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A complete physical mapping of the vitamin D receptor gene for dental implant loss: A pilot study

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Abstract

Objectives: The aim of this pilot case–control study was to investigate the association of clinical variables and genetic polymorphisms in the vitamin D receptor gene (*VDR*) with dental implant loss.

Material and Methods: This study was carried out with 244 individuals with mean age 51.90 ± 11.28 (81 cases and 163 controls matched by age, sex, and smoking habit). Also, the clusterization phenomenon was investigated stratifying the sample into two groups: (a) 34 patients with multiple losses (presenting two or more lost implants) and (b) 210 without multiple losses (up to one implant loss). Sociodemographic, clinical, and periodontal parameters were analyzed. The tagSNPs in the *VDR* gene were analyzed by real-time PCR. Univariate and multivariate analyses were performed ($p < .05$).

Results: Edentulism, number of implants installed, and Gingival, Plaque, and Calculus Indexes were associated with implant loss in the univariate analysis. After the multivariate analysis, the allele G of rs3782905 in the recessive model, together with number of installed implants and Gingival Index, was associated with implant failure.

Conclusion: It is suggested that the allele G of rs3782905 in the recessive model may be a new genetic risk marker for dental implant loss in patients who lost two or more dental implants. In addition, number of implants installed and Gingival Index were also associated. Replication is mandatory to confirm these findings, due to the modest sample size of this work.

KEYWORDS

clinical parameters, dental implant loss, genetic polymorphisms, *VDR*

1 | INTRODUCTION

The global epidemiological status of common oral diseases reports notable numbers of prevalence such as untreated cavities in permanent teeth (2.5 billion people), chronic severe periodontitis (538 million people), and edentulism (276 million people) worldwide (Kassebaum et al., 2017). This panorama can contribute to an increasing demand for dental replacement, and, in this context, the treatment with dental implants proves to be an excellent option, due to its high success rates (Moraschini, Poubel, Ferreira, & Barboza, 2015).

The failure rate in the implant treatment is small in percentage; however, it becomes relevant in absolute values, once the demand for implant indication is increasing (Guillaume, 2016).

The literature points to several risk factors for implant loss, such as smoking (Ghanem et al., 2017), bone characteristics (Sakka,

Baroudi, & Nassani, 2012), systemic diseases (Dawson & Jasper, 2015), peri-implantitis, and occlusal overload (Sakka et al., 2012). Nevertheless, some patients present losses even without the identification of a clinical cause.

Additionally, there is scientific evidence that some implant complications (such as implant loss) tend to cluster in subsets of individuals (Tonetti, 1999; Weyant & Burt, 1993). This suggests that genetic factors may be involved in the physiopathology of dental implant loss (Alvim-Pereira, Montes, Mira, & Trevilatto, 2008).

Vitamin D is considered a multifunctional steroid hormone, mainly involved in osteomineral homeostasis, with emphasis on the metabolism of calcium and phosphorus, being its biological effects promoted by the binding with its receptor, called vitamin D receptor (VDR; Castro, 2011).

Genetic polymorphisms represent common alterations in the gene sequences that may impact the function of DNA (Cargill et al., 1999), affecting protein synthesis. Single nucleotide polymorphisms (SNPs) in the *VDR* gene have been associated with diseases in which bone loss is a classic sign (Dastgheib, Gartland, Tabei, Omrani, & Teare, 2016), such as osteoporosis (Wu et al., 2014) and periodontal disease (Martelli, Martelli, Rosati, & Fanti, 2014). However, its association with dental implant loss is not clear yet. In addition, several of whole-genome transcriptome studies suggest complex molecular pathways that may play putative roles in osseointegration, particularly through the vitamin D—VDR axis (Nishimura, 2013). One of the main molecular mechanisms proposed is related to a cascade in which intermediate vitamin D metabolites act as transcription factors for genes linked to osseointegration, such as *HAPLN1*, *Col2*, *Col9*, *Col10*, and *Col11* (genes related to bone extracellular matrix formation), and genes that induce osteoclastogenesis (such as *RANKL*). Thus, genetic variations in *VDR* may have as a consequence the decrease in its biological effects, compromising the performance of the metabolic intermediates of the endocrine system of vitamin D, which can be important to the success for osseointegration (Castro, 2011; Nishimura, 2013), particularly if other risk factors are present.

Then, the aim of this study was to search for association of clinical, sociodemographic and periodontal aspects, and polymorphisms in the *VDR* gene, with dental implant loss, performing a complete physical mapping of this gene. This approach becomes especially interesting considering that SNPs associated with failures could act as risk genetic biomarkers, contributing to early detection of more susceptible individuals, before the surgical installation.

2 | MATERIAL AND METHODS

2.1 | Ethical approval

This pilot case–control study was approved by the Ethics Committee on Research of the Pontifical Catholic University of Paraná (PUCPR) (No. 0003772/10—Protocol No. 323). This study was characterized as a pilot study because its sample size was modest for genetic association studies.

2.2 | Sampling

The individuals who composed the sample were recruited from the universe of patients treated with dental implants in the Faculdade do Instituto Latino Americano de Pesquisa e Ensino Odontológico (Faculdade ILAPEO), Curitiba, Paraná, from 1996 to 2006. Of all treated individuals, 3.5% presented implant loss (126/3578 subjects). Early loss was the majority of cases, 88.2% (187/212 implants). Of these 126 patients, 81 were analyzed (45 were not included due to death, change of address or presence of exclusion criteria) and established the study group (S). Regarding implant failure, early losses (up to 180 days) and late losses (after 180 days) were considered. The control group (C) was composed of 163 patients with at least one healthy implant in function for at least 6 months and no failed implant (rate 2:1 control/study, to increase the statistical power of the analysis; Rosenbaum, 2013), matched by age, sex, and smoking habits. Thus, the sample consisted of 244 unrelated Caucasian individuals, older than 25 years old (mean age 51.90 ± 11.28) undergoing oral rehabilitation treatment with dental implants (NEODENT™*ImplanteOss eointegrável*). Only patients without disturbance during the surgical or prosthetic procedure were included in the sample. The exclusion criteria were as follows: individuals with HIV/AIDS, malignant neoplasm, history of radiotherapy or chemotherapy, pregnancy or lactation, and patients presenting necrotizing ulcerative gingivitis/periodontitis.

The patients were from the state of Paraná in the south region of Brazil. According to the Brazilian Institute of Geography and Statistics (2005), most Paraná State population was Caucasians (73%), being the remaining distributed in mixed ancestry (23.3%), Afro-Americans (2.5%), and Asiatic (1.2%) descents.

All individuals were submitted to an interview to fill out a clinical file containing identification data, anamnesis, medical and dental history, and periodontal clinical examination. The patients answered a questionnaire to identify the socioeconomic profile according to Brazilian Economical Classification Criteria—2003 (ABEP, 2003). They signed an informed consent form of the research, following the rules of the Ethical Committee in Research at PUCPR.

2.3 | Power calculations

The calculation of the sample power was performed using the Genetic Power Calculator (Purcell, Cherny, & Sham, 2003). The sample power was 86%, considering the frequency of the rarer allele of 25% (detailed in Table 1).

2.4 | Periodontal status

The periodontal parameters evaluated in partially edentulous patients were as follows: Gingival Index (GI; Loe & Silness, 1963), Plaque Index (PI; Silness & Loe, 1964), Calculus Index (CI; Greene & Vermillion, 1964), probing pocket depth (PPD), clinical attachment loss (CAL), and mobility (absent or present). Periodontal analyses were performed by a single observer (F.A.P.), using a millimeter conventional U.N.C periodontal probe, Hu-Friedy™, measuring four sites for each tooth.

2.5 | DNA collection and purification

Epithelial buccal cells were collected according to Trevilatto and Line (2000). The study participants rinsed their mouths with mouthwash containing 5 ml 3% glucose solution for 1 min. Following mouth washing, a sterile wood spatula was used to scrape the buccal mucosa. The tip of the spatula was then shaken into the retained mouthwash solution (Trevilatto & Line, 2000). Oral epithelial cells were pelleted by centrifugation at 706 *g* for 10 min. The supernatant was discarded and the cell pellet resuspended in 1.3 ml of extraction buffer (10 mM Tris-HCl [pH 7.8], 5 mM ethylenediaminetetraacetic acid [EDTA], and 0.5% sodium dodecyl sulfate [SDS]). Ten microliters of proteinase K (20 mg/ml) was added to the solution, and this was incubated overnight at 65°C. DNA was purified by adding ammonium acetate 10 M, precipitated with isopropanol, and resuspended in 50 µl Tris 10 mM (pH 7.6) and EDTA 1 mM (Aidar & Line, 2007).

