Vitamin D receptor gene FokI but not TaqI, Apal, BsmI polymorphism is associated with Hashimoto’s thyroiditis: a meta-analysis

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Four VD receptor (VDR) gene polymorphisms (TaqI, Apal, FokI and BsmI) have been reported to influence Hashimoto’s thyroiditis (HT) risk. However, individual studies have produced inconsistent results. We conducted a comprehensive meta-analysis of eleven case-control studies to better understand roles of the four polymorphisms in HT development. The results showed only FokI polymorphism was significantly associated with the risk of HT (F vs f: OR = 1.44, 95% CI = 1.09–1.91, P = 0.010; FF vs Ff: OR = 1.72, 95% CI = 1.09–2.70, P = 0.019). Subgroup analyses demonstrated the significant effect was only present in Asian population (F vs f: OR = 1.45, 95% CI = 1.07–1.95, P = 0.016; FF vs f: OR = 1.64, 95% CI = 1.03–2.59, P = 0.036; FF + Ff vs ff: OR = 1.34, 95% CI = 1.00–1.80, P = 0.047; FF vs Ff + ff: OR = 1.64, 95% CI = 1.03–2.64, P = 0.039), but not in Caucasian. For TaqI, Apal and BsmI polymorphisms, no significant association was found in any model comparison. Based on the current literature, it appears that only VDR FokI polymorphism is associated with HT risk in Asian population, but not in Caucasians; and the TaqI, Apal and BsmI polymorphisms have not positive association neither in the overall population, nor when stratified by ethnicity. Further well-designed studies with larger sample sizes and different ethnic population are needed to clarify the present findings.

Hashimoto’s thyroiditis (HT) is an autoimmune thyroid disease (AITD), which has been reported to lead hypothyroidism in up to 5% of population1-3. It is characterized by diffuse infiltration of chronic lymphocytic cells and presence of high serum thyroid antibodies concentrations4-6. Accumulating evidence has demonstrated that HT may be an autoimmune disease triggered by both genetic and environmental factors7-9. Data on twins studies showed the concordance rates for HT were significantly higher among monozygotic twins than dizygotic twins10,11, which suggests that patients with HT have a substantial inherited susceptibility. Moreover, a number of studies have reported certain immunomodulatory genes polymorphisms, such as fork head box P3 (FOXP3), cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and human leukocyte antigen (HLA) family, were involved in the susceptibility to HT12-15. Thus, HT seems to be a polygenic disease with a complex mode of inheritance. However, the precise gene factors inciting the condition remain not fully comprehended.

Vitamin D receptor (VDR) is a ligand inducible transcription factor, which is harbored on many human immune cells16-18. The active vitamin D, an important immunomodulator, exerts its biological effects through binding to the VDR, and in this way to modulate immune cells activity, triggering innate and adaptive immune responses19-31. Certain single nucleotide polymorphisms (SNPs) of the VDR gene may modify vitamin D function. More than sixty SNPs of human VDR gene have been reported32-33. Among them, four common VDR SNPs: TaqI (rs731236, exon 9, +65058 T > C), Apal (rs7975232, intron 8, +64978 C > A), FokI (rs2228570, exon 2, +30920 C > T) and BsmI (rs1544410, intron 8, +63980 G > A), were studied intensively for association with various human traits. They were reported to affect the risk of several autoimmune disorders, including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, diabetes mellitus and the other AITD (Graves’ diseases, GD)34-37. Recently, several studies have also investigated the association of the four VDR SNPs and HT susceptibility38-39, but their results were inconsistent.
Therefore, it is necessary to carry out a meta-analysis of the available evidence to clarify this inconsistency and provide a much comprehensive and quantitative understanding of the association of VDR gene polymorphisms with HT risk.

Results
Study characteristics. As shown in Fig. 1, the search strategy retrieved 136 articles. After further evaluation, only eleven relevant studies28–38 finally fulfilled the inclusion criteria, including 1338 cases and 1303 controls. All were case-control studies. Nine studies published in English and two in Chinese. There were six studies involving Asians28,29,31,32,34,36 and the other five studies involving Caucasians30,33,35,37,38. The VDR gene was genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) in all studies, excepting one study used Matrix assisted laser desorption ionization–time of flight mass spectrometer (MALDI-TOF-MS)36. The NOS scores of included studies ranged from 6 to 7 stars, with a median 7 stars. All studies but two28,36 was scored as high quality studies (≥ 7 stars). Table 1 summarizes the characteristics of these studies. The following four VDR SNPs were studied: TaqI (rs731236, alleles T/t), ApaI (rs7975232, alleles A/a), FokI (rs2228570, alleles F/f), and BsmI (rs1544410, alleles B/b). Genotypes are designated conventionally by the first letter of the name of restriction enzymes, with a lower case indicating the presence of restriction site, whereas an upper case letter indicating its absence. Table 2 shows the genotype distribution in the cases and controls, along with the test for genotype distribution and HWE in control group. HT is often diagnosed mainly on the basis of laboratory test for chi square test for genotype distribution and HWE in control group. HT is often diagnosed mainly on the basis of laboratory and ultrasonographic features, such as positive serum anti-thyroid antibodies, heterogeneous echo-structure with diffuse or patchy hypoechogenicity at ultrasonography, with hypothyroid or euthyroid metabolic state.

