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Research Paper
Orthdontic Mini Implants

Analysis of the association of *IL4* polymorphisms with orthodontic mini-implant loss

T. F. Lopes[1], C. M. Souza[1], A. M. Reichow[1], A. C. Melo[2], P. C. Trevilatto[1]
[1]School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brazil; [2]Latin American Institute of Dental Research and Education (ILAPEO), Curitiba, Paraná, Brazil

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Abstract. The aim of this study was to investigate the association of clinical characteristics and *IL4* tag single nucleotide polymorphisms (SNPs; rs2227284 and rs2243268) with orthodontic mini-implant (MI) failure. The sample included 135 subjects of both sexes, mean age 48.7 ± 10 years (range 20–76 years): 104 in the control group (patients without any MI loss) and 31 in the study group (patients presenting ≥1 MI loss). Genotypes were determined by real-time PCR. Bivariate and multivariate analyses were performed ($P < 0.05$). No association was found between the selected tag SNPs and MI loss. The C allele of the *IL4* rs2243268 polymorphism in the recessive model was more frequent in patients who had fewer MIs installed (≤2 vs. >2; $P = 0.043$, odds ratio 0.65, 95% confidence interval 0.58–0.74). On multivariate analysis, smoking habit was significantly associated with the group with multiple MIs installed ($P = 0.036$), however the significance of the association with rs2243268 was not maintained. No association was found between the socio-demographic, smoking, or genetic factors studied and MI loss. This study supports the interaction between host and environmental factors and its influence on susceptibility to orthodontic MI failure.

Key words: orthodontic mini-implant; mini-implant loss; polymorphisms; *IL4*.

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Throughout the history of orthodontics, there have been repeated efforts to improve the method of anchorage[1]. Initially, teeth were used as a means of obtaining anchorage; however the teeth selected for anchorage often move simultaneously with those for which movement is desired. Since Brånemark provided the biological basis for osseointegration, different skeletal anchorage options in orthodontics have been suggested[2,3]. Satisfactory clinical results in orthodontic treatment require adequate anchorage control.

Orthodontic mini-implants (MIs) are temporary skeletal anchorage devices used in situations where orthodontic biomechanics such as mass movement of teeth is required[4]. Since their introduction, the use of orthodontic MIs has become widespread and has increased in popularity, because of the convenience of the placement and removal procedures, their comparative low cost, and the possibility of immediate loading after surgery. Studies have shown that if maximum anchorage is required, MIs should be the system of choice[5,6].

MIs are effective as anchorage, and their success depends on numerous factors, including mechanical stability and loading quantity[7], patient age[8], vertical skeletal pattern[9], inflammation[10], and genetic factors[11]. The mean success rate of

MIs is 87.8%[12]. The unexpected loss of a MI will lead to a change in the original treatment plan, which can be unfavourable when anchorage is required. Inflammation has been shown to be a major predictor of MI failure, leading to bone tissue destruction and affecting the mechanical stability of MIs[13].

Interleukin 4 (IL-4) is a pleiotropic anti-inflammatory cytokine produced mainly by T helper type 2 (Th2) cells. This cytokine plays a central role in humoral and cell-mediated immunity, regulating cell proliferation, differentiation, apoptosis, activation of effector cells, and expression of numerous genes in various cell types[14–16]. In addition, IL-4 reduces the production of functionally important monokines[17], which are implicated in many inflammatory changes. IL-4 is responsible for the equilibrium in inflammation by suppressing pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α), IL-1, and prostaglandin E2 (PGE2), which are known to play a major role in inflammatory and immune reactions[18]. Nowzari et al.[19] investigated the presence of selected cytokines after the placement of dental implants and found that significant levels of IL-1β and TNF-α were present in the crevicular fluid up to 6 months after implant placement, which may have led to bone resorption. Furthermore, IL-4 inhibits the secretion of matrix metalloproteinases, which are mainly responsible for extracellular matrix degradation[20].

The human *IL4* gene (interleukin 4 [*Homo sapiens* (human)] gene; NCBI reference sequence NG_023252.1, OMIM 147780) is located on chromosome 5q31.1 and comprises four exons and three introns in approximately 10 kb[21].

Single nucleotide polymorphisms (SNPs) are variations in the DNA with alterations of a single nucleotide in the genome sequence. SNPs are the most common type of DNA variation between individuals, with a frequency higher than 1% for the rarer alleles in the population. A SNP located within the coding sequence of a gene may influence the corresponding protein, causing an alteration in its function or expression, and SNPs may therefore be associated with susceptibility to human diseases[22].

