Biochemistry Letters, 13(13) 2018, pages (164-170)

Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Pilot study for association of chemokine CCL2 rs13900 with the Susceptibility to HCV Infection

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ARTICLE INFO

SNP, gene polymorphism, RT-PCR,

CCR2, CCL2, CHC, HCV, allele

ABSTRACT

Background: In some genes, Polymorphisms may have an influence on the persistence of HCV infection, HCV replication, or spontaneous clearance of the virus. This study was carried out to investigate the association of chemokines CCL2 gene polymorphism with the susceptibility to HCV infection with high-resolution techniques in Egyptian families. **Methods:** A total of 75 Egyptian families with a total of 291 subjects in this study were recruited from different Egyptian populations from Dakahlia governorate and were classified into 2 groups: 146 infected persons with chronic hepatitis C (CHC), 145 subjects as a healthy control group. All subjects were genotyped for rs13900 C/T SNP of CCL2 gene using allelic discrimination real time PCR (RT-PCR) technique. **Results:** The allele carriage of at least one copy of C allele of CCL2 rs13900 C/T polymorphism was significantly lower in the positive group when compared to that of negative group (OR = 0.4169, 95% CI 0.1830 to 0.9496, p 0.0372) indicating that The C allele of rs13900 C/T polymorphism is protective allele against development of chronic HCV while the allele carriage of homozygotes TT of CCL2 rs13900 C/T polymorphism was significantly higher in the positive group when compared to that of negative group (OR = 2.3986, 95% CI 1.0530 to 5.4635, p 0.0372) indicating that The C allele of rs13900 C/T polymorphism is protective allele against development of chronic HCV. **Conclusions:** HCV infection is associated with rs13900 C/T SNP of CCL2 gene in the Egyptian families.

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INTRODUCTION

Hepatitis C virus (HCV) leads to acute and often chronic hepatitis[1]. Almost, 170 million people globally are evaluated to be infected with HCV [2]. The natural course of chronic disease is considered to have high variation and ranges from asymptomatic to mild disease to cirrhosis and hepatocellular carcinoma (HCC) and HCV-related co-diseases [3].

The high prevalence of chronic HCV infection suggest that both the innate and the adaptive immune system is not enough to eliminate the virus for most patients [4].

Chemokines are a huge family of tiny chemoattractant cytokines that have a crucial role in the cell trafficking regulation and in the induction of chemotaxis[5]. mRNA transcripts for CCL2 and CCR2 are significantly raised in HCV infected livers.[6]. Furthermore, an increase in serum CCL2 correlates with progressive liver inflammation in infected persons compared to healthy individuals [7].

Expression of CCR2 is on monocytes, T cells, DCs and macrophages. CCR2 ligands include CCL2 (also known as monocyte chemotactic protein-1, MCP-1), CCL13, CCL8 and CCL7, all of them are expressed in the hepatic environment. mRNA transcripts for CCL2 and CCR2 are significantly increased in HCV-infected livers [8]

PATIENTS AND METHODS

Study population

This study was carried out in the Molecular Genetic Unit in Endemic Medicine & Virological Diseases (MGEVD), Faculty of Medicine, Mansoura University, on Egyptian HCV patients and their families or close household contacts from different Egyptian populations from Dakahlia governorate between 2014 and 2015. Each family was selected on the basis of containing at least one positive HCV index patient, one positive HCV member and one other member who was HCV negative with no history of any liver complications or disorders.

A total of 75 Egyptian families were recruited in this study with a total of 291 individuals (146 infected persons with chronic hepatitis C (CHC), 145 controls (negative household contact to HCV: HCV antibody positive but HCV-RNA negative in two successive samples at least 6 months apart. The index cases in the family were selected with inclusion criteria that included HCV positive by PCR RNA for >6 months, adults (above 18 years) of both sexes and any stage of HCV-related liver diseases. Key exclusion criteria were as follows: patients co-infected with HIV or HBsAg, patients with anti-HCV antibodies positive and no detectable HCV-RNA in the serum.