Meta-analysis results. Table 3 provides the pooled results regarding the association of the four VDR gene polymorphisms and HT risk under five different genetic models, along with the P-value of Egger's test for publication bias.

FokI polymorphism. Eight studies including 978 cases and 938 controls examined the association of FokI polymorphism and HT risk. Pooled analyses showed a significant association in the allele model (F vs f: OR = 1.44, 95% CI = 1.09–1.91, P = 0.010) and the dominant model (FF vs Ff + ff: OR = 1.72, 95% CI = 1.09–2.70, P = 0.019), but not in the other models (Table 3, Fig. 2). Significant heterogeneity existed in these two models (F2 = 69.2%, and P = 0.002 for allele model; F2 = 75.7%, and P = 0.000 for dominant model). Then, Galbraith plot analyses were performed to further explore the sources of heterogeneity. As shown in Fig. 3A and C, the studies performed by Guleryuz et al.,38 and Meng et al.,36 might mainly contribute to the heterogeneity. With exclusion of these studies, the heterogeneity decreased significantly (F2 = 0% and P = 0.760 for F vs f; F2 = 0% and P = 0.738 for FF vs Ff + ff) while the overall association remained significant in these two models (F vs f: OR = 1.72, 95% CI = 1.42–2.07, P = 0.000; FF vs Ff + ff: OR = 2.32, 95% CI = 1.79–3.02, P = 0.000) (Fig. 3B and D). There was one study35 that the genotype distributions in controls departed from HWE. Sensitivity analyses by excluding this study did not change the pooled result of allele model (F vs f: OR = 1.37, 95% CI = 1.03–1.82, P = 0.030), but the P value of the dominant model was borderline (FF vs Ff + ff: OR = 1.54, 95% CI = 0.98–2.43, P = 0.060). Subgroup analyses by ethnicity indicated that the FokI F allele or FF genotype significantly increased the risk of HT in Asians (F vs f: OR = 1.45, 95% CI = 1.07–1.95, P = 0.016; FF vs Ff + ff: OR = 1.64, 95% CI = 1.03–2.59, P = 0.036; FF vs ff: OR = 1.34, 95% CI = 1.00–1.80, P = 0.047; FF vs Ff + ff: OR = 1.64, 95% CI = 1.03–2.64, P = 0.039), but the positive association was not found in Caucasians. However, significant heterogeneity were also detected in two models among studies with Asian population (F vs f: P = 0.027; FF vs Ff + ff: P = 0.020) (Table 4). Galbraith plot analyses indicated that Meng et al.36 might be the source of heterogeneity. With exclusion of this study, the pooled results remain significant (F vs f: OR = 1.64, 95% CI = 1.31–2.04, P = 0.000; FF vs Ff + ff: OR = 2.07, 95% CI = 1.50–2.86, P = 0.000), with no significant heterogeneity (F vs f: P = 0.718; FF vs Ff + ff: P = 0.889; and P = 0.940). Subgroup analyses by study quality suggested that this positive association only existed in pooled analyses of high-quality studies (F vs f: OR = 1.58, 95% CI = 1.10–2.68, P = 0.013; FF vs Ff + ff: OR = 1.92, 95% CI = 1.09–3.40, P = 0.025).
### Table 1. Studies characteristics of each article included in the meta-analysis. MALDI-TOF-MS: Matrix assisted laser desorption ionization-time of flight mass spectrometer; PCR-RFLP: Polymerase chain reaction–restriction fragment length polymorphism; PB: Population-based; HB: Hospital-based; NR: Not reported; NOS, Newcastle–Ottawa Scale.