Currently, hundreds of *IL4* SNPs have been detected in the human genome, with some influencing the expression of IL-4, leading to a weakened immune response and making the host vulnerable to inflammation-related diseases[23]. Host genetic variations in the *IL4* gene have been associated with several diseases, such as hepatitis C and hepatocellular carcinoma[24], rheumatoid arthritis and multiple sclerosis[25], and respiratory conditions[26]. SNPs in this gene have also been associated with implant loss[21] and progression from gingivitis to periodontitis[27].

The factors underlying the success rates of MIs have been studied extensively over recent years. However, it appears that there is only one study relating the loss of MIs to genetic factors, which studied the *IL6* gene[11]. The aim of this study was to investigate the association of clinical variables and *IL4* gene polymorphisms with MI failure.

Materials and methods

Study population

The records of a total of 487 patients attending the Latin American Institute of Dental Research and Education (ILAPEO, Curitiba, PR, Brazil) from 2004 to 2010 were reviewed. Of these, 148 patients were treated with orthodontic MIs (Neodent, Curitiba, PR, Brazil); these patients were recruited for this study. Eight patients with potential confounding factors (such as syphilis and current pregnancy or lactation) were excluded from the study and five refused to participate in the study. Informed consent was obtained from all individual participants included in the study. The population of this study is the same as that investigated in a previous study by Reichow et al.[11].

The study included a group of 135 subjects of both sexes, with a mean age of 48.7 ± 10 years (range 20–76 years). The control group comprised 104 patients without any MI loss, whose implants had been in function for at least 6 months. The study group consisted of 31 patients who presented at least one MI loss.

All patients answered a questionnaire about their socio-demographic characteristics and smoking habits, and signed an informed consent agreement approved by the Ethics Committee in Research at Pontifícia Universidade Católica do Paraná. The numbers of MIs placed and lost were obtained from the patient records. Patient socio-economic factors and medical and oral clinical variables, as well as clinical findings such as the MI location, position, and side of installation, diameter, length, type of neck, type of anchorage, and type of movement, can be found in the study published by Reichow et al.[11].

DNA collection

Cells were obtained through a mouthwash with 3% glucose solution and scraping of the oral mucosa with a sterile wooden spatula[28]. DNA was extracted from epithelial buccal cells with ammonium acetate (10 M) and ethylenediaminetetraacetic acid (EDTA, 1 mM)[29].

Analysis of *IL4* tag SNPs

Adjacent SNPs are often highly correlated in terms of linkage disequilibrium (LD). When those SNPs are in strong LD they can capture the information of others in the same block (or bin) and are called tag SNPs. The strategy of genotyping tag SNPs intends to capture the information of a whole gene, reducing genotyping costs and time[30]. Genotyping these avoids the need to genotype all of the SNPs of a given gene. Thus, *IL4* gene tag SNPs were selected according to the information available on the International HapMap Project website, release 24 (http://www.hapmap.org). All selected markers presented a minimum allele frequency (MAF) of 0.05 in the CEU population (Northern and Western European ancestry). The CEU population was chosen because the patients selected for this study were from the south region of Brazil, which is considered a heterogeneous population with a European ancestry. According to the Brazilian Demographic Census in 2010, 70% of the population from Paraná State were Caucasian.

The cut-off parameter to define LD between two markers was a multi-marker $r^2 > 0.8$, considered a high level of LD. Using this criterion, the following tag SNPs were included: reference SNPs rs2243268 and rs2227284 (Fig. 1); these captured the complete information for the *IL4* gene.

Patients were genotyped for the tag SNPs by real-time polymerase chain reaction (PCR) technique (7500 Real-Time PCR System; Applied Biosystems, Foster City, CA, USA), with the use of TaqMan Genotyping Master Mix technology (Applied Biosystems).

Statistical analysis

Statistical analyses were performed and the sample power calculated using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). Odds ratios (OR) and 95% confidence intervals (95% CI) were determined when possible. Haploview 4.2 software (Broad Institute, Cambridge, MA, USA) was used to evaluate Hardy–Weinberg equilibrium and LD.

