test and presented as means ± standard deviation (SD) or median and range. Comparison of qualitative variables was done by chi-squared ($\chi^2$) and shown as percentage. Regression analysis was used to identify the hazard factors for severe liver fibrosis. $P$ value should be less than 0.05 in order to be regarded as statistically significant.

**Results**

**Grade of hepatic fibrosis:**

According to results of FibroScan® and FibroTest®, there were 62 CHC patients with no/low liver fibrosis level (F0-F1), 56 patients with moderate/sever level of hepatic fibrosis (F2-F3), and 58 CHC candidates with cirrhosis (F4). Detailed laboratory data of the group is shown in supplementary table 1.

**Genotyping**

After genotyping, minor allele frequency (MAF) of different genotypes for control and CHC patients was calculated to be in Hardy-Weinberg equilibrium (HWE) equal to 0.293 for control while equal to 0.381, 0.344, and 0.386 for rs12979860, rs4803217 and rs368234815 respectively in CHC patients. Table 1 summarizes the distribution of genotype of the 3 SNPs in control and CHC patients.

**Table 1:** The genotype distribution of the 3 SNPs in controls and CHC patients

<table>
<thead>
<tr>
<th>SNP</th>
<th>Control</th>
<th>CHC patient</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>[C]</th>
<th>[T]</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12979860</td>
<td></td>
<td></td>
<td>49 (49.8%)</td>
<td>42 (42.4%)</td>
<td>8 (8.19%)</td>
<td>70.7 %</td>
<td>29.3 %</td>
</tr>
<tr>
<td>rs4803217</td>
<td></td>
<td></td>
<td>62 (35.2%)</td>
<td>94 (53.4%)</td>
<td>20 (11.4%)</td>
<td>61.9 %</td>
<td>38.1 %</td>
</tr>
<tr>
<td>rs368234815</td>
<td></td>
<td></td>
<td>71 (40.3%)</td>
<td>89 (50.6%)</td>
<td>21 (11.9%)</td>
<td>65.6 %</td>
<td>34.4 %</td>
</tr>
</tbody>
</table>
As previously reported [30,31,37] we did the genetic association statistics for the 3 SNPs considering a dominant model for the minor allele as a protective allele for fibrosis and cirrhosis; however, the genetic association statistics considering other models (e.g., recessive and additive) are in the supplementary data (supplementary tables 2-7).

**Linkage Disequilibrium.**

We explored the linkage disequilibrium (LD) between the three variants in Egyptian HCH patients participated in this study. The LD between rs12979860 and rs368234815 was stronger in the participants ($r^2=0.97$) than the LD between rs12979860 and rs4803217 ($r^2= 0.91$), while the LD between rs368234815 and rs4803217 was the weakest ($r^2= 0.88$). Table 2 summarizes the LD between the three SNPs.

**Table 2.** Pair-wise linkage disequilibrium ($r^2$) between three variants in *IFNL3/IFNL4* region

<table>
<thead>
<tr>
<th></th>
<th>rs12979860</th>
<th>rs368234815</th>
<th>rs4803217</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12979860</td>
<td>0.97</td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td>rs368234815</td>
<td>0.97</td>
<td>0.91</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Minor alleles of rs12979860, rs4803217, and rs368234815 variants in *IFNL3-IFNL4* region decrease the risk of liver fibrosis and cirrhosis.

We investigated the association between different alleles of the 3 examined variants and hazard of hepatic fibrosis and cirrhosis in CHC Egyptian patients. The minor (protective) alleles of the three examined SNPs are present in a higher frequency in the CHC participants group with no/low (F0/F1) hepatic fibrosis than in those with moderate/sever liver fibrosis (F2-F4). This difference was statistically significant under the dominant model of inheritance ($P=0.05$, $0.05$, and $0.03$ for rs12979860, rs4803217, and rs368234815 respectively). Interestingly, the association of the major alleles in the 3 examined variants with transition to fibrosis (F0 against $\geq$ F1) was more significant ($P=0.015$, $0.026$, and $0.007$) than their association to the severity of liver fibrosis (F0-F1 against F2-F4) (tables 3 and 4) (figures 1 and 2). Moreover, the major (risk) alleles of the 3 SNPs are present in higher frequency in CHC patients with cirrhosis (F4) as described in table 5 and figure 3. The association of the major alleles in rs4803217 and rs368234815 with cirrhosis was significant ($P=0.03$ and $0.047$ respectively); however, association of the major allele of rs12979860 with cirrhosis was in trend of significance ($P=0.06$).

