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Research paper

The association of *CCL3* and *CCL4* polymorphisms with HCV clearance in Chinese Han population

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ABSTRACT

Aim: To explore the association of *CCL3* (rs1063340) and *CCL4* (rs1049807) polymorphisms with hepatitis C virus (HCV) clearance and sustained virologic response (SVR).

Methods: Two populations were enrolled in the current study; one was a general population including 1585 untreated individuals, with HCV infection and the other was a treatment population comprising 353 HCV-infected patients treated with pegylated interferon-α and ribavirin (pegIFN-α/RBV). Two single nucleotide polymorphisms (SNPs) were genotyped, and the relationship between HCV clearance and treatment outcome was analysed.

Results: The general population comprised 995 persistent HCV cases (both HCV RNA and anti-HCV were positive) and 590 spontaneous clearance cases (HCV RNA was negative, but anti-HCV was positive). An association between the SNPs and HCV clearance was not found in our study. The treatment population consisted of 235 patients who achieved SVR and 118 non-responders. Variants of both SNPs (rs1063340-C and rs1049807-G) were associated with a reduction in SVR following IFN treatment (dominant model: P = 0.026 for rs1063340 and P = 0.048 for rs1049807). In addition, the ancestral alleles of rs1063340 and rs1049807 increased the likelihood of virus clearance by 62% compared to both the derived and minor alleles of the two SNPs (P = 0.040).The interaction analysis showed that the level of glucose interacted with the association of rs1063340 and SVR.

Conclusions: Our results suggested that genetic variants at the *CCL3* and *CCL4* loci may be marker SNPs for risk of HCV treatment outcome.

1. Introduction

Hepatitis C virus (HCV) is one of the most common blood-borne disseminate diseases with approximately 3% of the population infected worldwide and 700,000 deaths annually (Ferlay et al., 2010; Rowe, 2017). It is estimated that at least 25 million are individuals infected with HCV in China, and the number of cases of HCV infection has been increasing steadily since 2003 (Bian et al., 2017; Duan et al., 2014; Gower et al., 2014). Because of the latent nature of HCV, patients infected with HCV tend to progress as chronic HCV infection, and nearly 71 million people suffer from chronic HCV worldwide as of 2015 (Collaborators, 2017). Chronic HCV infection is one of the most common causes of hepatocellular carcinoma (Bruden et al., 2017), so it is urgent to determine the factors that affect the HCV spontaneous clearance and treatment response.

The main route of HCV transmission is blood; for instance, intravenous drug use, renal dialysis, and paid blood donations can be behaviours with a high risk of HCV infection (Lavanchy, 2011). A review by Gao shows that the prevalence of HCV infection among individuals with blood donation behaviour is 8.68%, which is

Abbreviations: HCV, hepatitis C virus; SVR, sustained virologic response; pegIFN-α, pegylated interferon-α; RBV, ribavirin; SNPs, single nucleotide polymorphisms; WT, wild-type; GLU, glucose; GWAS, genome-wide association study; MIP-1α, macrophage inflammatory protein 1 alpha; ORs, odds ratios; CIs, confidence intervals; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, α-fetal protein; TP, total protein; ALB, albumin; TC, total cholesterol; WBC, white blood cell; HB, hemoglobin

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significantly higher than that the 3.2% noted among the general population in China (Gao et al., 2011). Since no specific vaccine against HCV is available, the therapy for HCV tends to be important. Although direct-acting antiviral (DAA) regimens have revolutionized the treatment method with their low rate of side effects and high rate of sustained virological response (SVR), the primary therapeutic method in China is pegylated interferon-α combined with ribavirin (pegIFN-α/RBV) (Sulkowski et al., 2014). The primary indicator for evaluating the efficacy of treatment is SVR, so the current study uses SVR to identify the indicators that influence treatment outcomes.

Persistent HCV infection and HCV clearance are influenced by complex factors such as viral load and genotype, therapy, genetic makeup environmental factors, and host factors including age, gender, and fibrosis degree (Asselah et al., 2010; Mosley et al., 2005). Some studies have reported susceptibility loci related to HCV infection and clearance in genome-wide association studies (GWAS), such as rs1063478 in *HLA-DMA*, rs4273729 in *HLA* class II region, and rs4803217 in *IFNL3* (Huang et al., 2014; Xu et al., 2015; Świątek-Kościelna et al., 2017). However, very few studies have reported an association between variants in *CCL3/CCL4* and HCV progression and treatment outcome. *CCL3* is a cytokine belonging to the CC chemokine family that participates in the acute inflammatory state in polymorphonuclear cells (Wolpe et al., 1988). The locus of *CCL3* represents a small inducible cytokine, and the encoded protein, also known as macrophage inflammatory protein 1 alpha (MIP-1α), plays a role in inflammatory responses by binding to the receptors *CCR1*, *CCR4* (MIP-1β) and *CCR5* (Sherry et al., 1988).

