

Association of The Vitamin D Receptor Gene Bsml and FokI Polymorphisms with Systemic Sclerosis Risk in a Tunisian Population

Association des polymorphismes du gène Bsml et FokI des récepteurs de vitamine D avec le risque de sclérose systémique dans une population tunisienne

Daouda Dia^{1,2}, Amani Kallel^{1,2}, Rami Gasmi^{2,4}, Maissa Bouzidi³, Moncef Feki^{1,2}, Imed ben Ghorbel^{1,3}, Monia Smiti^{1,3}, Haifa Sanhaji^{1,2}

1. University of Tunis El Manar, Faculty of Medicine of Tunis, LR99ES11, Tunis, Tunisia

2. Laboratory of Biochemistry, Rabta Hospital, Tunis, Tunisia

3. Department of Internal Medicine, Rabta Hospital, Tunis, Tunisia

4. University of Tunis El Manar, Faculty of Science of Tunis, Tunis, Tunisia

ABSTRACT

Introduction: Systemic sclerosis is a rare autoimmune disease characterized by microcirculation abnormalities and fibrosis of the skin and/or internal organs. Both genetic and environmental factors are recognized contributors to AID development. Vitamin D, through its receptor, plays a role in immune regulation, and the VDR gene has been identified as a candidate for AID susceptibility. However, studies examining the relationship between VDR polymorphisms and AIDs have yielded conflicting results, often varying by ethnic background.

Objectif: The purpose of this study was to investigate the association of FokI and Bsml polymorphisms of the VDR gene with systemic sclerosis in a Tunisian population.

Methods: A case-control study was conducted, involving 68 SSc patients and 190 healthy controls. Genotyping of the two polymorphisms was performed using restriction fragment length polymorphism polymerase chain reaction, and data analysis was conducted via SPSS for Windows (version 28.0).

Results: Results showed significant differences between SSc patients and controls for both polymorphisms. The Bsml bb genotype (0.28 vs. 0.12; $p=0.004$) and b allele (0.49 vs. 0.36; $p=0.014$) were more frequent in SSc patients. Conversely, the FokI Ff (0.54 vs. 0.41; $p=0.007$) and ff (0.15 vs. 0.06; $p=0.008$) genotypes, along with the f allele (0.42 vs. 0.26; $p<0.001$), were more common in the control group.

Conclusion: The Bsml polymorphism was associated with increased genetic predisposition to SSc, whereas the FokI polymorphism appeared to confer protection in the studied population.

Key words: Systemic sclerosis, vitamin D receptor, polymorphism, SNP, rs2228570 (FokI), rs1544410 (Bsml), Tunisian population.

RÉSUMÉ

Introduction : La sclérose systémique est une maladie auto-immune rare caractérisée par des anomalies de microcirculation et une fibrose de la peau et/ou des organes internes. Les facteurs génétiques et environnementaux sont tous deux des contributeurs reconnus au développement de l'AID. La vitamine D, par son récepteur, joue un rôle dans la régulation immunitaire, et le gène VDR a été identifié comme un candidat à la susceptibilité à l'AID. Cependant, des études examinant la relation entre les polymorphismes VDR et les SIDA ont donné des résultats contradictoires, souvent variant selon l'origine ethnique.

Objectif : Le but de cette étude était d'étudier l'association des polymorphismes FokI et Bsml du gène VDR avec la sclérose systémique chez une population tunisienne.

Méthodes : Une étude cas-témoins a été réalisée, impliquant 68 patients atteints de SSc et 190 témoins en bonne santé. Le génotypage des deux polymorphismes a été réalisé à l'aide de la polymorphisme polymorphisme de longueur de restriction par polymérase, et l'analyse des données a été réalisée via SPSS pour Windows (version 28.0).

