Arch Biol Sci. 2025;77(1):71-82 https://doi.org/10.2298/ABS241227007M

A pilot case-control study of the association of vitamin D-related gene variants with peri-implantitis

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Received: December 27, 2024; **Revised**: February 14, 2025; **Accepted**: February 17, 2025; **Published online**: April 14, 2025

Abstract: This pilot case-control study investigated whether variants in vitamin D-related genes can be used to predict the occurrence of peri-implantitis. Eighty-two patients with at least one dental implant were enrolled. Thirty patients (36.6%) were diagnosed with peri-implantitis, whereas 52 (63.4%) had healthy peri-implant tissues. Clinical parameters, risk factors (history of periodontitis, smoking), and genetic factors were assessed. A buccal mucosa swab was taken for DNA analysis. Four variants in three vitamin D-related genes, *VDR* (rs2228570), retinoid X receptor alpha (*RXRA*) (rs3118523, rs7864987), and cytochrome P450 family 27 subfamily B member 1 (*CYP27B1*) (rs4646536) were genotyped by real-time PCR. There was no difference in the genotype distribution of the variants *VDR* rs2228570 (P=0.410), *RXRA* rs3118523, rs7864987 (P=0.789 and P=0.144, respectively), and *CYP27B1* rs4646536 (P=0.562) between patients with healthy peri-implant tissues and those affected by peri-implantitis. History of periodontitis, a modified plaque index (mPI), modified bleeding index (mBI), peri-implant pocket depth, and implant location were associated with peri-implantitis incidence. In patients with peri-implantitis, *CYP27B1* rs4646536 was associated with mBI (P=0.040), and *RXRA* rs7864987 was associated with implant position (P=0.009). We conclude that the variants of vitamin D-related genes, *VDR*, *RXRA*, and *CYP27B1*, cannot be used as molecular markers for peri-implantitis.

Keywords: vitamin D, vitamin D receptor, *RXRA*, *CYP27B1*, peri-implantitis, genetic polymorphism

INTRODUCTION

Dental implant placement is a highly predictable treatment option for replacing missing teeth, with high survival and a success rate greater than 95% [1]. Despite the favorable outcome of implant treatment, biological complications known as peri-implant diseases (peri-implant mucositis and peri-implantitis) may affect the health of osseointegrated implants and their long-term stability leading to implant loss. Peri-implantitis is a pathological condition in the tissues surrounding the dental implant, characterized by inflammation of the peri-implant mucosa and progressive bone loss [2]. The incidence of peri-implantitis varies from 1.4% to 53.5% [3]. The etiology of peri-implantitis is multifactorial and includes local, systemic, and implant-based factors. Poor oral hygiene, a history of periodontitis, smoking, diabetes mellitus, and osteoporosis contribute to peri-implantitis occurrence [2-4]. Despite exposure to known risk factors, the predisposition of certain patients to peri-implantitis suggests that genetic variability among individuals may play a role in their susceptibility. To date, gene association studies of peri-implantitis mainly focused on assessing the variants in genes involved in immune response, bone growth, and gene expression regulation [4,5].

The process of osseointegration primarily depends on bone metabolism. Vitamin D ($1\alpha25(OH)_2D_3$) is an important regulator of calcium and phosphate metabolism and bone matrix mineralization [6,7]. In addition, vitamin D exerts anti-inflammatory effects by regulating

immune cells, and the prostaglandin, p38-MAPK, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways [8]. Moreover, vitamin D reduces the production of pro-inflammatory cytokines, key mediators of osteoclastogenesis, which are found in higher concentrations in the peri-implant cervical fluid of patients with peri-implantitis. It has been reported that low vitamin D levels may lead to decreased osseointegration rates, resulting in a higher risk of dental implant failure [9-12]. In contrast, another study failed to confirm low serum levels of vitamin D as an indicator of early implant failure [13]. Calcidiol, the major circulating form of vitamin D, was found to be lower in patients with peri-implantitis than in patients with healthy peri-implant tissue [14,15]. The biosynthesis and biological action of vitamin D is complex and involves the activation of multiple genes. The vitamin D receptor, encoded by the *VDR* gene, is considered to play the most crucial role. It binds to the retinoid X receptor encoded by the *RXRA* gene. Vitamin D bound to a VDR-RXR complex regulates the transcription of many genes. The *CYP27B1* gene encodes for the cytochrome P450 family 27 subfamily B member 1, which plays a central role in calcidiol conversion to a biologically active form of vitamin D, calcitriol. Variants in the sequence of vitamin D-related genes may affect both function and vitamin D levels. Therefore, specific gene variants in vitamin D-related genes may be linked to an increased risk of inflammatory processes, including peri-implantitis.

To ensure the long-term success of dental implants, it is of interest to identify the potential genetic risk indicators that might lead to peri-implantitis occurrence. These factors would help to identify patients susceptible to peri-implantitis at an early stage and to develop appropriate preventive treatments and individualized follow-up. The current study aimed to investigate whether the variants of vitamin D-related genes, *VDR*, *RXRA,* and *CYP27B1* are associated with peri-implantitis.

MATERIALS AND METHODS

Study design and participants

This pilot case-control study was conducted at the Department of Implantology, Clinic of Dentistry, Military Medical Academy following the Helsinki Declaration of Human Studies (2013). The procedures were approved by the Ethics Committee of the School of Dental Medicine, University of Belgrade (No. 36/28). The study group consisted of 82 patients (40 females and 42 males, aged 23 to 80 years, median age 59) recruited between January 2015 and June 2017. The patients were fully informed about all relevant aspects of the study, and their informed consent was obtained prior to its commencement. The study included patients with one or more dental implants that had been functional for at least one year and had undergone prosthetic rehabilitation for more than six months. Exclusion criteria were uncontrolled systemic diseases (diabetes, osteoporosis), treatment with drugs affecting bone metabolism, peri-implant therapy within the last 6 months, peri-implant mucositis, pregnancy, and breastfeeding.

Case definition

Thirty patients (36.6%) were diagnosed with peri-implantitis, while 52 patients (63.4%) had healthy peri-implant tissue. The study group was homogeneous, as all participants were Caucasians, of the same Serbian ethnicity. Since most patients had been treated in the same department, comprehensive baseline documentation with radiographs was available. All implants included were 2-piece dental, conventionally loaded implants (median 5, range 1 to 17 years in function) with a standard diameter (3.5-5.0 mm) and length (8-16 mm). The following case definitions were applied to assess the presence of the disease [16]: (i) peri-implant health, defined by the absence of bleeding on probing (BOP) and suppuration (SUP), with no additional bone loss for at least one year after implant placement and a minimum of six months of prosthetic rehabilitation. Depending on the implant type (bone or tissue level), the peri-implant probing depth (PPD) should not exceed 5 mm. (ii) peri-implantitis, characterized by the presence of BOP and/or SUP, a PPD ≥5 mm, and radiographic evidence of pathological bone loss of ≥2 mm [16].