Nominal variables were expressed as frequencies and percentages. The Pearson χ^2 test was used to assess the association

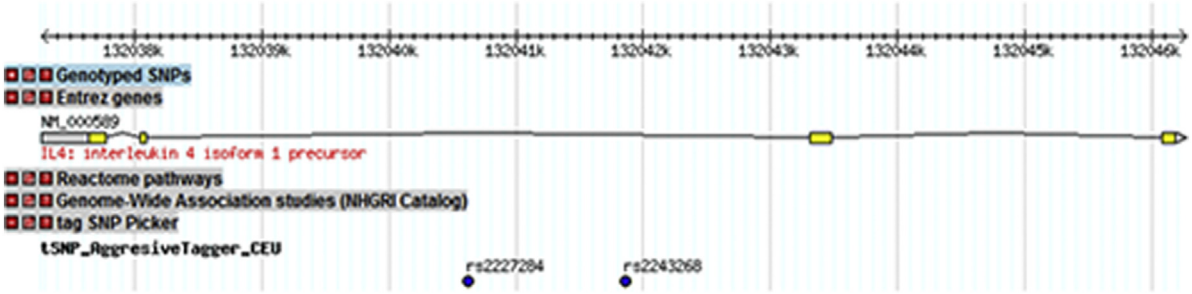

Fig. 1. The *IL4* gene with tag SNPs presenting a minimum allele frequency (MAF) of 0.05 (Font: HapMap).

between binary variables. Continuous variables were first classified by distribution as normal or non-normal. Non-normal continuous variables were analyzed by Mann–Whitney *U*-test. The following genetic models of the association between markers (tag SNPs) were assessed: additive, dominant, and recessive; these were examined by Pearson χ^2 test. For the multivariate analysis, the binary logistic regression model was adjusted to analyze genotypic frequencies, including variables with $P < 0.20$ significance in the bivariate tests as co-variables.

Results

A total of 135 individuals were genotyped for the study. Table 1 describes the demographic and clinical characteristics of the participants. There was no statistically significant difference between the groups with regard to age, sex, ethnic group, or smoking habit (Mann–Whitney *U*-test, or Pearson χ^2 test).

The MI success rate in this study was 77.0%. Analysis showed that 23.0% of the patients in the sample (31 out of 135 patients) presented at least one MI loss, and of these 31 patients, eight (5.9% of the total sample) presented two or more MI losses (multiple losses). Moreover, when more than two MIs were installed, the failure rate was 77.4%. This higher number of MIs installed per patient was associated with MI failure ($P = 0.000$; OR 2.03, 95% CI 1.46–2.82). The sample power test was done for the variable number of MIs installed per patient, and by accepting the alternative hypothesis (H1), the test power was above 99%.

The tag SNPs rs2227284 and rs2243268 selected for this study were not in high LD ($r^2 < 0.8$), confirming their independence in the study population (Fig. 2).

The genotype distribution for the two SNPs tested was in Hardy–Weinberg equilibrium in the control group. Neither of the two *IL4* tag SNPs was significantly associated with MI loss. The genotype distribution according to the genetic models is shown in Tables 2 and 3.

Since subjects who had more than two MIs installed concentrated showed increased MI loss (77.4%), an investigation was performed to determine whether an association with specific alleles of the two tested polymorphisms existed (Table 4). The C allele of rs2243268 in the recessive model was significantly more frequent in the group with two or fewer MIs installed ($P = 0.043$; OR 0.65, 95% CI 0.58–0.74).

On multivariate analysis, smoking habit was significantly associated with the group with multiple MIs installed ($P = 0.036$); however the significance of the association with rs2243268 was not maintained.

Discussion

Since the introduction of MIs as an intraoral anchorage system, they have become an important part of the orthodontic treatment plan. Although studied for several years, MI failure is a complex trait and inconclusive findings are shown in the literature concerning its aetiological aspects.

MIs are considered successful when they are maintained in the bone until the

Table 1. Results of the bivariate analysis of socio-demographic and clinical variables for the control and study groups.