Résultats : Les résultats ont montré des différences significatives entre les patients SSc et les témoins pour les deux polymorphismes. Le génotype Bsml bb (0,28 vs. 0,12 ; $p=0,004$) et l'allèle b (0,49 vs. 0,36 ; $p=0,014$) étaient plus fréquents chez les patients SSc. Inversement, les génotypes FokI Ff (0,54 contre 0,41 ; $p=0,007$) et ff (0,15 contre 0,06 ; $p=0,008$), ainsi que l'allèle f (0,42 contre 0,26 ; $p<0,001$), étaient plus fréquents dans le groupe témoin.

Conclusion : Le polymorphisme Bsml était associé à une prédisposition génétique accrue au SSc, tandis que le polymorphisme FokI semblait conférer une protection dans la population étudiée.

Mots-clés : Sclérose systémique, récepteur de la vitamine D, polymorphisme, SNP, rs2228570 (FokI), rs1544410 (Bsml), population tunisienne

Correspondance

Amani Kallel

University of Tunis El Manar, Faculty of Medicine of Tunis, LR99ES11, Tunis. Laboratory of Biochemistry, Rabta Hospital, Tunis, Tunisia

Email: amani.kallel@fmt.utm.tn

INTRODUCTION

Systemic sclerosis (SSc) is a rare autoimmune connective tissue disease characterized by widespread fibrosis (thickening and scarring) of the skin and internal organs and vasculopathy affecting small blood vessels (microcirculation), which can impair blood flow and contribute to tissue damage. This condition often involves immune system dysfunction, leading to excessive collagen production and the subsequent hardening and tightening of tissues (1). SSc is a complex and debilitating autoimmune disease (AID), whose heterogeneity complicates both diagnosis and management. This condition profoundly impacts patients' quality of life, affecting their physical, psychological, and social well-being, and limiting their autonomy in daily activities (2). The pathophysiology of the disease remains not fully elucidated. It is believed that exposure to environmental factors combined with a predisposed genetic background may play a significant role in its development. Environmental triggers such as infections, chemicals, or radiation could potentially activate an abnormal immune response in genetically susceptible individuals. This interplay between genetic and environmental factors might lead to dysregulation of the immune system, resulting in excessive collagen production and the progression of the disease (3).

Vitamin D (Vit-D) is a secosteroid hormone that plays a key role in various physiological processes, especially in maintaining bone health and regulating mineral metabolism. It enhances the absorption of phosphorus and calcium in the intestines and reduces their excretion by the kidneys, thereby aiding in bone formation (4,5). The active form of vit-D, known as calcitriol or 1,25-hydroxy vitamin D, interacts with its nuclear receptor, the vitamin D receptor (VDR), to regulate the expression of target genes (6). Research has demonstrated that calcitriol influences the expression of hundreds of genes, including those involved in the immune system (7). The presence of the VDR in nearly all immune cells (8) has prompted researchers to investigate the role of vit-D in the immune system. Numerous studies have established that calcitriol plays a role in the activation and proliferation of immune cells as well as in cytokines production (9,10). On one hand, calcitriol enhances the innate immune response, safeguarding the body against foreign pathogens. On the other hand, it downregulates the adaptive immune system, thereby helping to prevent AIDs. Vit-D is known to enhance the antibacterial activity of human monocytes and macrophages. Furthermore, it plays a crucial role in innate immunity by stimulating the production of pattern recognition receptors (PRRs), antimicrobial peptides, and cytokines in immune cells. Vit-D plays a role in the prevention of AIDs through several mechanisms. It inhibits the proliferation and differentiation of B cells and helper T cells and decreases the production of pro-inflammatory cytokines. Additionally, it stimulates the proliferation and differentiation of regulatory T cells, thereby enhancing immune tolerance (6,11,12).

Low vitamin D levels or altered function caused by poor receptor interaction can result in various conditions

including infectious and autoimmune disorders (11). Genetic variations, particularly Single Nucleotide Polymorphisms (SNPs) in the gene encoding VDR may impact its transcription, expression, or structure, affecting its interaction with vit-D. These alterations may reduce the effectiveness of vit-D, potentially leading to diseases such as autoimmune disorders due to disrupted physiological processes (13).