To assess the extent of crestal bone changes, periapical radiographs were obtained using standardized intraoral radiography with the long-cone paralleling technique. Bone loss was assessed visually and compared with previous radiograms, giving insights into implant health conditions and disease presence with a clinical estimate.

Data collection and inter-examiner calibration

Data collection was in two parts: clinical and radiographic examination and comparison to earlier radiography records, and demographic, implant, and implant-related factors (the interview). The following clinical parameters of the study population were recorded: PPD, modified plaque index (mPI), modified bleeding index (mBI) [17], the presence of suppuration, the gingival phenotype (thick or thin) determined by the periodontal probe [18], smoking habits (non-smoker/light smoker or smoker), history of early treated periodontitis (presence or absence), the timing of implant placement (immediate or delayed), time in function (in years), implant position (maxilla or mandible, anterior or posterior), implant collar design (machined or rough), and implant mobility (measured by palpation). All data were recorded in a case-record form.

If a patient had multiple implants with healthy peri-implant tissue, one implant was randomly selected for further analysis. If only one of the multiple implants was diagnosed with peri-implantitis, that implant was chosen for analysis. If peri-implantitis was diagnosed in multiple implants, the implant with the most severe clinical and radiological findings was selected.

A clinical and radiographic examination was conducted by two trained professionals (MM and DR), who reviewed the clinical parameters of 5 healthy implants and 5 implants with diagnosed peri-implantitis that were not included in the study, to ensure the consistency of their assessments. The inter- and intra-examiner reproducibility were assessed on two separate occasions 48 h apart. The calibration was accepted if >90% of the recordings between the first and second measurements could be reproduced within 1 mm of each other with an interclass correlation coefficient of 0.91 [19].

Sample collection, DNA isolation, and *VDR*, *RXRA,* and *CYP27B1* gene variant genotyping

Buccal swab samples were taken from each subject. The patients rinsed their mouths with clear water before the samples were taken. Swabs were taken with a sterile swab brush (FLOQSwabs® Flocked Swabs 552C, Copan, USA) by moving the brush up and down, left and right on the inside of the cheek for at least 30 s. The collected swabs were air-dried for at least 5 min and then returned to the original tube. Buccal swabs were stored at -40°C until further use and DNA isolation.

DNA was isolated from the collected buccal swabs using the PureLink™ Genomic DNA mini kit (Invitrogen, USA) according to the manufacturer's instructions. The isolated DNA was stored at -40°C until further use.

Since vitamin D biosynthesis and function are complex processes, *VDR*, *RXRA*, and *CYP27B1* genes were chosen based on their critical biological roles in vitamin D signaling (*VDR, RXRA*) and metabolism (*CYP27B1*). At least one gene variant in *VDR*, *RXRA*, and *CYP27B1* genes was selected for analysis. The criteria for the selection of gene variants were as follows: (i) frequency of the alternative allele of more than 5% in European populations; (ii) association of the selected variants with peri-implantitis or other inflammatory diseases reported in the literature. Details on the selected variants are presented in Supplementary Table S1. The gene variants in *VDR* (rs2228570), *RXRA* (rs3118523, rs7864987), and *CYP27B1* (rs4646536) genes were determined by allelic discrimination and the real-time PCR method (Real Time 7500, Applied Biosystems, USA). Predesigned TaqMan™ SNP genotyping assays (Assay ID: C_12060045_20, C_2002263_20, C_28976210_20, C_25623453_10) and 2xTaqMan™ Universal PCR Master Mix (Applied Biosystems, USA) were used to prepare the PCR mixture with isolated DNA. The real-time PCR reaction mixture contained 5µL 2xTaqMan™ Universal PCR Master Mix, 0.25 µL 40× TaqMan™ SNP genotyping assay, 20 ng DNA, and dH_2O to the total volume of 10 µL. The temperature conditions were as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Statistical analysis

SPSS v.20.00 software (IBM, USA) was used for statistical analysis. The Pearson chi-square test (χ^2) or Fisher's exact test was used to analyze categorical data and assess differences in genotype and allele distributions of the examined gene variants between patients with peri-implantitis and those with healthy peri-implant tissue. The normality of the data distribution was tested using the Shapiro-Wilk test. As there were no normally

distributed variables, parametric statistical tests were not used. Differences between continuous variables that were not normally distributed were tested using the non-parametric Mann-Whitney U test. The association of the studied variants in the *VDR*, *RXRA*, and *CYP27B1* genes with peri-implantitis risk was assessed by calculating the odds ratio (OR). An OR with a 95% confidence interval (CI) was determined by binary logistic regression. The adjusted OR was calculated accounting for the history of periodontitis. Different genetic models (pairwise, dominant, recessive, and overdominant) were tested. To estimate statistical epistatic

Table 1. Association of demographic factors, implant and implant-related factors, and clinical characteristics of the study group with peri-implantitis.

Demographic and clinical characteristics		Patients with dental implants				P	OR (95% CI)	P
		with healthy peri-implant tissue (n=52)		with peri-implantitis (n=30)				
		n	%	n	%			
Gender	male	27	51.9	15	50	0.867	Ref.	0.867
	female	25	48.1	15	50		1.080 (0.440-2.654)	
Age (years)	mean± SD	54.52 ± 15.355		60.20 ± 10.284		0.145 †	1.033 (0.996-1.070)	0.079
Age (years, median)	≤61	32	61.5	17	56.7	0.665	Ref.	0.665
	>61	20	38.5	13	43.3		1.224 (0.491-3.049)	
Smoking status	non-smoker	36	69.2	19	63.3	0.584	Ref.	0.584
	smoker	16	30.8	11	36.7		1.303 (0.505-3.361)	
History of periodontitis	no	37	71.2	13	43.3	**0.013**	Ref.	**0.014**
	yes	15	28.8	17	56.7		3.226 (1.262-8.248)	
Modified plaque index (mPI)	0	27	51.9	6	20	**0.005**	Ref.	**0.006**
	1+2+3	25	48.1	24	80		4.320 (1.516-12.308)	
Modified bleeding index (mBI)	0+1	44	84.6	9	30	**<0.0001**	Ref.	**<0.0001**
	2+3	8	15.4	21	70		12.833 (4.336-37.980)	
Peri-implant pocket depth	mean± SD	3.259 ± 0.588		6.463 ± 1.997		**<0.0001** †	105.352 (7.143-1553.786)	**0.001**
Peri-implant pocket depth (median)	≤4mm	49	94.2	2	6.7	**<0.0001**	Ref.	**<0.0001**
	>4mm	3	5.8	28	93.3		228.667 (36.010-1452.042)	
Gingiva phenotype	thin	17	32.7	7	23.3	0.370	Ref.	0.372
	thick	35	67.3	23	76.7		1.596 (0.572-4.450)	
Number of implants	one	14	26.9	2	6.7	**0.026**	Ref.	**0.039**
	two or more	38	73.1	28	93.3		5.158 (1.084-24.544)	
Number of implants with peri-implantitis	one	-	-	13	43.3	-	-	-
	two or more	-	-	17	56.7			
Timing of implant placement	immediate / early	17	32.7	12	40	0.505	Ref.	0.506
	delayed	35	67.3	18	60		0.792 (0.287-1.851)	
Jaw	maxilla	37	71.2	12	40	**0.006**	Ref.	0.007
	mandible	15	28.8	18	60		3.700 (1.438-9.522)	
Location of implant	anterior	21	40.4	7	23.3	0.117	Ref.	0.121
	posterior	31	59.6	23	76.7		2.226 (0.810-6.119)	
Implant collar design	machined	40	76.9	19	63.3	0.187	Ref.	0.190
	rough	12	23.1	11	36.7		1.930 (0.722-5.161)	