General data	Control group		Study group		Bivariate	
	$n = 104$	%	$n = 31$	%	*P*-value	OR (95% CI)
Sex						
Male	29	27.9	7	22.6	0.558[a]	1.32 (0.52–3.41)
Female	75	72.1	24	77.4		
Age (years), mean ± SD	48.69 ± 10.31		48.77 ± 9.11		0.888[b]	–
Ethnic group						
Caucasian	73	70.2	22	71.0	0.934[a]	0.96 (0.40–2.33)
Non-Caucasian	31	29.8	9	29.0		
Smoking habit						
Smoker + ex-smoker	26	25.0	12	38.7	0.136[a]	1.17 (0.93–1.49)
Non-smoker	78	75.0	19	61.3		
Number of MIs installed per patient						
1 or 2	84	80.8	7	22.6	0.000[a,*]	2.03 (1.46–2.82)
3, 4, or 5	20	19.2	24	77.4		

CI, confidence interval; OR, odds ratio; MI, mini-implants; SD, standard deviation.
[a] Pearson χ^2 test.
[b] Mann–Whitney *U*-test.
[*] Significant, $P < 0.05$.

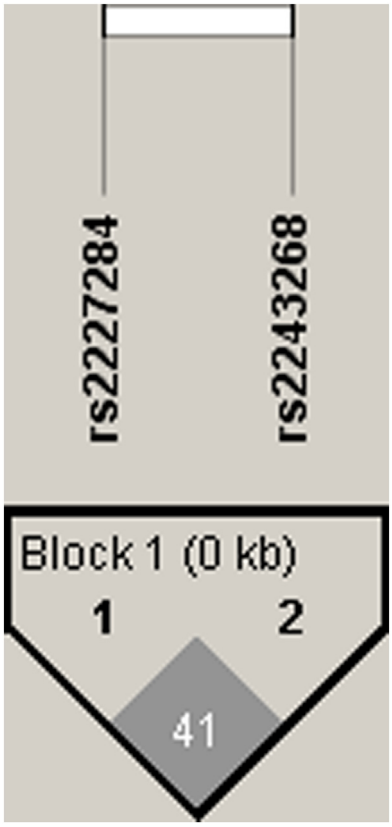

Fig. 2. Analysis of linkage disequilibrium (LD) between the selected *IL4* tag SNPs. The number inside the square shows the proportion of LD as a percentage (41%). The LD between the tag SNPs is $r^2 < 0.8$.

end of treatment or intentional removal[10]. The overall MI success rate varies dramatically according to clinical procedures such as the implant placement technique[11], mobility and side of placement[10], and host factors[31]. The clinical characteristics related to the MIs in this sample were analyzed and the findings can be found in the study published by Reichow et al.[11]. However, there is one common condition for MI success – no sign of inflammation[12]. In this study, the MI success rate was 77.0%, lower than that reported in other studies[32,33].

Orthodontic treatment is usually sought by a young population, therefore MIs are commonly used in patients around 20 years old, and it has not yet been determined conclusively whether age interferes in MI loss[34]. In the present study, the mean age of the patients in both groups was approximately 48 years, which is an uncommon age when compared to other reports. This is explained by the fact that most of the selected patients were being prepared for prosthetic implant treatment.

The lack of association between sex and MI loss has been reported previously in the literature[10]. The present study is in agreement with this finding.

It is not appropriate to analyze Brazilians in separate groups based on ethnic characteristics because Brazilian individuals have overlapping genotypes due to miscegenation[35]. Nevertheless, a comparison of the groups according to ethnic group was performed and no difference was found.

In this study, having more than two MIs installed doubled the risk of presenting MI loss. Indeed, it was found that there was an increased failure rate after a second MI was installed (77.4%). Yao et al.[34] evaluated the stability of MIs for orthodontic anchorage and found an association between the number of MIs installed per patient and higher failure rates. In the present study, when the group with up to two MIs and the group with more than two MIs installed were analyzed, the C allele of rs2243268 of the *IL4* gene in the recessive model was significantly more frequent in the group with fewer MIs installed. As the group with multiple MIs installed presented a higher failure rate, it could be that the C allele is associated with protection against MI loss.

When the multivariate analysis was applied to the groups according to the number of MIs installed, the indicated association of the rs2243268 polymorphism was not maintained. This could be explained by the fact that genetic polymorphisms in different genes may contribute individually with a small proportion of the immune-inflammatory modulation. However, smoking was found to be associated with the placement of multiple MIs, which was associated significantly with MI failure. Smoking is known to cause implant failure and it has been demonstrated that smokers have a 2.5 times greater chance of losing an implant when compared to non-smokers[36]. Thus, smoking could mute the influence of a genetic

Table 2. Genotypic analysis of the tag SNPs in the *IL4* gene in the additive model.

