BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2020, VOL. 77, NO. 2, 91–93
https://doi.org/10.1080/09674845.2019.1683310

BIOMEDICAL SCIENCE IN BRIEF

Single nucleotide polymorphisms rs12979860 and rs8099917 in IL-28B and spontaneous clearance of hepatitis C genotype 4

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ARTICLE HISTORY Received 18 August 2019; Accepted 9 October 2019
KEYWORDS IL28B; hepatitis C; rs12979860; rs8099917

Hepatitis C virus (HCV) is associated with liver disease worldwide, an estimated 170 million are infected. The majority of the infected patients develop chronic liver disorders that range from fibrosis, and cirrhosis to hepatocellular carcinoma. Genotype 4 is the main form of HCV in Africa and Middle East, and around 25% of the infected patients clear the virus spontaneously within 24 weeks without treatment as defined by undetectable level of HCV RNA in plasma. The mechanism of spontaneous eradication depends on the host immune response mainly through the interferon-mediated pathway [1–3]. Among cytokines involved in the antiviral pathway through interferon is interleukin-28 (IL-28), which has two isoforms: IL-28A and IL-28B. IL-28 has been shown to play a role in the adaptive immune response against viruses through induction of two proteins 2′,5′-oligoadenylate synthetase and ISGF3G Interferon Stimulated Gene Factor 3 [4]. The variation of the host immune responses affects the clearance of the viruses and the genetic polymorphism of cytokines has a vital role. Several studies have reported that single nucleotide polymorphisms (SNP) of IL-28 B have an effect on the response of HCV to therapy [5,6]. A recent study linked SNPs in IL-28B (rs12979860) with cirrhosis, fibrosis and hepatocellular carcinoma [7]. We hypothesized differences in SNP_S for IL-28B at two sites (rs12979860 and rs8099917) can distinguish active infection from the spontaneous clearance of HCV.

The study was conducted in Mansoura University Hospital, Egypt between January 2017 and March 2018, recruiting 100 patients with positive HCV antibodies at two or more times with positive HCV-RNA by real-time PCR at two or more times over 6 months, and elevated alanine aminotransferases (ALT). These were compared to 100 subjects positive for HCV-IgG at two or more times and negative HCV –RNA by real-time PCR at two or more times within 6 months and with normal ALT. Inclusion criteria were age >18 years, negative for hepatitis B virus, HIV and with no other hepatic disease such as malignancy, alcohols or non-viral hepatitis. The study was approved by Mansoura ethical committee and informed consent was obtained from each participating subject. From each subject, 10 mL of blood was obtained under sterile condition and separated into two tubes one without anticoagulant and the other with EDTA for peripheral blood mononuclear cells (PBMCs) separation. The blood samples in the plain tubes were separated for determination of liver functions tests alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and albumin by Dialab autoanalyzer system. DNA was extracted from PBMCs by the use of QIAamp DNA blood mini kit provided from Qiagen according to the manufacture's protocol. The amplification of rs8099917 and rs12979860 was carried by the use of the primers: for IL28B-rs12979860: F AGCAGGA CAGATTGGCAAAG, R CACAATTCCCACCACGAGAC. For IL28B-rs8099917: F CTGGAACAAATCGTCCCAAT, RTT CCTTTAGGCCTGTGGATG. The amplification process was performed by a Qiagen amplification kit (Qiagen-Germany). The total amplification volume was 20µl including 2.0 µl 10x PCR/buffer liquid, 1.5 µl $MgCl_2$, 1.0 µl dNTP, 0.5 µl forward primer, 0.5 l reverse primer, 0.5 l Taq DNA polymerase, 2.0 µl template DNA, and the rest was filled with sterile water. PCR reaction condition included the following cycles, predenaturation at 95°C for 6 min; denaturation at 94°C for 50 s, annealing at 56°C for 50 s, and extension at 72°C for 50 s (35 cycles); and extension at 70°C for 5 min. RFLP of the amplified product (694-bp product) was performed by digestion with Hpy1661 for rs12979860 genotype differentiated into; CC (509 + 185 bp), CT (509 + 185 + 155 + 300 bp), TT (509 + 155 + 30 bp). Identification by BseMI (BsrDI-Thermofisher- USA) enzyme to differentiate IL-28B genotypes of rs8099917 into TT (496 bp), TG (496 + 272 + 224 bp) and GG (272 + 224 bp). The restriction pattern of IL-28B genotype was analysed on 2% agarose gel. Which showed bands of different sizes. Data were analysed with SPSS v24. Continuously

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variable data are presented as mean with standard deviation [SD] or median with interquartile range [IQR]. The comparison between the groups was done by One Way Analysis of Variance (ANOVA). The HCV-RNA units are presented as median/interquartile range (IQR) and analysed by Kruskal Wallis test to assess the statistical significance of the difference between more than two groups of non-parametric variables. Categorical data are expressed as percentage and compared by the Chi–Square test. P-value will be considered significant at < 0.05.

