Vitamin D Receptor Gene Polymorphisms Are Associated With Risk Of Hashimoto's Thyroiditis in Chinese Patients in Taiwan

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The etiology of the autoimmune thyroid, Hashimoto's thyroiditis (HT) is not very clear. However, genetic susceptibility is thought to play a critical role. The vitamin D receptor (VDR)-related endocrine system has been demonstrated to be able to carry out modulation of the immune response. Here, we investigated whether single nucleotide polymorphisms (SNPs) of VDR are associated with HT patients. VDR SNP was detected by polymerase chain reaction (PCR)-based restriction analysis in 109 patients with HT and 90 normal controls. Significant differences were found in the genotype distribution of VDR SNP between

Hashimoto's thyroiditis patients and controls ($P = 0.0458$). Allelic frequency of the VDR gene distinguished HT patients from controls ($P = 0.0089$). The results revealed a significant difference between HT patients and normal controls in VDR SNP and a statistic correlation between VDR-FokI polymorphisms and HT formation. It could be concluded that patients who carry the C/C homozygote of the VDR-FokI gene polymorphism in exon 2 may have a higher risk of developing HT in Chinese patients in Taiwan. *J. Clin. Lab. Anal.* 20:109–112, 2006. © 2006 Wiley-Liss, Inc.

Key words: Hashimoto's thyroiditis; single nucleotide polymorphisms (SNPs); vitamin D receptor; autoimmune thyroid

INTRODUCTION

Hashimoto's thyroiditis (HT) and Graves' disease (GD) are common chronic autoimmune thyroid diseases (AITD) (1,2). The defining characteristic of HT is that there is lymphocytic infiltration in the thyroid that can cause hypothyroidism, while GD is the production of thyroid stimulating hormone (TSH) receptor-stimulating antibodies that induce hyperthyroidism (3–5). Both diseases are mediated by organ-specific T-cells against thyroid, and are thought to be related to patients' immunogenetic background (5). Several reports showed that development of AITD is an inherited condition that runs in a family (5). Therefore, many researchers tried to identify genes involved in HT pathogenesis by studying single nucleotide polymorphisms (SNPs), and remarkable advances have been achieved. Those disease-related genes include human leukocyte antigen (HLA), cytotoxic T-lymphocyte associated-4 (CTLA-4), TSH recep-

tor (TSHR) gene, and others (3,5). Interestingly, previous studies showed that vitamin D receptor (VDR) SNPs were associated with the susceptibility to AITD (6–8). The correlation between VDR SNPs and HT pathogenesis is under investigation.

Human VDR gene includes six 5' noncoding exons and eight coding exons (9). VDR protein has been found to be expressed in most lymphocytic cells involved in immune systems, such as dendritic cells, CD4⁺ lymphocytes, CD8⁺ T-lymphocytes (10), and monocytic-macrophage lineage cells (11). VDR ligands binding to VDR

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have been indicated to carry out immunomodulatory activities through different mechanisms (12,13). Administration of active vitamin D could prohibit the development of thyroiditis in animal models (14). However, this treatment could not prevent but even enhance the development of AITD in humans (10). These data show that some detail mechanisms of HT formation are still controversial. For examples, associations of the VDR SNP with AITD reported in previous studies could not be observed in different populations (15). Moreover, the same SNP but with distinct genetic backgrounds could either increase or decrease the risk to disease susceptibility. Recently, the connection between VDR SNP and susceptibility to HT has been reported in Japanese patients (8). Nevertheless, this finding could not answer whether VDR SNP could link to the risk of developing HT in the Chinese population. To address this question, we researched the distribution and potential effects of VDR SNP on HT susceptibility in Chinese patients from Taiwan.

MATERIALS AND METHODS

The HT group included 109 patients (nine males, 98 females; age range 17–69 years; mean age 35.8 ± 11.9 years) treated at the China Medical University Hospital, Taichung, Taiwan. A total of 90 irrelevant healthy individuals from the same area were used as controls.

Blood leukocytes were used as the source of DNA in HT patients and controls. DNA was isolated using a Genomaker reagent kit. Genotyping was performed using polymerase chain reaction (PCR) and carried out in a total reaction volume of 50 μ L containing genomic DNA, 2–6 pmol of each primer, $1 \times$ Taq polymerase buffer (1.5 mM MgCl_2) and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA). The PCR analyses were performed using a GeneAmp PCR system 2400 (Perkin Elmer, Wellesley, MA). The PCR conditions comprised an initial denaturation step at 94°C for 5 min; 35 amplification cycles at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec; and a final extension step at 72°C for 7 min. The primers (5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3' and 5'-ATGGAACACCTTGCTTCTTCTCCCTC-3') were used for PCR amplification of exon 2 of the VDR gene. PCR products were digested with the restriction enzyme *FokI* (New England Biolabs, Beverly, MA) overnight at 37°C , separated on 3% agarose gel, and detected by ethidium bromide staining. The enzyme digested the first ATG to yield two products, 69 and 196 bp. The polymorphism was divided into digestible (TT homozygote), indigestible (CC homozygote), and TC heterozygote.