SD – standard deviation; P<0.05 is presented in bold; †Mann-Whitney U test OR (95% CI) – Odds Ratio (95% confidence interval); mPI: 0 – no plaque, 1 – plaque recognized by running a probe, 2 - plaque seen by the eye, 3 – abundance of plaque; mBI: 0 – no bleeding, 1 – isolated bleeding spots visible, 2 – blood forms a confluent red line, 3 – heavy bleeding
Ref. – referent

interactions among the gene variants of interest in the current study, the generalized multifactorial dimensionality reduction (GMDR) method was performed using GMDR v.0.9 software [20]. Pathogenicity and functional effects of selected variants in *VDR*, *RXRA*, and *CYP27B1* genes were analyzed by *in silico* software SIFT (for nonsynonymous variants), regSNP-intron, PD-SNP^{g}, HaploReg 4.2 and RegulomeDB$^{2.2}$ (for intronic variants). G*Power 3.1 software was used to estimate the post-hoc power of the study [21,22]. The post-hoc power was calculated with a significance level of 0.05 and an effect size of 0.3, indicating small to medium effects [23]. Associations were considered significant if the P value was less than 0.05.

RESULTS

Association of demographic, implant, implant-related factors, and clinical characteristics of the study group with peri-implantitis

The association of demographic, implant, implant-related factors, and clinical characteristics of the study group with peri-implantitis is shown in Table 1. A positive periodontitis history (patients with early treated periodontitis) was more common in patients with peri-implantitis (56.7%) than in healthy patients (28.8%) (P=0.013). A history of periodontitis was associated with a 3.2-fold increased risk of peri-implantitis. mPI (P=0.006) and mBI (P<0.0001), along with increased PPD (P<0.0001), were significantly higher in patients with peri-implantitis compared to those with healthy peri-implant tissue (Table 1). A 4.3-fold increased risk for peri-implantitis was observed in the presence of plaque, and a 12.8-fold increased risk was observed with a higher mBI. The distribution of the number of implants placed differed significantly between the study groups (P=0.026). Patients with two or more implants had a 5.1-fold higher risk of peri-implantitis. There was a significant difference in the distribution of implants between arches for the two groups of patients (P=0.006). Healthy peri-implant tissue was observed more frequently in the maxilla, and peri-implantitis was observed more often in the mandible, with this location being associated with a 3.7-fold higher risk of peri-implantitis. No difference was observed between implants placed in the anterior and posterior regions of the jaws. There were no differences between cases and controls as regards gender, age, smoking status, gingival phenotype, timing of implant placement, and implant collar design (P>0.05).

Genotype and allele distribution of *VDR*, *RXRA*, and *CYP27B1* gene variants

The variants in the *VDR* (rs2228570), *RXRA* (rs3118523, rs7864987), and *CYP27B1* (rs4646536) genes were in Hardy-Weinberg equilibrium (HW) in both groups of studied patients. There were no significant differences in the genotypes and allelic distribution of the analyzed rs2228570, rs3118523, and rs4646536 variants between patients with and without peri-implantitis (Table 2). A mutated C allele of the *RXRA* variant rs7864987 was significantly more frequent in patients with peri-implantitis than in those with healthy peri-implant tissue (P=0.019).

Table 2. Distribution of genotypes and allele of analyzed variants in *VDR*, *RXRA* and *CYP27B1* genes in patients with healthy peri-implant tissue and those with peri-implantitis.

		Patients with dental implants				P
		with healthy peri-implant tissue (n=52)		with peri-implantitis (n=30)		
Gene variant	**Genotype**	**n**	**%**	**n**	**%**	
***VDR* rs2228570**	AA	11	21.15	4	13.3	0.410
	AG	24	46.15	12	40	
	GG	17	32.7	14	46.7	
	A	46	44.2	20	33.3	0.170
	G	58	55.8	40	66.7	
***RXRA* rs3118523**	GG	2	3.85	2	6.7	0.789
	GA	14	26.92	9	30	
	AA	36	69.23	19	63.3	
	G	18	17.3	13	21.7	0.492
	A	86	82.7	47	78.3	
***RXRA* rs7864987**	TT	15	28.85	4	13.33	0.144
	TC	15	28.85	7	23.33	
	CC	22	42.3	19	63.34	
	T	45	43.3	15	25	**0.019**
	C	59	56.7	45	75	
***CYP27B1* rs4646536**	AA	35	67.3	20	66.66	0.562
	AG	12	23.1	5	16.67	
	GG	5	9.6	5	16.67	
	A	82	78.8	45	75	0.570
	G	22	21.2	15	25	

P<0.05 is significant and indicated in bold.

Table 3. Association of *VDR* (rs2228570), *RXRA* (rs3118523, rs7864987), and *CYP27B1* (rs4646536) gene variants with peri-implantitis risk.