| Study | Year | Country | Ethnicity | Genotyping method | Control sources | Sample size (case/control) | Age (case/control) | % Female (case/control) | SNPs | Matched factors | NOS score (*) |
|-------|------|---------|-----------|------------------|----------------|---------------------------|------------------|-----------------------|------|---------------|---------------|
| Ban24 | 2001 | Japan   | Asian     | PCR-RFLP         | NR             | 130/150                   | NR/NR            | 100/100               | FokI | NR            | 6             |
| Lin24  | 2006 | China   | Asian     | PCR-RFLP         | PB             | 109/90                    | 36 ±12/NR        | 89/99/NR              | FokI | Region        | 7             |
| Stefani30 | 2008 | Croatia | Caucasian | PCR-RFLP         | PB             | 145/145                   | 44 ±14/42 ±14    | 93.1/93.1             | TaqI, ApaI, BsmI | Age, sex, ethnicity, region | 9             |
| Huo31  | 2010 | China   | Asian     | PCR-RFLP         | PB             | 115/120                   | 38 ±13/37 ± 6.2  | 80.9/75.0            | BsmI | Region        | 8             |
| Ban28  | 2001 | Japan   | Asian     | PCR-RFLP         | PB             | 82/80                     | NR/NR            | 64.6/75.0             | FokI | NR            | 7             |
| Yazici33 | 2013 | Turkey  | Caucasian | PCR-RFLP         | PB             | 111/159                   | 48 ±13/31 ± 6.3  | 86.8/95.5             | TaqI, ApaI, FokI | NR            | 7             |
| Inoue34 | 2014 | Japan   | Asian     | PCR-RFLP         | PB             | 116/76                    | NR/28.9 ±11      | 64.5                  | TaqI, ApaI, FokI, BsmI | NR            | 7             |
| Djurovic35 | 2015 | Serbia  | Caucasian | PCR-RFLP         | PB             | 44/32                     | 38 ± 5.4/NR      | 100/100               | TaqI, ApaI, FokI, BsmI | Age, sex, region | 9             |
| Meng36  | 2015 | China   | Asian     | MALDI-TOF-MS     | HB             | 250/301                   | 31.9 ±13/33.6 ±13| 84.4/69.8            | TaqI, ApaI, FokI, BsmI | NR            | 6             |
| Giovannazzo37 | 2016 | Italy   | Caucasian | PCR-RFLP         | PB             | 100/100                   | 42 ±15/40 ±13    | 87/88                 | TaqI, ApaI, BsmI | Age, sex, region | 9             |
| Galeryua38 | 2016 | Turkey  | Caucasian | PCR-RFLP         | PB             | 136/50                    | 39 ±9.9/35 ±11   | 91.2/90.0             | TaqI, FokI | Sex           | 8             |

BsmI polymorphism. Six studies including 837 cases and 901 controls evaluated the association of BsmI polymorphism and HT risk. Pooled results indicated that there was no significant correlation between BsmI polymorphism and HT risk in all genetic models (B vs b: OR = 0.95, 95% CI = 0.72–1.26, P = 0.727; BB vs bb: OR = 0.84, 95% CI = 0.46–1.52, P = 0.554; Bb vs bb: OR = 0.99, 95% CI = 0.76–1.29, P = 0.930; BB + Bb vs bb: OR = 0.96, 95% CI = 0.73–1.27, P = 0.764; BB vs Bb + bb: OR = 0.84, 95% CI = 0.49–1.45, P = 0.538) in the overall population (Table 3). Similar results were also observed in the subgroup analyses by ethnicity (Table 4). Moreover, sensitivity analyses showed the results did not change meaningfully by excluding two studies31,34 departed from HWE or one study with low-quality36. There was no significant heterogeneity for all models except the allele model (I² = 52.1% and P = 0.064). A Galbraith plot analysis suggested that Stefanic et al.30 might be the source of heterogeneity for the allele model. Omitting this study, the pooled result was still not statistically significant (B vs b: OR = 1.06, 95% CI = 0.85–1.31, P = 0.615), with no significant heterogeneity (I² = 0% and P = 0.621).

ApaI polymorphism. Six studies including 766 cases and 813 controls evaluated the association of ApaI polymorphism and HT risk. The meta-analyses demonstrated no positive relationship of ApaI polymorphism and HT risk in the overall population (A vs a: OR = 0.98, 95% CI = 0.82–1.19, P = 0.869; AA vs aa: OR = 0.90, 95% CI = 0.60–1.36, P = 0.615; Aa vs aa: OR = 1.06, 95% CI = 0.82–1.36, P = 0.670; AA + Aa vs aa: OR = 1.01, 95% CI = 0.78–1.32, P = 0.916; AA vs Aa + aa: OR = 0.92, 95% CI = 0.65–1.29, P = 0.620). No significant heterogeneity was found in all the comparisons (all P > 0.05, Table 3). Similar results were found in the subgroup analyses by ethnicity; ApaI polymorphism was not associated with HT risk in Asian or Caucasian populations (Table 3). Sensitivity analyses, by excluding these two studies31,33, not in HWE or one study with low-quality36, suggested that the results were consistent with those of the primary analyses (all P > 0.05).

