Significance of Vitamin D Receptor Gene Polymorphisms for Risk of Hepatocellular Carcinoma in Chronic Hepatitis C

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Abstract

BACKGROUND/AIMS: Biological and epidemiological data suggest that vitamin D levels may influence cancer development. Several single nucleotide polymorphisms have been described in the vitamin D receptor (VDR) gene in association with cancer risk. We aimed to investigate the association of VDR gene polymorphisms with hepatocellular carcinoma (HCC) development in chronic hepatitis C patients.

METHODS: In a cross-sectional, hospital-based setting, 340 patients (201 chronic hepatitis, 47 cirrhosis and 92 HCC) and 100 healthy controls receiving VDR genotyping (bat-haplotype: BsmI rs1544410 C, ApaI rs7975232 C and TaqI rs731236 A) were enrolled.

RESULTS: Patients with HCC had a higher frequency of ApaI CC genotype ($P = 0.027$) and bAt[CCA]-haplotype ($P = 0.037$) as compared to control subjects. There were no differences in BsmI and TaqI polymorphisms between two groups. In patients with chronic hepatitis C, HCC subjects had a higher frequency of ApaI CC genotype and bAt[CCA]-haplotype than those with chronic hepatitis ($P = 0.001$ and $0.002$, respectively) and cirrhosis ($P = 0.019$ and $0.026$, respectively). After adjusting age and sex, logistic regression analysis showed that ApaI CC genotype (odds ratio: 3.02, 95% confident interval: 1.65-5.51) was independently associated with HCC development.

CONCLUSION: VDR ApaI polymorphism plays a role in the development of HCC among chronic hepatitis C patients. Further explorations of this finding and its implications are required.

Introduction

Hepatitis C virus (HCV) infection is one of the major public health problems worldwide [1]. Chronic HCV infection is characterized by a high rate of progression to fibrosis, chronic hepatitis, leading to cirrhosis and ultimately to hepatocellular carcinoma (HCC) [2–4]. Although the relationship between HCV and the development of HCC is well established, the pathogenetic mechanism of hepatocarcinogenesis, including host- and viral-related factors, is still unknown. It is prudent to affirm that differences in the incidence rates and the strong gender distribution in HCC are not entirely due to differences in the exposure to the causative agents [5,6]. Of great importance, genetic factors can also contribute, particularly gene polymorphisms of inflammatory cytokines and growth factor ligands and receptors [7].

Vitamin D is involved in the metabolism of skeleton as a systemic hormone but also has important roles in the regulation of host immune responses, fibrogenesis and development of cancer through vitamin D receptor (VDR) [8–14]. Previous data have suggested that vitamin D levels may influence cancer development. In particular, several single nucleotide polymorphisms have been described in the VDR gene, and some polymorphisms are associated with tumor occurrence [12–16]. For instance, VDR polymorphisms have been related to cancers of the breast, prostate, skin, colon-rectum, bladder and kidney, although with conflicting observations [12–16]. VDR polymorphisms have also been investigated in the context of some chronic liver diseases, such as chronic hepatitis B, primary biliary cirrhosis and autoimmune hepatitis [17–19]. In a recent published study, VDR polymorphism may be used as a molecular marker to predict the risk and to evaluate the disease severity of HCC in patients with chronic hepatitis B [20].

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So far, there are limited data in the literature on the association between VDR polymorphisms and the occurrence of HCC. In this present study, we investigated the role of VDR gene polymorphisms in the susceptibility and clinicopathological status of HCC in Chinese subjects with chronic HCV infection.

Patients and Methods

Patients
From August 2011 to July 2013, a total of 340 patients with chronic HCV infection receiving long-term follow up in a single center were enrolled. They included 201 chronic hepatitis, 47 cirrhosis and 92 HCC patients. All patients were seropositive for HCV antibody (by third-generation enzyme-linked immunosorbent array (ELISA) and HCV RNA (Amplicor™, Roche Diagnostics, Branchburg, NJ, USA). Patients were excluded if they were positive for serum hepatitis B surface antigen or anti-human immunodeficiency virus antibody, or exhibited other causes of hepatocellular injury (e.g., any history of alcoholism, autoimmune hepatitis, primary biliary cirrhosis and severe nonalcoholic liver disease with metabolic syndrome). During the same period, 100 healthy volunteers were collected as controls.

Pathologic diagnoses of chronic hepatitis or cirrhosis were made by percutaneous liver biopsies according to the modified Knodell histologic activity index [21], which were analyzed by pathologists who were blind to the patients’ characteristics. Diagnosis of HCC was based on either the histopathologic findings in tumor tissues or typical HCC features of dynamic images if the nodules were larger than 1 cm in cirrhotic livers [22]. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethical committees of Chang Gung Memorial Hospital. All patients gave written informed consent before enrollment.