In the current study, to further test the association of *CCL3* and *CCL4* variants with the risk of HCV persistence, spontaneous clearance and treatment outcome, we genotyped two single-nucleotide polymorphisms (SNPs) in 1585 untreated patients with HCV infection and 353 treated with IFN/RBV in Chinese Han population.

2. Methods

2.1. Study subjects

Two populations were enrolled in this study. One of the populations was a general population including 1585 HCV-infected individuals, who were recruited from six hospital haemodialysis centres and Nanjing compulsory detoxification centres or were former plasma donors in Jiangsu Province from May 2006 to January 2013. All the subjects had not been treated, and the details were described in our previous study (Huang et al., 2015). Persistent infection cases were defined as those that were positive for both anti-HCV and HCV-RNA on three biochemical tests within the consecutive six months during follow-up, and spontaneous clearance cases were defined those that were anti-HCV-positive while HCV-RNA-negative for at least six months.

The other population was a treatment population comprising 353 HCV-infected patients recruited from Jurong People's Hospital (Zhenjiang, China) from April 2011 to January 2016. All of the enrolled subjects in the treatment population were chronic hepatitis C (CHC) patients with genotype 1 and had a history of paid-blood donation behaviours. Detailed information about the subjects in treatment population has been described in our previous study (Huang et al., 2014). Sustained virological response (SVR) was defined as the absence of serum HCV RNA at least 24 weeks after the treatment of pegylated interferon-α and ribavirin (pegIFN-α/RBV). The protocol in this study was checked and approved by the institutional review board of Nanjing Medical University (Nanjing, China). All subjects provided written informed consent.

All individuals were over 18 years old without other liver diseases such as hepatitis B virus or human immunodeficiency virus co-infection. Information on demographic characteristics and history of environmental exposure were collected by face-to-face interviews by trained interviewers using a structured and standardized questionnaire. After the interview, a venous blood sample of approximately 10 ml was collected from each subject. The peripheral blood mononuclear cells and sera were separately stored in a −40 °C refrigerator until assay.

2.2. Laboratory testing

Anti-HCV was detected by enzyme-linked immunosorbent assay (ELISA, Beijing Wantai Biological Pharmacy Engineering Co., Ltd., Beijing, China) in the serum, following the manufacturer's instructions. Blood biochemical tests were performed by Roche Module P800 Automatic Biochemical Analyzer (Roche Diagnostics GmbH, Basel, Switzerland). TRIzol LS Reagent was used to extract total RNA from serum, and RT-PCR was used to detect HCV RNA with specific primers using PrimeScript RT-PCR kit (DRR014S; Takara Biotechnology Co., Ltd., Dalian, China).

2.3. Genotyping

Information about the two SNPs (rs1063340 and rs1049807) was accessed from the Chinese Han population database of Hap Map (http://www.hapmap.org) and from the NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP). The minor allele frequencies (MAFs) of the two SNPs were above 0.05. Genomic DNA was extracted from leucocyte pellets using proteinase K digestion followed by phenol-chloroform purification. The polymorphisms in *CCL3* rs1063340 (G > C) and *CCL4* rs1049807 (A > G) were genotyped by a TaqMan allelic discrimination assay on an ABI PRISM 7900HT Sequence Detection system (Applied Bio systems, San Diego, CA, USA). PCR was performed by the following thermal profile: 50 °C for 2 min to preheat, 95 °C for 10 min (preincubation), then 40 cycles at 95 °C for 15 s to denature and 60 °C for 1 min (annealing). The primers and probes (Nanjing Bio Steed Biotechnologies Co., Ltd., Nanjing, China) used in this study are shown in Supplementary Table 1. Two blank controls were assigned to a 384-well format for quality control with a randomly selected 10% of samples as repeat samples, yielding 100% concordance.

2.4. Statistical analysis

The statistical analysis was performed using Stata/MP version 13.0 (Stata Crop LP, College Station, Texas, USA) for Windows 64 bit. The differences in the demographic characteristics and clinical features in the different groups were calculated by using the chi-square (χ^2) test for categorical variables and one-way analysis of variance or Student's *t*-test for continuous variables with normal distribution. Hardy–Weinberg equilibrium (HWE) for each SNP was estimated by the χ^2 goodness of fit test. The association of SNPs with HCV clearance and treatment outcome was estimated by calculating the odds ratios (ORs) and the 95% confidence intervals (CIs) from two genetic models (dominant and additive) and multivariate logistic regression analysis. The cumulative effects of the number of the protective haplotypes in IFN/RBV (interferon/ribavirin) treatment outcomes and the interaction effect between genetic variants and risk factors, were estimated by multivariate logistic regression analysis. The Cochran-Armitage test was used for trend analysis. A P-value < 0.05 in a two-sided test was considered to indicate a significant difference.

3. Results

3.1. Baseline demographic and clinical characteristics of the study population

The baseline characteristics of the general population are shown in Table 1. All enrolled subjects, comprising 995 persistent HCV cases and 590 spontaneous clearance cases, were divided into two groups based on their HCV RNA and anti-HCV status. There were no differences in the distribution of gender between the two groups. Older patients were