TaqI polymorphism. A total of 902 cases and 863 controls from seven studies investigated the relationship between TaqI polymorphism and HT risk. The genotype distribution was consistent with HWE in the controls of all studies (all P > 0.05, Table 2). The pooled results showed that the TaqI polymorphism wasn’t significantly associated with HT risk (T vs t: OR = 1.16, 95% CI = 0.83–1.62, P = 0.372; TT vs tt: OR = 1.55, 95% CI = 0.87–2.76, P = 0.139; Tt vs tt: OR = 1.19, 95% CI = 0.79–1.81, P = 0.386; TT + Tt vs tt: OR = 1.42, 95% CI = 0.98–2.04, P = 0.064; TT vs Tt + tt: OR = 1.23, 95% CI = 0.77–1.96, P = 0.379, Table 3). There was significant heterogeneity for comparison of T vs tt and TT vs Tt + tt (I² = 70.8%, P = 0.002 and I² = 75.4%, P = 0.000, respectively). In the Galbraith plots, two studies33,35 were outside of the 95%CI from the log OR, causing the heterogeneity in the results. When these two studies were excluded, the heterogeneity decreased significantly, but the pooled results were not changed significantly (T vs t: OR = 1.16, 95% CI = 0.95–1.41, P = 0.147; I² = 0% and P = 0.635 for heterogeneity; TT vs Tt + tt: OR = 1.16, 95% CI = 0.90–1.50, P = 0.262; I² = 0% and P = 0.788 for heterogeneity). Subgroup analyses by ethnicity found the similar results in Caucasian or in Asian (all P > 0.05) (Table 4).

Publication bias. No evidence of publication bias was detected by visual inspections of these funnel plots and Egger’s test in all the models regarding the FokI, TaqI and ApaI polymorphism (all P Egger’s > 0.05). However, significant publication bias was detected in two models regarding BsmI polymorphism (P Egger’s = 0.001 for BB vs bb and P Egger’s = 0.005 for BB + Bb vs bb) (Table 3, Fig. 4A and C). We used the trim and fill method incorporating the hypothetical studies to recalculate the pooled risk estimate. The pooled analyses continued to show no significant association between BsmI polymorphism and HT risk (BB vs bb: OR = 0.90, 95% CI = 0.71–1.15, P = 0.397;
and BB + Bb vs bb: OR = 0.80, 95% CI = 0.59–1.08, P = 0.141). The imputed studies produced symmetrical funnel plots (Fig. 4B and D).

Discussion
To our knowledge, this is the first meta-analysis specially focused on the association of VDR polymorphism with HT risk. A significant association between the BsmI and TaqI polymorphisms and AITD risk has been reported by a previous meta-analysis. However, in that study, the AITD, including GD and HT, was regarded as an entirety to analyze and only two studies concentrated on HT alone among all the contained studies. Although GD and HT shared similar immune-mediated mechanisms characterized by the production of thyroid autoantibodies and by thyroid lymphocytic infiltration, a number of studies has indicated that the two diseases might harbor different susceptibility genes. Thus, it is necessary to perform a meta-analysis specially focused on HT. Recently, several individual studies have been conducted to investigate the association between the VDR gene polymorphisms and HT risk, but results from these studies remain conflictive and inconclusive. The reasons for this discrepancy may be small sample size, extensive geographic variations and difference in lifestyle and ethnicities. Therefore, in order to overcome the potential limitations of individual studies, we performed a meta-analysis and found that VDR FokI but not TaqI, ApaI and BsmI polymorphism was significantly associated with the risk of HT. Furthermore, the positive association of FokI polymorphism was only detected in Asians, not in Caucasians by subgroup analyses based on ethnicity.

