

# Lack of Association of VDR Gene Polymorphisms with Thyroid Autoimmune Disorders: Familial and Case/Control Studies

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**Abstract** We study the association between three Vitamin D receptor gene polymorphisms (rs10735810, rs1544410, rs731236) and susceptibility to thyroid autoimmune diseases. Seventy-six affected subjects, belonging to a large family, as well as one hundred unrelated Tunisian patients and one hundred healthy Tunisian controls were genotyped. A family-based association test and a standard chi-square test were used to assess association in family and case-control data, respectively. Our results showed no significant association of the Vitamin D receptor gene polymorphisms with the susceptibility to thyroid autoimmune diseases in the family. Moreover, allele frequencies for the three

polymorphisms in the Tunisian population were similar to those reported in the Tunisian control population and none was associated with the disease. These results suggest a lack of association between the Vitamin D receptor gene polymorphisms and susceptibility to thyroid autoimmune diseases in Tunisian population, in agreement with data from the UK, but in conflict with studies from the Far East.

**Keywords** Vitamin D receptor · autoimmune thyroid diseases · association study · gene polymorphism

## Introduction

The autoimmune thyroid diseases (AITDs) include a number of disorders, Graves' disease (GD), and Hashimoto's thyroiditis (HT) and atrophic thyroiditis. The pathogenesis of these diseases involves a complex interaction between genetic and environmental factors. The role of heredity is revealed by the increased prevalence of AITDs among family members and a high concordance rate in monozygotic twins (20–30%) [1] as compared to dizygotic twins (0–7%) [2]. When looking for the genetic factors underlying the development of AITDs, most attention has been oriented on the HLA region. Graves' disease and Hashimoto's thyroiditis showed mainly association with the –DR3 and –DR5 of the HLA complex [3–9]. However, the relative weakness of the contribution of HLA genes to susceptibility in AITDs is underlined by the lower concordance (7%) for Graves' disease in HLA-identical siblings, compared to 20 to 30% for monozygotic twins [1] and the weak or absent linkage of HLA with Graves' disease and Hashimoto's thyroiditis in family studies [10–

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12]. Therefore, a major component of the genetic susceptibility to AITDs lies outside the HLA region [13].

To explore other genetic influences contributing to the development of AITDs, recent studies were focused on the vitamin D receptor (VDR) that plays an immunoregulatory role, based on the fact that the activation of human leucocytes causes the expression of the vitamin D receptor (VDR). Vitamin D is also involved in interleukin-2 (IL-2) inhibition and antibody production and in lymphocyte proliferation suppression and the generation of cytotoxic lymphocytes [14, 15]. In addition, monocytes constitutively express VDR, as well as activated but not resting lymphocytes [16, 17]. The VDR gene, lies on chromosome 12q12-14 and harbours several polymorphisms and was found to be associated with several autoimmune diseases as Addison's disease, Type 1 diabetes, Rheumatoid arthritis, and systemic lupus erythematosus [18–21]. However, other genetic linkage studies failed to confirm VDR as a susceptibility locus in systemic lupus erythematosus [20]. In this study, we investigated the distribution of three VDR polymorphisms: *FokI* T>C (rs 10735810) located in exon 2, *BsmI* A>G (rs 1544410) located in intron 8 and *TaqI* C>T (rs 731236) located in exon 9 (Fig. 1). Our study concerned a large consanguineous multiplex family affected with GD and HT as well as unrelated sporadic AITDs patients and controls to explore a possible existence of association. Our results did not support the involvement of VDR gene polymorphisms in AITDs pathogenesis in Akr family and general Tunisian population.

## Materials and Methods

### Sample

The Akr family is composed of more than 400 members (76 affected) extending over ten generations from which 242 (from three generations) were sampled: 40 with GD, 13 with HT, 23 with Primary Idiopathic Myxoedema (PIM), and 166 unaffected. The female/male ratio was 1 in GD patients, 3.3 in HT patients, and 4.2 for patients with PIM. Patient ages at the onset of the disease ranged from 12 to

58 years with a mean age of 35 years [22, 23]. Blood samples were available for 123 individuals.

Moreover, 100 unrelated randomly recruited Tunisian patients subdivided into 55 GD and 45 HT were included in this study. GD patients included 43 women and 12 men. HT patients included 30 women and 15 men. A total of 100 healthy controls living in the same geographic area were used as controls including 47 men and 53 women.

