The Journal of Rheumatology Volume 39, no. 9

Vitamin D Receptor Polymorphism rs2228570 (Fok1) Is Associated with Rheumatoid Arthritis in North American Natives

CAROL A. HITCHON, YE SUN, DAVID B. ROBINSON, CHRISTINE A. PESCHKEN, CHARLES N. BERNSTEIN, KATHERINE A. SIMINOVITCH and HANI S. EL-GABALAWY

J Rheumatol 2012;39;1792-1797
http://www.jrheum.org/content/39/9/1792

Vitamin D Receptor Polymorphism rs2228570 (Fok1) Is Associated with Rheumatoid Arthritis in North American Natives

CAROL A. HITCHON, YE SUN, DAVID B. ROBINSON, CHRISTINE A. PESCHKEN, CHARLES N. BERNSTEIN, KATHERINE A. SIMINOVITCH, and HANI S. EL-GABALAWY

ABSTRACT. ***Objective.*** Vitamin D (VitD) has immunomodulatory activity relevant to rheumatoid arthritis (RA) and acts by binding nuclear receptors that regulate gene transcription. VitD receptor polymorphisms have been variably associated with RA. Because North American Native (NAN) populations have a high prevalence of RA with a strong genetic contribution, we studied potential associations of the rs2228570 (Fok1) VitD receptor polymorphism in a Canadian NAN population.

Methods. The single-nucleotide polymorphism (SNP) Fok1 was tested by sequencing NAN patients with RA (n = 448) and unrelated NAN controls (n = 704). Associations were tested using genotypic, dominant, and recessive models.

Results. The minor allele frequency (F/C) in the NAN control population was 0.44 and lower than reported in white subjects of the same geographical area. The Fok1 VitD receptor SNP was significantly associated with RA. Comparing patients with RA to unaffected NAN controls, the Fok1 SNP was associated with RA using both genotypic [FF vs Ff vs ff: RA 20%, 54%, 26% vs control 22%, 44%, 34% (chi-square 13.35, p = 0.003)] and dominant models [FF/Ff vs ff: RA 74% vs 26% control 66% vs 34% (OR 1.5, 95% CI 1.16–1.96, p = 0.003)]. This association was strongest in shared-epitope-positive RA.

Conclusion. VitD receptor polymorphisms may contribute to the high prevalence of RA in NAN populations. (First Release Aug 1 2012; J Rheumatol 2012;39:1792–7; doi:10.3899/jrheum.120387)

Key Indexing Terms:
VITAMIN D RECEPTOR
RHEUMATOID
SINGLE-NUCLEOTIDE POLYMORPHISM
NORTH AMERICAN NATIVES

Vitamin D (VitD) has multiple effects on immune cell function, is important for normal host responses to infection, and influences bone metabolism and tumor proliferation. These mechanisms are all potentially important for the pathogenesis of inflammatory arthritis. The activity of 1,25 OH VitD is mediated through VitD nuclear receptors that, in complex with other nuclear proteins, regulate transcription of VitD-responsive genes[1]. VitD receptor binding sites are enriched in gene loci associated with autoimmunity and rheumatoid arthritis (RA)[2]. Both a deficiency of VitD and altered VitD receptor function have been variably linked to susceptibility to infection, malignancy, and autoimmune conditions including RA[3,4,5]. Several polymorphisms of the VitD receptor have been described. Only the Fok1 VitD receptor polymorphism has been associated with the development of RA in some populations[6,7], while other polymorphisms may associate with early disease onset[8] or activity[9].

North American Native (NAN) populations have high RA prevalence rates, early onset of severe seropositive disease[10], and a high frequency of HLA-DRB1 shared-epitope (SE) alleles in the background population[11]. Environmental factors associated with RA risk are also prevalent in this population, and population studies have documented low serum VitD levels in multiple North American populations including some NAN communities[12,13].

We examined the association of the rs2228570 (Fok1) polymorphism of the VitD receptor gene with RA susceptibility in the NAN population of central Canada. The Fok1 F(C) allele was associated with RA in this NAN population, particularly in the setting of shared epitope.

From the Department of Internal Medicine, University of Manitoba, Winnipeg, Manitoba; Samuel Lunefeld Research Institute, Mount Sinai Hospital, Toronto, Ontario; Toronto General Research Institute, Mount Sinai Hospital, Toronto, Ontario; and Department of Medicine, University of Toronto, Toronto, Ontario, Canada.