Gene variant	Genotype / Genetic model		OR (95%CI)	P	aOR (95%CI)	P
VDR rs2228570	AA		1.000	Ref.	1.000	Ref.
	AG vs. AA		1.375 (0.361-5.240)	0.641	1.024 (0.248-4.236)	0.973
	GG vs. AA		2.265 (0.590-8.695)	0.234	1.868 (0.464-7.521)	0.379
	Dominant	AG+GG vs. AA	1.744 (0.502-6.059)	0.381	1.353 (0.370-4.949)	0.648
	Recessive	GG vs. AG+AA	1.801 (0.716-4.530)	0.211	1.782 (0.683-4.651)	0.238
	Over-dominant	AG vs. GG+AA	0.778 (0.313-1.935)	0.589	0.670 (0.256-1.751)	0.413
RXRA rs3118523	GG		1.000	Ref.	1.000	Ref.
	GA vs. GG		0.643 (0.076-5.417)	0.685	0.560 (0.041-7.639)	0.664
	AA vs. GG		0.528 (0.069-4.048)	0.539	0.536 (0.072-4.396)	0.584
	Dominant	GA+AA vs. GG	0.560 (0.075-4.195)	0.573	0.615 (0.075-5.022)	0.650
	Recessive	AA vs. GA+GG	1.021 (0.375-2.781)	0.968	1.044 (0.364-2.993)	0.937
	Over-dominant	GA vs. GG+AA	1.163 (0.431-3.139)	0.765	1.010 (0.357-2.856)	0.985
RXRA rs7864987	TT		1.000	Ref.	1.000	Ref.
	TC vs. TT		1.750 (0.422-7.253)	0.440	1.814 (0.420-7.830)	0.425
	CC vs. TT		3.239 (0.917-11.443)	0.068	3.333 (0.902-12.308)	0.071
	Dominant	TC+CC vs. TT	2.635 (0.785-8.851)	0.117	2.737 (0.780-9.597)	0.116
	Recessive	CC vs. TC+TT	2.355 (0.935-5.935)	0.069	2.373 (0.907-6.206)	0.078
	Over-dominant	TC vs. CC+TT	0.751 (0.266-2.118)	0.588	0.768 (0.262-2.255)	0.631
CYP27B1 rs4646536	AA		1.000	Ref.	1.000	Ref.
	AG vs. AA		0.729 (0.224-2.371)	0.600	0.515 (0.142-1.871)	0.314
	GG vs. AA		1.750 (0.451-6.790)	0.419	1.994 (0.483-8.234)	0.340
	Dominant	AG+GG vs. AA	1.029 (0.396-2.675)	0.953	0.931 (0.344-2.526)	0.889
	Recessive	GG vs. AG+AA	1.880 (0.497-7.117)	0.353	2.275 (0.562-9.213)	0.249
	Over-dominant	AG vs. GG+AA	0.667 (0.210-2.120)	0.492	0.507 (0.148-1.734)	0.279

OR (95% CI) – odds ratio (95% confidence interval). Adjustment was done for the history of periodontitis; Ref. – referent

Association of *VDR*, *RXRA,* and *CYP27B1* gene variants with peri-implantitis and clinical characteristics

No association was found between variants in the *VDR*, *RXRA*, and *CYP27B1* genes and the risk of peri-implantitis for any of the variants studied or genetic models tested (pairwise, dominant, recessive, over-dominant). (Table 3). In the case of *RXRA* (rs7864987), a statistical trend of increased risk of peri-implantitis was observed in the mutated CC genotype compared to the TT wild type (OR=3.239, 95% CI: 0.917-11.443, P=0.068) and in the recessive genetic model (CC vs. TT and TC genotypes combined, OR=2.355, 95% CI: 0.935-5.935, P=0.069). After adjustment, none of the genotypes and genetic models of the studied variants in the *VDR*, *RXRA*, and *CYP27B1* genes were found to be associated with the risk of peri-implantitis. After adjustment for periodontitis history, a statistical trend remained for increased risk of peri-implantitis in the tested mutated CC genotype compared to wild-type TT (OR =3.333, 95% CI: 0.902-12.308, P=0.071) and recessive genetic model (OR =2.373, 95% CI: 0.907-6.206, P=*0.078*) of *RXRA* variant rs7864987.

Clinical characteristics (history of periodontitis, mPI, mBI, PPD), as the main indicators of periodontal status, were studied relative to variants in the *VDR*, *RXRA,* and *CYP27B1* genes in patients with peri-implantitis (Table 4). Dental implant location was associated with the *RXRA* rs7864987 variant (P=0.009). Patients with the CC genotype of the *RXRA* rs7864987 variant were more likely to have peri-implantitis and dental implants placed in the mandible. mBI was associated with the *CYP27B1* rs4646536 variant as patients with wild-type TT genotype were more likely to have unfavorable mBI (P=0.040). Other clinical characteristics were not associated with the gene variants studied

Table 4. Association of *VDR*, *RXRA* and *CYP27B1* gene variants with epidemiological and clinical characteristics of patients with peri-implantitis.

Demographic and clinical characteristics		*VDR* rs2228570			P	*RXRA* rs3118523			P	*RXRA* rs7864987			P	*CYP27B1* rs4646536			P
		AA	AG	GG		GG	GA	AA		TT	TC	CC		AA	AG	GG	
Gender	male	1	7	7	0.513	1	5	9	0.921	3	3	9	0.550	10	3	2	0.819
	female	3	5	7		1	4	10		1	4	10		10	2	3	
Age (years, mediana)	≤61	3	5	9	0.372	1	6	10	0.768	0	4	13	**0.043**	12	2	3	0.712
	>61	1	7	5		1	3	9		4	3	6		8	3	2	
Smoking status	non-smoker	3	9	7	0.366	2	3	14	0.063	3	4	12	0.839	13	3	3	0.965
	smoker	1	3	7		0	6	5		1	3	7		7	2	2	
History of periodontitis	no	3	4	6	0.346	1	1	11	0.064	2	3	8	0.959	8	1	4	0.140
	yes	1	8	8		1	8	8		2	4	11		12	4	1	
Modified plaque index (mPI)	0	2	2	2	0.270	1	1	4	0.453	0	2	4	0.513	4	2	0	0.287
	1+2+3	2	10	12		1	8	15		4	5	15		16	3	5	
Modified bleeding index (mBI)	0+1	1	3	5	0.815	0	4	5	0.392	2	3	4	0.361	3	3	3	**0.040**
	2+3	3	9	9		2	5	14		2	4	15		17	2	2	
Peri-implant pocket depth	≤4mm	0	1	1	0.842	0	0	2	0.538	1	1	0	0.124	1	1	0	0.392
	>4mm	4	11	13		2	9	17		3	6	19		19	4	5	
Gingiva phenotype	thin	1	4	2	0.517	0	4	3	0.178	0	2	5	0.492	5	1	1	0.954
	thick	3	8	12		2	5	16		4	5	14		15	4	4	
Number of implants with peri-implantitis	one	1	6	6	0.682	1	5	7	0.635	1	1	11	0.101	7	3	3	0.428
	two or more	3	6	8		1	4	12		3	6	8		13	2	2	
Timing of implant placement	immediate / early	0	5	7	0.196	2	3	7	0.197	1	5	6	0.148	8	2	2	1.000
	delayed	4	7	7		0	6	12		3	2	13		12	3	3	
Jaw position	maxilla	1	4	7	0.554	2	3	7	0.197	0	6	6	**0.009**	8	2	2	1.000
	mandible	3	8	7		0	6	12		4	1	13		12	3	3	
Position of placement	anterior	1	2	4	0.771	1	2	6	0.119	2	2	3	0.316	5	1	1	0.954
	posterior	3	10	10		1	9	13		2	5	16		15	4	4	
Implant collar design	machined	2	6	11	0.269	2	5	12	0.498	3	6	10	0.262	13	4	2	0.408
	rough	2	6	3		0	4	7		1	1	9		7	1	3	