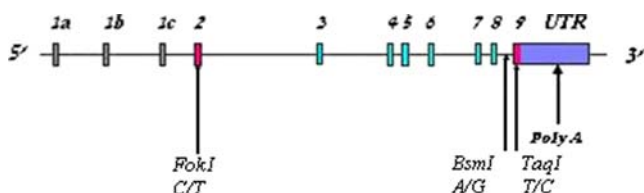
The diagnosis of GD was based on the presence of biochemical hyperthyroidism, as indicated by a decrease in TSH, an increase in T4 levels and positive anti-TSH receptor antibodies evaluated using the TRAK assay (Henning, Berlin, Germany), in association with diffuse goiter or the presence of exophthalmos. The level of anti-TSHR varied between 4.5 and 85% of inhibition of binding of radiolabelled TSH to the purified and solubilized receptor [24]. The diagnosis of HT was based on the presence of a palpable goiter and the presence of thyroid hormone-replaced primary hypothyroidism as defined as a TSH level above the upper limits associated with positive titers of thyroid autoantibodies (anti-thyroid peroxidase or anti-thyroglobulin) using the Immunofluorescence and ELISA methods [22]. The level of anti-TPO was in the range 1/10–1/500 and 1/20–1/200 for GD and HT, respectively. The level of anti-Tg varied between 1.14 and 20 mU/ml for GD and 1.11 and 17.41 mU/ml for HT. PIM was diagnosed by the presence of hypothyroidism requiring T3 or T4 replacement. Patients with PIM have an atrophic gland.

### Genotyping

DNA was isolated from whole blood according to standard protocols. Genotypes for three restriction polymorphic sites (*FokI*, *BsmI*, and *TaqI*) were identified by the polymerase chain reaction followed by restriction fragment length polymorphism (PCR/RFLP) method. The *FokI*, *BsmI*, and *TaqI* polymorphisms were studied using primers described by Pani et al. [21]. PCR amplifications were performed on each sample in a 25  $\mu$ l reaction volume using Techne thermocycler. PCR-RFLP conditions were as described by Maalej A et al. [25].

### Statistical Analysis

FBAT software was used to test for association between the intragenic SNPs of the VDR gene and the disease. Both single and multiple marker (haplotypic) tests were performed. This test implements a general family-based association test that allows the use of multiple nuclear families from a single pedigree. This test, called FBAT, accommodates phenotype-unknown subjects, incorporate di- or multi-allelic marker data and allows additive,



**Fig. 1** Structure of the vitamin D receptor gene, numbered boxes represent the 11 VDR exons. Localisation of VDR gene polymorphisms (*FokI*, *BsmI*, and *TaqI*) is represented with arrows.

**Table I** Family-based Association Test (FBAT-e) Under Recessive Model for the *FokI*, *BsmI* and *TaqI* Polymorphisms of VDR Gene in Akr Family

Alleles	Frequencies	Z	p value
F	0.528	0.284	0.77
B	0.692	-1.773	0.07
T	0.654	0.608	0.54

dominant, or recessive models and results in correct type I error probabilities regardless of population admixture, the true genetic model, and the sampling strategy [26–28]. We calculated FBATs under both additive and dominant models and for both the biallelic mode using 45 nuclear families with 167 individuals (extracted automatically by FBAT from the Akr family). To ensure its validity as a test of association in the pedigree, the FBAT statistic was calculated using empirical variance (the e-option).

Results from control subjects and AITDs unrelated patients were compared using the  $\chi^2$  test (2×2 contingency tables) for statistical significance. The distribution of the VDR gene polymorphisms in each group was evaluated. Tests with p value less than 0.05 were considered as statistically significant. Haplotype frequencies and linkage disequilibrium from population data (cases and controls) were estimated using the program PHASE [29] and compared using  $\chi^2$  test and if necessary Fisher Exact test.

**Results**

We calculated FBATs for the Akr family under different genetic models, and we did not find any significant association with any polymorphism. The smallest p value was obtained for *BsmI* polymorphism under recessive model (p=0.07) and all p values for other SNPs were larger than 0.28 (Table I).