Patients with autoimmune hepatitis, HCC and metabolic liver diseases were excluded. Healthy

control household contacts were included in this study with inclusion criteria of age >18 years of both sexes, first- and second-degree consanguinity to the index case living and sharing usual family activity with the index case with at least 15 years exposure to HCV infection and having no serological evidence of HCV, HBV or HCC and with no history of liver disease. Each participant was subjected to molecular diagnosis and HCV-RNA analysis to confirm HCV infection. Written informed consent was obtained from each patient included in the study, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution’s human research committee in Mansoura University.

Procedures

Collection of blood samples

Three ml blood sample was withdrawn from each patient. One ml was delivered to k_2EDTA vacutainer tubes for DNA host analysis. The EDTA blood was then aliquoted and stored at -50 C. The remaining blood sample was delivered into plain tubes centrifuged at 4000 rpm for 10 min. Then the serum was separated and aliquoted into 250 µl volume and stored at -50 until used.

Serological markers

Anti-HCV Abs was assessed using an ELIZA immunoassay technique (Abbott Laboratories, Abbott Park, IL, USA), quantitative PCR for positive anti HCV patients was done**.**

Molecular diagnosis

DNA Extraction

Genomic DNA was extracted and purified from leucocytes of peripheral blood samples using a commercial Qiagen DNA isolation kit (Qiamp® DNA Minikit, Qiagen, Germany), according to the manufacturer's instructions.

Allelic discrimination with real time PCR

One Single-nucleotide polymorphisms (SNP) of the Chemokine ligand 2 (CCL2) was studied on the patients of this study the allelic genotyping of each DNA sample with real-time PCR reaction on the apparatus (model 7500; Applied Biosystems) using a ready-made fluorescein-amidite–labeled SNP primers and probes (purchased from Applied Biosystems) for one SNP of CCL2 for gene CCL2 rs13900 and TaqMan® Universal Master Mix II (2x):Supplied from (Applied Biosystems) .also DNA Template , RNase free water and optical plate (MicroAmp® Optical 96-Well Reaction Plate, Applied Biosystems), figure 1.

RESULTS:

The current study 75 Egyptian families was recruited for this study. Laboratory data are

presented in table 1. All individuals of these families (291) were genotyped for SNP CCL2 rs13900 C/T polymorphism.

Table 1 show Clinical & laboratory Characteristics of studied groups. All samples were successfully genotyped for rs13900. Figure 2 show the distribution of the allele carriage of CCL2 rs13900 among different groups.

Table (2) and figure (2) show the distribution of allele carriage and allele frequency, Hardy Weinberg and heterozygosity of different SNPs among different groups. All groups for all SNPs are in hardy Weinberg equilibrium. P value is greater than 0.5 indicating that the groups of current study represent our population for genetic analysis.

The heterozygosity of all SNPs in different groups is ranged from 0.194 to 0.51 indicating that this SNPs are suitable for performing genetic analysis in the current groups.

The allele carriage of at least one copy of C allele of CCL2 rs13900 C/T polymorphism was significantly lower in the positive group when compared to that of negative group (OR = 0.4169, 95% CI 0.1830 to 0.9496, p 0.0372) indicating that The C allele of rs13900 C/T polymorphism is protective allele against development of chronic HCV, Table (3).

On other hand the allele carriage of homozygotes TT of CCL2 rs13900 C/T polymorphism was significantly higher in the positive group when compared to that of negative group (OR = 2.3986, 95% CI 1.0530 to

5.4635, p 0.0372) indicating that The T allele of rs13900 C/T polymorphism is risk

allele for development of chronic HCV, Table (4).

DISCUSSION:

CCL2 is considered one of the member of the CC chemokine subfamily the responsibility for the mononuclear cells movement is due to the concentration gradient of CCL2, mainly monocytes/macrophages, to the sites of inflammation. A several cell types produce CCL2, including macrophages, lymphocytes, neutrophils, fibroblasts, keratinocytes, vascular endothelial cells and various cancer cell lines. These immune and non-immune cells produce CCL2 as a result of stimulation by a large group of mediators that include cytokines, lipopolysaccharides and growth factors referring the role of CCL2 in host defense against bacteria [8].