Table 2. Distribution of VDR genotype and allele in Hashimoto’s thyroiditis patients and controls. HWE: Hardy-Weinberg equilibrium.
Eight previous studies investigated the distributional difference of FokI polymorphism in patients with HT and controls, and six found a positive association, but another two studies did not. By pooling these results, our meta-analysis demonstrated that the F allele might be a risk factor for susceptibility of HT (OR = 1.44, P = 0.010) and the incidence of HT was significantly higher in FF genotype individuals than that of Ff genotype individuals in overall population (OR = 1.72, P = 0.019). In addition, results from subgroup analyses stratified by ethnic group indicated that HT risk was increased in Asians with FF genotype (OR = 1.64, P = 0.039), but not in Caucasians. This inconsistent result in these two ethnicities may be due to the influence of different genetic backgrounds, lifestyle and environment factors (such as sunlight exposure and diet). In addition, an insufficient number of samples for analysis might lead to unreliable conclusions with deviation in Caucasians.

**Table 3.** Meta-analyses of the association between VDR gene polymorphisms and Hashimoto's thyroiditis risk. Sample size refers to the total number of genotype for cases and controls; n number of involved studies; Bold indicating P < 0.05.

| SNPs         | Sample size* (case/control) | Genetic models | Test for association | Test for heterogeneity |
|--------------|----------------------------|----------------|----------------------|------------------------|
|              |                            |                | OR (95% CI)          | P          | (%)  | P  |
|              |                            | F vs f         | 1.44 (1.09–1.91)    | 0.010      | 69.2 | 0.002 | 0.158 |
| Fokl rs2228570 (n = 8) | 978/938          | FF vs ff       | 1.43 (0.99–2.08)    | 0.059      | 20.9 | 0.264 | 0.526 |
|              |                            | Ff vs ff       | 1.09 (0.82–1.45)    | 0.566      | 0    | 0.485 | 0.594 |
|              |                            | FF vs Ff vs ff | 1.25 (0.95–1.63)    | 0.107      | 0    | 0.574 | 0.793 |
|              |                            | FF vs FF + ff  | 1.72 (1.02–2.70)    | 0.019      | 75.7 | 0.000 | 0.290 |
| BsmI rs1544410 (n = 6) | 837/901          | B vs b         | 0.95 (0.72–1.26)    | 0.727      | 52.1 | 0.064 | 0.121 |
|              |                            | BB vs bb       | 0.84 (0.46–1.52)    | 0.554      | 43.5 | 0.115 | 0.380 |
|              |                            | Bb vs Bb       | 0.99 (0.76–1.29)    | 0.930      | 0    | 0.672 | 0.001 |
|              |                            | BB + Bb vs bb  | 0.96 (0.73–1.27)    | 0.764      | 18.5 | 0.293 | 0.005 |
|              |                            | AA vs a        | 0.98 (0.82–1.19)    | 0.869      | 33.2 | 0.187 | 0.896 |
| Apal rs7975232 (n = 6) | 766/813          | AA vs aa       | 0.90 (0.60–1.36)    | 0.615      | 33.2 | 0.187 | 0.999 |
|              |                            | Aa vs aa       | 1.06 (0.82–1.36)    | 0.670      | 5.7  | 0.380 | 0.438 |
|              |                            | AA + Aa vs aa  | 1.01 (0.78–1.32)    | 0.916      | 18.3 | 0.295 | 0.607 |
|              |                            | AA vs Aa + aa  | 0.92 (0.65–1.29)    | 0.620      | 37.4 | 0.157 | 0.719 |
| TaqI rs731236 (n = 7)  | 902/863          | T vs t         | 1.16 (0.83–1.62)    | 0.372      | 70.8 | 0.002 | 0.052 |
|              |                            | TT vs Tt       | 1.55 (0.87–2.76)    | 0.139      | 40.9 | 0.118 | 0.147 |
|              |                            | Tt vs tt       | 1.19 (0.79–1.81)    | 0.386      | 0    | 0.687 | 0.208 |
|              |                            | TT + Tt vs tt  | 1.42 (0.98–2.04)    | 0.064      | 0    | 0.440 | 0.330 |
|              |                            | TT vs Tt + tt  | 1.23 (0.77–1.96)    | 0.379      | 75.4 | 0.000 | 0.113 |

VDR 3′-RFLP haplotypes have been positioned within the regulatory element spanning-3′-untranslated region which contains polymorphic sequences affecting either VDR mRNA stability or VDR transcriptional activity. Thus, **BsmI, Apal** and **TaqI**, although functionally most likely anonymous, have been associated with total and allele-specific VDR mRNA expression. Given these three variants strong LD with each other, it is rational to assess the haplotypes effects of VDR polymorphism on HT risk. Meng et al. reported three common haplotypes (ab, Ab and AB) of **Apal-BsmI** LD block were not associated with Chinese patients with HT (P > 0.05). Giovinazzo et al. found the distribution of Bat and baT, the two most common BsmI–Apal–TaqI haplotypes, was not significantly different in HT patients and controls from Italy. In another study conducted in Croatia, the bT and BT of **BsmI-TaqI** haplotypes were found to be the predisposing and protective haplotypes, respectively. Similarly, common baT as well as the rare BaT haplotypes was associated with increased and decreased risk, respectively. However, we couldn't do meta-analysis due to insufficient published data in these studies. These effects, including effects associated with rare variants or specific stimuli need further research.