To identify a genetic relation between VDR SNPs and clinical phenotype of AITDs (Graves’ disease and Hashi-

**Table II** Family-based Association Test (FBAT) for the *FokI/BsmI/TaqI* Haplotypes in Akr Family

Haplotypes	Frequencies	Z	p value
FBT	0.053	0.588	0.55
FBt	0.413	-1.726	0.084
FbT	0.104	-1.229	0.219
Fbt	0.016	-	-
fBT	0.107	-0.701	0.483
fBt	0.114	0.143	0.886
fbT	0.168	1.564	0.117
fbt	0.024	-0.669	0.503

**Table III** Allele Frequencies for the *FokI*, *BsmI*, and *TaqI* Polymorphisms of VDR Gene in AITDs and Control Subjects

Alleles	AITDs (N=100)	Controls (N=100)	$\chi^2$ (p value)
F	161	156	0.106 (0.74)
B	109	117	0.65 (0.41)
T	123	140	3.20 (0.073)

to’s thyroiditis) FBAT were recalculated considering each phenotype separately. No significant result was observed in either GD or HT. However, for the GD phenotype, a small p value (although nonsignificant after Bonferroni correction) was found for *FokI* (p=0.03, corrected p=0.09).

Haplotypes were inferred from family data. Six different haplotypes with a frequency higher than 5% were found (Table II). The fbT was the most frequent haplotype. No significant association was found with any of the haplotypes under all models (p values ranged from 0.08 to 0.88).

Similar results were found in case-control study. All three polymorphisms were in Hardy-Weinberg equilibrium in the control group. No significant differences in *FokI*, *BsmI*, and *TaqI* were detected between cases and controls (p>0.05; Tables III and IV).

**Discussion**

This study is the first to investigate the involvement of the VDR SNPs (*FokI*, *BsmI*, and *TaqI*) in genetic susceptibility to AITDs in the Tunisian population. Our results suggest that these SNPs, despite their associations with many other autoimmune disorders [18–21] were not associated with AITDs in a large consanguineous family as well as the general Tunisian population. Our results were similar to a previous study that showed a lack of association of the vitamin D receptor gene with Graves’ disease in UK Caucasians [30].

**Table IV** Haplotypes Frequencies for the *FokI*, *BsmI*, and *TaqI* Polymorphisms of VDR Gene in AITDs and Controls Subjects

Genotypes	Controls	AITDS	$\chi^2$ (p value*)	Family
FBT	0.188	0.244	2.58 (0.60)	0.050
FBt	0.200	0.178	0.26 (0.99)	0.349
FbT	0.333	0.262	1.99 (0.75)	0.109
Fbt	0.053	0.119	5.27 (0.16)	0.021
FBT	0.076	0.063	0.15 (0.99)	0.129
fBT	0.05	0.058	0.19 (0.99)	0.110
FbT	0.087	0.049	1.94 (0.76)	0.203
Fbt	0.005	0.023	2.70 (0.57)	0.029

\*Corrected p value (Bonferroni correction for multiple testing)

To assess the informativeness of our case-control sample, we computed the power of detecting, using this sample, a susceptibility variant with a genotypic relative risk of 1.5 (minor contributor) and different allele frequencies (from 0.3 to 0.7) under both multiplicative and recessive models. We found that power varies from 61.2% to 99.9% indicating a good informativeness and supporting the fact that failure to detect association with VDR gene in the case-control study was not due to limitation in sample size.

In contrast to these negative results, VDR gene was reported by Ban et al. [23] to be associated with AITDs on 180 GD patients and 195 controls, which showed a significant association of B allele and Bb genotype ( $p=0.008$  and  $p=0.023$ ), respectively. More recently, the analysis of four dimorphisms (*Apal*, *TaqI*, *BsmI*, and *FokI*) in patients with GD and controls from three European populations (German, Polish, and Serbian) showed that the variant “b” was found to be associated with GD in the Polish population ( $p=0.007$ ) whereas the *FokI* variant “f” was found to be associated with GD in Germans and “F” in Polish patients ( $p=0.0024$  and  $p=0.0049$ ). However, no association was found in the patients from Serbia [31].

It is worth noting that the analysis of case-control and family-based studies of six PTPN22 tag SNPs showed that in contrast to type 1 diabetes, susceptibility to GD is only limited to polymorphism in a segment of this gene, supporting the hypothesis that the mechanisms by which susceptibility genes confer susceptibility to autoimmune diseases may in part be disease specific [32].

Despite extensive efforts, the results in the identification and inference of genetic effects for complex traits from independent association studies often failed to reach consensus [33, 34]. In one meta-analysis, significant allelic effects were observed in 11 of 25 examined gene association studies in the direction opposite to the original report of association [33]. The findings suggest genetic heterogeneity within the VDR gene in different diseases and populations, possibly due to divergent evolutionary lineages resulting in separate clusters of distinct geography [35, 36].

