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ORIGINAL ARTICLE

The association between vitamin D receptor polymorphisms and serum 25-hydroxyvitamin D levels with ulcerative colitis in Chinese Han population

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Summary There is now growing evidence suggesting that Vitamin D is playing a critical role in modulating the innate and adaptive immune responses. Several polymorphisms have been identified in the vitamin D receptor (*VDR*) gene but their association with ulcerative colitis (UC) susceptibility remained controversy. In the current study, we examined the association between *VDR* polymorphisms and serum level of 25-hydroxyvitamin D [25(OH)D] with UC in Chinese Han population. Polymorphisms of FokI (rs2228570)/BsmI (rs1544410)/ApaI (rs7975232)/TaqI (rs731236) in the *VDR* gene were assessed in a case–control study comprising 404 UC patients and 612 controls. Moreover, 25(OH)D levels were measured by electro-chemiluminescence immunoassay in 75 UC patients and 120 controls. Our results suggested that *Bsm*I polymorphism frequency was significantly lower in UC patients ($P = 0.028$), and the frequency of AAC haplotype formed by *Bsm*I, *Apa*I and *Taq*I was also significantly lower in UC patients ($P = 0.012$). Moreover, *Fok*I polymorphism was more frequently observed in patients with mild and moderate UC as compared to those with severe UC ($P = 0.001$, $P < 0.001$, respectively). Average 25(OH)D level was lower in UC patients than in controls (19.3 ± 6.8 *vs.* 21.8 ± 7.3 ng/mL, $P = 0.017$), and

Abbreviations: IBDs, inflammatory bowel diseases; UC, ulcerative colitis; CD, Crohn's disease; SLE, systemic lupus erythematosus; VDR, vitamin D receptor; 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein; WBC, white blood cell count; Hb, hemoglobin; PLT, platelet count; Alb, albumin; ESR, erythrocyte sedimentation rate; BMI, body mass index; PCR, polymerase chain reaction; SD, standard deviation; LD, linkage disequilibrium; OR, odds ratio; CI, confidence interval.

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was significantly correlated with hemoglobin ($\beta = 0.49$, $P < 0.001$), C-reactive protein ($\beta = -0.36$, $P < 0.001$), severity of UC ($\beta = -0.21$, $P = 0.025$) and *Fok*I polymorphism ($\beta = -0.20$, $P = 0.031$) in UC patients. Interestingly, there was a significant correlation between *Fok*I polymorphism and vitamin D deficiency (<20 ng/mL) in UC patients ($P = 0.006$). Together, these results supported that *VDR* polymorphisms and 25(OH)D level were significantly correlated with UC risk and severity in Chinese Han population.

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are two main forms of inflammatory bowel disease (IBD) characterized by a chronic, relapsing inflammatory process of the gastrointestinal tract. It is generally accepted that a combination of genetic predisposition, environmental triggers, luminal flora disharmony and immunological imbalance ultimately leads to intestinal inflammation in patients with IBD, although the specific etiology of IBD is still ambiguous [1,2].

Recently, developing evidence supports that vitamin D is playing important roles in anti-inflammation and immunoregulation [3,4]. Previous studies showed that vitamin D interacts with NF-κB, nuclear factor of activated T-cells as well as glucocorticoid receptor, resulting in down-expressions of TNF-α, IL-6 and IL-17 [3]. Through regulating the expression levels of some transcription factors such as Smad, GATA and FoxP3, vitamin D also shifts T cell immune responses more toward anti-inflammatory Th2 and regulatory T cell responses [5,6]. Growing evidence from epidemiological, immunological and genetic studies has implicated vitamin D in many autoimmune diseases including systemic lupus erythematosus (SLE), multiple sclerosis and type 1 diabetes [7–9]. Furthermore, vitamin D deficiency in murine colitis models has been shown to exacerbate the severity of colitis, whereas vitamin D supplementation ameliorates colitis symptoms [10,11].

As a member of steroid-hormone receptor superfamily, the vitamin D receptor (VDR) plays a crucial role in regulating immune response and inflammation through binding with its counter ligand vitamin D. In theory, most of the biological activities of the VDR are mainly controlled and modulated by its own gene polymorphisms. *Fok*I (rs2228570), *Bsm*I (rs1544410), *Apa*I (rs7975232) and *Taq*I (rs731236) are identified as the most important functional loci of the *VDR* gene [12]. So far, numerous genetic association researches have investigated the association between the *VDR* polymorphisms and UC susceptibility, however, the conclusions overall from different ethnic groups were lack of consistency [13–16]. Notably, the influence of *VDR* gene polymorphisms on several related disorders, such as colorectal adenoma [17], prostate cancer [18] and tuberculosis [19], is affected by serum 25-hydroxyvitamin D [25(OH)D] levels, which reflects vitamin D level in clinical practice [20]. In order to determine whether *VDR* genotypes might interact with 25(OH)D levels in UC patients, we examine *VDR* (*Fok*I, *Bsm*I, *Apa*I, *Taq*I) genotypes and 25(OH)D levels in a cohort of UC patients in Chinese Han population from Southeast China.

Materials and methods

Study subjects

From July 2004 to July 2014, a total of 404 UC patients were recruited from The Second and The First Affiliated Hospitals of Wenzhou Medical University, The Central Hospital of Wenzhou City as well as The Wenzhou People Hospital in Zhejiang Province of Southeast China. The diagnosis of UC was established on clinical, radiological, endoscopic and histological data in accordance with Lennard–Jones criteria [21]. The severity of UC was evaluated by Truelove & Witts' severity index, and the location was assessed by the result of colonoscopy as described in the Montreal classification [22]. For each UC patient, the following laboratory parameters were recorded simultaneously: C-reactive protein (CRP), white blood cell count (WBC), hemoglobin (Hb), platelet count (PLT) and albumin (Alb). During the corresponding period, 612 healthy controls were recruited at the Health Examination Center of The Second Affiliated Hospital of Wenzhou Medical University. The exclusion criteria for each study subject were as follows: age less than 18 years older, obesity [body mass index (BMI) > 28 kg/m^2], recent pregnancy or breast feeding (within 6 months), severe dysfunction of liver and kidney as well as other immunological and infectious diseases involved in vitamin D metabolism. In particular, subjects who had received vitamin D supplementation or some drugs interfering with vitamin D metabolism, such as antiepileptics and anticoagulants [23], within 6 months were excluded from our study. All participants are genetically-unrelated Han Chinese. The study protocol was approved by Ethics Committees of the four hospitals mentioned above and the informed consents were obtained from all study subjects.

Genotyping

Genomic DNA was extracted from the peripheral blood leukocytes by the DNeasy Blood & Tissue kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The genotypes of *Fok*I, *Bsm*I, *Apa*I and *Taq*I were examined by ABI *SNaPshot* method (Applied Biosystems, CA, USA) as described previously [24]. In brief, a total of 1 μL genetic DNA was added to a 10 μL polymerase chain reaction (PCR) protocol containing 1 μL dNTPs (Promega, Wisconsin, USA), 1 μL $MgCl_2$ (Roche, Basel, Switzerland), 0.5 U Taq polymerase (Roche, Basel, Switzerland) as well as a defined concentration of each primer [*Fok*I: 0.05 μmol/L, (*Bsm*I,

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