Infection, Genetics and Evolution 41 (2016) 146–152

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Vitamin D level and vitamin D receptor genetic variations contribute to HCV infection susceptibility and chronicity in a Chinese population

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ARTICLE INFO

Article history:
Received 15 January 2016
Received in revised form 26 March 2016
Accepted 30 March 2016
Available online 8 April 2016

Keywords:
Vitamin D
Vitamin D receptor
Polymorphism
Hepatitis C virus

ABSTRACT

Vitamin D and vitamin D receptor (VDR) are involved in multiple immune-mediated disorders including chronic hepatitis C virus (HCV) infection. The aim of this study was to determine the association between plasma vitamin D level, *VDR* genetic polymorphisms and risk of HCV infection susceptibility and chronicity. Seven single nucleotide polymorphisms (SNPs) in *VDR* gene were genotyped and plasma 25-hydroxyvitamin D [25(OH)D] levels were measured in a Han Chinese population of 898 HCV persistent infection cases, 558 spontaneous clearance subjects and 1136 uninfected controls with high risk of HCV infection. In this case–control study, the average plasma 25(OH)D level in persistent infection patients was significantly lower than that in spontaneous clearance cases ($P = 0.039$) and controls ($P = 0.005$). Logistic analyses indicated that rs7975232-C, rs2239185-T and rs11574129-T alleles were significantly associated with a decreased risk of HCV infection susceptibility (all $P_{Bonferroni} < 0.05$, in additive/dominant models; $P_{trend} = 9.000 \times 10^{-4}$, combined effects in a locus-dosage manner). The protective effects of three favorable alleles were more evident among males, females and subjects aged ≤50 years (all $P < 0.05$). Haplotype analyses suggested that compared with the most frequent haplotype $A_{rs7975232}T_{rs731236}C_{rs11574129}$, CTT was correlated with a reduced risk of HCV infection susceptibility ($P = 2.200 \times 10^{-3}$). These findings implied that low vitamin D levels might be associated with an increased risk for HCV infection and chronicity, and favorable *VDR* variants (rs7975232-C, rs2239185-T and rs11574129-T) might contribute to a decreased susceptibility to HCV infection in a high-risk Chinese population.

1. Introduction

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV) that leads to both acute and chronic infection. According to recent estimates from the World Health Organization (WHO) (http://www.who.int/mediacentre/factsheets/fs164/en/), the majority (55%–85%) of infected individuals develop persistent (chronic) infection. The most affected regions are Africa and Central & East Asia. Approximately 29.8 million Chinese (Lavanchy, 2011) and >150 million people worldwide are chronically infected with HCV. One-third of those develop progressive liver cirrhosis, liver cancer and/or premature death (Bialek and Terrault, 2006). It remains unknown why some patients clear HCV during acute infection, while most of them proceed to chronic infection. The course and outcome of HCV infection is most likely determined by a complex interplay of viral and host factors (Neumann-Haefelin and Thimme, 2007). Host factors range from individual genetic differences to the vigor and quality of the immune response to HCV.

Vitamin D and its receptor have increasingly been recognized for their effects on the regulation of the immune system as well as calcium–phosphorous homeostasis and bone metabolism (Baur et al., 2012a). Accumulating evidence suggests that 1, 25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], the activated hormonal form of vitamin D, is a key immune modulator regulating the innate and adaptive immune pathways (von Essen et al., 2010). It has been identified to be crucial for spontaneous HCV clearance and success of antiviral therapy (Lange et al., 2011). Meanwhile, *in vitro* assays indicate that vitamin D exerts an anti-HCV effect (Yano et al., 2007; Gal-Tanamy et al., 2011). Recently, decreased 25-hydroxyvitamin D [25(OH)D] levels have been described in various forms of chronic liver disease, and associated with advanced fibrosis and poor response to interferon-based therapy in chronic HCV patients (Arteh et al., 2010; Petta et al., 2010; Lange