In order to analyze the concentration of DNA obtained by extraction, the genetic material was subjected to spectrophotometric reading in NanoDrop 2000® equipment (Thermo Fisher Scientific). This method makes use of the principle of selective absorption of ultraviolet light by DNA molecules in 260 nm, while proteins do this absorption in 280 nm. Thus, it is possible to quantify the DNA and proteins that make up the extracted sample by reading at these two wavelengths. To calculate the DNA concentration, the absorbance value found at 260 nm was used and the A260/280 ratio indicates the purity of the sample. DNA samples with a 260/280 nm ration ranging from 1.5 to 2.0 were considered adequate (Morey et al., 2013). After extraction, the DNA was not diluted in any working concentration. One µl of the extracted DNA was used for the PCR process.

TABLE 1 Calculation of the sample power, using the Genetic Power Calculator tool (Purcell et al., 2003), setting as parameters: D' = 1, rare allele frequency ranging from 0.2% to 50.4% (based on the marker whose rare allele was less and most frequent in the sample, respectively), prevalence of implant loss of 3.5%, and level of significance of 0.05, with the power variation being dependent on the relative risk of rare allele

Effect size	Power			*N* cases for 80% power		
	A	B	C	a	b	c
1.2	0.05	0.07	0.05	67,980	3,506	10,538
1.5	0.05	0.16	0.08	13,292	645	1,905
2	0.06	0.37	0.16	5,117	233	673
3	0.08	0.76	0.35	2,151	89	250
3.5	0.09	**0.86**	0.45	1,683	**67**	187
4	0.10	0.92	0.54	1,397	54	149

Note: A: Power of the sample when the rarer allele frequency is 0.2%; a: n cases for 80% power when the rarer allele frequency is 0.2%; B: Power of the sample when the rarer allele frequency is 25% (minimum to be considered ideal for the rarer allele); b: n cases for 80% power when the rarer allele frequency is 25% (minimum to be considered ideal for the rarer allele); C: Power of the sample when the rarer allele frequency is 50.4%; c: n cases for 80% power when the rarer allele frequency is 50.4%.
The bold values are significative values.

2.6 | Selection of genetic polymorphisms and genotyping

The approach proposed in this study was the investigation of tagSNPs, which integrally represent the functional candidate *VDR* gene, by means of high linkage disequilibrium (LD). The key *VDR* gene polymorphisms (40 tagSNPs) were chosen based on the International HapMap Project, release24/phase 2_Nov08 (http://www.hapmap.org), following the LD criterion calculated by r^2 (>.8), multimarker, and minimum allele frequency (MAF) of 0.05 in the African population (YRI), the ancestral population.

The amplification reactions to obtain the genotypes were performed by the real-time PCR technique (Applied Biosystems 7500 Real-Time PCR System) using TaqMan® Genotyping Master Mix technology (Applied Biosystems; Ranade et al., 2001).

2.7 | Genetic models tested

The additive, dominant, and recessive genetic models were evaluated in this study. To verify the association of polymorphisms with the dental implant loss, we first determined the most frequent allele in the study group, through cross-reference tables obtained by the IBM SPSS Statistics 20.0.

2.8 | Statistical analysis

Frequencies and percents were used to express nominal variables. To verify the existence of association of nominal variables with the outcome, binary logistic regression, Pearson chi-square (χ^2), Fisher's exact, and chi-square with likelihood ratio correction were the tests performed.

Continuous variables were expressed as mean, median, and standard deviation. The conditions of normality were evaluated by the Kolmogorov–Smirnov test. When the distribution of quantitative variables was non-normal, the Mann–Whitney *U* non-parametric test was used. When the distribution was normal, the parametric test used was Student's *t* test.

For univariate genetic analyses, the correction for multiple tests (Bonferroni correction) was performed. Thus, the level of significance adopted in this situation was $p < .00041$ (0.05/120—number of tagSNPs × number of genetic models).

Multivariate analysis was performed using binary logistic regression, by the backward method. Clinical, periodontal, and genetic variables were analyzed altogether (p-value < .20 in the univariate analysis). The interaction between risk factors such as diabetes, smoke, and periodontal disease status (GI, PI, CI, and PPD) was also verified in the multivariate analysis. After that, results were considered statistically significant with p-value < .05.

Statistical analysis was also performed for the implant population (a total of 1,193 placed implants). Those implants were classified as healthy (n = 1,057) or lost (n = 136). The survival time of the installed implants was plotted in a Kaplan–Meier curve, in accordance with genotypes of rs3782905 polymorphism. The logrank test

was performed to investigate the association between the genetic marker and the survival time of dental implants.

Analyses were performed with the statistical software IBM SPSS Statistics 20.0. The Hardy–Weinberg equilibrium and LD map were obtained by Haploview 4.2.

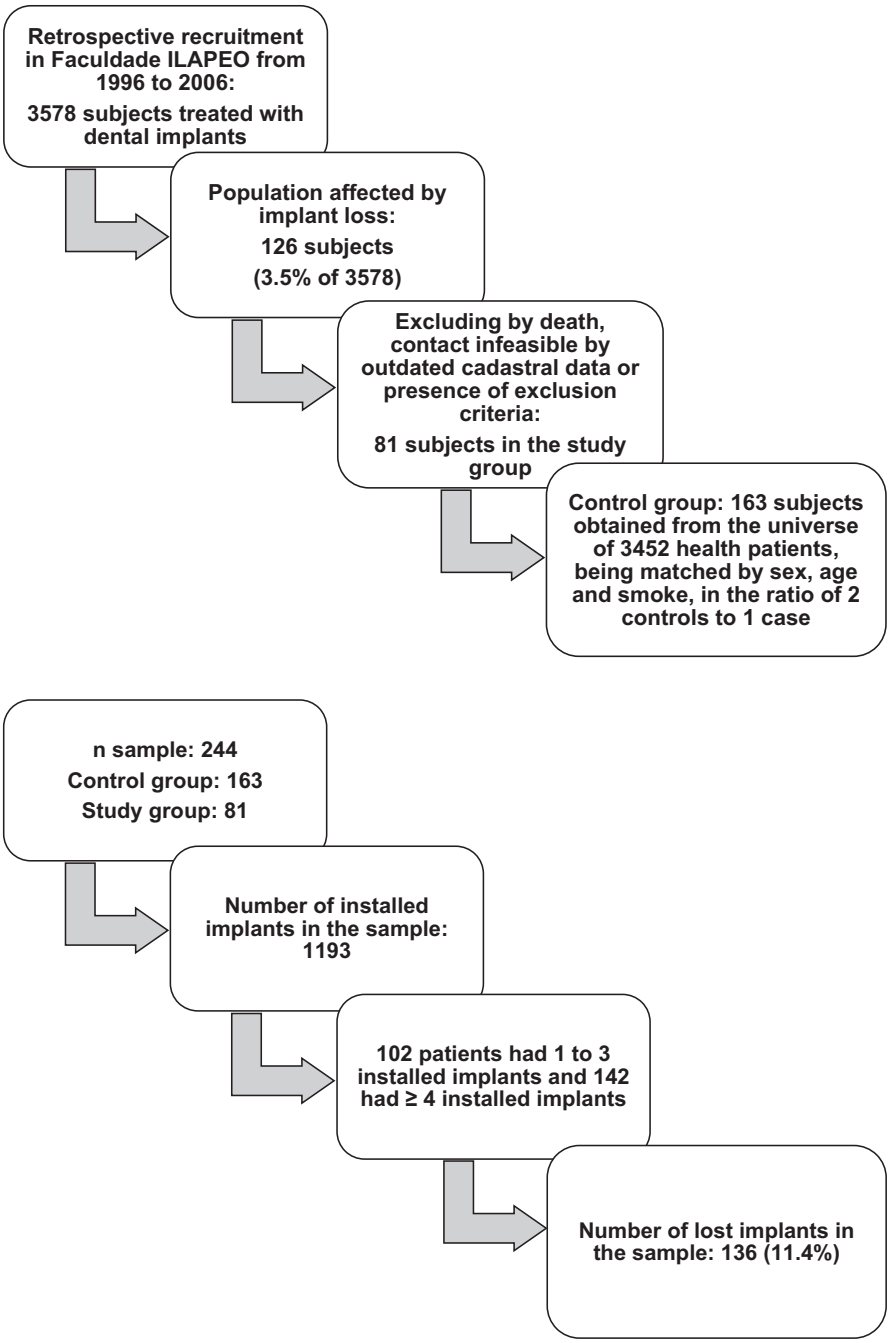
This work followed the STROBE guidelines (described in Table 2).