The reasons for nonreplication of association studies are numerous [37] and largely reflect inadequate sample sizes, although differences may also be attributed to population stratification, variation in study design, confounding sampling bias and misclassification of phenotypes [38, 39].

The VDR SNPs showed different frequencies of alleles and haplotypes in the Akr family as compared to the general population. Particularly allele F of *FokI* is more prevalent in the family but the most remarkable difference occurs for haplotype FBT. Similar findings have been reported concerning the IgVH gene family and T cell receptor (TCR) C $\beta$  gene [40] and more generally on 103 STR markers [41].

This different pattern of polymorphism in the Akr family could be explained by the high level of consanguinity in the family [9] and the high rate of endogamy in the village of BirElhfaï population from which the Akr family originates.

The result in this study contrasts with our previous findings on RA [25] and might not support the hypothesis that VDR is a common susceptibility gene for different autoimmune diseases. It remains that the VDR was reported to be a susceptibility gene to many autoimmune disorders. We think that further association studies on many different populations with large samples, as well as functional investigations, are needed to shed light on this issue.

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## References

1. Stenszky V, Kozma L, Balaczs C, Rochlitz S, Bear JC, Farid NR. The genetics of Graves’ disease: HLA and disease susceptibility. *J Clin Endocrinol Metab* 1985;61:735–40.
2. Brix TH, Christensen K, Holm NV, Harvald B, Hegedus L. A population based study of Graves’ disease in Danish Twins. *Clin Endocrinol* 1998;48:397–400.
3. Farid NR, Sampson L, Noel EP, Barnard JM, Mandeville R, Larsen B, et al. A study of human leukocyte D locus related antigens in Graves’ disease. *J Clin Invest* 1979;63:108–13.
4. Allanic H, Fauchet R, Lorcy Y, Heim J, Gueguen M, Leguerrier AM, et al. HLA and Graves’ disease: An association with HLA-DRw3. *J Clin Endocrinol Metab* 1980;51:863–7.
5. Moens H, Farid NR, Sampson L, Noel EP, Barnard JM. Hashimoto’s thyroiditis is associated with HLA-DRw3. *N Engl J Med* 1978;299:133–4.
6. Weissel M, Hofer R, Zasmata H, Mayr WR. HLA-DR and Hashimoto’s thyroiditis. *Tissue Antigens* 1980;16:256–7.
7. Farid NR, Sampson L, Moens H, Barnard JM. The association of goitrous autoimmune thyroiditis with HLA-DR5. *Tissue Antigens* 1981;17:265–8.
8. Thomsen M, Ryder LP, Bech K, bliddal H, Feldt-Rasmussen U, Molholm J, et al. HLA-D in Hashimoto’s thyroiditis. *Tissue Antigens* 1983;21:173–5.
9. Bougacha-Elleuch N, Rebai A, Mnif M, Makni H, Bellassouad M, Joudia J, et al. Analysis of MHC genes in a Tunisian isolate with autoimmune thyroid diseases: implication of TNF  $\gamma$ -308 gene polymorphism. *J Autoimmun* 2004;23:75–80.
10. Roman SH, Greenberg D, Rubinstein P, Wallenstein S, Davies TF. Genetics of autoimmune thyroid disease: lack of evidence for linkage to HLA within families. *J Clin Endocrinol Metab* 1992;74:496–503.