Abbreviations: HCV, hepatitis C virus; WHO, World Health Organization; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 25(OH)D, 25-hydroxyvitamin D; SVR, sustained viral response; APCs, antigen-presenting cells; DCs, dendritic cells; VDR, vitamin D receptor; HBV, hepatitis B virus; SNP, single nucleotide polymorphism; HD, hemodialysis; HIV, human immunodeficiency virus; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription-polymerase chain reaction; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium; ANOVA, analysis of variance; OR, odds ratio; CI, confidence interval; LD, linkage disequilibrium; AST, aspartate aminotransferase; ALT, alanine transaminase; mRNA, messenger ribonucleic acid; UTR, untranslated region.

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http://dx.doi.org/10.1016/j.meegid.2016.03.032
1567-1348/

et al., 2011; Ladero et al., 2013). In addition, high levels of vitamin D or supplementation are strongly associated with a sustained viral response (SVR) among HCV-infected individuals (Villar et al., 2013). Meta-analyses support these findings and provide the most comprehensive empirical evidence to date that basal serum 25(OH)D levels and vitamin D supplementation improve the SVR to HCV infection. These results highlight the influence of vitamin D deficiency on the antiviral response in chronic HCV infection.

Early animal, epidemiologic and clinical studies have demonstrated a potential role for vitamin D in maintaining immune system balance. Vitamin D modulates the adaptive immune system through the direct effect on T cells activated by antigen-presenting cells (APCs), such as dendritic cells (DCs) (Kamen and Tangpricha, 2010). Vitamin D regulates immune responses and inflammation through binding to vitamin D receptor (VDR) (Di Rosa et al., 2011). *VDR* genetic variants (rs1544410, rs7975232 and rs731236) could affect the function of *VDR*, modulating the biological effects of vitamin D (Jurutka et al., 2001; Uitterlinden et al., 2004; Baur et al., 2012a). Genetic variations in *VDR* have been implicated in many immune-mediated diseases, such as tuberculosis (Salimi et al., 2015), systemic lupus erythematosus (Carvalho et al., 2015), autoimmune hepatitis, primary biliary cirrhosis (Vogel et al., 2002; Fan et al., 2005; Tanaka et al., 2009), hepatocellular carcinoma (Yao et al., 2013), and hepatitis B virus (HBV) infection (Suneetha et al., 2006; Falleti et al., 2012a). Moreover, these variants are related to rapid fibrosis progression and treatment response in chronic HCV infection (Baur et al., 2012a, 2012b; Lange et al., 2012; Garcia-Martin et al., 2013). However, there is little information available on the association between *VDR* polymorphisms and the outcomes of HCV infection. The aim of this case–control study is to explore the incidence of vitamin D deficiency in chronic HCV patients. In addition, we systemically evaluated the relationships between seven single nucleotide polymorphisms (SNPs) (rs7975232, rs1544410, rs731236, rs11568820, rs2107301, rs2239185 and rs11574129) in *VDR* gene, plasma vitamin D levels, and HCV infection susceptibility and chronicity in 2592 Han Chinese subjects.

2. Patients and methods

2.1. Study population and specimen collection

The study protocol was approved by the institutional review board of Nanjing Medical University (Nanjing, China). Written informed consent was obtained before enrolling the Han Chinese participants for conducting various blood tests and genetic analysis. Clinical and biochemical assessments of the patients were carried out.

A total of 2592 subjects were recruited from October 2008 to May 2015, including 816 hemodialysis (HD) subjects from nine hospitals in southern China, 510 drug users from a compulsory detoxification center in Nanjing, and 1266 paid blood donors from six villages in Zhenjiang. Eligible participants with a high risk of HCV exposure had on-the-spot interviews with a structured questionnaire including demographic and relevant exposure information. Meanwhile, a 10-mL blood sample from each subject was collected by venipuncture after an overnight fast. All plasma samples were separated and frozen at −80 °C until further serological tests, genotyping assays and virus detection. The serological data were confirmed by three independent experiments over six consecutive months. Patients who had antiviral therapies and those co-infected with HBV or human immunodeficiency virus (HIV), or with other types of liver diseases (*e.g.* autoimmune, alcoholic or metabolic liver diseases) were excluded.