3 | RESULTS

3.1 | Characterization and recruitment of the sample

3.2 | Clinical findings

In the univariate analysis, no significant statistical difference (SSD) was observed between groups for the following parameters: sex, age, socioeconomic profile, smoking habit, systemic disease (both dichotomized and stratified), medical treatment, continuous medication use, medication used in the last trimester, frequency of visits to the dentist in the previous year, daily brushing frequency, use of mouthwash, use of dental floss, and number of present teeth ($p > .05$). However, edentulism and number of installed implants showed SSD between groups ($p = .038$ and $p = .010$, respectively). Edentulism was associated with protection, 17.2% (C) × 7.4% (S) (OR: 2.593, CI: 1.027–6.543), and a higher number of installed implants



were associated with risk, with mean of 5.59 (S) × 3.99 (C) implants installed (Table 3).

When the periodontal characteristics were compared between the groups, GI, PI, and CI showed SSD (p = .038, p = .002, p = .014, respectively), being associated with implant loss (higher indices in the study group). In contrast, it was not possible to find an association (protection or susceptibility) of PPD, CAL, and mobility with dental implant loss (p > .05; Table 4).

3.3 | Genetic findings

For a complete mapping of the *VDR* gene, 40 tagSNPs were evaluated in this study. The genotypic frequencies observed were compatible with expected genotypic frequencies, indicating that all polymorphisms representing the *VDR* gene were in Hardy–Weinberg equilibrium in the control group. The LD map was performed for the study population (Figure 1).

To avoid false-positive results (type I error), the Bonferroni correction was performed (0.05/120 genetic tests), which resulted in a significance level of p < .00041. There was no SSD between the 40 SNPs evaluated and implant loss (p > .00041) for all genetic models tested (additive, dominant, and recessive) in the univariate analysis, when the study and the control groups were compared (Tables S1-S6).

Next, a multivariate analysis was carried out. Clinical, periodontal, and genetic variables with p < .20 in the univariate analysis were included in this analysis. To adjust the multivariate model, the variables diabetes, periodontal status, and smoke were also included, due to their known clinical importance. The interaction between risk factors (diabetes, smoke, and periodontal status—GI, PI, CI, and PPD) was also verified in the multivariate analysis. As result, a higher number of installed implants (p = .006), a higher GI (p = .010), and a smaller PPD (p = .015) were associated with implant failure as well as the allele G of tagSNP rs3782905 in the recessive model (p = .049, OR: 1.840, CI: 1.001–3.381; Table 5).

In order to show that the tendency of dental implant losses is concentrated in some individuals, and that this condition in part is due to the genetic characteristics of the patients, the individuals of the sample were then allocated into two groups: (a) 34 individuals with multiple losses (two or more lost implants) and (b) 210 without multiple losses (up to one loss). In this univariate analysis, 11 of the 40 tagSNPs studied presented p < .05, but not p < .00041 (Table 6). Among these markers, three tagSNPs presented significant odds ratio values (OR ≥ 4.000): rs7136534 in the dominant model for allele C (p = .047, OR: 4.000, CI: 1.103–14.511), rs886441 in the dominant model for allele A (p = .030, OR: 4.857, CI: 1.291–18.280), and rs3782905 in the recessive model for allele G (p = .00046, OR: 4.506, CI: 1.941–10.461).

3.4 | Survival curve for the installed implants

Considering each implant independently (n = 1,193), a Kaplan–Meier survival curve was made, considering different genotypes of rs3782905 polymorphism, due to their possible influence on the survival time of dental implants (Figure 2). The logrank test did not show an association between the study SNP and the implants survival time (p = .290). It was found that 75% of dental implants were lost up to 6 weeks after been installed.

4 | DISCUSSION

Several factors influence dental implant loss, since it is considered a complex condition, dependent on the interaction of environmental and host factors (Alvim-Pereira, Montes, Thomé, Olandoski, & Trevilatto, 2008). However, the main known causes for failure are not able to explain all cases of dental implant losses. In addition, there is a tendency of concentration of dental implant losses in some individuals (Montes et al., 2009), the clustering phenomenon. This points to the existence of host genetic susceptibility factors.

Sex, age, and smoking did not show differences between the groups. This probably occurred because of the sample matching for these conditions. Such matching was performed to minimize the possible influences of these variables in the results. Similar results were found by Mangano, Mortellaro, Mangano, and Mangano (2016) when they did not detect an association between these variables and a higher incidence of dental implant losses (Mangano et al., 2016).

Edentulism was more frequent in patients who did not suffer dental implant losses (17.2% in C × 7.4% in S). In our findings, edentulous patients were about 2.5 times less prone to a dental implant loss, similar to what was found previously (Alvim-Pereira, Montes, Thomé, et al., 2008; Dirschnabel et al., 2011; Montes et al., 2009; Pigossi, Alvim-Pereira, Alvim-Pereira, Trevilatto, & Scarel-Caminaga, 2014). Edentulism is associated with a smaller likelihood of a dental implant loss probably due to less bacterial biofilm adhered to remaining teeth, which is considered a risk factor for dental implant failure (Tallarico, Canullo, Caneva, & Özcan, 2017). Biofilm of dentate individuals develops more rapidly and is quantitatively and qualitatively more complex than the biofilm formed in edentulous users of total dentures (Teles et al., 2012).

A higher number of implants installed were observed in the study group (mean 3.99 in C × 5.59 in S) and seem to be a variable associated with a higher risk of dental implant loss. The higher the number of implants installed, the bigger the amount of surgical wounds, which may lead to more inflammatory response necessary to tissue repair and may justify the association found.

In relation to the periodontal variables, GI, PI, and CI were higher in the study group. These parameters may be suggestive of increased bacterial contamination and local inflammation in the patients with dental implant losses. Probing Pocket Depth did not associate with implant loss in the univariate analysis; however, it showed an association after the multivariate analysis. Nevertheless, the difference in mean probing pocket depth between the C and S groups was not clinically relevant (only 0.13 mm).

TABLE 2 STROBE statement guidelines

STROBE guidelines to be adopted in observational case-control studies		
Title and abstract		
	Indicate the study's design with a commonly used term in the title or the abstract	[a]
	Provide in the abstract an informative and balanced summary of what was done and what was found	[a]
Introduction		
Background/rationale	Explain the scientific background and rationale for the investigation being reported	[a]
Objectives	State specific objectives, including any prespecified hypotheses	[a]
Methods		
Study design	Present key elements of study design early in the paper	[a]
Setting	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	[a]
Participants	Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	[a]
	For matched studies, give matching criteria and the number of controls per case	[a]
Variables	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	[a]
Data sources/ measurement	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	[a]
	Describe any efforts to address potential sources of bias	[a]
Study size	Explain how the study size was arrived at	[a]
Quantitative variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	[a]
Statistical methods	Describe all statistical methods, including those used to control for confounding	[a]
	Describe any methods used to examine subgroups and interactions	[a]
	Explain how missing data were addressed	[a]
	If applicable, explain how matching of cases and controls was addressed	[a]
	Describe any sensitivity analyses	[b]
Results		
Participants	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	[a]
	Give reasons for non-participation at each stage	[a]
	Consider use of a flow diagram	[a]
Descriptive data	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	[a]
	Indicate number of participants with missing data for each variable of interest	[a]
Outcome data	Report numbers in each exposure category, or summary measures of exposure	[a]
Main results	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	[a]
	Report category boundaries when continuous variables were categorized	[a]
	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	[b]
Other analyses	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	[a]
Discussion		
Key results	Summarise key results with reference to study objectives	[a]
Limitations	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	[a]
Interpretation	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	[a]
Generalisability	Discuss the generalisability (external validity) of the study results	[a]
Other information		
Funding	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	[a]

[a]Adhered.
[b]Not adhered/Not applicable.