Genome-wide association studies (GWAS) have identified numerous loci, which predispose to AIDs. Among these loci, the HLA genes are most frequently implicated (14). Regarding non-HLA candidate genes, the VDR gene has garnered significant interest due to the crucial role of vit-D in the immune system. Several studies have investigated the association between VDR gene SNPs and AIDs, but the data remained inconsistent (15,16). Studies on the Tunisian population have not yet been published in SSc. The current study aimed to examine the association of the BsmI and FokI polymorphisms of the VDR gene with SSc in the Tunisian population.

POPULATION AND METHODS

We conducted a case-control study involving 68 patients with SSc (mean age, 48.2 ± 12.5 years) recruited from the Internal Medicine Department at Rabta Hospital (Tunis, Tunisia) and 190 healthy volunteer controls recruited from the laboratory staff and the patients' companions (mean age 49.3 ± 9.6 years). SSc diagnosis was established based on the 2013 classification by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) (16). The eligibility criteria for both patients and controls were as follows: participants of both sexes, aged over 18, provided informed consent. Exclusion criteria included current viral infections (such as HIV or hepatitis), recent blood transfusion (within the past three months), and, for control subjects, familial history of autoimmune or chronic inflammatory diseases. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The protocol was approved by the Ethics Committee of Rabta hospital, and all subjects gave written informed consent to participate to the study.

Fasting blood was drawn for all participants on EDTA-containing tubes. DNA was extracted from peripheral blood cells using the salting-out method, and the genotyping of the BsmI and FokI polymorphisms was conducted by RFLP-PCR. For the BsmI polymorphism, the primers were 5'-CAA CCA AGA CTA CAA GTA CCG C-3' (forward) and 5'-AAC CAG CGG AAG AGG TCA AGG-3' (reverse), with an annealing temperature of 68°C. The resulting PCR product was 850 bp. Upon digestion with the BsmI restriction enzyme, the following fragment patterns were observed: the BB genotype yielded a single 850 bp fragment (indicating the absence of a restriction site), the Bb genotype produced three fragments of 850 bp, 650 bp, and 200 bp, and the bb genotype resulted in two fragments of 650 bp and 200 bp. For the FokI polymorphism, the primers used were 5'-AGC TGG CCC

TGG CAC TGA CTC TGC TCT-3' (forward) and 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3' (reverse), with an annealing temperature of 71°C. The PCR product size was 265 bp. After digestion with the FokI restriction enzyme (BseGI), the digestion patterns were as follows: the FF genotype resulted in a single 265 bp fragment (indicating the absence of a restriction site), the Ff genotype produced three fragments of 265 bp, 196 bp, and 69 bp, and the ff genotype generated two fragments of 196 bp and 69 bp.

Statistical analysis was performed using SPSS for Windows (version 28.0). Chi-square tests were used to assess deviation from Hardy-Weinberg equilibrium and to compare genotype distributions between groups. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated as estimators of the relative risk of SSc for the BsmI and FokI genotypes, alleles, and/or haplotypes. A p-value of <0.05 was considered the threshold for statistical significance.

RESULTS

There were no significant differences between SSc patient and control groups according to gender and age. BMI and smoking prevalence were lower whereas diabetes ($p<0.001$) and hypertension ($p<0.001$) were more frequent in patients (Table 1). Cutaneous involvement, sclerodactyly, Raynaud's phenomenon, digestive involvement and microangiopathy, were the most frequent clinical manifestations in patients (Figure 1).

Table 1. Anthropometric and clinical characteristics of the study population

	SSc patients (n=68)	Controls (n=190)	p
Gender (man/woman)	3/65	10/180	NS
Age (year)	48,2 ± 12,5	49.3 ± 9,6	NS
BMI (Kg/m ²)	25,31 ± 3,74	27,77 ± 5,48	<0,001
Smoking (%)	20.5	26,7	<0,001
Diabetes (%)	9.1	3,7	<0,001
Hypertension (%)	10.3	4,2	<0,001

BMI, body mass index; SSc, systemic sclerosis; NS, no significant.