Tag SNPs dbSNP ID[a]	Alleles[b]	Groups	Genotypes, *n* (%)			*P*-value[c]
			GG	GT	TT	
rs2227284[d]	[G/T]	Control, *n* (%)	33 (33.0)	51 (51.0)	16 (16.0)	0.683
		Study, *n* (%)	9 (31.0)	17 (58.6)	3 (10.3)	
			AA	AC	CC	
rs2243268[e]	[A/C]	Control, *n* (%)	61 (58.7)	35 (33.7)	8 (7.7)	0.280
		Study, *n* (%)	20 (64.5)	11 (35.5)	0 (0.0)	

SNP, single nucleotide polymorphism.
[a] SNP identifier based on the NCBI SNP database.
[b] The first allele is designated as the major allele, and the second allele is designated as the minor allele.
[c] Pearson χ^2 test.
[d] Control group $n = 100$, study group $n = 29$.
[e] Control group $n = 104$, study group $n = 31$.

Table 3. Genotypic analysis of the tag SNPs in the *IL4* gene in the dominant and recessive models.

Tag SNPs dbSNP ID[a]	Alleles[b]	Groups	Genotypes		*P*-value[c]	OR (95% CI)
			TT + GT	GG		
rs2227284[d] (Dom T)	[G/T]	Control, *n* (%)	67 (67.0)	33 (33.0)	0.842	0.914 (0.38–2.23)
		Study, *n* (%)	20 (69.0)	9 (31.0)		
			GT + GG	TT		
rs2227284[d] (Rec T)	[G/T]	Control, *n* (%)	84 (84.0)	16 (16.0)	0.449	0.606 (0.16–2.24)
		Study, *n* (%)	26 (89.7)	3 (10.3)		
			AC + CC	AA		
rs2243268[e] (Dom C)	[A/C]	Control, *n* (%)	43 (41.3)	61 (58.7)	0.559	1.282 (0.56–2.95)
		Study, *n* (%)	11 (35.5)	20 (64.5)		
			AA + AC	CC		
rs2243268[e] (Rec C)	[A/C]	Control, *n* (%)	96 (92.3)	8 (7.7)	0.111	–
		Study, *n* (%)	31 (100)	0 (0.0)		

CI, confidence interval; Dom, dominant; OR, odds ratio; Rec, recessive; SNP, single nucleotide polymorphism.
[a] SNP identifier based on the NCBI SNP database.
[b] The first allele is designated as the major allele, and the second allele is designated as the minor allele.
[c] Pearson χ^2 test.
[d] Control group $n = 100$, study group $n = 29$.
[e] Control group $n = 104$, study group $n = 31$.

Table 4. Genotypic analysis of the tag SNPs in the *IL4* gene in the group with up to two mini-implants installed (≤2) and the group with three to five mini-implants installed (>2) in the dominant and recessive models.

Tag SNPs dbSNP ID[a]	Alleles[b]	Groups	Genotypes		*P*-value[c]	OR (95% CI)
			TT + GT	GG		
rs2227284[d] (Dom T)	[G/T]	≤2, *n* (%)	61 (70.9)	25 (29.1)	0.232	1.18 (0.89–1.57)
		>2, *n* (%)	26 (60.5)	17 (39.5)		
			GT + GG	TT		
rs2227284[d] (Rec T)	[G/T]	≤2, *n* (%)	71 (82.6)	15 (17.4)	0.219	0.49 (0.15–1.56)
		>2, *n* (%)	39 (90.7)	4 (9.3)		
			AC + CC	AA		
rs2243268[e] (Dom C)	[A/C]	≤2, *n* (%)	39 (42.9)	52 (57.1)	0.330	1.12 (0.89–1.42)
		>2, *n* (%)	15 (34.1)	29 (65.9)		
			AA + AC	CC		
rs2243268[e] (Rec C)	[A/C]	≤2, *n* (%)	83 (91.2)	8 (8.8)	0.043*	0.65 (0.58–0.74)
		>2, *n* (%)	44 (100)	0 (0.0)		

CI, confidence interval; Dom, dominant; OR, odds ratio; MI, mini-implant; Rec, recessive; SNP, single nucleotide polymorphism.
[a] SNP identifier based on the NCBI SNP database.
[b] The first allele is designated as the major allele, and the second allele is designated as the minor allele.
[c] Pearson χ^2 test.
[d] MIs installed ≤2 $n = 86$, MIs installed >2 $n = 43$.
[e] MIs installed ≤2 $n = 91$, MIs installed >2 $n = 44$.
* Significant, $P < 0.05$.