TABLE 3 Patients' clinical and sociodemographic findings (n = 244)

Variables	Control group n (%) (II = 734)	Study group n (%) (II = 459)	p-value	OR (CI 95%)
Sex[a]				
Male	52 (31.9)	30 (37.0)	.424[b]	0.796 (0.456–1.392)
Female	111 (68.1)	51 (63.0)		
Age[c]				
	51.26 ± 11.37	53.16 ± 11.05	.216[d]	–
Socioeconomic profile[a]				
A1/A2/B1	84 (51.5)	40 (49.4)	.752[b]	1.090 (0.640–1.857)
B2/C/D/E	79 (48.5)	41 (50.6)		
Smoking[a]				
Non-smoking	131 (80.4)	66 (81.5)	.835[b]	0.930 (0.471–1.838)
Smoking	32 (19.6)	15 (18.5)		
Systemic disease[a]				
Absence	52 (31.9)	21 (25.9)	.337[b]	1.338 (0.737–2.430)
Presence	111 (68.1)	60 (74.1)		
Diabetes[a]				
Absence	154 (94.5%)	80 (98.8%)	.172[e]	0.214 (0.027–1.)
Presence	9 (5.5%)	1 (1.2%)		
Rheumatoid diseases[a]				
Absence	131 (80.4%)	60 (74.1%)	.262[b]	1.433 (0.763–2.689)
Presence	32 (19.6%)	21 (25.9%)		
Osteoporosis[a]				
Absence	160 (98.2%)	79 (97.5%)	1.000[e]	1.350 (0.221–8.245)
Presence	3 (1.8%)	2 (2.5%)		
Hypothyroidism[a]				
Absence	147 (90.2%)	72 (88.9%)	.753[b]	1.148 (0.484–2.725)
Presence	16 (9.8%)	9 (11.1%)		
Cardiovascular diseases[a]				
Absence	153 (93.9%)	74 (91.4%)	.469[b]	1.447 (0.530–3.954)
Presence	10 (6.1%)	7 (8.6%)		
Systemic arterial hypertension[a]				
Absence	132 (81.0%)	61 (75.3%)	.305[b]	1.396 (0.737–2.644)
Presence	31 (19.0%)	20 (24.7%)		
Under medical treatment[a]				
No	100 (61.3)	44 (54.3)	.293[b]	1.335 (0.779–2.288)
Yes	63 (38.7)	37 (45.7)		
Continuous use medication[a]				
No	97 (59.5)	44 (54.3)	.440[b]	1.236 (0.722–2.116)
Yes	66 (40.5)	37 (45.7)		
Medication in the last quarter[a]				
No	107 (65.6)	51 (63.0)	.680[b]	1.124 (0.645–1.958)
Yes	56 (34.4)	30 (37.0)		
Visits to the dentist in the previous year[a]				
More than two times	125 (76.7)	67 (82.7)	.279[b]	0.687 (0.348–1.358)
1–2 times	38 (23.3)	14 (17.3)		

(Continues)

TABLE 3 (Continued)

Variables	Control group *n* (%) (II = 734)	Study group *n* (%) (II = 459)	*p*-value	OR (CI 95%)
Brushing daily[a]				
More than three times	119 (73.0)	63 (77.8)	.420[b]	0.773 (0.413–1.448)
1–3 times	44 (27.0)	18 (22.2)		
Mouth washing daily[a]				
No	92 (56.4)	43 (53.1)	.620[b]	1.145 (0.671–1.955)
Yes	71 (43.6)	38 (46.9)		
Dental floss daily[a]				
No	130 (79.8)	61 (75.3)	.428[b]	1.292 (0.686–2.433)
Yes	33 (20.2)	20 (24.7)		
Present teeth[b]				
	16.82 ± 9.61 (20.00)	17.26 ± 8.43 (20.00)	.959[f]	–
Edentulous[a]				
Yes	28 (17.2)	6 (7.4)	**.038**[b]	2.593 1.027–6.543)
No	135 (82.8)	75 (92.6)		
Placed implants[b]				
	3.99 ± 2.94 (3.00)	5.59 ± 3.75 (5.00)	**.010**[f]	–

Note: II = Number of installed implants in the respective group. Socioeconomic profile: defined by a punctuation based on the possession of assets, schooling of the head of the household, and other indicators that provide a final score for the groups A1, A2, B1, B2, C, D, and E. These points range from 0 (class E) to 34 (class A1).
Abbreviations: CI, confidence interval; OR: odds ratio.
[a]Number (Frequency).
[b]Chi-square test.
[c]Mean ± Standard Deviation (median).
[d]Student's *t* test.
[e]Fisher's exact test.
[f]Mann–Whitney *U* test.
The bold values are significative values.

TABLE 4 Periodontal characteristics of partially edentulous patients (*n* = 210)

Variables	Control group (*n* = 135) (II = 538)	Study group (*n* = 75) (II = 417)	*p*-value	OR (CI 95%)
Gingival Index[a]	0.20 ± 0.42 (0.00)	0.45 ± 0.97 (0.00)	**.038**[b]	–
Plaque Index[a]	0.13 ± 0.86 (0.00)	0.24 ± 0.88 (0.00)	**.002**[b]	–
Calculus Index[a]	0.05 ± 0.60 (0.00)	0.23 ± 1.14 (0.00)	**.014**[b]	–
Probing pocket depth[a,c]	2.24 ± 0.51 (2.00)	2.11 ± 0.53 (2.00)	.073[b]	–
Clinical attachment loss[a,c]	3.19 ± 0.94 (3.00)	3.21 ± 1.17 (3.00)	.616[b]	–
Mobility[d]				
Presence *n* (%)	117 (86.7)	59 (78.7)	.132[e]	1.763 (0.839–3.704)
Absence *n* (%)	18 (13.3)	16 (21.3)		

Note: II = Number of installed implants in the respective group.
Abbreviations: CI, confidence interval; OR, odds ratio.
[a]Mean ± Standard Deviation (median).
[b]Mann–Whitney *U* test.
[c]Unit of measurement in millimeters (mm).
[d]Number (frequency).
[e]Chi-square test.
The bold values are significative values.

Individual immune-inflammatory response is highly variable, both at systemic and local levels. An amount of bacterial biofilm can generate varying degrees of inflammatory response and tissue damage (Meyle & Chapple, 2015), suggesting that individual genetic background is capable of influencing the immunological and inflammatory action in the periodontal/peri-implant tissues.