Supported in part by funding from the Canadian Institutes of Health Research (MOP7770 and IIN 84040). Prof. Siminovitch is supported by a Canada Research Chair and holds the Sherman Family Chair in Genomic Medicine.

C.A. Hitchon, MD, Associate Professor of Medicine, University of Manitoba; Y. Sun, PhD, Mount Sinai Hospital; D.B. Robinson, MD, Associate Professor of Medicine, University of Manitoba; C.A. Peschken, MD, Associate Professor of Medicine, University of Manitoba; C.N. Bernstein, MD, Professor of Medicine, University of Manitoba; H.S. El-Gabalawy, MD, Professor of Medicine, University of Manitoba; K.A. Siminovitch, PhD, Professor of Medicine, University of Toronto.

Address correspondence to Dr. C.A. Hitchon, RR149, 800 Sherbrook St., Winnipeg, Manitoba R3A 1M4, Canada. E-mail: chitchon@hsc.mb.ca

Accepted for publication May 28, 2012.

MATERIALS AND METHODS

Subjects and procedures. Cree/Ojibway NAN patients with RA (n = 448) were recruited from clinic populations. All met American College of Rheumatology criteria for RA[14]. Cree/Ojibway controls (n = 704) were recruited from the same geographic areas. Controls were interviewed and examined by a rheumatologist (DBR or HSG) or trained research nurse and did not have clinical inflammatory arthritis or another autoimmune condition. All subjects had at least 3 of 4 grandparents of NAN origin (99% had 4 of 4) by self-report.

Blood samples were obtained at enrollment, stored at –80°C, and processed according to a standard procedure.

All study subjects provided informed consent. All aspects of the study were approved by the Research Ethics Board of the University of Manitoba and by the Band Councils of the study communities.

Rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPA) testing. Blood samples were tested for RF by nephelometry or ELISA. Positive cutoffs were preset to be < 5% of a control white population (IgM RF > 50 IU/ml). Serum samples from RA patients and controls were tested for the presence of anticyclic citrullinated protein antibodies (ACPA) using a commercial anti-ACPA2 ELISA (Inova Diagnostics, San Diego, CA, USA).

HLA-DRB1 allele testing. HLA-DRB1 typing was performed by polymerase chain reaction using sequence-specific oligonucleotide primers in 353 patients with RA and 392 controls. The following DRB1 alleles were included as SE-bearing alleles: DRB1*0101, 0102, 0401, 0404, 0405, 0408, 0410, 1001, and 1402[11].

VitD receptor single-nucleotide polymorphism (SNP) testing. Genotyping was undertaken in the laboratory of Dr. Siminovitch. The multiplexed SNP assays were performed on the Sequenom Mass Array iPLEX platform (Sequenom, San Diego, CA, USA). Allele-specific extension products were plated onto a SpectroCHIP (Sequenom), subjected to mass spectrometric analysis, and the genotypes identified using SpectroCaller software (Sequenom). The VitD receptor gene is located on chromosome 12 (12q13.11). The Fok1 polymorphism is located at a translation initiation site in exon 2 at the 5' gene region and is not in linkage disequilibrium with several other known VitD receptor polymorphisms located in noncoding introns and exon 9 at the 3' gene region[15].

Statistical analysis. Associations of VitD receptor gene polymorphisms and RA phenotype were analyzed using genotypic (FF vs Ff vs ff), allelic (F vs f), dominant (FF, Ff vs ff), and recessive (FF vs Ff, ff) models. Associations were tested by chi-square and regression methods using SPSS[15]. Attributable risk of Fok1 to RA was determined as the incidence of RA in the population with at least 1 dominant Fok1 allele minus the incidence of RA in the population without a dominant Fok1 allele[16].

RESULTS

The clinical characteristics of subjects are shown in Table 1. As reported in this population, NAN have a high frequency of RF and ACPA-positive RA and there is a high frequency of RF and SE in the background population[10,11]. Patients with RA were older than the controls [mean 47 yrs (SD 15) vs 35 yrs (SD 12); p < 0.0001] and more likely to be female (82% vs 59%, respectively; p < 0.0001).