11. Shields DC, Ratanachaiyavong S, McGregor AM, Collins A, Morton NE. Combined segregation and linkage analysis of graves' disease with a thyroid autoantibody diathesis. *Am J Hum Genet* 1994;55:540–54.
12. Tomer Y, Barbesino G, Keddache M, Greenberg DA, Davies TF. Mapping of a major susceptibility locus for Graves' disease (GD1) to chromosome 14q31. *J Clin Endocrinol Metab* 1997;82:1645–8.
13. Ayadi H, Hadj Kacem H, Rebai A, Farid NR. The genetics of autoimmune thyroid disease. *Trends Endocrinol Metab* 2004;15:234–9.
14. Manolagas SC, Provvedini DM, Tsoukas CD. Interactions of 1,25-dihydroxyvitamin D3 and the immune system. *Mol Cell Endocrinol* 1985;43:113–22.
15. Rigby WF. The Immunobiology of Vitamin D. *Immunol Today* 1988;9:54–8.
16. Bhalla AK, Amento EP, Clemens TL, Holick MF, Krane SM. Specific high affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J Clin Endocrinol Metab* 1983;57:1308–10.
17. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-dihydroxyvitamin D3 receptors in human leukocytes. *Science* 1983;221:1181–3.
18. McDermott MF, Ramachandran A, Ogunkolade BW, Aganna E, Curtis D, Boucher BJ, et al. Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. *Diabetologia* 1997;40:971–5.
19. Pani MA, Knapp M, Donner H, Braun J, Baur MP, Usadel KH, et al. Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. *Diabetes* 2000;49:504–7.
20. Huang CM, Wu MC, Wu JY, Tsai FJ. Association of vitamin D receptor gene BsmI polymorphisms in Chinese patients with systemic lupus erythematosus. *Lupus* 2002;11:31–4.
21. Pani MA, Seissler J, Usadel KH, Badenhop K. Vitamin D receptor genotype is associated with Addison's disease. *Eur J Endocrinol* 2002;147:635–40.
22. Makni H, Maalej A, Ayadi F, Abid M, Jouida J, Ayadi H. Clinical and biological follow up of a family with high prevalence of thyroid autoimmune disease. *Tunis Med* 1996;74:433–8.
23. Ban Y, Taniyama M, Ban Y. Vitamin D receptor gene polymorphism is associated with Graves' disease in the Japanese population. *J Clin Endocrinol Metab* 2000;85:4639–43.
24. Filetti S, Foti D, Costante G, Rapoport B. Recombinant human TSH receptor in a radioreceptor assay for the measurement of receptor autoantibodies. *J Clin Endocrinol Metab* 1991;72:1096–101.
25. Maalej A, Petit-Teixeira E, Michou L, Rebai A, Cornelis F, Ayadi H. Association study of VDR gene with rheumatoid arthritis in the French population. *Genes Immun* 2005;6:707–11.
26. Laird NM, Horvath S, Xu X. Implementing a unified approach to family-based tests of association. *Genet Epidemiol* 2000;19 (suppl 1):S36–42.
27. Rabinowitz D, Laird NM. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Hum Hered* 2000;50:211–23.
28. Horvath S, Xu X, Lake SL, Silverman EK, Weiss ST, Laird NM. Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. *Genet Epidemiol* 2004;26:61–9.
29. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001;68:978–89.
30. Collins JE, Heward JM, Nithiyanthan R, Nejentsev S, Todd JA, Franklyn JA, et al. Lack of association of the vitamin D receptor gene with Graves' disease in UK caucasians. *Clin Endocrinol* 2004;60:618–24.
31. Ramos-Lopez E, Kurylowicz A, Bednarczuk T, Paunkovic J, Seidl C, Badenhop K. Vitamin D receptor polymorphisms are associated with Graves' disease in German and Polish but not in Serbian patients. *Thyroid* 2005;15:1125–30.
32. Heward JM, Brand OJ, Barrett JC, Carr-Smith JD, Franklyn JA, Gough SC. Association of PTPN22 haplotypes with Graves' disease. *J Clin Endocrinol Metab* 2007;92:685–90.
33. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003;33:177–82.
34. Redden DT, Allison DB. Nonreplication in genetic association studies of obesity and diabetes research. *J Nutr* 2003;133:3323–6.
35. Vogel A, Strassburg CP, Manns MP. Genetic association of vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. *Hepatology* 2002;35:126–31.
36. Semino O, Passarino G, Oefner PJ, Lin AA, Arbuzova S, Beckman LE, et al. The genetic legacy of paleolithic Homo sapiens sapiens in extant Europeans: a Y chromosome perspective. *Science* 2000;290:1155–9.
37. Tabor HK, Risch NJ, Myers RM. Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nat Rev Genet* 2002;3:391–7.
38. Cardon LR, Palmer LJ. Population stratification and spurious allelic association. *Lancet* 2003;361:598–604.
39. Colhoun HM, McKeigue PM, Davey Smith G. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003;361:865–72.
40. Fakhfakh F, Maalej A, Makni H, Abid M, Jouida J, Zouali M, et al. Analysis of Immunoglobulin VH and TCR C $\beta$  polymorphisms in a large family with thyroid autoimmune disorder. *Exp Clin Immunogenet* 1999;16:185–91.
41. Maalej A, Rebai A, Ayadi A, Jouida J, Makni H, Ayadi H. Allelic structure and distribution of 103 STR loci in a southern Tunisian population. *J Genet* 2004;83:65–71.