**Table 3:** Genotype frequencies and link of *INFL3-INFL4* SNPs with fibrosis occurrence ($\geq$ F1) in dominant model

<table>
<thead>
<tr>
<th><em>INFL3-INFL4</em> polymorphisms</th>
<th>CHC patients</th>
<th>OR (95% CI)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12979860</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT-TT</td>
<td>40 (35.1%)</td>
<td>74 (64.9%)</td>
<td>Reference</td>
</tr>
<tr>
<td>CC</td>
<td>11 (17.7%)</td>
<td>51 (82.3%)</td>
<td>2.5 (1.18-5.34)</td>
</tr>
<tr>
<td>rs4803217</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GT-TT</td>
<td>37 (35.2%)</td>
<td>68 (64.8%)</td>
<td>Reference</td>
</tr>
<tr>
<td>GG</td>
<td>14 (19.7%)</td>
<td>57 (80.3%)</td>
<td>2.2 (1.1-4.5)</td>
</tr>
<tr>
<td>rs368234815</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT/ΔG - ΔG/ΔG</td>
<td>41 (35.7%)</td>
<td>74 (64.3%)</td>
<td>Reference</td>
</tr>
<tr>
<td>TT</td>
<td>10 (16.4%)</td>
<td>51 (83.6%)</td>
<td>2.8 (1.3-6.2)</td>
</tr>
</tbody>
</table>

Figure 1. Genotype frequencies and linkage of *INFL3-INFL4* SNPs to fibrosis occurrence ($\geq$ F1) in dominant models. (A) rs12979860, (B) rs4803247, and (C) rs368234815. Significant association was present when $P < 0.05$. 

In the current work, we studied the association of genetic variants in \textit{INFL3-INFL4} region (e.g., rs12979860, rs4803217, and rs368234815) to hepatic fibrosis, severity of liver fibrosis as well as cirrhosis in CHC Egyptian cohort. Based on our results, the 3 examined variants are highly linked disequilibrium in CHC Egyptian patients and are linked to: 1) the occurrence of hepatic fibrosis, 2) to the level of hepatic fibrosis, and 3) to cirrhosis in a dominant model of inheritance for the minor alleles (the protective alleles). Liver fibrosis is a consequence of persistent inflammation and is a prevalent result of different hepatic illnesses such as viral infections [43]. It happens over years or decades of prolonged or recurrent organ injury and destruction with a continuous immune response [43]. It is manifested by an excessive change in the liver architecture leading to impairment of organ function, change intrahepatic blood flow and might lead to liver cirrhosis [43]. Therefore,

Table 4. Genotype frequencies and linkage of \textit{INFL3-INFL4} SNPs to moderate/severe fibrosis (≥F2) in dominant model.

<table>
<thead>
<tr>
<th>\textit{INFL3-INFL4} polymorphisms</th>
<th>CHC patients</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fibrosis level &lt; F2 (n = 62)</td>
<td>Fibrosis level ≥F2 (n = 114)</td>
<td></td>
</tr>
<tr>
<td>rs12979860</td>
<td>CT-CT 46 (40.4%)</td>
<td>68 (59.7%)</td>
<td>Reference 0.05</td>
</tr>
<tr>
<td></td>
<td>CC 16 (25.8%)</td>
<td>46 (74.2%)</td>
<td>1.94 (1.01-3.84)</td>
</tr>
<tr>
<td>rs4803217</td>
<td>GT-TT 43 (41%)</td>
<td>62 (59%)</td>
<td>Reference 0.05</td>
</tr>
<tr>
<td></td>
<td>GG 19 (26.8%)</td>
<td>52 (73.2%)</td>
<td>1.9 (1.02-3.65)</td>
</tr>
<tr>
<td>rs368234815</td>
<td>TT/ΔG - ΔG/ΔG 47 (40.9%)</td>
<td>68 (59.1%)</td>
<td>Reference 0.03</td>
</tr>
<tr>
<td></td>
<td>TT 15 (24.6%)</td>
<td>46 (75.4%)</td>
<td>2.1 (1.06-4.2)</td>
</tr>
</tbody>
</table>

Figure 2. Genotype frequencies and linkage of \textit{INFL3-INFL4} SNPs to moderate/severe fibrosis (≥F2) in dominant models. (A) rs12979860, (B) rs4803247, and (C) rs368234815. Significance was considered when \( P < 0.05 \).