polymorphism[37]. Kornman et al.[38] suggested that smoking is a strong risk factor and that this might have had an effect on *IL1β* polymorphism, modulating susceptibility to chronic periodontitis. In the presence of smoking, a strong and well-known risk factor that significantly impairs the host response, the influence of the polymorphic protection allele should have been masked. Another consideration is that the association might have been spurious because of the low frequency of the rarer allele (C allele) of rs2243268. The sample size should be increased to confirm this association. Moreover, replication in other populations is essential before any conclusions can be drawn regarding rs2243268 as a genetic marker for susceptibility to MI loss.

This study was novel in investigating the association of *IL4* polymorphism with MI loss. The tag SNP rs2243268 has been reported previously in the literature, associated with resistance to extrapulmonary tuberculosis in Chinese Han children[39] and resistance to adverse events after smallpox vaccination[40]. However, the A allele was found to be the protective allele, which differs from the findings of the present study. This discrepancy in findings might be explained in part by different racial genetic backgrounds and aetiopathological molecular mechanisms underlying these complex diseases and traits. In this context, the results of this study strongly suggest that host and environmental factors interact deeply to modulate susceptibility to complex traits, which characterize them as multifactorial.

In conclusion, no association was found between *IL4* tag SNPs and MI failure. Nonetheless, an association was observed between patients with multiple MIs installed and *IL4* rs2243268 polymorphism. On multivariate analysis, smoking habit was associated with the group with multiple MI placements, but the association with the rs2243268 polymorphism disappeared. This study supports the interaction between host and environmental factors and its influence on susceptibility to certain conditions.

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Competing interests

All authors declare that they have no conflict of interest.

Ethical approval

This study was approved by the Ethics Committee in Research at Pontifícia Universidade Católica do Paraná (protocol 5693/10).

Patient consent

Not required.