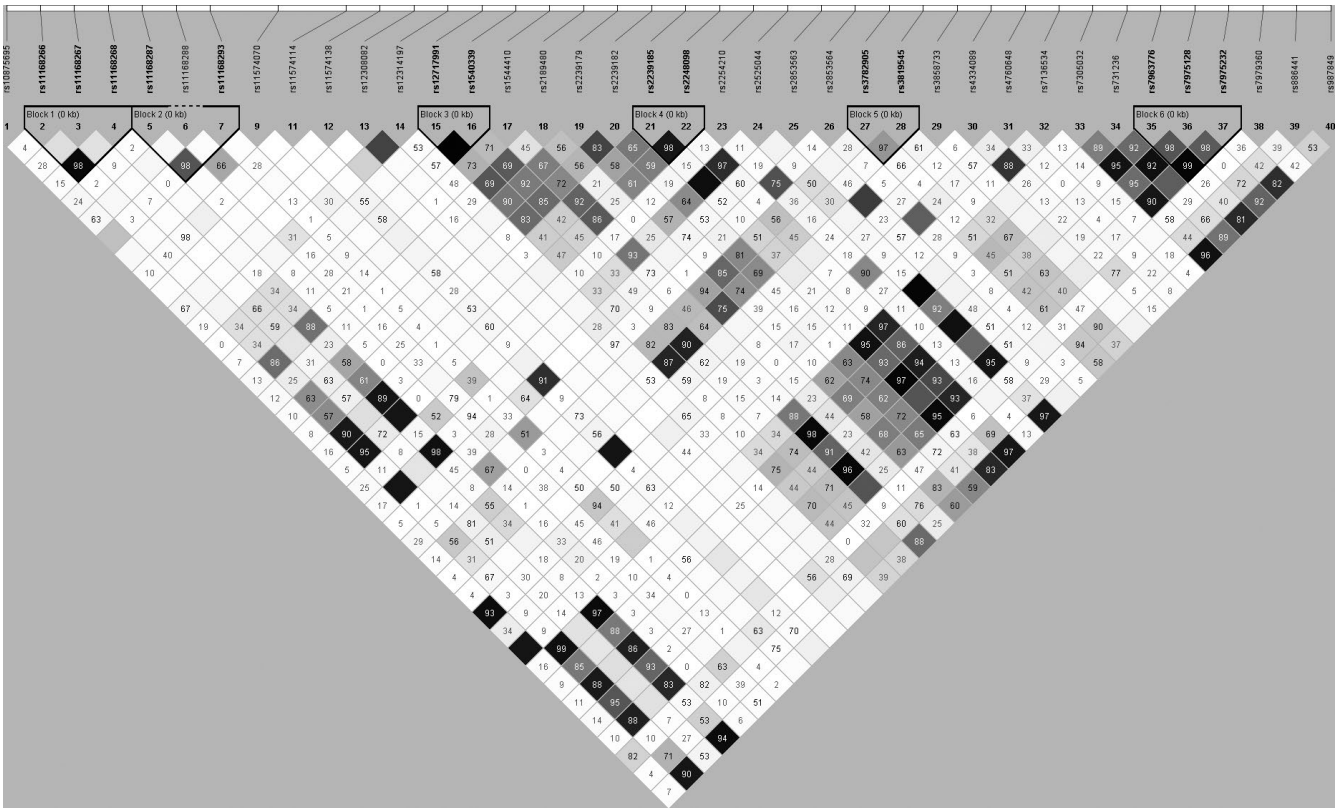


FIGURE 1 Analysis of LD between tagSNPs of the *VDR* gene for the study population. The number inside the squares indicates the ratio of LD in %. The intensity of the color inside the squares reflects the degree of LD between two *loci*; that is, the darker squares represent the highest LD between two SNPs

TABLE 5 Outcome of multivariate analysis considering the dentate patients (n = 210)

Variables	Control group (n = 135) (II = 538)	Study group (n = 75) (II = 417)	p-value[a]	OR (CI 95%)
Implants Installed[b]	3.99 ± 2.94	5.59 ± 3.75	**.006**	–
Gingival Index[b]	0.20 ± 0.42	0.45 ± 0.97	**.010**	–
Probing pocket depth[b,c]	2.24 ± 0.51	2.11 ± 0.53	**.015**	–
rs3782905[d]				
CC + CG *n* (%)	79 (58.5)	35 (46.7)	**.049**	**1.840 (1.001–3.381)**
GG *n* (%)	56 (41.5)	40 (53.3)		

Note: II = Number of installed implants in the respective group. rs3782905: tagSNP in the recessive model for the allele G.
Abbreviations: CI, confidence interval; OR, odds ratio.
[a]Binary logistic regression.
[b]Mean ± Standard Deviation.
[c]Unit of measurement in millimeters (mm).
[d]Number (frequency).
The bold values are significative values.

In this context, vitamin D, which acts through binding with its receptor VDR (Castro, 2011), is important in promoting regulation of immunity (Jiménez-Sousa, Martínez, Medrano, Fernández-Rodríguez, & Resino, 2018). This molecule and its set of precursor molecules, such as 1.25 $(OH)_2$ D_3 (cholecalciferol), have the ability to increase chemotaxis, autophagy, and phagolysosomal fusion of innate immune cells and increase the antimicrobial activity of macrophages and monocytes (Sassi, Tamone, & D'Amelio, 2018). In addition, the vitamin D-VDR complex acts as a transcription factor in about 3% of the human genome (Wang et al., 2005). Therefore, it is possible that it controls many pathophysiological processes, influencing the osteomineral balance (Insua, Monje, Wang, & Miron, 2017; Naja, Dardenne, Arabian, & St Arnaud, 2009) and the immune-inflammatory response (Verstuyf, Carmeliet, Bouillon, & Mathieu, 2010). For this reason, SNPs in the *VDR* gene could impact the individual immunological ability to establish and/or maintain the osseointegration of dental implants (Nishimura, 2013).

TABLE 6 The table below shows all SNPs with *p*-value < .05 in the clusterization analysis (*n* = 244, multiple losses: 34 and non-multiple losses: 210)

tagSNPs[a]	Variation[b] [1/2]	Groups	Genotypes *n* (%)		*p*-value[c]	OR (CI 95%)
rs11168268 Dom T	[C/T]		**TT + CT**	**CC**	**.032**	2.375 (1.059–5.329)
		No multiple losses (*n* = 207)	171 (82.6)	36 (17.4)		
		Multiple losses (*n* = 33)	22 (66.7)	11 (33.3)		
rs2239185 Dom A	[G/A]		**AA + AG**	**GG**	**.017**	2.571 (1.165–5.675)
		No multiple losses (*n* = 209)	171 (81.8)	38 (18.2)		
		Multiple losses (*n* = 33)	21 (63.6)	12 (36.4)		
rs2525044 Dom G	[A/G]		**GG + AG**	**AA**	**.012**	2.750 (1.216–6.218)
		No multiple losses (*n* = 208)	176 (84.6)	32 (15.4)		
		Multiple losses (*n* = 33)	22 (66.7)	11 (33.3)		
rs3819545 Dom T	[C/T]		**TT + CT**	**CC**	**.011**	3.000 (1.244–7.234)
		No multiple losses (*n* = 208)	184 (88.5)	24 (11.5)		
		Multiple losses (*n* = 32)	23 (71.9)	9 (28.1)		
rs7136534 Dom C	[T/C]		**CC + CT**	**TT**	**.047**[d]	4.000 (1.103–14.511)
		No multiple losses (*n* = 210)	203 (96.7)	7 (3.3)		
		Multiple losses (*n* = 33)	29 (87.9)	4 (12.1)		
rs7305032 Dom T	[C/T]		**TT + CT**	**CC**	**.014**	2.703 (1.195–6.114)
		No multiple losses (*n* = 205)	173 (84.4)	32 (15.6)		
		Multiple losses (*n* = 33)	22 (66.7)	11 (33.3)		
rs7963776 Dom A	[G/A]		**AA + AG**	**GG**	**.013**	2.656 (1.202–5.872)
		No multiple losses (*n* = 209)	172 (82.3)	37 (17.7)		
		Multiple losses (*n* = 33)	21 (63.6)	12 (36.4)		
rs7975232 Dom T	[G/T]		**TT + TG**	**GG**	**.008**	2.857 (1.288–6.338)
		No multiple losses (*n* = 210)	175 (83.3)	35 (16.7)		
		Multiple losses (*n* = 33)	21 (63.6)	12 (36.4)		
rs886441 Dom A	[G/A]		**AA + AG**	**GG**	**.030**[d]	4.857 (1.291–18.280)
		No multiple losses (*n* = 210)	204 (97.1)	6 (2.9)		
		Multiple losses (*n* = 32)	28 (87.5)	4 (12.5)		
rs987849 Dom T	[C/T]		**TT + CT**	**CC**	**.031**	2.389 (1.065–5.359)
		No multiple losses (*n* = 208)	172 (82.7)	36 (17.3)		
		Multiple losses (*n* = 33)	22 (66.7)	11 (33.3)		
rs2239185 Rec A	[G/A]		**GG + AG**	**AA**	**.014**	0.306 (0.113–0.826)
		No multiple losses (*n* = 209)	132 (63.2)	77 (36.8)		
		Multiple losses (*n* = 33)	28 (84.8)	5 (15.2)		
rs2525044 Rec G	[A/G]		**AA + AG**	**GG**	**.021**	0.367 (0.152–0.884)
		No multiple losses (*n* = 208)	120 (57.7)	88 (42.3)		
		Multiple losses (*n* = 33)	26 (78.8)	7 (21.2)		
rs3782905 Rec G	[C/G]		**CC + CG**	**GG**	**.000**	4.506 (1.941–10.461)
		No multiple losses (*n* = 210)	124 (59.0)	86 (41.0)		
		Multiple losses (*n* = 33)	8 (24.2)	25 (75.8)		
rs7305032 Rec T	[C/T]		**CC + CT**	**TT**	**.049**	0.421 (0.174–1.000)
		No multiple losses (*n* = 205)	125 (61.0)	80 (39.0)		
		Multiple losses (*n* = 33)	26 (78.8)	7 (21.2)		