P<0.05 is significant and indicated in bold; mPI: 0 – no plaque, 1 – plaque recognized by running a probe, 2 – plaque seen by eye, 3 – abundance of plaque; mBI: 0 – no bleeding, 1 – isolated bleeding spots visible, 2 – blood forms a confluent red line, 3 – heavy bleeding

(Table 4). The data presented should be interpreted with consideration of the limited sample size of the peri-implantitis patient group.

Epistatic interactions between the analyzed variants in the *VDR*, *RXRA*, and *CYP27B1* genes

Given that *VDR*, *RXRA*, and *CYP27B1* are key genes involved in the function and synthesis of vitamin D, we conducted a statistical epistatic interaction analysis to explore potential combinations of genes and their variants that could be associated with peri-implantitis. None of the models tested proved to be significant and associated with peri-implantitis (Table 5).

In silico analysis of pathogenicity and functional effects of analyzed variants in *VDR*, *RXRA*, and *CYP27B1* genes

According to the *in silico* prediction of pathogenicity for the analyzed variants, the *VDR* gene variant rs2228570 was found to be deleterious, while rs3118523 and rs7864987 in *RXRA*, as well as rs4646536 in *CYP27B1*, were identified as benign. *RXRA* rs3118523 was identified as altering regulatory motifs for Maf binding, rs7864987 as affecting AP-1, NF-Y, and TATA binding, and *CYP27B1* rs4646536 as influencing DMRT7 regulatory elements. *RXRA* rs3118523 changes the binding site of the CTCF transcription site, while

Table 5. Models of statistical epistatic interactions of analyzed *VDR* rs2228570, *RXRA* (rs3118523, rs7864987), and *CYP27B*1 (rs4646536) variants and their association with peri-implantitis.

Tested epistatic model	Training balanced accuracy	Testing balanced accuracy	Cross validation consistency	P
rs7864987	0.6061	0.5269	9/10	0.1719
rs2228570-rs7864987	0.6504	0.5043	8/10	0.9453
rs2228570-rs7864987-rs4646536	0.7112	0.4199	7/10	0.9896
rs2228570-rs3118523-rs7864987-rs4646536	0.7693	NaN	10/10	0.9453

NaN – not a number

rs7864987 alters the binding sites of POLR2A, ZFX, GABPA, STAT3, and *CYP27B1* rs4646536 of EZH2 transcription factor (Supplementary Table S2).

DISCUSSION

This case-control study aimed to investigate whether variants in vitamin D-related genes could be used to predict the occurrence of peri-implantitis. Our results suggest that the variants analyzed in *VDR, RXRA*, and *CYP27B1* genes cannot be used as molecular predictors of peri-implantitis. In patients with peri-implantitis, *CYP27B1* variant rs4646536 was associated with a modified bleeding index (P=0.040), and *RXRA* rs7864987 with the implant jaw position (P=0.009).

The genetic background of peri-implantitis is largely unknown. Due to the increasing number of patients with integrated dental implants and the occurrence of biological complications, such as peri-implantitis, there is a growing need for further clarification of the etiology, pathology, and genetic predisposition to peri-implantitis. Identifying individuals at risk for peri-implantitis could help develop targeted implant treatment and placement strategies, including appropriate follow-up and care for dental implants. Studies have examined the association of different gene variants with peri-implantitis to identify new genetic markers. Most studies focus on genes involved in the immune response, while there is a lack of association studies exploring other genes [24,25]. Vitamin D has multiple biological functions, including anti-inflammatory effects. For example, vitamin D lowers the pro-inflammatory cytokines elevated in peri-implantitis [8]. The biological function of vitamin D might be affected by variants in genes that are important for vitamin D function and synthesis. To the authors' knowledge, this is the first study reporting an association between vitamin D-related gene variants and the occurrence of peri-implantitis.

None of the analyzed variants in the vitamin D-related genes *VDR* (rs2228570), *RXRA* (rs3118523, rs7864987), and *CYP27B1* (rs4646536) were associated with the occurrence of peri-implantitis after adjusting for the history of periodontitis as one of the main and the most important confounding factors. The lack of association might be due to the limited size of our study group, the functional effects of the variants, and the specific role of the analyzed variants in the pathology under investigation. Although our study group was limited in size, it was ethnically homogeneous, which is important for gene association studies. Furthermore, all the variants studied were in Hardy-Weinberg equilibrium in both the peri-implantitis cases and the controls with healthy peri-implant tissues. In gene association studies, HW equilibrium in a group of patients indicates no association between common gene variants with the disease, which is consistent with our results. The power of the study was 68% for a significance level of 0.05 and an effect size of 0.3, indicating modest power to detect differences between the peri-implantitis group and the group with healthy peri-implant tissue.

The rs2228570 variant, also known as FokI, is one of the most studied and functionally confirmed variants in the *VDR* gene. The presence of mutated allele C results in the synthesis of a VDR protein that is three amino acids shorter, but has a higher transactivation capacity in binding vitamin D [26]. This variant in the *VDR* gene is associated with various inflammatory conditions, such as periodontitis [27-29], but, according to our results, it is not linked to peri-implantitis in the Serbian group of patients with dental implants. Although peri-implantitis is an inflammatory condition, it is clear that the *VDR* variant rs2228570 may have different associations across various inflammatory

pathologies. In addition to the studied variants, there are numerous identified single nucleotide changes in the *VDR* gene, and some of these have been studied in the context of dental implant losses. A previous study reported that variant rs731236 (*VDR* TaqI) was not associated with dental implant loss [30], while *VDR* rs3782905 was found to be a potential risk factor for multiple dental implant loss [31]. These studies focused on the association of *VDR* gene polymorphism with implant loss, not peri-implantitis.

The results of association studies linking *VDR* gene polymorphisms to periodontitis suggest that certain genotypes may be associated with increased disease susceptibility. However, differences in findings may arise due to variations in study designs, sample sizes, or heterogeneous populations [32-34]. For instance, the *VDR* variant rs2228570 has been associated with periodontitis [28,29].