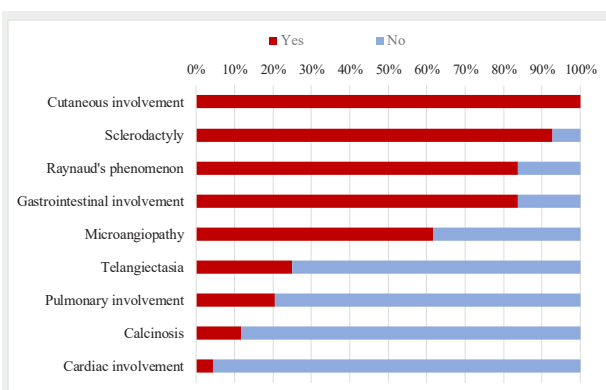


Figure 1. Frequencies of clinical manifestations in systemic sclerosis patients

In the Hardy-Weinberg equilibrium (HWE) analysis, both control and SSc patient groups showed no significant deviation from equilibrium for the two SNPs ($p = 0.898$ in controls and 0.468 in SSc patients and 0.231 in controls and 0.827 in SSc patients respectively for BsmI and FokI). The study showed differential genotype distribution and allele frequencies for the two SNPs between SSc patients and controls. The BsmI bb genotype and b allele were significantly more frequent whereas the FokI ff genotype and f allele were less frequent in SSc patients than controls. The minor allele frequencies (MAF) of the BsmI and FokI SNPs were 0.36 and 0.42 , respectively, in healthy controls. (Table 2). Haplotype analyses showed no association between SSc and the BsmI/FokI haplotypes in the studied population (Table 3).

Table 2. Genotype distributions and allele frequencies of BsmI and FokI polymorphisms in systemic sclerosis patients and controls

	SSc patients (n=68)	Controls (n=190)	p	OR [CI=95%]
BsmI polymorphism				
Genotype, n (%)				
BB	20 (0.29)	77 (0.41)	-	-
Bb	29 (0.43)	90 (0.47)	0.624	1.24 [0.65-2.37]
bb	19 (0.28)	23 (0.12)	0.004	3.18 [1.46-6.95]
Allelic frequency (%)				
B	0.51	0.64		
b	0.49	0.36	0.014	1.67 [1.12-2.49]
FokI polymorphism				
Genotype, n (%)				
FF	36 (0.53)	58 (0.31)		
Ff	28 (0.41)	104 (0.54)	0.007	0.43 [0.24-0.78]
ff	4 (0.06)	28 (0.15)	0.008	0.23 [0.08-0.71]
Recessive model				
FF	36 (0.53)	58 (0.305)		
Ff+ff	32 (0.47)	132 (0.695)	<0.001	0.39 [0.221-0.689]
Allelic frequency (%)				
F	0.74	0.58		
f	0.26	0.42	<0.001	0.50 [0.32-0.76]

SSc, systemic sclerosis; OR, odd ratio; CI, confident interval.

Table 3. Haplotype analysis of BsmI/FokI polymorphisms in systemic sclerosis patients and controls

BsmI/FokI haplotypes n (%)				
Haplotype	SSc patients n=68	Controls n=190	p	OR [CI=95%]
BB/FF	12 (0.18)	25 (0.13)	-	reference
BB/Ff	21 (0.31)	66 (0.35)	0.378	0.66 [0.26-1.54]
BB/ff	11 (0.16)	21 (0.11)	0.999	1.09 [0.40-2.97]
Bb/FF	12 (0.18)	46 (0.24)	0.231	0.54 [0.21-1.39]
Bb/ff	10 (0.14)	30 (0.16)	0.614	0.69 [0.26-1.87]
Bb/ff	2 (0.03)	2 (0.013)	0.596	2.08 [0.26-16.63]

SSc, systemic sclerosis; OR, odd ratio; CI, confident interval.

Discussion

The study findings demonstrate a clear association between the BsmI and FokI polymorphisms and SSc in the studied population. Specifically, the increased prevalence of the BsmI bb genotype and b allele in SSc patients suggests a genetic predisposition for the disease. Conversely, the lower frequencies of the FokI Ff and ff genotypes and the f allele in SSc patients suggest these variants are protective from SSc in the studied population.