(Continues)

TABLE 6 (Continued)

tagSNPs[a]	Variation[b] [1/2]	Groups	Genotypes *n* (%)		*p*-value[c]	OR (CI 95%)
rs7963776 Rec A	[G/A]		**GG + AG**	**AA**	**.024**	0.333 (0.123–0.898)
		No multiple losses (*n* = 209)	136 (65.1)	73 (34.9)		
		Multiple losses (*n* = 33)	28 (84.8)	5 (15.2)		
rs7975232 Rec T	[G/T]		**GG + GT**	**TT**	**.033**	0.376 (0.149–0.951)
		No multiple losses (*n* = 210)	132 (62.9)	78 (37.1)		
		Multiple losses (*n* = 33)	27 (81.8)	6 (18.2)		

Note: The dominant and recessive model was designed taking into account the most frequent allele in the study group. The different *n* values refer to failure in genotyping.
Abbreviations: CI, confidence interval; OR, odds ratio.
[a]SNP identifier based on NCBI dbSNP.
[b]The first allele is the least frequent, and the second allele is the most frequent in the study group.
[c]*p*-value for Pearson chi-square test.
[d]*p*-value for Fisher chi-square test.
The bold values are significative values.

In this study, the allele G of rs3782905 in the recessive model was associated with dental implant failure, together with number of installed implants, Gingival Index, and probing pocket depth, after the multivariate model. Although only this marker (among 40 tagSNPs studied) was formally associated with dental implant loss, several polymorphisms studied might be considered as genetic risk markers for multiple losses (Table 6), once these markers showed nominal *p*-values between <.05 and <.00041. It was demonstrated before (Vieira, McHenry, Daack-Hirsch, Murray, & Marazita, 2008) that known true associations are missed when a correction for multiple testing as strict as Bonferroni's is implemented.

It is noteworthy that although the study included only 81 individuals affected by dental implant loss, the sample power was 86%, considering the frequency of the rarer allele of 25%, as shown in Table 1 (Purcell et al., 2003).

When sample was stratified according to the presence or not of multiple losses (clusterization analysis), various polymorphisms presented nominal $p < .05$, but higher than 0.00041. Among them, rs7136534 (OR: 4.000), rs886441 (OR: 4.857), and rs3782905 (OR: 4.506) as shown in Table 6. It is interesting to note that these markers are in intronic regions, which initially leads us to believe that they are not functional SNPs. However, there is current evidence that polymorphisms in non-coding regions are important because

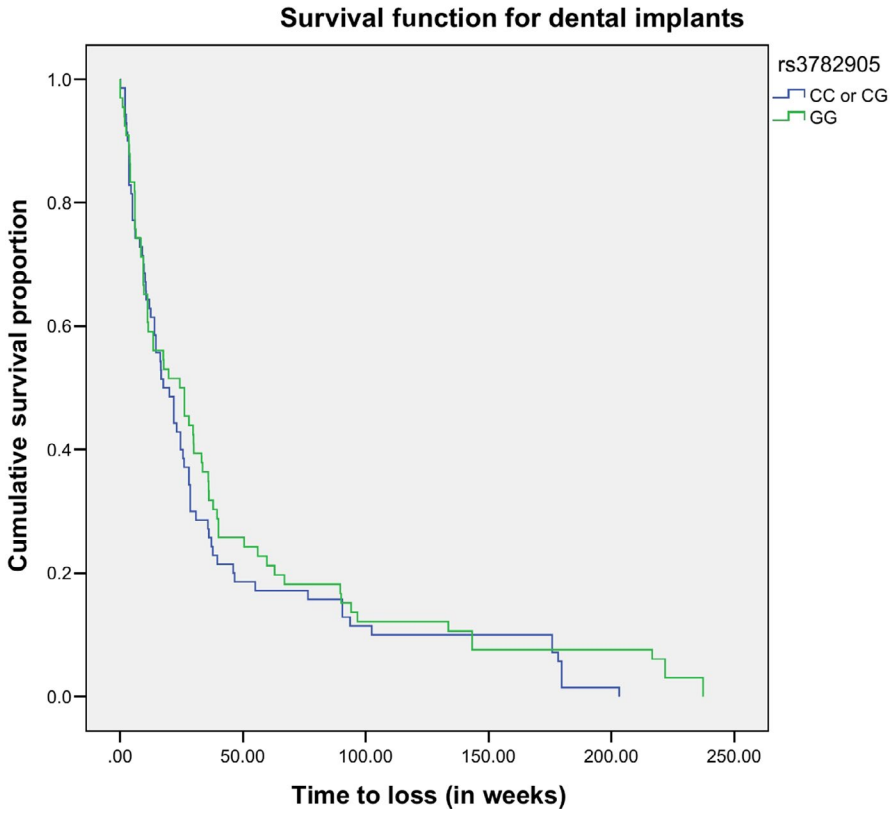


FIGURE 2 Kaplan–Meier curve indicating the cumulative survival proportion of the installed dental implants over time, considering the genotypes of rs3782905 polymorphism as a possible risk factor. The survival time does not differ significantly according to the patients' genotypes

they may impact mRNA processing, modifying the action of spliceosome in the removal of introns and union of exons (Anna & Monika, 2018). This mechanism may bring as a consequence impacts to the protein synthesis. Still about these three SNPs mentioned above, to the best of our knowledge, no other study identified association of the rs7163534 with another condition/disease. In contrast, the rs886441 was associated with non-Hodgkin's lymphoma (Kelly et al., 2012). However, we did not find reports associating this marker with some oral or dental disease, or conditions involving bone metabolism. In relation to the rs3782905, this SNP was already associated with an increased risk of asthma (Han et al., 2016) and osteoporosis in physically inactive women (Wu et al., 2014). Thus, these findings reinforce the *VDR* SNPs action mainly in chronic inflammatory response and in bone physiology.

In relation to the other markers for the clustering phenomenon (Table 6), there is in literature indication of associations mainly with autoimmune diseases (Silva et al., 2013), susceptibility to infections (Wu et al., 2016), and some types of cancer (Grant et al., 2013; Orlow et al., 2012). Only the rs7975232 was previously investigated for an oral condition (periodontitis; Tanaka, Miyake, Hanioka, & Arakawa, 2013). Although the authors failed to find a direct association between this marker and periodontitis, they detected a biological interaction of the SNP with the smoking habit on the risk of periodontal disease (Tanaka et al., 2013). This finding concurs with that of a more recent study, which detected interaction between rs7975232 and age in the pathogenesis of chronic periodontitis (Tobón-Arroyave, Isaza-Guzmán, & Pineda-Trujillo, 2017). It is worth mentioning that rs7975232 is located at the 3' untranslated of the *VDR* gene and may be involved in the regulation of gene expression by modulating the mRNA stability (Valdivielso & Fernandez, 2006).

This study presents the following main limitations. First, the sample size is modest, and larger samples are needed to provide adequate power to avoid false negatives (when there is an association, it cannot be detected). However, the recruitment of the sample involved the participation of all patients treated during 10 years at Faculdade ILAPEO. Second, the selection of tagSNPs was based on a population different from the one studied (the African population, YRI), once the Brazilian population is not cataloged. The Brazilian population is not a "pure" Caucasian population, but is of mixed-race ancestry, which means that it can present an overlapping of genotypes/alleles to some extent with Africans (which represent the oldest and most fragmented population in terms of LD). Thus, the structure of the LD in the YRI population requires more SNPs for a complete physical mapping of the gene. Therefore, there is a chance we did not completely covered the *VDR* gene, although it is more likely that some of the SNPs chosen were redundant. Another limitation refers to the inclusion of both early and late implant loss in the sample. Ideally, a more homogeneous sample, consisting of only early losses, would be more appropriate. This inclusion was performed because late losses were also detected in patients who presented early losses, and these cases were kept to allow for a larger sample number. Also in regard to the homogeneity of the sample, it is worth emphasizing that in genetic studies, the recruitment of a sample as homogeneous as possible is fundamental. Regarding the ethnical characteristics of the patients, it is important to say that the study sample was mainly Caucasoid. However, the Brazilian white population is heterogeneous. For this reason, it is not recommended grouping Brazilians into ethnic groups based on color, race, and geographical origin because Brazilian individuals classified as white or black have significantly overlapping genotypes due to miscegenation (Parra et al., 2003). To overcome this limitation, the patients were recruited from the same institute and with very similar socioeconomic status.