In HCV-infected livers, there are markedly raised in mRNA transcripts for CCL2 and CCR2. In addition, an increase in serum CCL2 associates with progressive liver inflammation in infected persons compared to healthy persons. Kupffer cells release IFN-inducible CCL2 in the early phase of infection and its secretion stimulates the first wave of infiltrating monocytes including CCR2+ plasmacytoid DCs. Also, CCR2 expressing CD8+ T cells are enriched in the inflamed liver during persistent infection with HCV [9].

Genetic analysis in the current study assure the contribution of CCL2 in the Pathogenesis of HCV. We genotype one SNP namely CCL2 rs13900 in 75 Egyptian family with at least one member affected with HCV.

The allele frequency of the existing study are consistent with the published global frequency. In brief the allele T of CCL2 rs13900 is a risk allele while the allele C of CCL2 rs13900 is a protective allele.

During different stages of HCV infection, several chemokines play sophisticated roles which may illustrate some of the conflicting findings from studies analyzing the influence of chemokine gene mutations on HCV pathogenesis.

It explain to what extent targeting chemokines therapeutically is so complex [10].

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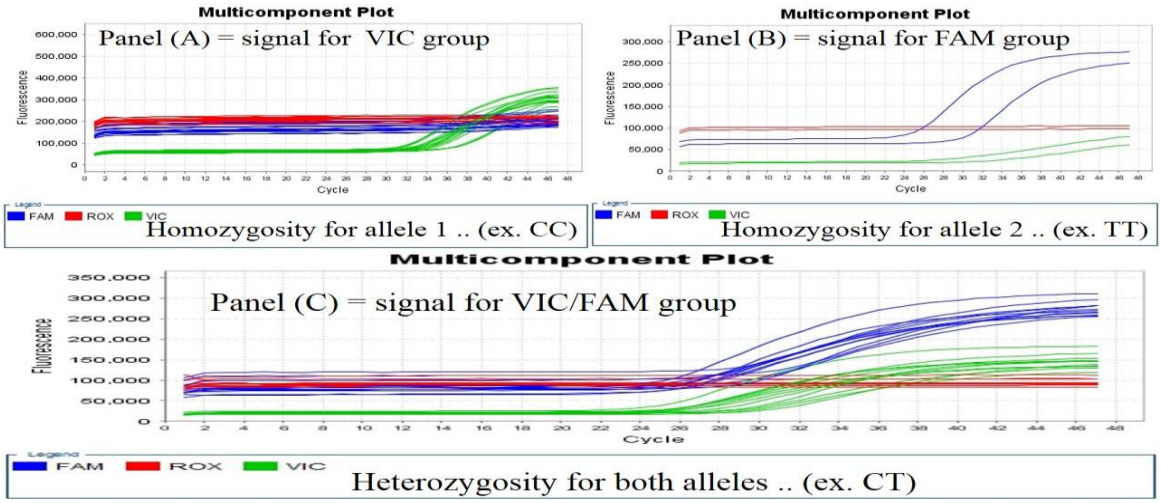

Figure 1: Real Time PCR charts for different genotypes of (rs 13900 C/T) SNP of CCL2 gene

Life Technologies follows the pattern of assigning FAM™ dye as the reporter for the target assay and assigning VIC® dye as the reporter for the normalizer assay. (Applied Biosystems provides common endogenous control assays for human, mouse, and rat that are available primer-limited and labeled with the VIC® dye, which is recommended for multiplexing with FAM™ dye). The allele specific probes were labeled with a fluorescent dye (VIC and FAM) and used in the allele typing of each DNA sample with real-time PCR reaction on the apparatus (model 7500; Applied Biosystems) using a readymade fluorescein-amidite–labeled SNP primers and probes.

Heterozygosity: having two different alleles of the same gene.

Homozygosity: having two identical alleles of the same gene.