The lack of association with peri-implantitis risk was also observed for analyzed *RXRA* rs3118523 and rs7864987 variants. Mutated C allele of *RXRA* intron variant rs7864987 was more frequent in patients with peri-implantitis than in patients with healthy peri-implant tissue. However, risk analysis adjusted for history of periodontitis showed a trend toward an increased risk of peri-implantitis in the recessive genetic model (CC versus TC and TT combined). Because the association showed a statistical trend, it may be possible that enlarging the size of the study group could lead to the discovery of a significantly increased risk. If this were the case, the variant rs7864987 could be used to identify patients at risk for peri-implantitis after implant placement. However, this assumption needs to be further tested in a larger group of patients with peri-implantitis. A recent study reported that the expression of *RXRA* and *VDR* was lower in patients with peri-implantitis compared to patients with healthy peri-implant tissue and individuals with periodontitis [35]. It would be of great interest to measure the expression of *VDR* and *RXRA* in the context of gene variants in patients with peri-implantitis.

In our study, there was no association between the studied variant rs4646536 in the *CYP27B1* gene and peri-implantitis risk. Previous reports indicate that the presence of the T allele (according to HGVS *A* allele) of rs4646536 in the *CYP27B1* gene is associated with vitamin D deficiency [36]. In the current study, we did not measure the vitamin D level of the participants, which may be of interest for future research.

Smoking status was not found to be associated with peri-implantitis risk in our study group. The role of smoking habits in peri-implantitis remains a topic of ongoing debate [2]. A greater rate of tooth loss was displayed in smokers compared to non-smokers and the incidence of dental implants in smoking patients was increased [37]. However, the current meta-analysis revealed a significant increase in marginal bone loss around implants, leading to subsequent bone loss, in smokers compared to non-smokers [38]. This was in contrast to a previous study in which smoking was identified as a risk factor in peri-implantitis development in the Serbian population [25]. Nevertheless, similar to our findings, smoking was not a significant predictor for peri-implantitis, suggesting that other factors, such as a history of early treated periodontitis and poor plaque control, play a more crucial role in the onset of peri-implantitis, representing an increased risk for its development [2,3].

According to our results, the location of dental implants, especially in the mandible, might be considered a potential indicator of increased risk for peri-implantitis. The current literature is inconsistent in determining which specific implant position poses a risk for peri-implantitis. Some authors found a significant correlation between implants placed in the mandible and the prevalence of peri-implantitis [39,40], while others found no association between implant location and peri-implantitis [41,42]. The timing of implant placement was not a risk for peri-implantitis. Studies examining the risk for peri-implantitis in immediately and conventionally placed implants also found no difference between the two placement protocols [43,44]. Our findings agree with a recent systematic review describing the relative risk of peri-implantitis for two-piece implants with machined and rough collars [45].

Apart from well-known and commonly considered risk factors, microbial composition is widely recognized as a driving force for the occurrence and severity of peri-implantitis [46,47]. A recent study on the Thai population found that the VDR-FokI polymorphism influences subgingival microbial composition and the severity of periodontitis [33]. Therefore, genetic association studies should consider microbial analysis as an important confounding factor for peri-implantitis.

One of the main limitations of our study is the relatively small size of the study group, which may have affected the reliability and generalizability of the results. We performed a post-hoc power analysis, and the obtained results should be interpreted in light of the modest power of the study. Small sample sizes may limit the ability to detect true associations or differences between groups, increasing the risk of type II (false negative) and type I (false positive) errors in interpreting the results. In addition, the small sample size limits the generalizability of the results as they may not necessarily apply to other populations. For these reasons, our results should be considered as preliminary. Future studies should involve a larger participant sample to confirm the results and validate the conclusions. The procedure for recruiting patients with peri-implantitis and healthy peri-implant tissue proved feasible and provides a basis for future studies.

Based on the above and considering the limitations of the current study, variants in vitamin D-related genes, *VDR* (rs2228570), *RXRA* (rs3118523, rs7864987), and *CYP27B1* (rs4646536) cannot be used as predictors of peri-implantitis in the Serbian patient group. The next steps for future research will include a larger study group, measurement of the active form of vitamin D (calcidiol), expression of vitamin D-related genes, and analysis of gene and environmental interactions. It is important to take a comprehensive approach that encompasses genetic, transcriptomic, epigenetic, and microbial analyses of peri-implant tissue to elucidate the complex etiopathogenesis of peri-implantitis.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-65/2024-03/200178).

Author contributions: conceptualization, MM and KZ; Methodology, MM, SJ, and KZ; validation, MM and KZ; formal analysis, MM, ZL, and KZ; investigation, MM, DR, SJ, and KZ; resources, MM, ZL and DR; data curation, MM and ZL; writing – original draft preparation, MM and KZ; Writing – review and editing, MM, SJ, ZL, DR and KZ; visualization – MM and KZ; supervision, MM and KZ. All authors have read and agreed to the published version of the manuscript.

Conflict of interest disclosure: The authors declare no conflicts of interest.

Data availability: The data underlying this article are available in the online supplementary material: https://www.serbiosoc.org.rs/NewUploads/Uploads/Magic%20et%20al_Dataset.pdf