Vitamin D, well-known for its role in calcium and bone metabolism, has important effects on immune regulation by binding to the VDR localized in T lymphocytes and macrophages. A number of studies have found the serum vitamin D level was lower in subjects with HT than that of healthy controls. This inverse association indicated that vitamin D deficiency might be a causal factor leading to HT. Therefore, vitamin D level might be a significant confounder which should be considered when analyzing the association of VDR and HT risk.
However, a different point of view has also been postulated, which suggested that the low level of serum vitamin D seen in disease is a secondary phenomenon of VDR dysfunction rather than the reason for autoimmunity. Although vitamin D level is seen as playing an important role, it is VDR dysfunction that is proposed to be the key factor in the autoimmune diseases process. Because VDR is key to innate immune response which is important in the pathogenesis of autoimmune diseases, VDR dysregulation greatly compromises the innate immune response. The 25-hydroxyvitamin D3 (25-OHD) level is a reliable parameter reflecting the vitamin D level of the body and usually measured as the level of vitamin D. When VDR dysregulation, the expression of CYP24A1, an enzyme that inactivating 1,25-dihydroxyvitamin D (1,25-OHD) was inhibited. Increased 1,25-OHD will decrease 25-OHD by reducing gene expression and inhibiting expression of CYP27A1 which is an enzyme involved in conversion of vitamin D into 25-OHD.

Among our included studies, only two studies concurrently provided the information on vitamin D levels and VDR in patients with HT. One study found that the prevalence of vitamin D deficiency in HT patients was significantly higher than that in the control group (70% vs 18.2%; \( P = 0.0001 \)), but VDR BsmI, ApaI, and TaqI polymorphisms were not associated with HT risk. The other study indicated that the prevalence of vitamin D insufficiency in HT cases was significantly higher than controls (\( P = 0.02 \)) while VDR TaqI, but not FokI polymorphisms is associated with HT. It is unfortunate that neither study analyzed the

![Figure 2. Meta-analysis of the association of FokI polymorphism and HT risk based on different gene models.](image-url)
distributional difference of VDR polymorphisms stratified by vitamin D levels. Therefore, the mechanism and effect for the interaction of vitamin D and VDR in patients with HT need further investigations.

Several limitations should be discussed when explaining the results of our meta-analysis. First, lack of adjustments for some factors, such as age, gender, thyroid functional status, circulating vitamin D levels, or dietary vitamin D intake, which may influence the association between VDR variants and risk of HT, might bias the present results. Second, because of unpublished data or limited number of studies, significant publication bias was found in two models regarding \( BsmI \) polymorphism, which might have some impact on the final outcome. However, we used trim and fill method to assess the influence of publication bias and found that the results were not significantly changed with or without the addition of hypothetical missing studies. Heterogeneity among studies was also detected in some analyses due to ethnic difference, geographic characteristics and lifestyle. However, our sensitivity analysis showed that studies that contribute to heterogeneity did not significantly alter the conclusions of the overall OR. Third, the statistical power to detect the association may be lower because number of studies included in our meta-analysis is relatively small. However, Ioannidis et al. estimated the median sample size required to detect the observed summary effects in each population addressed in 752 studies is 3,535, which is 13.3-fold more subjects than in each original study. These sample size requirements can be inflated considerably if trying to account for potential bias or heterogeneity. These estimates may be difficult to address even by very large biobanks and observational cohorts. Therefore, meta-analysis is an effective way to explore the truth before the emergence of large sample data. Further studies should be focusing on innovative study designs and strong collaborative efforts.

In conclusion, our meta-analysis suggests that the VDR \( FokI \) polymorphism is associated with HT risk in overall population or in Asians, but not in Caucasians. The \( TaqI, Apal \) and \( BsmI \) polymorphisms are not associated with HT risk. Further well-designed studies with larger sample sizes and different ethnic population are needed to clarify the present findings. Furthermore, the exact causality and mechanism for the interaction of VDR and HT development need further experimental or animal mechanism studies.