VitD receptor Fok1 polymorphism is associated with RA in NAN. The minor allele frequency (F/C) in the NAN control population was 0.44, similar to that of previous reports of Fok1 in the Manitoba NAN population (0.43)[17], and the allele frequencies were in Hardy-Weinberg equilibrium. The observed minor allele frequency in NAN was lower than that reported in the Manitoba white population (0.64) and in other ethnic groups[18,19,20]. Comparing RA patients to unaffected NAN controls, the Fok1 SNP distribution was associated with RA using both genotypic [FF vs Ff vs ff: RA 20%, 54%, 26% vs control 22%, 44%, 34% (chi-square 13.35, p = 0.003)] and dominant models [FF/Ff vs ff: RA 74%, 26% vs control 66%, 34% (OR 1.5, 95% CI 1.16–1.96, p = 0.003; Table 2]. The FF or Ff genotype was more common in NAN patients with RA than in controls. No associations were seen with the allelic or recessive models.

Table 1. Clinical characteristics of North American Native patients with rheumatoid arthritis (RA) and Native controls. Results are reported as percentage positive, mean (SD), or median (interquartile range; IQR) as indicated. All comparisons between RA and controls, p < 0.0001.

Characteristic	RA, n = 448	Controls, n = 704
Sex, % female	82	59
Age, yrs	47 (15)	35 (12)
RF-positive, %	86	32
RF titer, median (IQR)	171 (400)	19 (3)
ACPA-positive*, %	73	2.8
Shared epitope†, %	85	66

* Anticitrullinated protein antibody (ACPA) tested in 252 patients with RA, 529 controls. † One or more copies of shared-epitope (HLA-DRB1 alleles 0101, 0102, 0401, 0404, 0405, 0408, 0410, 1001, 1402) tested in 348 patients with RA and 330 controls. RF: rheumatoid factor.

Associations between RA and Fok1 were significant for SE-positive but not SE-negative subjects (Table 3), regardless of sex. This was seen for subjects with at least 1 copy of any SE allele in genotypic (FF vs Ff vs ff: chi-square 11.3, p = 0.003) and dominant models (FF or Ff vs ff: chi-square 9.8, p = 0.002), but not in recessive models. No additive or multiplicative interactions between SE and Fok1 were present in genotypic, dominant, or recessive models testing the association of Fok1 and RA. In multivariate binary logistic models controlling for age, sex, and SE, having at least 1 copy of the dominant Fok1 allele (FF or Ff) was independently associated with RA (OR 1.7, 95% CI 1.2–2.5, p = 0.002). The attributable risk of having at least 1 copy of the dominant Fok1 allele for RA in this NAN population was 0.098 (10%). In the NAN subset population with at least 1 copy of SE, the attributable risk was 0.084 (8%). In contrast, the attributable risk of at least 1 copy of SE to RA in this population was 0.22 (22%).

Small sample size reduced the power of the study to detect significant associations of SE alleles and Fok1. In this population (both controls and patients with RA), the presence of any SE allele was associated with the Fok1 allele in genotypic (ff/SE-negative 46/169, Ff/SE-negative 90/169, FF/SE-negative 33/169 vs ff/SE-positive 202/576, Ff/SE-positive 247/576, FF/SE-positive 127/576; chi-square 5.9, p = 0.052) but not dominant models (ff/SE-negative 46/169, Ff or FF/SE-negative 123/169 vs ff/SE-positive 202/576, FF or Ff/SE-positive 374/576; chi-square 3.6, p = 0.06). A modest association was found between having at least 1 copy of the HLA-DRB1*0101 SE allele and Fok1

Table 2. Vitamin D receptor single-nucleotide polymorphism rs2228570 (Fok1) is increased in North American Native patients with rheumatoid arthritis (RA) compared to unaffected Native controls. Values reported are n (% of RA or control); OR (95% CI).

Model	RA, n = 448	Control, n = 705	
Genotypic model			
CC (FF)	90 (20)	156 (22)	Chi-square 13.3, p = 0.003
CT (Ff)	243 (54)	308 (44)	
TT (ff)	115 (26)	241 (34)	
Dominant model			
CC, CT (FF, Ff)	330 (74)	464 (66)	OR 1.5 (95% CI 1.16–1.96),
TT (ff)	118 (26)	241 (34)	p = 0.003
Recessive model			
CC (FF)	90 (20)	157 (22)	OR 1.13 (95% CI 0.84–1.51),
CT, TT (Ff, ff)	358 (80)	548 (78)	p = NS
Allelic model			
C (F)	423 (47)	620 (44)	OR 1.14 (95% CI 0.74–1.04),
T (f)	473 (53)	790 (56)	p = NS

NS: nonsignificant.