REFERENCES

1. Buser D, Sennerby L, De Bruyn H. Modern implant dentistry based on osseointegration: 50 years of progress, current trends and open questions. Periodontol 2000. 2017;73(1):7-21. https://doi.org/10.1111/prd.12185
2. Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. J Periodontol. 2018;89 Suppl 1:S267-s90. https://doi.org/10.1002/jper.16-0350
3. Renvert S, Quirynen M. Risk indicators for peri-implantitis. A narrative review. Clin Oral Implants Res. 2015;26 Suppl 11:15-44. https://doi.org/10.1111/clr.12636
4. Fourmousis I, Vlachos M. Genetic Risk Factors for the Development of Periimplantitis. Implant Dent. 2019;28(2):103-14. https://doi.org/10.1097/id.0000000000000874
5. Eguia Del Valle A, López-Vicente J, Martínez-Conde R, Aguirre-Zorzano LA. Current understanding of genetic polymorphisms as biomarkers for risk of biological complications in implantology. J Clin Exp Dent. 2018;10(10):e1029-e39. https://doi.org/10.4317/jced.55141
6. Lin R. Crosstalk between Vitamin D Metabolism, VDR Signalling, and Innate Immunity. BioMed research international. 2016;2016:1375858. https://doi.org/10.1155/2016/1375858
7. Martelli FS, Martelli M, Rosati C, Fanti E. Vitamin D: relevance in dental practice. Clin Cases Miner Bone Metab. 2014;11(1):15-9.
8. Chen J, Tang Z, Slominski AT, Li W, Żmijewski MA, Liu Y, Chen J. Vitamin D and its analogs as anticancer and anti-inflammatory agents. Eur J Med Chem. 2020;207:112738. https://doi.org/10.1016/j.ejmech.2020.112738
9. Bryce G, MacBeth N. Vitamin D deficiency as a suspected causative factor in the failure of an immediately placed dental implant: a case report. J R Nav Med Serv. 2014;100(3):328-32.
10. Insua A, Monje A, Wang HL, Miron RJ. Basis of bone metabolism around dental implants during osseointegration and peri-implant bone loss. J Biomed Mater Res A. 2017;105(7):2075-89. https://doi.org/10.1002/jbm.a.36060
11. Kelly J, Lin A, Wang CJ, Park S, Nishimura I. Vitamin D and bone physiology: demonstration of vitamin D deficiency in an implant osseointegration rat model. J Prosthodont. 2009;18(6):473-8. https://doi.org/10.1111/j.1532-849X.2009.00446.x
12. Pourshahidi S, Yousefain M. The Relationship Between Serum Level of Vitamin D3 and Osseointegration Around the Dental Implant. J Oral Implantol. 2021;47(1):88-90. https://doi.org/10.1563/aaid-joi-D-19-00341
13. Guido Mangano F, Ghertasi Oskouei S. Low serum vitamin D and early dental implant failure: Is there a connection? A retrospective clinical study on 1740 implants placed in 885 patients. 2018;12(3):174-82. https://doi.org/10.15171/joddd.2018.027
14. Acipinar S, Karsiyaka Hendek M. Evaluation of FGF-23 and 25(OH)D(3) levels in peri-implant sulcus fluid in peri-implant health and diseases. Clin Implant Dent Relat Res. 2019;21(5):1106-12. https://doi.org/10.1111/cid.12832
15. Ustaoğlu G, Erdal E. Relationship between risk markers for cardiovascular disease and peri-implant diseases. Int J Implant Dent. 2020;6(1):73. https://doi.org/10.1186/s40729-020-00273-z

16. Sanz M, Chapple IL. Clinical research on peri-implant diseases: consensus report of Working Group 4. J Clin Periodontol. 2012;39 Suppl 12:202-6. https://doi.org/10.1111/j.1600-051X.2011.01837.x
17. Mombelli A, Lang NP. Clinical parameters for the evaluation of dental implants. Periodontol 2000. 1994;4:81-6. https://doi.org/10.1111/j.1600-0757.1994.tb00008.x
18. Kan JY, Rungcharassaeng K, Umezu K, Kois JC. Dimensions of peri-implant mucosa: an evaluation of maxillary anterior single implants in humans. J Periodontol. 2003;74(4):557-62. https://doi.org/10.1902/jop.2003.74.4.557
19. Rakasevic D, Lazic Z, Soldatovic I, Scepanovic M, Gabric D. Influence of titanium implant macrodesign on peri-implantitis occurrence: a cross-sectional study. Clin Oral Investig. 2022;26(8):5237-46. https://doi.org/10.1007/s00784-022-04492-z
20. Xu HM, Xu LF, Hou TT, Luo LF, Chen GB, Sun XW, Lou XY. GMDR: Versatile Software for Detecting Gene-Gene and Gene-Environ- ment Interactions Underlying Complex Traits. Curr Genomics. 2016;17(5):396-402. https://doi.org/10.2174/1389202917666160513102612
21. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009;41(4):1149-60. https://doi.org/10.3758/brm.41.4.1149
22. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175-91. https://doi.org/10.3758/bf03193146
23. Cohen J. Statistical Power Analysis for the Behavioral Sciences 2nd. ed: Lawrence Erlbaum Associates; 1988.
24. He K, Jian F, He T, Tang H, Huang B, Wei N. Analysis of the association of TNF-α, IL-1A, and IL-1B polymorphisms with peri-implantitis in a Chinese non-smoking population. Clin Oral Investig. 2020;24(2):693-9. https://doi.org/10.1007/s00784-019-02968-z
25. Petkovic-Curcin A, Zeljic K, Cikota-Aleksic B, Dakovic D, Tatic Z, Magic Z. Association of Cytokine Gene Polymorphism with Peri-implantitis Risk. Int J Oral Maxillofac Implants. 2017;32(5):e241-e8. https://doi.org/10.11607/jomi.5814
26. Whitfield GK, Remus LS, Jurutka PW, Zitzer H, Oza AK, Dang HT, Haussler CA, Galligan MA, Thatcher ML, Encinas Dominguez C, Haussler MR. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol Cell Endocrinol. 2001;177(1-2):145-59. https://doi.org/10.1016/s0303-7207(01)00406-3
27. Marian D, Rusu D, Stratul SI, Calniceanu H, Sculean A, Anghel A. Association of Vitamin D Receptor Gene Polymorphisms with Chronic Periodontitis in a Population in Western Romania. Oral Health Prev Dent. 2019;17(2):157-65. https://doi.org/10.3290/j.ohpd.a39738
28. Naito M, Miyaki K, Naito T, Zhang L, Hoshi K, Hara A, Masaki K, Tohyama S, Muramatsu M, Hamajima N, Nakayama T. Association between vitamin D receptor gene haplotypes and chronic periodontitis among Japanese men. Int J Med Sci. 2007;4(4):216-22. https://doi.org/10.7150/ijms.4.216
29. Park KS, Nam JH, Choi J. The short vitamin D receptor is associated with increased risk for generalized aggressive periodontitis. J Clin Periodontol. 2006;33(8):524-8. https://doi.org/10.1111/j.1600-051X.2006.00944.x
30. Alvim-Pereira F, Montes CC, Thomé G, Olandoski M, Trevilatto PC. Analysis of association of clinical aspects and vitamin D receptor gene polymorphism with dental implant loss. Clin Oral Implants Res. 2008;19(8):786-95. https://doi.org/10.1111/j.1600-0501.2008.01532.x
31. Munhoz Pereira T, Alvim-Pereira F. A complete physical mapping of the vitamin D receptor gene for dental implant loss: A pilot study. Clin Oral Implants Res. 2019;30(12):1165-78. https://doi.org/10.1111/clr.13529
32. Chantarangsu S, Sura T, Mongkornkarn S, Donsakul K, Torrungruang K. Vitamin D Receptor Gene Polymorphism and Smoking in the Risk of Chronic Periodontitis. J Periodontol. 2016;87(11):1343-51. https://doi.org/10.1902/jop.2016.160222
33. Torrungruang K, Chantarangsu S, Sura T, Thienpramuk L. Interplay between vitamin D receptor FokI polymorphism and smoking influences Porphyromonas gingivalis proportions in subgingival plaque. J Clin Periodontol. 2020;47(8):912-20. https://doi.org/10.1111/jcpe.13307
34. Wang C, Zhao H, Xiao L, Xie C, Fan W, Sun S, Xie B, Zhang J. Association between vitamin D receptor gene polymorphisms and severe chronic periodontitis in a Chinese population. J Periodontol. 2009;80(4):603-8. https://doi.org/10.1902/jop.2009.080465
35. Taskan MM, Gevrek F. PPAR-γ, RXR, VDR, and COX-2 Expressions in gingival tissue samples of healthy individuals, periodontitis and peri-implantitis patients. Niger J Clin Pract. 2020;23(1):46-53. https://doi.org/10.4103/njcp.njcp_349_19
36. Yu S, Li X, Wang Y, Mao Z, Xie Y, Zhang L, Wang C, Li W. Family-based Association between Allele T of rs4646536 in CYP27B1 and vitamin D deficiency. J Clin Lab Anal. 2019;33(6):e22898. https://doi.org/10.1002/jcla.22898
37. Jansson L, Lavstedt S. Influence of smoking on marginal bone loss and tooth loss--a prospective study over 20 years. J Clin Periodontol. 2002;29(8):750-6. https://doi.org/10.1034/j.1600-051x.2002.290812.x
38. Chrcanovic BR, Albrektsson T, Wennerberg A. Smoking and dental implants: A systematic review and meta-analysis. J Dent. 2015;43(5):487-98. https://doi.org/10.1016/j.jdent.2015.03.003
39. Ferrantino L, Tironi F, Pieroni S, Sironi A, Simion M. A Clinical and Radiographic Retrospective Study on 223 Anodized Surface Implants with a 5- to 17-Year Follow-up. Int J Periodontics Restorative Dent. 2019;39(6):799-807. https://doi.org/10.11607/prd.4330
40. Rakašević D, Lazić Z, Rakonjac B, Soldatović I, Janković S, Magić M, Aleksić Z. Efficiency of photodynamic therapy in the treatment of peri-implantitis – A three-month randomized controlled clinical trial. Srp Arh Celok Lek. 2016;144(9-10):478-84.
41. Pimentel SP, Shiota R, Cirano FR, Casarin RCV, Pecorari VGA, Casati MZ, Haas AN, Ribeiro FV. Occurrence of peri-implant diseases and risk indicators at the patient and implant levels: A multilevel cross-sectional study. J Periodontol. 2018;89(9):1091-100. https://doi.org/10.1002/jper.17-0599