Figure 3. Evaluation of heterogeneity among studies on FokI polymorphism. Galbraith plot analyses for the comparisons of allele model (A) and recessive model (C); Pooled risk estimates with its 95% CIs for the allele model (B) and recessive model (D) after removing studies that contribute most to heterogeneity. \( b = \ln(OR) \); \( se(b) = \) standard error of \( \ln(OR) \).
Methods

Search strategy. We identified all the studies regarding the relationship of VDR gene polymorphisms and HT by searching PubMed, Embase, China National Knowledge Internet (CNKI), and Wanfang databases without language restrictions (the last search update performed on September 30, 2016). The following key words and search terms were used to identify relative publications: "Vitamin D receptor", "VDR", "ApaI", "BsmI", "FokI", "TaqI" and "Hashimoto's thyroiditis". The reference lists of identified articles and related reviews were reviewed for additional studies.

Inclusion and exclusion criteria. Studies meeting all of the following inclusion criteria were included: (1) case-control study or cohort study; (2) investigating the association between VDR gene polymorphisms (ApaI, BsmI, FokI and TaqI) and HT risk; and (3) providing the frequencies of the variants in cases and controls or providing sufficient data to calculate the estimation of odds ratios (ORs) with 95% confidence interval (95% CI). Exclusion criteria were as follows: (1) overlapping data; (2) studies without genotype frequency and genotype distribution or insufficient information for data extraction; (3) family-based study design; and (4) abstracts, reviews,

Table 4. Subgroup analyses of the association between VDR gene polymorphisms and Hashimoto's thyroiditis risk based on ethnicity. *Sample size refers to the total number of genotype for cases and controls; n number of involved studies; Bold indicating P < 0.05.
comments or editorial articles lack of necessary raw data. In the case of overlapping data, only the study with the largest population was selected for this meta-analysis.

**Data extraction.** Two investigators (XF Wang and WL Cheng) extracted data independently. Any disagreement was resolved through discussion. The extracted data included: name of the first author, year of publication, country, ethnicity, number of cases and controls, genotyping method, control sources, and genotype distribution in cases and controls.

**Quality Assessment.** The quality of included studies was assessed by two independent reviewers (XF Wang and Y Ma) using the Newcastle-Ottawa Scale (NOS)\(^6\). The NOS judged a study based on three perspectives: selection, comparability and exposure/outcome. The full score was 9 stars. Study that scored above six stars was considered as high quality.