Table 3. Vitamin D receptor single-nucleotide polymorphism rs2228570 (Fok1) and shared-epitope (SE) status in patients with rheumatoid arthritis (RA) and controls. Values reported are n (% of RA or control); OR (95% CI).

Model	RA, n = 348	Control, n = 330	
Genotypic model			
SE-negative			
CC (FF)	9 (18)	24 (20)	Chi-square 0.39, p = NS
CT (Ff)	29 (57)	61 (52)	
TT (ff)	13 (25)	33 (28)	
SE-positive			
CC (FF)	67 (22)	60 (22)	Chi-square 11.3, p = 0.003
CT (Ff)	147 (49)	100 (36)	
TT (ff)	88 (29)	114 (42)	
Dominant model			
SE negative			
CC, CT	38 (75)	85 (72)	OR 1.19 (95% CI 0.54–2.60),
TT	13 (25)	33 (28)	p = NS
SE positive			
CC, CT	214 (71)	160 (58)	OR 1.74 (95% CI 1.22–2.48),
TT	88 (29)	114 (42)	p = 0.002

NS: nonsignificant.

receptor status (using the dominant model) in the entire study population (RA and controls: ff/HLA*0101-negative 195/662 vs FF or Ff/HLA*0101-positive 29/51; chi-square 4.2, p = 0.04, without correction for multiple comparisons), but not in the subset that had at least 1 copy of any SE allele (ff/HLA*0101-negative 159/503 vs FF or Ff/HLA*0101-positive 29/51; chi-square 2.8, p = 0.09). The presence of 2 copies of DRB1*1402 was associated with the Fok1 allele using the dominant model (chi-square 6.1, $p < 0.05$, without correction for multiple comparisons; Table 4).

VitD receptor polymorphisms were not associated with RF or ACPA positivity in NAN patients with RA. However, in NAN controls with the SE, Fok1 was associated with RF: 14/18 (78%) FF, 18/31 (58%) Ff, and 23/61 (38%) ff were positive for RF (chi-square 10, p = 0.007) and 32/49 (65%) subjects with FF or Ff were positive for RF compared to 23/61 (38%) subjects with ff (chi-square 8.3, p = 0.004). No associations were seen with ACPA in NAN controls.

DISCUSSION

The VitD receptor Fok1 polymorphism was shown to be associated with RA in our NAN study population. Compared to the NAN control population, NAN patients with RA were more likely to be homozygous or heterozygous for the F(C) allele. Previous studies in white populations have also shown an association with the F(C) allele and RA, and that this association may be strongest for individuals with HLA-DRB1 SE alleles[6]. The frequency of the Fok1 F(C) allele in our NAN

Table 4. Associations between individual HLA-DRB1 shared-epitope (SE) alleles and Fok1 polymorphisms in the North American Native (NAN) population including patients and controls, and the subset of NAN with at least 1 HLA-DRB1 SE allele.

	FF (CC)	Ff (CT)	ff (TT)	
All, n = 745				
Any SE –[†]	33	90	46	chi-square 5.9, p = 0.05
Any SE+	127	247	202	
0101–	152	315	195	NS
0101+	8	21	22	
0104–	160	336	217	NA
0104+	0	0	0	
0401–	152	320	204	NS
0401+	8	16	13	
0404–	93	205	127	NS
0404+	67	131	90	
0405–	159	334	215	NS
0405+	1	2	2	
0408–	160	330	212	NS
0408+	0	6	5	
0413–	160	335	217	NS
0413+	0	1	0	
0416–	160	336	217	NA
0416+	0	0	0	
1001–	160	336	216	NS
1001+	0	0	1	
1402–	108	234	151	NS
1402+	52	104	66	
SE+, n = 554				
0101–	119	225	159	NS
0101+	8	21	22	
0104–	127	246	181	NA
0104+				
0401–	119	230	168	NS
0401+	8	16	13	
0404–	60	115	91	NS
0404+	67	131	90	
0405–	126	244	179	NS
0405+	1	2	2	
0408–	127	240	176	NS
0408+	0	6	5	
0413–	127	245	181	NS
0413+	0	1	0	
0416–	127	246	181	NA
0416+	0	0	0	
1001–	127	246	180	NS
1001+	0	0	1	
1402–	75	144	115	NS
1402+	52	102	66	

[†] Specific HLA-DRB1 allele known for 713/745 tested (missing for 22 SE-positive and 10 SE-negative controls). NS: nonsignificant; NA: not available.

control population was lower than that reported in unaffected white subjects from the same geographical area (0.64)[17] and other European[21] and African American (0.78)[22] populations.