Detection of VDR Polymorphisms
The DNA was extracted from peripheral blood leukocytes using the Qiagen DNA isolation kit (Qiagen, Germany). The VDR genotype was determined by polymerase chain reaction (PCR) amplification and restriction length fragment polymorphisms (RFLP) as previously determined by polymerase chain reaction (PCR) amplication and Qiagen DNA isolation kit (Qiagen, Germany). The VDR genotype was determined by ethidium bromide-UVB illumination of the fragments separated on gels of 2% agarose. The presence of BsmI, Apal or TaqI restriction site was defined as the lower-case ‘b’, ‘a’ and ‘t’, respectively, and the absence of the site was defined as the upper-case ‘B’, ‘A’ or ‘T’. The BsmI restriction site resulted in two fragments (645 bp and 177 bp). Digestion with Apal produced two fragments of 531 bp and 214 bp when the restriction site was present. Digestion with TaqI resulted in three fragments of approximately 205, 290 bp and 245 bp in the presence of TaqI polymorphic site, and in fragments of 245 and 495 bp in the absence of a TaqI polymorphic site.

Statistical Analysis
Continuous data are expressed as mean ± standard deviation, and the categorical data are expressed as number (percentage). Comparisons of differences in the categorical date between groups were performed using the chi-square test. Distributions of continuous variables were analyzed by the Student’s t-test or one-way ANOVA test with least significant difference (LSD) post-hoc correction between groups where appropriate. Stepwise logistic regression analysis was performed to assess the influence of each factor on the risk of developing HCC. All analyses were carried out using SPSS software version 15.0 (SPSS Inc., Chicago, IL). All tests were 2-tailed, and a p-value of less than 0.05 was considered statistically significant.

Results
Baseline Features of the Studied Population
The basic demographic and clinical features of the patients are shown in Table 1. The mean age of HCC patients was significantly higher than those with cirrhosis, chronic hepatitis and controls (P < 0.001). Patients with HCC had a higher male-to-female ratio than other groups (P = 0.001). There was no significant difference in BMI among these groups. The HCC subjects had lower platelet count compared to those with chronic hepatitis; whereas the platelet count was comparable between cirrhosis and HCC groups.

The Distribution of Frequencies of VDR Genotype and Haplotype
Firstly, HCC patients were compared with a control cohort of 100 healthy volunteers with regard to allelic frequency. The distribution of the alleles of BsmI, Apal and TaqI polymorphisms was in accordance with the Hardy-Weinberg equilibrium (P > 0.05 for any). Patients with HCC had a higher frequency of Apal CC genotype (P = 0.027) and bAt[CCA]-haplotype consisting of BsmI

| Table 1. Baseline Characteristics of the Studied Population |
|----------------------------------------------------------|
| | Con (n = 100) | CH (n = 201) | LC (n = 47) | HCC (n = 92) | P-value |
|----------------|----------------|----------------|----------------|----------------|----------|
| Age (years) | 52.7 ± 15.6 | 52.4 ± 11.5 | 55.9 ± 10.0 | 64.5 ± 11.0 | <0.001 |
| Male gender (%) | 43 (43%) | 101 (50%) | 21 (45%) | 64 (70%) | 0.001 |
| BMI (kg/m²) | 24.6 ± 3.6 | 25.3 ± 3.6 | 24.2 ± 3.6 | 24.2 ± 3.6 | 0.239 |
| AST (U/L) | 42 ± 33abc | 77 ± 58abc | 107 ± 41abc | 102 ± 80abc | <0.001 |
| ALT (U/L) | 50 ± 53abc | 138 ± 131abc | 147 ± 76abc | 94 ± 76abc | <0.001 |
| Platelet (10⁴/μL) | 18.7 ± 5.4b | 13.7 ± 6.1b | 13.2 ± 7.1b | 0.239 |

Data are expressed as mean ± standard deviation or number (percentage). Strength of correlation: a Significant differences between Con and CH; b Significant differences between Con and LC; c Significant differences between CH and HCC; d Significant differences between LC and HCC; e Significant differences between Con and HCC with LSD post-hoc correction or χ² test.

Abbreviation: Con: healthy control; CH: chronic hepatitis; LC: liver cirrhosis; HCC: hepatocellular carcinoma; BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase.
Comparison Between Chronic HCV Patients with Apa I CC Type and CA/AA Type

Since ApaI CC genotype was a significant factor associated with developing HCC, we thus compared the chronic hepatitis C patients with ApaI CC type and CA/AA type. As shown in Table 4, patients carrying ApaI CC genotype had a higher prevalence of HCC and pre-existing cirrhosis and a higher ratio of BsmI CC type and TaqI AA type as compared to those with ApaI CA/AA type.

Discussion

Hepatocarcinogenesis is a complex and multi-factorial process, in which both environmental and genetic features interfere and contribute to malignant transformation [24]. The identification of genetic factors related to HCC susceptibility may improve our understanding of the various biological pathways involved in hepatocarcinogenesis.