**Statistical analysis.** A random-effects model was used to incorporate within- and between-study heterogeneity as this can provide more conservative result than a fixed effects model\(^6\). Pooled ORs and their respective 95% CIs were calculated to evaluate the association between the four VDR SNPs and HT risk under five genetic models: the allele model (eg, A vs a), the homozygous model (eg, AA vs aa), the heterozygous model (eg, Aa vs aa), the recessive model (eg, AA + Aa vs aa), and the dominant model (eg, AA vs Aa + aa). The Hardy-Weinberg equilibrium (HWE) in controls was tested using the goodness-of-fit \(\chi^2\) statistic with one degree of freedom\(^6\). Cochrane’s Q test and \(I^2\) test were used to assess heterogeneity among trials. Q-test reported a \(P\) value < 0.1 or \(I^2\) > 50% was defined as significant heterogeneity\(^6\). In case of substantial heterogeneity, a Galbraith plot was created to graphically identify the potential outlier studies that might cause the heterogeneity. Then, a meta-analysis was rerun after excluding the outlier studies\(^6\). Subgroup analyses were performed based on ethnicity and quality of included studies to avoid the potential bias influence. Sensitivity analyses were performed by excluding each individual study or the studies with controls inconsistent with HWE to evaluate the impact of individual study on the pooled risk estimate. Publication bias was evaluated by visual inspection of funnel plot and Egger’s test\(^6\). If publication bias was indicated, the “trim and fill” method which conservatively imputes hypothetical negative unpublished studies to mirror the positive studies that cause funnel plot asymmetry was performed to further assess the possible effect of publication bias\(^6\). All \(P\)-values were two-tailed. All analyses were performed using Stata 11.0 (Stata Corporation, College Station, TX, USA). This article follows the PRISMA statement\(^6\) and the Cochrane Collaboration guidelines for reporting meta-analysis.
41. Whitfield, G. K. et al. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. *Mol. Cell. Endocrinol.* **177**, 145–159 (2001).
42. Kanan, R. M., Varanasi, S. S., Francis, R. M., Parker, L. & Datta, H. K. Vitamin D receptor gene start codon polymorphism (FokI) and bone mineral density in healthy male subjects. *Clinical endocrinology* **53**, 93–98 (2000).
43. Jurutka, P. W. et al. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. *Mol. Endocrinol.* **14**, 401–420, doi: 10.1210/mend.14.3.0435 (2000).
44. Colin, E. M. et al. Consequences of vitamin D receptor gene polymorphism in cultured human peripheral blood mononuclear cells by 1, 25-dihydroxyvitamin D3. *Clinical endocrinology* **52**, 211–216 (2000).
45. Saito, T. et al. A unique mutation in the vitamin D receptor gene in three Japanese patients with vitamin D-dependent rickets type II: utility of single-strand conformation polymorphism analysis for heterozygous carrier detection. *Am. J. Hum. Genet.* **49**, 668–673 (1991).
46. van Eten, E. et al. The vitamin D receptor gene FokI polymorphism: functional impact on the immune system. *Eur. J. Immunol.* **37**, 395–405, doi: 10.1002/eji.200636043 (2007).
47. Jurutka, P. W. et al. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. *Rev. Endocr. Metab. Disord.* **2**, 203–216 (2001).
48. Pastinen, T. et al. Mapping common regulatory variants to human haplotypes. *Hum. Mol. Genet.* **14**, 3963–3971, doi: 10.1093/hmg/ddi420 (2005).
49. Deluca, H. F. Overview of general physiologic features and functions of vitamin D. *Am. J. Clin. Nutr.* **80**, 1689S–1696S (2004).
50. Deluca, H. F. & Cantorna, M. T. Vitamin D: its role and uses in immunology. *FASEB J.* **15**, 2579–2585, doi: 10.1096/fj.01-0433rev (2001).
51. Bozkurt, N. C. et al. The association between severity of vitamin D deficiency and Hashimoto's thyroiditis. *Endocrin. Pract.* **19**, 479–484, doi: 10.4158/EP12376.OR (2013).
52. Mansournia, N., Mansournia, M. A., Saeedi, S. & Dehghan, J. The association between serum 25OHD levels and hypothyroid Hashimoto's thyroiditis. *Journal of endocrinological investigation* **37**, 473–476, doi: 10.1007/s40618-014-0064-y (2014).
53. Mazokopakis, E. E. et al. Is vitamin D related to pathogenesis and treatment of Hashimoto's thyroiditis? *Hell. J. Nucl. Med.* **18**, 222–227 (2015).
54. Wang, J. et al. Meta-analysis of the association between vitamin D and autoimmune thyroid disease. *Nutrients* **7**, 2485–2498, doi: 10.3390/nu7042485 (2015).
55. Proal, A. D., Albert, P. J. & Marshall, T. G. Dysregulation of the vitamin D nuclear receptor may contribute to the higher prevalence of some autoimmune diseases in women. *Ann. N. Y. Acad. Sci.* **1173**, 252–259, doi: 10.1111/j.1749-6632.2009.04672.x (2009).
56. Waterhouse, J. C., Perez, T. H. & Albert, P. J. Reversing bacteria-induced vitamin D receptor dysfunction is key to autoimmune disorders. *Rev. Immunol.* **25**, 225–250 (2005).
57. Yoshizawa, T. et al. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nature genetics* **36**, 391–396, doi: 10.1038/ng0897-391 (1997).
58. Ioannidis, J. P., Trikalinos, T. A. & Khoury, M. J. Implications of small effect sizes of individual genetic variants on the design and quality of nonrandomised studies in meta-analyses. *Clin. Genet.* **72**, 203–216 (2007).
59. Waterhouse, J. C., Perez, T. H. & Albert, P. J. Reversing bacteria-induced vitamin D receptor dysfunction is key to autoimmune disease. *Ann. N. Y. Acad. Sci.* **1173**, 757–765, doi: 10.1111/j.1749-6632.2009.04673.x (2009).
60. Marshall, T. G. Vitamin D discovery outpaces FDA decision making. *Bioessays* **30**, 173–182, doi: 10.1002/bies.20708 (2008).
61. Wang, J. et al. Vitamin D receptor gene FokI but not TaqI, ApaI, BsmI polymorphism is associated with Hashimoto's thyroiditis: a meta-analysis. *Sci. Rep.* **7**, 41540; doi: 10.1038/srep41540 (2017).

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X.F. Wang, W.L. Cheng, Y. Ma and J.Q. Zhu were responsible for the conception and design, acquisition of data, analysis and interpretation of data, drafting the initial manuscript and revising it critically for important intellectual content. X.F. Wang wrote the final draft. All authors read and approved the final manuscript.

**Additional Information**

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