References

1. Costello BJ, Ruiz RL, Petrone J, Sohn J. Temporary skeletal anchorage devices for orthodontics. *Oral Maxillofac Surg Clin North Am* 2010;**22**:91–105. http://dx.doi.org/10.1016/j.coms.2009.10.011.
2. Roberts WE, Smith RK, Zilberman Y, Mozsary PG, Smith RS. Osseous adaptation to continuous loading of rigid endosseous implants. *Am J Orthod* 1984;**86**:95–111.
3. Block MS, Hoffman DR. A new device for absolute anchorage for orthodontics. *Am J Orthod Dentofacial Orthop* 1995;**107**:251–8.
4. Alves Jr M, Baratieri C, Mattos CT, Araujo MT, Maia LC. Root repair after contact with mini-implants: systematic review of the literature. *Eur J Orthod* 2013;**35**:491–9. http://dx.doi.org/10.1093/ejo/cjs025.
5. Feldmann I, Bondemark L. Anchorage capacity of osseointegrated and conventional anchorage systems: a randomized controlled trial. *Am J Orthod Dentofacial Orthop* 2008;**133**(339):e19–28. http://dx.doi.org/10.1016/j.ajodo.2007.08.014.
6. Sandler J, Murray A, Thiruvenkatachari B, Gutierrez R, Speight P, O'Brien K. Effectiveness of 3 methods of anchorage reinforcement for maximum anchorage in adolescents: a 3-arm multicenter randomized clinical trial. *Am J Orthod Dentofacial Orthop* 2014;**146**:10–20. http://dx.doi.org/10.1016/j.ajodo.2014.03.020.
7. Chen Y, Kyung HM, Zhao WT, Yu WJ. Critical factors for the success of orthodontic mini-implants: a systematic review. *Am J Orthod Dentofacial Orthop* 2009;**135**:284–91. http://dx.doi.org/10.1016/j.ajodo.2007.08.017.
8. Lee SJ, Ahn SJ, Lee JW, Kim SH, Kim TW. Survival analysis of orthodontic mini-implants. *Am J Orthod Dentofacial Orthop* 2010;**137**:194–9. http://dx.doi.org/10.1016/j.ajodo.2008.03.031.
9. Moon CH, Park HK, Nam JS, Im JS, Baek SH. Relationship between vertical skeletal pattern and success rate of orthodontic mini-implants. *Am J Orthod Dentofacial Orthop* 2010;**138**:51–7. http://dx.doi.org/10.1016/j.ajodo.2008.08.032.
10. Park HS, Jeong SH, Kwon OW. Factors affecting the clinical success of screw implants used as orthodontic anchorage. *Am J Orthod Dentofacial Orthop* 2006;**130**:18–25. http://dx.doi.org/10.1016/j.ajodo.2004.11.032.
11. Reichow AM, Melo AC, de Souza CM, Castilhos BB, Olandoski M, Alvim-Pereira CC, Alvim-Pereira F, Trevilatto PC. Outcome of orthodontic mini-implant loss in relation to interleukin 6 polymorphisms. *Int J Oral Maxillofac Surg* 2016;**45**:649–57. http://dx.doi.org/10.1016/j.ijom.2015.11.012.
12. Rodriguez JC, Suarez F, Chan HL, Padial-Molina M, Wang HL. Implants for orthodontic anchorage: success rates and reasons of failures. *Implant Dent* 2014;**23**:155–61. http://dx.doi.org/10.1097/ID.0000000000000048.
13. Chen YJ, Chang HH, Lin HY, Lai EH, Hung HC, Yao CC. Stability of miniplates and miniscrews used for orthodontic anchorage: experience with 492 temporary anchorage devices. *Clin Oral Implants Res* 2008;**19**:1188–96. http://dx.doi.org/10.1111/j.1600-0501.2008.01571.x.
14. Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. *Annu Rev Immunol* 1999;**17**:701–38. http://dx.doi.org/10.1146/annurev.immunol.17.1.701.
15. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, Keegan AD, Garcia KC. Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* 2008;**132**:259–72. http://dx.doi.org/10.1016/j.cell.2007.12.030.
16. Mueller TD, Zhang JL, Sebald W, Duschl A. Structure, binding, and antagonists in the IL-4/IL-13 receptor system. *Biochim Biophys Acta* 2002;**1592**:237–50.
17. te Velde AA, Huijbens RJ, Heije K, de Vries JE, Figdor CG. Interleukin-4 (IL-4) inhibits secretion of IL-1 beta, tumor necrosis factor alpha, and IL-6 by human monocytes. *Blood* 1990;**76**:1392–7.
18. Hart PH, Vitti GF, Burgess DR, Whitty GA, Piccoli DS, Hamilton JA. Potential antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor alpha, interleukin 1, and prostaglandin E2. *Proc Natl Acad Sci U S A* 1989;**86**:3803–7.
19. Nowzari H, Yi K, Chee W, Rich SK. Immunology, microbiology, and virology following placement of NobelPerfect scalloped dental implants: analysis of a case series. *Clin Implant Dent Relat Res* 2007;**10**:157–65. http://dx.doi.org/10.1111/j.1708-8208.2007.00075.x.
20. Wojdasiewicz P, Poniatowski LA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm* 2014;**2014**:561459. http://dx.doi.org/10.1155/2014/561459.
21. Pigossi SC, Alvim-Pereira F, Alvim-Pereira CC, Trevilatto PC, Scarel-Caminaga RM. Association of interleukin 4 gene polymorphisms with dental implant loss. *Implant Dent* 2014;**23**:723–31. http://dx.doi.org/10.1097/ID.0000000000000157.
22. Koberle B, Koch B, Fischer BM, Hartwig A. Single nucleotide polymorphisms in DNA repair genes and putative cancer risk. *Arch Toxicol* 2016;**90**(October (10)):2369–88. http://dx.doi.org/10.1007/s00204-016-1771-2.
23. Belopolskaya OB, Smelaya TV, Moroz VV, Golubev AM, Salnikova LE. Clinical associations of host genetic variations in the genes of cytokines in critically ill patients. *Clin Exp Immunol* 2015;**180**:531–41. http://dx.doi.org/10.1111/cei.12592.
24. Wu Z, Qin W, Zeng J, Huang C, Lu Y, Li S. Association between IL-4 polymorphisms and risk of liver disease: an updated meta-analysis. *Medicine (Baltimore)* 2015;**94**:e1435. http://dx.doi.org/10.1097/MD.0000000000001435.
25. Qiu LJ, Ni J, Cen H, Wen PF, Zhang M, Liang Y, Pan HF, Mao C, Ye DQ. Relationship between the IL-4 gene promoter −590C/T (rs2243250) polymorphism and susceptibility to autoimmune diseases: a meta-analysis. *J Eur Acad Dermatol Venereol* 2015;**29**:48–55. http://dx.doi.org/10.1111/jdv.12435.
26. Patarcic I, Gelemanovic A, Kirin M, Kolcic I, Theodoratou E, Baillie KJ, de Jong MD, Rudan I, Campbell H, Polasek O. The role of host genetic factors in respiratory tract infectious diseases: systematic review, meta-analyses and field synopsis. *Sci Rep* 2015;**5**:16119. http://dx.doi.org/10.1038/srep16119.
27. Shapira L, van Dyke TE, Hart TC. A localized absence of interleukin-4 triggers periodontal disease activity: a novel hypothesis. *Med Hypotheses* 1992;**39**:319–22.
28. Trevilatto PC, Line SR. Use of buccal epithelial cells for PCR amplification of large DNA fragments. *J Forensic Odontostomatol* 2000;**18**:6–9.
29. Aidar M, Line SR. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. *Braz Dent J* 2007;**18**:148–52.
30. Liu G, Wang Y, Wong L. FastTagger: an efficient algorithm for genome-wide tag SNP selection using multi-marker linkage disequilibrium. *BMC Bioinformatics* 2010;**11**:66. http://dx.doi.org/10.1186/1471-2105-11-66.
31. Romano FL, Consolaro A. Why are mini-implants lost: the value of the implantation technique! *Dental Press J Orthod* 2015;**20**:23–9. http://dx.doi.org/10.1590/2176-9451.20.1.023-029.oin.
32. Kuroda S, Sugawara Y, Deguchi T, Kyung HM, Takano-Yamamoto T. Clinical use of miniscrew implants as orthodontic anchorage: success rates and postoperative discomfort. *Am J Orthod Dentofacial Orthop* 2007;**131**:9–15. http://dx.doi.org/10.1016/j.ajodo.2005.02.032.
33. Moon CH, Lee DG, Lee HS, Im JS, Baek SH. Factors associated with the success rate of orthodontic miniscrews placed in the upper and lower posterior buccal region. *Angle Orthod* 2008;**78**:101–6. http://dx.doi.org/10.2319/121706-515.1.