Table 5. Genotype frequencies and linkage of \textit{INFL3-INFL4} SNPs to cirrhosis (= F4) in dominant model.

<table>
<thead>
<tr>
<th>\textit{INFL3-INFL4} polymorphisms</th>
<th>CHC patients</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO cirrhosis F0-F3 (n = 118)</td>
<td>Cirrhosis F4 (n = 58)</td>
<td></td>
</tr>
<tr>
<td>rs12979860</td>
<td>CT-CT 82 (71.9%)</td>
<td>32 (28.1%)</td>
<td>Reference 0.06</td>
</tr>
<tr>
<td></td>
<td>CC 36 (58.1%)</td>
<td>26 (41.9%)</td>
<td>1.85 (0.97-3.54)</td>
</tr>
<tr>
<td>rs4803217</td>
<td>GT-TT 77 (73.3%)</td>
<td>28 (26.7%)</td>
<td>Reference 0.03</td>
</tr>
<tr>
<td></td>
<td>GG 41 (57.8%)</td>
<td>30 (42.2%)</td>
<td>2.01 (1.06-3.8)</td>
</tr>
<tr>
<td>rs368234815</td>
<td>TT/ΔG - ΔG/ΔG 83 (72.2%)</td>
<td>32 (27.8%)</td>
<td>Reference 0.047</td>
</tr>
<tr>
<td></td>
<td>TT 35 (57.4%)</td>
<td>26 (42.6%)</td>
<td>1.93 (1.0-3.69)</td>
</tr>
</tbody>
</table>

Figure 3. Genotype frequencies and linkage of \textit{INFL3-INFL4} SNPs to cirrhosis (= F4) in dominant models. (A) rs12979860, (B) rs4803247, and (C) rs368234815. The significance level was set at \( P < 0.05 \).
inflammation and fibrosis are strongly associated, as proved in different chronic hepatic illnesses [43]. Single nucleotide polymorphisms in the \textit{IFNL3-IFNL4} loci have been linked to liver inflammation and fibrosis from different etiologies in different ethnicities, however; alleles that are associated to enhanced HCV clearance relates to serious inflammation and fibrosis[44–47] Study of \textit{IFNL3-IFNL4} polymorphisms is important now because individuals with homozygous major alleles genotype (rs12979860CC, rs368234815TT, and rs4803217CC) have shown sever clinical conditions, including severe fibrosis, hepatic cirrhosis, hepatocellular carcinoma (HCC), and finally, death.[30,35,45,48] Thus, inclusion of \textit{IFNL3-IFNL4} variants in detection and prognostication analysis offers a good tool for determining the level of liver fibrosis and foreseeing the hazard of severe clinical consequences [49], confirming the demand for early antiviral treatment in individuals carrying the \textit{IFNL3-IFNL4} variants risk alleles in order to decrease the hazard of rapid fibrosis and cirrhosis progression.

The explanation for the effects of these variants was introduced by Eslam and his colleagues who showed that the occurrence of this \textit{IFNL3-IFNL4} risk haplotypes leads to elevation of INF-λ3 expression more than INF-λ4 [30], that has been linked to elevated concentrations of plasma and hepatic inflammatory and fibro-genic biomarkers [21], mainly sCD163 (a marker for macrophage activation) [30]. This leads to a complicated chains of inflammatory reactions including hepatic migration of T cells [30,50,51], stimulation of macrophages (the main controller of liver inflammation and fibrosis) [50,52], and activation of cellular apoptosis [53]. These eventually appear as hepatic inflammation and fibrosis. Our results help in correct choice and classification of patients for further clinical experiments of current anti-fibrotic treatment. Moreover, help in discovery of new anti-inflammatory and antifibrotic drug targets depending on control of IFN-λ expression [13]. To sum up, for the first time we have shown the highly linked disequilibrium between 3 variants in \textit{IFNL3-IFNL4} region (rs12979860, rs4803217, and rs368234815). Additionally, we have extended the linkage of these SNPs to liver fibrosis and cirrhosis in a new cohort of CHC Egyptian patients, which may be a valuable predictor, helping therapeutic choices in local clinical centers.

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Conflict of interests
The authors declare no conflict of interest.

References