Factors Associated with Developing HCC by Logistic Regression Analysis

As shown in Table 3, univariate analysis revealed that age, male gender, lower platelet count (<15 x 10^4/μL), the carriage of bAt[CCA]-haplotype and ApaI CC genotype were factors significantly associated with developing HCC. Stepwise logistic regression analysis showed that age (odds ratio (OR): 1.10, 95% confidence interval (CI): 1.07-1.14, P < 0.001), male gender (OR: 3.90, 95% CI: 2.07-7.35, P < 0.001), low platelet count (<15 x 10^4/μL)(OR: 4.20, 95% CI: 2.26-7.83, P < 0.001) and the carriage of ApaI CC genotype (OR: 2.77, 95% CI: 1.47-5.21, P = 0.002) were the independent predictors.

Table 2. The Distribution of Frequencies of VDR Genotype and Haplotype Among Controls and Different Clinical Stages of Chronic HCV Infection

| Genotype   | Con (n = 100) | CH (n = 201) | LC (n = 47) | HCC (n = 92) |
|------------|---------------|--------------|-------------|--------------|
| BsmI       |               |              |             |              |
| TT         | 0 (0%)        | 0 (0%)       | 0 (0%)      | 0 (%)        |
| TC         | 11 (11%)      | 13 (6%)      | 6 (13%)     | 7 (8%)       |
| CC         | 89 (89%)      | 108 (54%)    | 41 (87%)    | 35 (38%)     |
| 5' vs. 3'  | 11:189        | 13:389       | 6:88        | 7:177        |
| ApaI       |               |              |             |              |
| CC         | 55 (55%)ab     | 102 (51)b     | 24 (51)b    | 65 (71)cde  |
| CA         | 40 (40)%abc    | 82 (41%)cde   | 19 (40%)    | 24 (26)cde   |
| AA         | 5 (5%)        | 17 (8%)      | 4 (9%)      | 3 (3%)       |
| 5' vs. 3'  | 130:59bc      | 286:116de    | 67:27bc    | 154:30bc    |
| TaqI       |               |              |             |              |
| GG         | 0 (0%)        | 0 (0%)       | 0 (0%)     | 0 (0%)       |
| AG         | 11 (11%)      | 15 (7%)      | 7 (15%)    | 6 (7%)       |
| AA         | 86 (86%)      | 186 (93%)    | 40 (85%)   | 86 (93%)     |
| G vs. A    | 14:186        | 15:387       | 7:87       | 6:178        |
| BsmI–ApaI–TaqI |   |              |             |              |
| TAG (bAt)  | 10 (10%)      | 12 (6%)      | 5 (11%)    | 5 (5%)       |
| CCA (bAt)  | 54 (54%)abc   | 102 (51)abc  | 24 (51)abc | 64 (70)abc  |
| CA (bAt)   | 31 (31%)      | 83 (41%)cde  | 15 (32%)   | 21 (23)cde  |
| CAG (bAt)  | 4 (4%)        | 3 (1%)       | 2 (4%)     | 0 (0%)       |
| TAA (bAt)  | 0 (0%)        | 1 (1%)       | 1 (2%)     | 1 (1%)       |
| TCG (bAt)  | 0 (0%)        | 0 (0%)       | 0 (0%)     | 1 (1%)       |
| TCA (bAt)  | 1 (1%)        | 0 (0%)       | 0 (0%)     | 0 (0%)       |
| CCA vs. TAG & CAA | 54:44 | 102:95 | 24:20 | 64:26 |
Table 4. Comparison Between Chronic HCV Patients with Apal CC Type and CA/AA Type

|                | Apal CC (N = 191) | Apal CA/AA type (N = 149) | P-value |
|----------------|-------------------|---------------------------|---------|
| Age (years)    | 55.8 ± 12.7       | 56.6 ± 12.2               | 0.600   |
| Male gender (%)| 106 (55%)         | 80 (54%)                  | 0.412   |
| BMI (kg/m²)    | 24.6 ± 3.2        | 24.6 ± 4.1                | 0.908   |
| AST (U/L)      | 83 ± 57           | 96 ± 78                   | 0.139   |
| ALT (U/L)      | 131 ± 133         | 123 ± 83                  | 0.522   |
| Platelet (10⁹/µL)| 16.1 ± 6.3       | 17.0 ± 6.8                | 0.153   |
| Bmllt CC type  | 190 (99%)         | 124 (83%)                 | <0.001  |
| TaqI AA type   | 190 (99%)         | 122 (82%)                 | <0.001  |

Data are expressed as mean ± standard deviation or number (percentage). P-value by Student's t-test or χ² test. *Analysis included 47 LC patients without HCC and 88 HCC patients with underlying LC.

Abbreviations: HCV: hepatitis C virus; BMI: body mass index; LC: liver cirrhosis; HCC: hepatocellular carcinoma; AST: aspartate transaminase; ALT: alanine transaminase.

The development of HCC depends on the progression of liver fibrosis and the presence of cirrhosis. In addition, vitamin D levels and history regarding vitamin D intake (dietary or supplemental) were not available, this could be justified since VDR gene variants modulate biological effects of vitamin D without influencing vitamin D plasma levels [29,35]. In conclusion, the present study suggests a significant association of VDR Apal polymorphism with the development of HCC in chronic HCV infection. The characterization of VDR genetic polymorphisms in HCV carriers may help to identify those who are at high risk of developing HCC. This observation needs to be validated in further studies.

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