SSc is a rare, non-organ-specific AID characterized by tissue fibrosis, and obliterative and proliferative microvascular damage. It is regarded as a complex disorder, heavily influenced by both genetic and epigenetic factors (3). Vit-D has been proven to regulate the immune system. Low vit-D status or impairment in its function can lead to immune system dysfunction, potentially triggering the onset of AIDs (17). The VDR gene is located on chromosome 12q13.11 (NC_000012.12, positions 47,841,537–47,904,994), spanning approximately 63.5 kb of genomic DNA. The coding sequence encodes a nuclear receptor protein of 427 amino acids, which functions as a ligand-activated transcription factor mediating the biological effects of 1,25-dihydroxyvitamin D₃ (18). Multiple polymorphisms of this gene have been implicated in a wide range of pathologies, including certain cancers (19 and 20), bone disorders (21), and autoimmune diseases (22 and 23). The BsmI polymorphism is located in intron 8, within the 3' untranslated region of the VDR gene. Although it does not directly affect the protein sequence, it may influence VDR expression through post-transcriptional mechanisms, particularly by affecting mRNA stability (15). The FokI polymorphism is located in exon 2 of the VDR gene, where it alters the translation initiation codon, leading to the production of a shorter protein by three amino acids (424 residues instead of 427) which has low affinity to the vitamin (15).

In our control group, the VDR BsmI polymorphism MAF of 0.36 is comparable to those observed in European (0.39) and global (0.38) populations but higher than those reported in African (0.26) and Asian (0.06) populations (24). The alternative allele "f" of the FokI polymorphism of 0.42 in our control group is lower compared to the frequency of 0.56 to 0.725 reported in different populations the ALFA database (25). These inter-population variabilities may reflect genetic and environmental differences.

Recent research has identified various genes associated with susceptibility to SSc, including HLA, IRF5, and STAT4. The genes CD247 and BANK1 also play an important role in the pathogenesis of SSc (14,26,27). While the VDR gene is well-studied in autoimmune diseases such as lupus and arthritis (22 and 28), few studies examine its link to SSc (29 and 30). A Chinese study found no association between the BsmI and FokI polymorphisms of the VDR gene and SSc (29), and another Iranian study on the ApaI and TaqI polymorphisms of the VDR gene similarly revealed no link with the disease (30).

Due to the lack of data on SSc, it is relevant to compare

our findings with those of other AIDs such as rheumatoid arthritis (RA), Behçet's disease (BD), and systemic lupus erythematosus (SLE), for which the BsmI and FokI polymorphisms have been more extensively studied (31–33). Our results showed several similarities with available literature data on other AIDs. Bagheri-Hosseinabadi et al. through meta-analysis study demonstrated that BsmI polymorphism was associated with an increased risk of RA in an African population, while FokI has a protective effect against the disease (31). Accordingly, Wu et al. reported that BsmI is linked to an increased risk of BD in a Caucasian population, and that FokI provides protection against BD in an African population (32). These results reinforced the idea that BsmI polymorphism is associated with an increased risk of AIDs, while FokI may have a protective immunomodulatory effect against autoimmune diseases. However, some divergences were notable when compared to the meta-analysis by Yang et al., which indicates a protective effect of BsmI against SLE in a Caucasian population, contrasting with our findings in SSc (33). In an African population, however, Yang et al. find that BsmI polymorphism increases the risk of SLE, a trend consistent with our results showing a deleterious effect of BsmI in SSc in Tunisian population (33). Furthermore, our finding that FokI has a protective effect against SSc contrasts with Yang et al., who report that FokI is a risk factor for SLE in an African population (33). This may suggest distinct pathogenic mechanisms depending on the autoimmune diseases, that may be different between patients and population, despite the common involvement of the VDR gene.