Vignoletti F, Di Domenico GL, Di Martino M, Montero E. Prevalence and risk indicators of peri-implantitis in a sample of university-based dental patients in Italy: A cross-sectional study. J Clin Periodontol. 2019;46(5):597-605. https://doi.org/10.1111/jcpe.13111
42. Kordbacheh Changi K, Finkelstein J, Papapanou PN. Peri-implantitis prevalence, incidence rate, and risk factors: A study of electronic health records at a U.S. dental school. Clin Oral Implants Res. 2019;30(4):306-14. https://doi.org/10.1111/clr.13416
43. Rodrigo D, Martin C, Sanz M. Biological complications and peri-implant clinical and radiographic changes at immediately placed dental implants. A prospective 5-year cohort study. Clin Oral Implants Res. 2012;23(10):1224-31. https://doi.org/10.1111/j.1600-0501.2011.02294.x
44. Messias A, Nicolau P, Guerra F. Titanium dental implants with different collar design and surface modifications: A systematic review on survival rates and marginal bone levels. Clin Oral Implants Res. 2019;30(1):20-48. https://doi.org/10.1111/clr.13389
45. Belibasakis GN, Manoil D. Microbial Community-Driven Etiopathogenesis of Peri-Implantitis. Journal of dental research. 2021;100(1):21-8. https://doi.org/10.1177/0022034520949851
46. Ghensi P, Manghi P, Zolfo M. Strong oral plaque microbiome signatures for dental implant diseases identified by strain-resolution metagenomics. NPJ Biofilms Microbiomes. 2020;6(1):47. https://doi.org/10.1038/s41522-020-00155-7

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Details of analyzed variants in *VDR, RXRA,* and *CYP27B1* genes

Gene	Chromosome location	dbSNP ID	Nucleotide change (GRCh38.p14, HGVS nomenclature)	Position within gene and functional effect	Position within gene and functional effect	Referent and alternative allele	Allele frequences in European population*
VDR	12q13.11	rs2228570	NC_000012.12:g.47879112A>G	first exon initiator codon variant missense	first exon initiator codon variant missense	Ref: A Alt: G	A=0.388696 G=0.612598
RXRA	9q34.2	rs3118523	NC_000009.12:g.134443675G>A	2KB upstream	2KB upstream	Ref: G Alt: A	G=0.19043 A=0.80957
		rs7864987	NC_000009.12:g.134356320T>C	intron 1	intron 1	Ref: T Alt: C	T=0.75194 C=0.24806
CYP27B1	12q14.1	rs4646536	NC_000012.12:g.57764205A>G	intron between exon 6 and 7	intron between exon 6 and 7	Ref: A Alt: G	A=0.68287 G=0.31713

† GRCh38.p14 – Genome Reference Consortium Human Build 38 patch release 14; HGVS – Human Genome Variation Society. Ref – referent allele; Alt – alternative allele. *Based on data provided in the dbSNP database for the European population

Supplementary Table S2. Prediction of pathogenicity and functional effects of analyzed variants in *VDR, RXRA,* and *CYP27B1* genes

		Pathogenicity predictors*			Functional effects predictors		
Gene	dbSNP ID	SIFT	regSNP-intron	PhD-SNP^{g}	HaploReg 4.2 (regulatory motifs altered)	RegulomeDB$^{2.2}$	
						TF binding sites (ChIP-seq)	Prediction score
VDR	rs2228570	deleterious	-	-	-	-	0.22271
RXRA	rs3118523	-	-	benign	Maf	CTCF	0.55436
	rs7864987	-	benign	benign	AP-1 NF-Y TATA	POLR2A ZFX GABPA STAT3	0.66703
CYP27B1	rs4646536	-	benign	benign	DMRT7	EZH2	0.55436

*SIFT software can be used only for missense variants; regSNP-intron and PhD-SNP^{g} are software for pathogenicity characterization of intronic gene variants; RegulomeDB$^{2.2}$ provides functional context to variants located within the non-coding regions. The prediction score [0-1] indicates the probability of the variant having real functional significance.

ONLINE SUPPLEMENTARY MATERIAL

The data underlying this article are available in the online supplementary material: https://www.serbiosoc.org.rs/NewUploads/Uploads/Magic%20et%20al_Dataset.pdf