Polymorphisms at the Fok1 site, located in a coding exon at the 5' region of the VitD receptor gene, result in different protein structures. The Fok1 f(T) allele encodes a 427-amino acid receptor, and has been variably associated with an increased risk of several malignancies, especially in the context of environmental factors[23,24]. The F(C) allele encodes a smaller (424 amino acids) possibly more active protein product[25] that appears to be associated with greater bone mineral density. However, NAN have lower site-specific bone mineral density[26] and low serum VitD compared to whites[27]. The biological effects of the Fok1 VitD receptor polymorphisms may differ across VitD-responsive genes and cell types and may be influenced by serum VitD levels and other VitD receptor polymorphisms[18,23].

Other VitD receptor polymorphisms have been identified in the promoter, exons 2–9, introns, and the 3' region of the gene[23] and were initially identified using restriction enzymes. Bsm, Apa1, EcoRV, and Tru9I restriction fragment length polymorphisms (RFLP) are located in noncoding introns at the 3' region of the VitD receptor gene. Taq1 is located in exon 9, also at the 3' region. These polymorphisms (Bsm1, Apa1, EcoRV, Tru91, and Taq1) are in linkage disequilibrium with each other, but not with Fok1. The Cdx2 polymorphism is located in the 5' promotor region. To date, no functional consequences of these RFLP have been reported, although they may affect RNA stability and thus gene expression[21].

The Bsm1, Taq1, and Apa1 VitD receptor RFLP have been studied in RA[6,28]. While no clear associations with disease prevalence have been demonstrated, there are suggestions that these RFLP may influence disease characteristics. In a Spanish population, although no differences in the distribution of RFLP between patients with RA and controls were observed, the combination of homozygous absence of Bsm1 and presence of Taq1 was associated with slightly earlier onset of RA, particularly for SE-positive women[8]. A separate study reported an association between Bsm1 RFLP and RA disease activity[9]. Multiple polymorphisms may influence disease features or susceptibility[21]. Further genotyping studies are planned to investigate the association of Fok1 and other VitD receptor polymorphisms in RA.

VitD is a key regulator of immune responses and has potent pro- and antiinflammatory effects on multiple immune cells[1]. Although the mechanisms for these effects are not fully understood, excess VitD receptor activity might evoke more robust innate immune responses to bacteria, resulting in a greater potential to break self-tolerance. Increased activity of this receptor may also alter the balance between immunostimulatory and immunoregulatory cells. RF titers have been shown to correlate modestly with immune responses to common pathogens in early inflammatory arthritis[29], and in NAN controls with the SE, Fok1 appeared to associate with RF, possibly reflecting exposure to pathogens. In contrast, low serum levels of VitD have been associated with RA activity[30] and with increased risk of developing RA in some but not all studies, and animal models suggest a functioning VitD receptor is important to limit synovial inflammation[31].

Genome-wide association studies (GWAS) conducted pri-

marily in whites of European descent have identified the HLA-DRB1 locus as the most significant genetic contributor to RA disease risk, although several other loci with lower contributions have been identified[32,33]. While no study has identified a specific association with the VitD receptor gene locus, an association with chromosome 12q13 and RA in the setting of HLA SE[34,35] is interesting. The VitD receptor gene is located in this region[36], as are the genes for small ubiquitin-like modifier 1-specific protease (SENP1; 12.13.1q), which influences expression of matrix metalloproteinase-1 in RA synoviocytes[37] and collagen type 2 (COL2a; 12.13.1-13.2), although the latter was found not to be associated with RA in a small study[38]. Thus, the observed associations of Fok1 and RA may be due to linkage disequilibrium with other RA-associated genes.

Our finding of an association between the Fok1 F allele and disease risk primarily in SE-positive individuals, while potentially related to the low numbers of SE-negative subjects in this population, also raises the possibility that the autoimmune effects of the variant VitD receptor are enhanced against the background of SE positivity. This is supported by studies of RA family trios in which F(C) transmission was enhanced in the setting of the SE[6]. VitD receptor polymorphisms have also been shown to cosegregate with disease-specific HLA-DRB1 alleles in other autoimmune conditions such as multiple sclerosis[39]. In addition, binding sites for the VitD receptor have been found in gene regions associated with autoimmune disease[2]. Further studies are required to explore this hypothesis, to evaluate the extent to which the polymorphism correlates with VitD levels and function in both f/f homozygotes and F/f heterozygotes, and thereby to delineate the pathway linking this polymorphism to increased risk for RA in the NAN population[12].