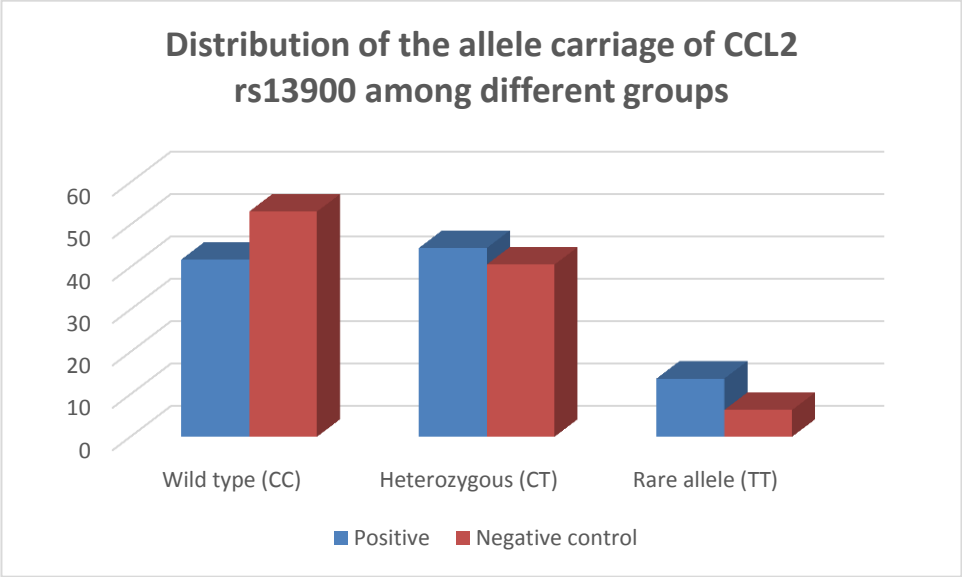

Figure 2: Distribution of the allele carriage of CCL2 rs13900 among different groups

Table 1: Clinical & laboratory Characteristics of studied groups

	Positive (146)	Negative (145)
Sex: M /F	84/62	62/83
	Mean (±SD)	Mean (±SD)
Age	39.5 (15.97)	33.2 (23.9)
S. Bilirubin (mg/dl)	1.4 (0.68)	0.8 (0.2)
S. Albumin (gm/dl)	3.8 (0.6)	4.6 (0.5)
SGOT (IU/L)	45 (11)	19.0 (6)
SGPT	52 (11)	20.0 (8)
ALP U/L	77.8 (15.9)	48.0 (18.0)
AFP ng/mL	25 (16.8)	2.5 (2.5)
ANA (U)	0.59 (0.20)	0.6 (0.25)
S. Creatinine	0.8 (0.35)	0.8 (0.39)

S. : Serum, S. Bilirubin (N: <1.1 mg/dl); S. Albumin (N 3.9 – 5.1 gm/dl); SGOT (aspartate aminotransferase) (N< 40 IU/L); (SGPT) alanine aminotransferase (N< 45 IU/L); ALP = Alkaline phosphatase (N: 37 – 116 U/L); Alpha Fetoprotein (AFP) (N < 10 ng/ml): Antinuclear Antibodies (ANA) (N <1.1 U); S. creatinine (N<1.4 mg/dl); S. Cholesterol (N< 200 mg/dl)

Table 2: Allele carriage and allele frequency of CCL2 rs13900 among different groups

	Negative control (145)	Positive (146)
Wild type (CC)	77 (53.1%)	61 (41.8%)
Heterozygous (CT)	59 (40.6%)	65 (44.5%)
Rare allele (TT)	9 (6.2%)	20 (13.7%)
Allele frequencies **C** **T**	 0.734 0.266	 0.640 0.360
PIC	0.334	0.349
Heterozygosity	0.407	0.442
No of alleles	290	292
Hardy-Weinberg **x2** **P**	 3.66981607 0.055406	 2.672123425 0.102120

PIC: Polymorphism information content.

Table 3: The risk of allele C of CCL2 rs13900 carriage

	Positive vs. negative
Odds ratio (OR)	0.4169
95% CI	0.1830 to 0.9496
z statistic	2.083
P	0.0372

95% CI: Confidence intervals

Table 4: The risk of allele TT of CCL2 rs13900 carriage

	Positive vs. negative
Odds ratio (OR)	2.3986
95% CI	1.0530 to 5.4635
z statistic	2.083
P	0.0372