In conclusion, a complete physical mapping of *VDR* gene was carried out in a Brazilian population for the first time, suggesting allele G of rs3782905 in the recessive model as a possible new genetic risk marker for dental implant loss, along with number of implants installed and Gingival Index. Moreover, we also suggest that the clustering phenomenon may be underlined by genetic factors.

Nevertheless, replications in other populations are mandatory to better elucidate the role of this gene in influencing individual susceptibility to dental implant loss.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Fabiano Alvim-Pereira and Paula Cristina Trevilatto conceived the ideas. Fabiano Alvim-Pereira and Cláudia Cristina Alvim-Pereira collected the data. Thaís Munhoz Pereira, Cleber Machado de Souza, and Sérgio Aparecido Ignácio analyzed the data. Thaís Munhoz Pereira and Paula Cristina Trevilatto led the writing.

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REFERENCES

ABEP. (2003) Retrieved from http://www.abep.org/codigosguias/ABEP_CCEB.pdf

Aidar, M., & Line, S. R. (2007). A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. *Brazilian Dental Journal*, *18*, 148–152. https://doi.org/10.1590/S0103-64402007000200012

Alvim-Pereira, F., Montes, C. C., Mira, M. T., & Trevilatto, P. C. (2008). Genetic susceptibility to dental implant failure: A critical review. *International Journal of Oral and Maxillofacial Implants*, *23*(3), 409–416.

Alvim-Pereira, F., Montes, C. C., Thomé, G., Olandoski, M., & Trevilatto, P. C. (2008). Analysis of association of clinical aspects and vitamin D receptor gene polymorphism with dental implant loss. *Clinical Oral Implants Research*, *19*(8), 786–795. https://doi.org/10.1111/j.1600-0501.2008.01532.x

Anna, A., & Monika, G. (2018). Splicing mutations in human genetic disorders: Examples, detection, and confirmation. *Journal of Applied Genetics*, *59*(3), 253–268. https://doi.org/10.1007/s13353-018-0444-7

Cargill, M., Altshuler, D., Ireland, J., Sklar, P., Ardlie, K., Patil, N., … Lander, E. S. (1999). Characterization of single-nucleotide polymorphisms in coding regions of human genes. *Nature Genetics*, *22*(3), 231–238. https://doi.org/10.1038/10290

Dastgheib, S. A., Gartland, A., Tabei, S. M. B., Omrani, G. R., & Teare, M. D. (2016). A candidate gene association study of bone mineral density in an Iranian population. *Frontiers in Endocrinology*, *7*(OCT), 1–8. https://doi.org/10.3389/fendo.2016.00141

Dawson, D. R., & Jasper, S. (2015). Key systemic and environmental risk factors for implant failure. *Dental Clinics of North America*, *59*(1), 25–39. https://doi.org/10.1016/j.cden.2014.09.002

de Azevêdo Silva, J., Monteiro Fernandes, K., Trés Pancotto, J. A., Sotero Fragoso, T., Donadi, E. A., Crovella, S., & Sandrin-Garcia, P. (2013). Vitamin D receptor (*VDR*) gene polymorphisms and susceptibility to systemic lupus erythematosus clinical manifestations. *Lupus*, *22*(11), 1110–1117. https://doi.org/10.1177/0961203313500549

de Castro, L. C. G. (2011). The vitamin D endocrine system. *Arquivos Brasileiros de Endocrinologia e Metabologia*, *55*(8), 566–575.

Dirschnabel, A. J., Alvim-Pereira, F., Alvim-Pereira, C. C., Bernardino, J. F., Rosa, E. A. R., & Trevilatto, P. C. (2011). Analysis of the association of IL1B(C-511T) polymorphism with dental implant loss and the clusterization phenomenon. *Clinical Oral Implants Research*, *22*(11), 1235–1241. https://doi.org/10.1111/j.1600-0501.2010.02080.x

Ghanem, A., Abduljabbar, T., Akram, Z., Vohra, F., Kellesarian, S. V., & Javed, F. (2017). A systematic review and meta-analysis of pre-clinical studies assessing the effect of nicotine on osseointegration. *International Journal of Oral and Maxillofacial Surgery*, *46*(4), 496–502. https://doi.org/10.1016/j.ijom.2016.12.003

Grant, D. J., Hoyo, C., Akushevich, L., Iversen, E. S., Whitaker, R., Marks, J., … Schildkraut, J. M. (2013). Vitamin D receptor (VDR) polymorphisms and risk of ovarian cancer in Caucasian and African American women. *Gynecologic Oncology*, *129*(1), 173–178. https://doi.org/10.1016/j.ygyno.2012.12.027

Greene, J. C., & Vermillion, J. R. (1964). The simplified oral hygiene index. *Journal of the American Dental Association*, *1939*(68), 7–13. https://doi.org/10.14219/jada.archive.1964.0034

Guillaume, B. (2016). Dental implants: A review. *Morphologie: Bulletin De L'association Des Anatomistes*, *100*(331), 189–198. https://doi.org/10.1016/j.morpho.2016.02.002

Han, J.-C., Du, J., Zhang, Y.-J., Qi, G.-B., Li, H.-B., Zhang, Y. J., & Yu, X.-L. (2016). Vitamin D receptor polymorphisms may contribute to asthma risk. *The Journal of Asthma: Official Journal of the Association for the Care of Asthma*, *53*(8), 790–800. https://doi.org/10.3109/02770903.2016.1158267

Insua, A., Monje, A., Wang, H.-L., & Miron, R. J. (2017). Basis of bone metabolism around dental implants during osseointegration and peri-implant bone loss. *Journal of Biomedical Materials Research Part A*, *105*, 2075–2089, https://doi.org/10.1002/jbm.a.36060

Jiménez-Sousa, M. Á., Martínez, I., Medrano, L. M., Fernández-Rodríguez, A., & Resino, S. (2018). Vitamin D in human immunodeficiency virus infection: Influence on immunity and disease. *Frontiers in Immunology*, *9*, 458. https://doi.org/10.3389/fimmu.2018.00458

Kassebaum, N. J., Smith, A., Bernabé, E., Fleming, T. D., Reynolds, A. E., Vos, T., … Yonemoto, N. (2017). Global, regional, and national prevalence, incidence, and disability-adjusted life years for oral conditions for 195 countries, 1990–2015: A systematic analysis for the global burden of diseases, injuries, and risk factors. *Journal of Dental Research*, *96*(4), 380–387. https://doi.org/10.1177/0022034517693566

Kelly, J. L., Drake, M. T., Fredericksen, Z. S., Asmann, Y. W., Liebow, M., Shanafelt, T. D., … Cerhan, J. R. (2012). Early life sun exposure, vitamin D-related gene variants, and risk of non-Hodgkin lymphoma. *Cancer Causes and Control: CCC*, *23*(7), 1017–1029. https://doi.org/10.1007/s10552-012-9967-0

Loe, H., & Silness, J. (1963). Periodontal disease in pregnancy I. Prevalence and severity. *Acta Odontologica Scandinavica*, *21*, 533–551. https://doi.org/10.3109/00016356309011240

Mangano, F., Mortellaro, C., Mangano, N., & Mangano, C. (2016). Is low serum vitamin D associated with early dental implant failure? A retrospective evaluation on 1625 implants placed in 822 patients. *Mediators of Inflammation*, *2016*, 5319718. https://doi.org/10.1155/2016/5319718

Martelli, F. S., Martelli, M., Rosati, C., & Fanti, E. (2014). Vitamin D: Relevance in dental practice. *Clinical Cases in Mineral and Bone Metabolism*, *11*(1), 15–19. https://doi.org/10.11138/ccmbm/2014.11.1.015