The various parameters were compared using the χ^2 test; $P < 0.05$ was considered statistically significant. SPSS software (version 10.0; SPSS Inc., Chicago, IL) was used to perform the calculations.

RESULTS

DNA samples from HT patients and the controls were prepared and applied to PCR. The 265-bp PCR products were assayed by restriction fragment-length polymorphism (RFLP). Theoretically, two fragments of 69 and 196 bp should be present if the product can be digested by *FokI*. Figure 1 revealed digestible, indigestible homozygous, and heterozygous sites for the VDR gene SNP.

The genotype distribution and allelic frequencies for the VDR gene in the patients and controls were presented in Table 1. Significant differences were found in the genotype distribution of VDR SNP between the patients and controls ($P = 0.0458$). The distribution of the C/C homozygote in the HT patients (36.7%) was higher than that in controls (23.3%). Besides, the allelic frequency of the VDR gene showed statistically significant that can distinguish HT patients from controls ($P = 0.0089$; χ^2 test).

DISCUSSION

This study is the first analysis of the VDR gene in HT in the Chinese population. Our data indicate that VDR SNP is associated with HT in the Chinese population in Taiwan. In addition, because the significance was dominant, this association was not due to random chance event.

Fig. 1. The patterns of RFLP obtained from the three genotypes after *FokI* digestion of the 265-bp PCR product for the VDR gene. The RFLP indicated a heterozygous band in lane 1, a cuttable fragment in lane 2 (196 and 69 bp), and an indigestible fragment in lane 3 (265 bp). (M = Marker, 100-bp ladder).

TABLE 1. Distribution of genotypes among the Hashimoto disease patients & healthy control subjects

	Hashimoto Patients n (%)	Normal Controls n (%)	P*
Genotype			0.0458
C/C	40(36.7%)	21(23.3%)	
T/C	48(44.0%)	40(44.4%)	
T/T	21(19.3%)	29(32.3%)	
Allelic frequency			0.0089
Allele C	128(58.72%)	82(45.56%)	
Allele T	90(41.28%)	98(54.44%)	

*P values were calculated by χ^2 test.

HT results from a malfunction in the immune system and some immune-related genes have been suggested as possible candidates for the genetic cause for developing HT. In recent years, genomic abnormalities/variants are found linked to certain kinds of diseases by SNP assay. Indeed, SNP technologies have been proven as genetic tools for investigating complex genetic traits (16). It would be very important to look for new genetic markers of HT by this method. Especially discovering critical gene SNPs could help in identification of high-risk population, patient prognostication, and preventive measures. In addition, SNPs also offer an effective method for exploring candidate genes in population-based studies. The cause of AITD, including HT, is thought to be associated with multiple genetic and environmental factors. To date, over 20 potential candidate loci related to AITD have been found by means of genetic assay (17,18). Among those genes, immune-related genes are proposed to play a very important role, because AITD is a kind of autoimmune disease. They include genes in the major histocompatibility complex (MHC) region and HLA region, and those encoding CTLA-4, cytokines, immunoglobulins, and vitamin D receptor (VDR), which were found to be linked to AITD genetic susceptibility (18).

In addition to playing a role in immune responses, the vitamin D-mediated endocrine system has been also known to play a modulatory role in calcium homeostasis and cell proliferation. Additionally, it has been indicated that active vitamin D is able to regulate immune response via VDR (12). It could be proposed that any deficiency in the VDR gene could change immune responses that lead to increased risk of developing HT. Moreover, VDR SNPs have been identified with ethnic genotype variations and linked to several common diseases, including osteoarthritis, diabetes, cancer, cardiovascular disease, and tuberculosis (19). In this study, a higher C/C homozygote in exon 2 of the VDR gene was found in HT patients in comparison to the normal population. Significant differences were found in the VDR-*FokI* alleles between HT and healthy controls. Specifically, the VDR SNP

was associated with HT in the Chinese population. In addition, we found that the VDR-*FokI* genotype distribution in the Chinese population is similar to that in the Japanese population, which was previously reported by Ban and Taniyama (8). It could be concluded that the VDR-*FokI* SNP is also likely to have an influence on the risk of developing HT in Chinese population in Taiwan. That is, the VDR-*FokI* SNP should be able to serve as a candidate marker for screening the cause of HT for Asian population.

In summary, our data suggest that Chinese patients in Taiwan who carry the C/C homozygote of T/C polymorphism in the VDR-*FokI* gene may have a higher risk of developing HT. Because no HT patients have been studied for the VDR gene SNP in the Chinese population in Taiwan before, further studies will be required to confirm whether VDR genotyping is clinically efficient for identifying patients at risk of developing HT.

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