The association of Fok1 and RA observed in this population requires validation in a separate NAN population, preferably of Cree/Ojibway descent.

ACKNOWLEDGMENT

The authors thank the Assembly of Manitoba Chiefs and the Chiefs and Band Councils of the Norway House and St. Theresa Point communities for their role in facilitating this study. We also thank Donna Hart, RN, for assistance with the study and Drs. Kiem Oen (University of Manitoba, Winnipeg, Manitoba) and Janet Markland (University of Saskatchewan, Saskatoon, Saskatchewan) for assistance with subject recruitment.

REFERENCES

1. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: A helpful immuno-modulator. Immunology 2011;134:123-39.
2. Ramagopalan SV, Heger A, Berlanga AJ, Maugeri NJ, Lincoln MR, Burrell A, et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: Associations with disease and evolution. Genome Res 2010;20:1352-60.
3. Hewison M. An update on vitamin D and human immunity. Clin Endocrinol 2012;76:315-25.
4. Cutolo M, Pizzorni C, Sulli A. Vitamin D endocrine system involvement in autoimmune rheumatic diseases. Autoimmun Rev 2011;11:84-7.
5. Fleet JC, Desmet M, Johnson R, Li Y. Vitamin D and cancer: A review of molecular mechanisms. Biochem J 2012;441:61-76.
6. 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.
7. Karray EF, Ben Dhifallah I, Ben Abdelghani K, Ben Ghorbel I, Khanfir M, Houman H, et al. Associations of vitamin D receptor gene polymorphisms FokI and BsmI with susceptibility to rheumatoid arthritis and Behcet's disease in Tunisians. Joint Bone Spine 2012;79:144-8.
8. Garcia-Lozano JR, Gonzalez-Escribano MF, Valenzuela A, Garcia A, Nunez-Roldan A. Association of vitamin D receptor genotypes with early onset rheumatoid arthritis. Eur J Immunogenet 2001;28:89-93.
9. Gomez-Vaquero C, Fiter J, Enjuanes A, Nogues X, Diez-Perez A, Nolla JM. Influence of the BsmI polymorphism of the vitamin D receptor gene on rheumatoid arthritis clinical activity. J Rheumatol 2007;34:1823-6.
10. Peschken CA, Hitchon CA, Robinson DB, Smolik I, Barnabe CR, Prematilake S, et al. Rheumatoid arthritis in a North American Native population: Longitudinal followup and comparison with a white population. J Rheumatol 2010;37:1589-95.
11. El-Gabalawy HS, Robinson DB, Hart D, Elias B, Markland J, Peschken CA, et al. Immunogenetic risks of anti-cyclical citrullinated peptide antibodies in a North American Native population with rheumatoid arthritis and their first-degree relatives. J Rheumatol 2009;36:1130-5.
12. Lebrun JB, Moffatt ME, Mundy RJ, Sangster RK, Postl BD, Dooley JP, et al. Vitamin D deficiency in a Manitoba community. Can J Public Health 1993;84:394-6.
13. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81.
14. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
15. Nejentsev S, Godfrey L, Snook H, Rance H, Nutland S, Walker NM, et al. Comparative high-resolution analysis of linkage disequilibrium and tag single nucleotide polymorphisms between populations in the vitamin D receptor gene. Hum Mol Genet 2004;13:1633-9.
16. Fletcher RW, Fletcher SW. Clinical epidemiology: the essentials. 2nd ed. Baltimore: Williams and Wilkins; 1988.
17. Larcombe LA, Orr PH, Lodge AM, Brown JS, Dembinski IJ, Milligan LC, et al. Functional gene polymorphisms in Canadian aboriginal populations with high rates of tuberculosis. J Infect Dis 2008;198:1175-9.
18. Whitfield GK, Remus LS, Jurutka PW, Zitzer H, Oza AK, Dang HT, et al. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol Cell Endocrinol 2001;177:145-59.
19. Gennari L, Becherini L, Mansani R, Masi L, Falchetti A, Morelli A, et al. FokI polymorphism at translation initiation site of the vitamin D receptor gene predicts bone mineral density and vertebral fractures in postmenopausal Italian women. J Bone Miner Res 1999;14:1379-86.
20. Eccleshall TR, Garnero P, Gross C, Delmas PD, Feldman D. Lack of correlation between start codon polymorphism of the vitamin D receptor gene and bone mineral density in premenopausal French women: The OFELY study. J Bone Miner Res 1998;13:31-5.
21. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene 2004;338:143-56.
22. Harris SS, Eccleshall TR, Gross C, Dawson-Hughes B, Feldman D. The vitamin D receptor start codon polymorphism (FokI) and bone mineral density in premenopausal American black and white