Several recent studies using advanced genotyping platforms have reported associations between VDR polymorphisms and AID. For example, Cancela Díez et al. observed that the TT genotype of FokI, determined by real-time PCR with TaqMan probes, was associated with an increased risk of multiple sclerosis in a Spanish cohort (23). Similarly, a meta-analysis on Hashimoto's thyroiditis identified a significant association of the FokI polymorphism with disease susceptibility, particularly in Asian populations (34). Another meta-analysis on rheumatoid arthritis also reported associations between FokI and disease risk, especially in European populations (35). In contrast, large-scale GWAS on systemic sclerosis, including more than 26,000 individuals, have so far not identified coding variants of VDR among the major susceptibility loci (36). These findings suggest that while FokI and other VDR SNPs may contribute to the risk of several autoimmune diseases, their role in systemic sclerosis remains unconfirmed and requires replication in larger cohorts using modern high-throughput genotyping or sequencing technologies, together with functional assays.

Conclusions

In conclusion, this case-control study indicated that the VDR BsmI polymorphism may be associated with an increased risk of SSc, whereas the FokI polymorphism could exert a protective effect in the studied Tunisian population. However, these findings should be

interpreted with caution due to certain limitations, including the relatively small sample size and the use of the PCR-RFLP method, which, although simple and cost effective, does not provide detailed insights into the functional consequences of these polymorphisms at the protein level. These limitations may partly explain the discrepancies observed across different populations and autoimmune diseases. Future studies using larger cohorts, advanced genotyping or sequencing techniques, and functional analyses are needed to confirm or refute these associations and to clarify the biological mechanisms by which VDR polymorphisms may contribute to SSc pathogenesis.

Abbreviations

ACR: American College of Rheumatology ; **AID:** autoimmune diseases ; **BD:** Behçet's disease ; **bp:** base pair ; **DNA:** deoxyribonucleic acid ; **EULAR:** European League Against Rheumatism ; **GWAS:** Genome-wide association studies ; **HLA:** Human leukocyte antigen ; **HWE:** Hardy-Weinberg equilibrium ; **MAF:** minor allele frequencies ; **mRNA:** messenger ribonucleic acid ; **OR:** odds-ratio ; **PCR:** polymerase chain reaction ; **RA:** rheumatoid arthritis ; **RFLP:** restriction fragment length polymorphism ; **SLE:** systemic lupus erythematosus ; **SNP:** single nucleotide polymorphism ; **PRR:** pattern recognition receptor ; **SSc:** systemic sclerosis ; **Vit-D:** vitamin D ; **VDR:** vitamin D receptor.