Meyle, J., & Chapple, I. (2015). Molecular aspects of the pathogenesis of periodontitis. *Periodontology 2000*, *69*(1), 7–17. https://doi.org/10.1111/prd.12104

Montes, C. C., Alvim-Pereira, F., De Castilhos, B. B., Sakurai, M. L. L., Olandoski, M., & Trevilatto, P. C. (2009). Analysis of the association of IL1B (C+3954T) and IL1RN (intron 2) polymorphisms with dental implant loss in a Brazilian population. *Clinical Oral Implants Research*, *20*(2), 208–217. https://doi.org/10.1111/j.1600-0501.2008.01629.x

Moraschini, V., Poubel, L. A. D. C., Ferreira, V. F., & Barboza, E. D. S. P. (2015). Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: A systematic review. *International Journal of Oral and Maxillofacial Surgery*, *44*(3), 377–388. https://doi.org/10.1016/j.ijom.2014.10.023

Morey, M., Fernández-Marmiesse, A., Castiñeiras, D., Fraga, J. M., Couce, M. L., & Cocho, J. A. (2013). A glimpse into past, present, and future DNA sequencing. *Molecular Genetics and Metabolism*, *110*(1–2), 3–24. https://doi.org/10.1016/j.ymgme.2013.04.024

Naja, R. P., Dardenne, O., Arabian, A., & St Arnaud, R. (2009). Chondrocyte-specific modulation of Cyp27b1 expression supports a role for local synthesis of 1,25-dihydroxyvitamin D3 in growth plate development. *Endocrinology*, *150*(9), 4024–4032. https://doi.org/10.1210/en.2008-1410

Nishimura, I. (2013). Genetic networks in osseointegration. *Journal of Dental Research*, *92*(12 Suppl), 109S–S118. https://doi.org/10.1177/0022034513504928

Orlow, I., Roy, P., Reiner, A. S., Yoo, S., Patel, H., Paine, S., … GEM Study Group (2012). Vitamin D receptor polymorphisms in patients with cutaneous melanoma. *International Journal of Cancer*, *130*(2), 405–418. https://doi.org/10.1002/ijc.26023

Parra, F. C., Amado, R. C., Lambertucci, J. R., Rocha, J., Antunes, C. M., & Pena, S. D. J. (2003). Color and genomic ancestry in Brazilians. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(1), 177–182. https://doi.org/10.1073/pnas.0126614100

Pigossi, S. C., Alvim-Pereira, F., Alvim-Pereira, C. C. K., Trevilatto, P. C., & Scarel-Caminaga, R. M. (2014). Association of Interleukin 4 Gene Polymorphisms With Dental Implant Loss. *Implant Dentistry*, *23*(6), 1. https://doi.org/10.1097/ID.0000000000000157

Purcell, S., Cherny, S. S., & Sham, P. C. (2003). Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. *Bioinformatics (Oxford, England)*, *19*(1), 149–150. https://doi.org/10.1093/bioinformatics/19.1.149

Ranade, K., Chang, M.-S., Ting, C.-T., Pei, D., Hsiao, C.-F., Olivier, M., … Botstein, D. (2001). High-throughput genotyping with single nucleotide polymorphisms. *Genome Research*, *11*(7), 1262. https://doi.org/10.1101/GR.157801

Rosenbaum, P. R. (2013). Impact of multiple matched controls on design sensitivity in observational studies. *Biometrics*, *69*(1), 118–127. https://doi.org/10.1111/j.1541-0420.2012.01821.x

Sakka, S., Baroudi, K., & Nassani, M. Z. (2012). Factors associated with early and late failure of dental implants. *Journal of Investigative and Clinical Dentistry*, *3*(4), 258–261. https://doi.org/10.1111/j.2041-1626.2012.00162.x

Sassi, F., Tamone, C., & D'Amelio, P. (2018). Vitamin D: Nutrient, hormone, and immunomodulator. *Nutrients*, *10*(11), 1656. https://doi.org/10.3390/nu10111656

Silness, J., & Loe, H. (1964). Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica*, *22*, 121–135. https://doi.org/10.3109/00016356408993968

Tallarico, M., Canullo, L., Caneva, M., & Özcan, M. (2017). Microbial colonization at the implant-abutment interface and its possible influence on periimplantitis: A systematic review and meta-analysis. *Journal of Prosthodontic Research*, *61*(3), 233–241. https://doi.org/10.1016/j.jpor.2017.03.001

Tanaka, K., Miyake, Y., Hanioka, T., & Arakawa, M. (2013). VDR gene polymorphisms, interaction with smoking and risk of periodontal disease in Japanese women: The Kyushu Okinawa maternal and child health study. *Scandinavian Journal of Immunology*, *78*(4), 371–377. https://doi.org/10.1111/sji.12095

Teles, F. R., Teles, R. P., Sachdeo, A., Uzel, N. G., Song, X. Q., Torresyap, G., … Socransky, S. S. (2012). Comparison of microbial changes in early redeveloping biofilms on natural teeth and dentures. *Journal of Periodontology*, *83*(9), 1139–1148. https://doi.org/10.1902/jop.2012.110506

Tobón-Arroyave, S. I., Isaza-Guzmán, D. M., & Pineda-Trujillo, N. (2017). Association study of Vitamin D Receptor (VDR) – Related genetic polymorphisms and their haplotypes with chronic periodontitis in Colombian population. *Journal of Clinical and Diagnostic Research: JCDR*, *11*(2), ZC60. https://doi.org/10.7860/JCDR/2017/23967.9451

Tonetti, M. S. (1999). Determination of the success and failure of root-form osseointegrated dental implants. *Advances in Dental Research*, *13*(1), 173–180. https://doi.org/10.1177/08959374990130010801

Trevilatto, P. C., & Line, S. R. (2000). Use of buccal epithelial cells for PCR amplification of large DNA fragments. *The Journal of Forensic Odonto-Stomatology*, *18*(1), 6–9.

Valdivielso, J. M., & Fernandez, E. (2006). Vitamin D receptor polymorphisms and diseases. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *371*(1–2), 1–12. https://doi.org/10.1016/j.cca.2006.02.016

Verstuyf, A., Carmeliet, G., Bouillon, R., & Mathieu, C. (2010). Vitamin D: A pleiotropic hormone. *Kidney International*, *78*(2), 140–145. https://doi.org/10.1038/ki.2010.17

Vieira, A. R., McHenry, T. G., Daack-Hirsch, S., Murray, J. C., & Marazita, M. L. (2008). Candidate gene/loci studies in cleft lip/palate and dental anomalies finds novel susceptibility genes for clefts. *Genetics in Medicine: Official Journal of the American College of Medical Genetics*, *10*(9), 668–674. https://doi.org/10.1097/GIM.0b013e3181833793

Wang, T.-T., Tavera-Mendoza, L. E., Laperriere, D., Libby, E., Burton MacLeod, N., Nagai, Y., … White, J. H. (2005). Large-scale in silico and microarray-based identification of direct 1,25-dihydroxyvitamin D3 target genes. *Molecular Endocrinology (Baltimore, Md.)*, *19*(11), 2685–2695. https://doi.org/10.1210/me.2005-0106

Weyant, R. J., & Burt, B. A. (1993). An assessment of survival rates and within-patient clustering of failures for endosseous oral implants. *Journal of Dental Research*, *72*(1), 2–8. https://doi.org/10.1177/00220345930720010201

Wu, F.-Y., Liu, C.-S., Liao, L.-N., Li, C.-I., Lin, C.-H., Yang, C.-W., … Lin, C.-C. (2014). Vitamin D receptor variability and physical activity are jointly associated with low handgrip strength and osteoporosis in community-dwelling elderly people in Taiwan: The Taichung Community Health Study for Elders (TCHS-E). *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, *25*(7), 1917–1929. https://doi.org/10.1007/s00198-014-2691-8

Wu, M., Yue, M., Huang, P., Zhang, Y., Xie, C., Yu, R., … Wang, J. (2016). Vitamin D level and vitamin D receptor genetic variations contribute to HCV infection susceptibility and chronicity in a Chinese population. *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, *41*, 146–152. https://doi.org/10.1016/j.meegid.2016.03.032

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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