women. J Bone Miner Res 1997;12:1043-8.
23. Kostner K, Denzer N, Muller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: A review of the literature. Anticancer Res 2009;29:3511-36.
24. Tang C, Chen N, Wu M, Yuan H, Du Y. Fok1 polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: A meta-analysis. Breast Cancer Res Treat 2009;117:391-9.
25. Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, et al. A vitamin D receptor gene polymorphism in the translation initiation codon: Effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res 1997;12:915-21.
26. Leslie WD, Metge CJ, Weiler HA, Doupe M, Wood SP, O'Neil JD. Bone density and bone area in Canadian Aboriginal women: The First Nations Bone Health Study. Osteoporos Int 2006;17:1755-62.
27. Weiler HA, Leslie WD, Krahn J, Steiman PW, Metge CJ. Canadian Aboriginal women have a higher prevalence of vitamin D deficiency than non-Aboriginal women despite similar dietary vitamin D intakes. J Nutr 2007;137:461-5.
28. Lee YH, Bae SC, Choi SJ, Ji JD, Song GG. Associations between vitamin D receptor polymorphisms and susceptibility to rheumatoid arthritis and systemic lupus erythematosus: A meta-analysis. Mol Biol Rep 2011;38:3643-51.
29. Newkirk MM, Goldbach-Mansky R, Senior BW, Klippel J, Schumacher HR Jr, El-Gabalawy HS. Elevated levels of IgM and IgA antibodies to Proteus mirabilis and IgM antibodies to Escherichia coli are associated with early rheumatoid factor (RF)-positive rheumatoid arthritis. Rheumatology 2005;44:1433-41.
30. Patel S, Farragher T, Berry J, Bunn D, Silman A, Symmons D. Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. Arthritis Rheum 2007;56:2143-9.
31. Zwerina K, Baum W, Axmann R, Heiland GR, Distler JH, Smolen J, et al. Vitamin D receptor regulates TNF-mediated arthritis. Ann Rheum Dis 2011;70:1122-9.
32. Gregersen PK. Susceptibility genes for rheumatoid arthritis — A rapidly expanding harvest. Bull NYU Hosp Jt Dis 2010;68:179-82.
33. Okada Y, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, et al. Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. Nat Genet 2012;44:511-6.
34. Osorio YF, Bukulmez H, Petit-Teixeira E, Michou L, Pierlot C, Cailleau-Moindrault S, et al. Dense genome-wide linkage analysis of rheumatoid arthritis, including covariates. Arthritis Rheum 2004;50:2757-65.
35. Barton A, Thomson W, Ke X, Eyre S, Hinks A, Bowes J, et al. Rheumatoid arthritis susceptibility loci at chromosomes 10p15, 12q13 and 22q13. Nat Genet 2008;40:1156-9.
36. Labuda M, Fujiwara TM, Ross MV, Morgan K, Garcia-Heras J, Ledbetter DH, et al. Two hereditary defects related to vitamin D metabolism map to the same region of human chromosome 12q 13-14. J Bone Miner Res 1992;7:1447-53.
37. Maciejewska-Rodrigues H, Karouzakis E, Striethol S, Hemmatazad H, Neidhart M, Ospelt C, et al. Epigenetics and rheumatoid arthritis: The role of SENP1 in the regulation of MMP-1 expression. J Autoimmun 2010;35:15-22.
38. Pile KD, Wordsworth BP. Susceptibility to rheumatoid arthritis is not associated with a single common allele of the type 2 collagen gene (COL2A1). Br J Rheumatol 1993;32:456-7.
39. Niino M, Fukazawa T, Yabe I, Kikuchi S, Sasaki H, Tashiro K. Vitamin D receptor gene polymorphism in multiple sclerosis and the association with HLA class II alleles. J Neurol Sci 2000;177:65-71.