REFERENCES

- Rosendahl A, Schönborn K, Krieg T. Pathophysiology of systemic sclerosis (scleroderma). *Kaohsiung J Med Sci.* 2022;38(3):187-95.
- Almeida C, Almeida I, Vasconcelos C. Quality of life in systemic sclerosis. *Autoimmun Rev.* 2015;14(12):1087-96.
- Thoreau B, Chaigne B, Renaud A, Mouthon L. Pathophysiology of systemic sclerosis. *Presse Médicale.* 2021;50(1):104087.
- Janoušek J, Pilařová V, Macáková K, Nomura A, Veiga-Matos J, Silva DDD, et al. Vitamin D: sources, physiological role, biokinetics, deficiency, therapeutic use, toxicity, and overview of analytical methods for detection of vitamin D and its metabolites. *Crit Rev Clin Lab Sci.* 2022;59(8):517-54.
- Gasmi A, Bjørklund G, Peana M, Mujawdiya PK, Pivina L, Ongene A, et al. Phosphocalcic metabolism and the role of vitamin D, vitamin K2, and natto kinase supplementation. *Crit Rev Food Sci Nutr.* 2022;62(30):7062-71.
- Širbe C, Rednic S, Grama A, Pop TL. An Update on the Effects of Vitamin D on the Immune System and Autoimmune Diseases. *Int J Mol Sci.* 2022;23(17):9784.
- Carlberg C. Vitamin D and Its Target Genes. *Nutrients.* 2022;14(7):1354.
- Daryabor G, Gholijani N, Kahmini FR. A review of the critical role of vitamin D axis on the immune system. *Exp Mol Pathol.* 2023;132-133:104866.
- Hewison M. An update on vitamin D and human immunity. *Clin Endocrinol (Oxf).* 2012;76(3):315-25.
- Usategui-Martín R, De Luis-Román DA, Fernández-Gómez JM, Ruiz-Mambrilla M, Pérez-Castrillón JL. Vitamin D receptor (VDR) gene polymorphisms modify the response to vitamin D supplementation: A systematic review and meta-analysis. *Nutrients.* 2022;14(2):360.
- Charoenngam N, Holick MF. Immunologic effects of vitamin D on human health and disease. *Nutrients.* 2020;12(7):2097.
- Gallo D, Baci D, Kustrimovic N, Lanzo N, Patera B, Tanda ML, et al. How does vitamin D affect immune cells crosstalk in autoimmune diseases? *Int J Mol Sci.* 2023;24(5):4689.
- Tourkochristou E, Tsounis EP, Tzoupis H, Aggeletopoulou I, Tsintoni A, Lourida T, et al. The influence of single nucleotide polymorphisms on vitamin D receptor protein levels and function in chronic liver disease. *Int J Mol Sci.* 2023;24(14):11404.
- Caliskan M, Brown CD, Maranville JC. A catalog of GWAS fine-mapping efforts in autoimmune disease. *Am J Hum Genet.* 2021;108(4):549-63.
- Ruiz-Ballesteros AI, Meza-Meza MR, Vizmanos-Lamotte B, Parra-Rojas I, De La Cruz-Mosso U. Association of vitamin D metabolism gene polymorphisms with autoimmunity: Evidence in population genetic studies. *Int J Mol Sci.* 2020;21(29):9626.
- Van Den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against Rheumatism Collaborative Initiative. *Ann Rheum Dis.* 2013;72(11):1747-55.
- Latini A, De Benedittis G, Perricone C, Colafrancesco S, Conigliaro P, Ceccarelli F, et al. VDR Polymorphisms in autoimmune connective tissue diseases: Focus on Italian population. *Xu B, éditeur. J Immunol Res.* 2021;2021:1-6.
- Available on: <https://www.ncbi.nlm.nih.gov/gene/7421> (Last visit: september 19th, 2025).
- Pineda-Lancheros LE, Gálvez-Navas JM, Rojo-Tolosa S, Membrive-Jiménez C, Valverde-Merino MI, Martínez-Martínez F, Sánchez-Martín A, Ramírez-Tortosa M, Pérez-Ramírez C, Jiménez-Morales A. Polymorphisms in VDR, CYP27B1, CYP2R1, GC and CYP24A1 Genes as Biomarkers of Survival in Non-Small Cell Lung Cancer: A Systematic Review. *Nutrients.* 2023 Mar 21;15(6):1525. doi: 10.3390/nu15061525. PMID: 36986255; PMCID: PMC10057500.
- Patel SR, Patel KD, Patel JB, Patel PS, Shah FD. Association of vitamin D receptor gene polymorphisms with breast cancer risk. *J Cancer Res Ther.* 2023 Jan 1;19(Suppl 2):S677-S681. doi: 10.4103/jcrt.jcrt_60_22. Epub 2023 Apr 30. PMID: 38384038.
- Mondockova V, Kovacova V, Zemanova N, Babikova M, Martiniakova M, Galbavy D, Omelka R. Vitamin D Receptor Gene Polymorphisms Affect Osteoporosis-Related Traits and Response to Antiresorptive Therapy. *Genes (Basel).* 2023 Jan 11;14(1):193. doi: 10.3390/genes14010193. PMID: 36672934; PMCID: PMC9858724.
- Zhang WT, Jin TF, Chen L. Associations of four common VDR polymorphisms with rheumatoid arthritis and systemic lupus erythematosus: evidence from a meta-analysis. *Lupus.* 2020 Apr;29(4):364-370. doi: 10.1177/0961203320909432. Epub 2020 Mar 9. PMID: 32151180.
- Cancela Díez, Bárbara; Pérez-Ramírez, Cristinab; Maldonado-Montoro, María del Marc; Carrasco-Campos, María Isabela; Sánchez Martín, Almudena; Pineda Lancheros, Laura Elena et al. Association between polymorphisms in the vitamin D receptor and susceptibility to multiple sclerosis. *Pharmacogenetics and Genomics* 31(2):p 40-47, February 2021. | DOI: 10.1097/FPC.0000000000000420
- ALFA: MAF BsmI. Available on: https://www.ncbi.nlm.nih.gov/snp/rs1544410#frequency_tab (Last visit: september 19th, 2025).
- ALFA: MAF FokI. Available on: https://www.ncbi.nlm.nih.gov/snp/rs2228570#frequency_tab (Last visit: september 19th, 2025).
- Truchetet ME, Brembilla NC, Chizzolini C. Current concepts on the pathogenesis of systemic sclerosis. *Clin Rev Allergy Immunol.* 2021;64(3):262-83.
- El-Halwagi A, Agarwal SK. Insights into the genetic landscape of systemic sclerosis. *Best Pract Res Clin Rheumatol.* 2024;101981.
- Punceviciene E, Gaizevskia J, Sabaliauskaite R, Venceviciene L, Puriene A, Vitkus D, Jarmalaite S, Butrimiene I. Vitamin D and VDR Gene Polymorphisms' Association with Rheumatoid Arthritis in Lithuanian Population. *Medicina (Kaunas).* 2021 Apr 3;57(4):346. doi: 10.3390/medicina57040346. PMID: 33916688; PMCID: PMC8065838.
- Li J, Chen SY, Liu HH, Yin XD, Cao LT, Xu JH, et al. Associations of Vitamin D receptor single nucleotide polymorphisms with susceptibility to systemic sclerosis. *Arch Med Res.* 2019;50(6):368-76.
- Mirfeizi SZ, Nabizadeh-Marvast M, Jokar MH, Rafatpanah H, Hashemzadeh K, Mehrad-Majd H. Genetic association of VDR gene Apa1 and Taq1 variants with scleroderma in an Iranian northeast population. *Curr Rheumatol Rev.* 2023;19(1):49-57.
- Bagheri-Hosseiniabadi Z, Imani D, Yousefi H, Abbasifard M. Vitamin