34. Yao CC, Chang HH, Chang JZ, Lai HH, Lu SC, Chen YJ. Revisiting the stability of mini-implants used for orthodontic anchorage. *J Formos Med Assoc* 2015;**114**:1122–8. http://dx.doi.org/10.1016/j.jfma.2014.08.001.
35. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. *Proc Natl Acad Sci U S A* 2003;**100**:177–82. http://dx.doi.org/10.1073/pnas.0126614100.
36. Wilson Jr TG, Nunn M. The relationship between the interleukin-1 periodontal genotype and implant loss. Initial data. *J Periodontol* 1999;**70**:724–9. http://dx.doi.org/10.1902/jop.1999.70.7.724.
37. Greenstein G, Hart TC. A critical assessment of interleukin-1 (IL-1) genotyping when used in a genetic susceptibility test for severe chronic periodontitis. *J Periodontol* 2002;**73**:231–47. http://dx.doi.org/10.1902/jop.2002.73.2.231.
38. Kornman KS, Crane A, Wang HY, di Giovine FS, Newman MG, Pirk FW, Wilson Jr TG, Higginbottom FL, Duff GW. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997;**24**:72–7.
39. Qi H, Sun L, Jin YQ, Shen C, Chu P, Wang SF, Yin QQ, Qi Z, Xu F, Jiao WW, Wu XR, Tian JL, Xiao J. Shen AD. rs2243268 and rs2243274 of interleukin-4 (IL-4) gene are associated with reduced risk for extrapulmonary and severe tuberculosis in Chinese Han children. *Infect Genet Evol* 2014;**23**:121–8. http://dx.doi.org/10.1016/j.meegid.2014.01.031.
40. Reif DM, McKinney BA, Motsinger AA, Chanock SJ, Edwards KM, Rock MT, Moore JH, Crowe JE. Genetic basis for adverse events after smallpox vaccination. *J Infect Dis* 2008;**198**:16–22. http://dx.doi.org/10.1086/588670.

Address:
Paula Cristina Trevilatto
School of Life Sciences
Pontifícia Universidade Católica do Paraná
Rua Imaculada Conceição
1155
Prado Velho
Curitiba – PR
Post Code: 80215-901
Brazil
Tel.: +55 41 3271 2582
E-mail: paula.trevilatto@pucpr.br