The two groups were matched for age and sex but not (unsurprisingly) for liver function tests (Table 1). There were marked differences in genotypes and alleles in both rs12979860 and rs8099917. Those with the TT genotype and T allele in rs12979860 were more likely to have chronic hepatitis C, whilst those with the TT genotype and T allele in rs8099917 were more likely to have spontaneously cleared the virus. Of the patients with chronic hepatitis C infection, 13 had Child-Pugh stage 1 disease, 54 had stage 2 and 34 had stage C disease. Child-Pugh stages and genotypes are shown in Table 2. In both SNP groups, those with stages A or B were more likely to carry CC, whereas those with group C were more likely to carry TT (48.5% in rs12979860 and 54.5% in rs8099917). There were no differences in liver function tests, except that ALT was higher in rs8099917 GG versus TT. HCV-RNA was higher in rs12979860 TT and rs8099917 GG compared to other genotypes.

Hepatitis C virus infection is a serious public health issue in many parts of the world. We report differences in two SNPs in IL28B that are related to the presence or spontaneous clearance of this virus. Our extend those of others who linked the TT genotype in rs12979860 to fibrosis, cirrhosis and hepatocellular carcinoma [7]. Moreover, others found the CC genotype to be linked with sustained response to antiviral therapy in HCV genotype 4 [8,9]. The spontaneous clearance of the virus was also associated with predominance of TT genotype of rs8099917. This association was similar to previous report [10]. These results indicate the involvement of the same IL28B SNPs in both spontaneous and treatment-induced control of HCV infection [11]. Therefore, these results might indicate protective roles for C and T genotypes of rs12979860 and rs8099917, respectively, and its association with spontaneous clearance of HCV genotype 4. The mechanism of such association is not fully defined but it appears to be related to the differential responsiveness to interferon signalling between the CC and TT genotypes and other genotypes [12]. There were reduced levels of CC genotypes of rs12979860 and TT of rs8099917 in patients with chronic hepatitis C compared to those with spontaneous clearance of HCV 4. Similar findings were reported in other groups of patients with different ethnicity and different genotypes of HCV [13].

We also report the association of (unfavourable) genotypes in patients with chronic hepatitis C with advanced Child-Pugh classification, higher viraemia and elevated ALT. The stages of liver fibrosis affect the response to antiviral therapy [14]. A previous study found a link between IL28B and hepatic fibrosis and the favourable rs8099917 TT genotype had a protective effect against advanced hepatic fibrosis and the SNP should be evaluated before treatment [15]. We suggest our data promote the view that an early start of therapy in patients with an unfavourable IL28B genotype would be beneficial.

Table 1. Comparison between subjects with spontaneous viral clearance and patients with chronic hepatitis C.

Parameter	Spontaneous viral clearance (n = 100)	Chronic hepatitis C (n = 100)	*P*
Sex Male (No.-%)	56 (56%)	52 (52%)	0.7
Female (No.-%)	44 (44%)	48 (48%)	
Age (years)	54 [8.4]	54 [11.2]	0.7
Albumin (g/L)	40 [4]	30 [6]	0.0001
AST (IU/L)	27 [12.9]	59 [20.4]	0.0001
ALT (IU/L)	30 [13.8]	49 [20.3]	0.0001
Total bilirubin (μmoL/L)	10 (6.8–25)	34 (20–89)	0.0001
IL28B- rs12979860			
CC (No.-%)	80 (80%)	50 (50%)	
CT (No.-%)	10 (10%)	22 (22%)	
TT (No.-%)	10 (10%)	28 (28%)	0.0001
IL28B- rs 8099917			
TT (No.-%)	65 (65%)	50 (50%)	0.0001
TG (No.-%)	15 (15%)	30 (30%)	
GG (No.-%)	20 (20%)	20 (20%)	
C/T (No.)	171/29	101/99	0.0001
T/G (No.)	145/55	111/89	0.0003

Data mean [SD], median [IQR] or n (%)

Table 2. Frequency of different genotypes according to Child-Pugh classification and comparison between laboratory findings in patients with chronic hepatitis C and different genotypes of IL28B.

	IL28B- rs12979860				IL28B- rs 8099917			
	CC	CT	TT	P	TT	TG	GG	P
Child-Pugh A	13	0	0	0.0001	13	0	0	0.0001
Child-Pugh B	30	12	12		34	18	2	
Child-Pugh C	7	10	16		3	12	18	
Albumin g/L	30 [5]	29 [5]	31 [5]	0.5	29 [5]	31[6]	31 [4]	0.2
Total bilirubin μmoL/L	59 (34–69.3)	58 (31.6–66.1)	55 (45–64.6)	0.2	59.5 (34–85)	60 (49–89)	59 (51–77)	0.7
AST IU/L	59 [20.8]	56 [19.7]	62 [20.4]	0.6	58 [20.5]	64 [18.3]	53 [21.9]	0.2
ALT IU/L	47 [18.5]	49 [20.3]	52 [23.3]	0.5	43 [16.9]	51[22.4]	59 [20.9]	0.01
HCV-RNA ($X10^6$) IU/ml	0.50 (0.19–1.40)	0.52 (0.21–1.10)	0.72 (0.09–3.5)	0.001	0.34 (0.13–1.0)	0.42 (0.17–1.80)	0.95 (0.20–3.20)	0.001

Data mean [SD], median [IQR] or n (%)

These data are an advance in biomedical science as they highlight protective roles for C and T genotypes of rs12979860 and rs8099917, respectively, and its association with spontaneous clearance of HCV genotype 4.

Disclosure statement

No potential conflict of interest was reported by the authors.

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