- D receptor (VDR) gene polymorphism and risk of rheumatoid arthritis (RA): Systematic review and meta-analysis. *Clin Rheumatol*. 2020;39(12):3555-69.
32. Wu M, Li L, Tian L, Liu D, Jian J, Zhou Y, et al. Apal, Taql, FokI, and BsmI polymorphisms and the susceptibility of Behcet's disease: An updated meta-analysis. *Immunol Res*. 2022;70(6):781-92.
33. Yang SK, Liu N, Zhang WJ, Song N, Yang JP, Zhang H, et al. Impact of vitamin D receptor gene polymorphism on systemic lupus erythematosus susceptibility: A pooled analysis. *Genet Test Mol Biomark*. 2022;26(4):228-38.
34. Wang X, Cheng W, Ma Y, Zhu J. Vitamin D receptor gene FokI but not TaqI, Apal, BsmI polymorphism is associated with Hashimoto's thyroiditis: a meta-analysis. *Sci Rep*. 2017 Jan 30;7:41540. doi: 10.1038/srep41540. PMID: 28134349; PMCID: PMC5278388.
35. Song GG, Bae SC, Lee YH. Vitamin D receptor FokI, BsmI, and TaqI polymorphisms and susceptibility to rheumatoid arthritis : A meta-analysis. *Z Rheumatol*. 2016 Apr;75(3):322-9. doi: 10.1007/s00393-015-1581-6. PMID: 26358095.
36. López-Isac E, Acosta-Herrera M, Kerick M, Assassi S, Satpathy AT, Granja J et al. GWAS for systemic sclerosis identifies multiple risk loci and highlights fibrotic and vasculopathy pathways. *Nat Commun*. 2019 Oct 31;10(1):4955. doi: 10.1038/s41467-019-12760-y. PMID: 31672989; PMCID: PMC6823490.