Anti-HCV test in the serum was determined using a third generation enzyme-linked immunosorbent assay (ELISA; Diagnostic Kit for Antibody to HCV 3.0 ELISA, Intec Products Inc., Xiamen, China). HCV-RNA was extracted from serum using Trizol LS reagent (Takara Biotech, Tokyo, Japan), and 10 μL HCV-RNA extracting solution was further analyzed by employing a reverse transcription-polymerase chain reaction (RT-PCR) kit (Takara Biotech, Tokyo, Japan). The murex HCV serotyping 1–6 assay ELISA kit (Abbott, Wiesbaden, Germany) was used to determine various HCV genotypes corresponding to type-specific antibodies. All participants were divided into three groups for analysis based on the status of anti-HCV and HCV RNA, including 1136 uninfected subjects

Table 1
Distributions and comparisons of demographic and clinical characteristics among the three study groups.

Variables	HCV persistent infection (%) (n = 898)	HCV spontaneous clearance (%) (n = 558)	Controls (%) (n = 1136)	*P*
Age (years)				0.076[a]
≤50	502 (55.9)	278 (49.8)	604 (53.2)	
>50	396 (44.1)	280 (50.2)	532 (46.8)	
mean ± SD	49.73 ± 10.92	49.02 ± 13.47	49.56 ± 14.38	0.595[b]
Gender				0.467[a]
Male	343 (38.2)	226 (40.5)	425 (37.4)	
Female	555 (61.8)	332 (59.5)	711 (62.6)	
Route of infection				<0.001[a]
HD subjects	84 (9.4)	101 (18.1)	631 (55.5)	
Drug user	150 (16.7)	162 (29.0)	198 (17.4)	
Blood donor	664 (73.9)	295 (52.9)	307 (27.0)	
HCV genotype				<0.001[a]
1	243 (47.1)	141 (53.2)	–	
Non-1	38 (7.4)	82 (30.9)	–	
Mixed	235 (45.5)	42 (15.8)	–	
ALT (median (IQR), U/L)	36.00 (21.00, 59.25)	26.00 (16.00, 41.25)	14.00 (8.00, 21.00)	<0.001[c]
AST (median (IQR), U/L)	35.00 (25.00, 53.50)	28.00 (20.75, 40.00)	19.00 (12.00, 25.00)	<0.001[c]
Plasma 25(OH)D (μg/L)[d]				0.035[a]
<20	163 (72.8)	91 (64.5)	157 (61.8)	
≥20	61 (27.2)	50 (35.5)	97 (38.2)	
Median (IQR)	17.32 (14.61, 20.32)	17.97 (14.59, 21.44)	18.51 (15.09, 21.52)	0.013[*,c]

Abbreviations: HCV, hepatitis C virus; SD, standard deviation; ALT, alanine transaminase; AST, aspartate aminotransferase; HD, hemodialysis; non-1, genotypes 2 or 3; mixed, co-infected with genotypes 1, 2 and/or 3; 25(OH)D, 25-hydroxyvitamin D; IQR, interquartile range.

[*] All $P < 0.05$: HCV persistent infection *vs.* Controls; HCV persistent infection *vs.* HCV spontaneous clearance; HCV persistent infection and spontaneous clearance *vs.* Controls.

[a] χ^2-test among three groups.

[b] One-way ANOVA test among three groups.

[c] One-way Kruskal–Wallis test among three groups.

[d] Plasma 25(OH)D levels were measured in 365 HCV infection cases (141 spontaneous clearance and 224 persistent infection cases) and 254 uninfected controls, and plasma 25(OH)D < 20 μg/L was considered the threshold for identifying low levels of vitamin D.