Association of clinical variables and polymorphisms in *RANKL*, *RANK*, and *OPG* genes with external apical root resorption

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Objectives: The aim of this study was to investigate the association of clinical variables and polymorphisms in the RANKL, RANK, and OPG genes with external apical root resorption (EARR). **Methods:** The sample was composed of 338 unrelated patients of both sexes, average age 14.9 years (range 8-21) with Class II Division 1 malocclusion, orthodontically treated. Periapical radiographs of the maxillary central incisor with the longer root (reference tooth) were taken before treatment and 6 months after starting treatment. DNA was extracted from buccal epithelial cells with the use of 10 mol/L ammonium acetate and 1 mmol/L EDTA. The analysis of 42 polymorphisms in the RANKL, RANK, and OPG genes was performed by means of real-time polymerase chain reaction. Univariate and multivariate analyzes were performed to verify the association of clinical and genetic variables with EARR ($P < 0.05$). **Results:** The initial root length and patient age were associated with EARR. Considering the study of polymorphisms of *RANKL*, no significant association was found of genetic polymorphisms with EARR. For *RANK* polymorphisms, only rs12455775 was associated with EARR. Regarding *OPG* polymorphisms, an association of rs3102724, rs2875845, rs1032128, and rs3102728 with EARR was found. After multivariate analysis, the initial root length, rapid maxillary expansion, and rs3102724 of the *OPG* gene were associated with EARR. **Conclusions:** Longer roots of upper central incisors and rapid maxillary expansion, as well as allele A of the rs3102724 polymorphism of the OPG gene, were associated with EARR in the study population. (Am J Orthod Dentofacial Orthop 2019;155:529-42)

It is well known that external apical root resorption (EARR) is among the most common and undesirable side effects of orthodontic treatment.[1-3] Several studies have been designed to discover the etiologic factors related to EARR, but so far the issue is still unclear and it is difficult to predict who will develop it.[3,4] The frequency of severe EARR during orthodontic treatment is reported to range from 5% to 18% of cases.[5,6]

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The application of orthodontic forces induces a local process, with inflammatory characteristics.[7,8] This inflammation is essential for tooth movement, also being the main component responsible for the radicular resorption process.[7,9] The identification of factors involved in the initiation and progression of EARR during orthodontic treatment has been the focus of numerous studies. These factors include sex, radicular anatomy,[10] trauma,[11] endodontic treatment,[12] age, stage of root formation at the beginning of orthodontic treatment,[13] type of device,[14] forces applied, and treatment duration, in additiion to genetic background.[3,15,16] The difficulty in assessing the causes of EARR is to separate the contributions related to genetics and to environment.[15,16]

Newman et al[17] were the first to report a genetic basis for EARR. Harris et al[3] estimated the heritable component through a genetic model using sets of brothers. The first description of a genetic marker that identified individuals more likely to develop EARR was reported by Al Qawasmi et al,[1] who found the polymorphism in

the interleukin-1β gene (*IL1B* +3954) associated with EARR during orthodontic treatment.

Recent advances in the understanding of bone cell biology demonstrate the key role of the receptor activator of nuclear factor kappa B (RANK), ligand receptor activator of nuclear factor kappa B (RANKL), and osteoprostegerin (OPG) in the activation and differentiation of osteoclasts.[18] Among these biomarkers, both RANKL and OPG have shown to be key regulators of bone remodeling during orthodontic movement. RANKL is produced by osteoblasts, stromal cells, and activated T cells and represents an essential factor for the formation, activation, and survival of osteoclasts resulting in bone resorption and bone loss.[19] RANKL activates its specific receptor, RANK, located on osteoclast precursors and dendritic cells. The effects of RANKL are counteracted by OPG, which is a soluble receptor produced by osteoblasts and hematopoietic and immune cells that acts to inhibit osteoclast differentiation and induce apoptosis.[20] Consequently, bone remodeling is dependent on a balance in the RANK/RANKL/OPG system.

In humans, the cytokine RANKL is coded by a gene located in the long arm of chromosome 13 in the 13q14 region.[21] The RANKL gene is composed of 8 exons with about 58 kilobases (kb).[22] RANK is a transmembrane glycoprotein of type I, whose gene is located on chromosome 18 in the 18q22.1 region, with an extension of about 80 kb and composed by 12 exons. OPG is encoded by a single gene located on chromosome 8 in the 8q24 region composed of 5 exons and 6 introns.[23]

Polymorphisms refer to the existence of 2 or more alleles at a particular locus with a frequency greater than 1% in a population.[24] Polymorphisms in RANKL, RANK, and OPG genes have been related to pathologic conditions such as osteoporosis,[25,26] rheumatoid arthritis,[27] mineral density bone loss,[28] and agressive periodontitis.[29] The aim of the present study was to investigate the association of clinical variables and polymorphisms in the RANKL, RANK and OPG genes with external apical root resorption.

MATERIAL AND METHODS

The sample was composed of 338 unrelated white patients of both sexes (154 male and 184 female), mean age 14.9 years (range 8-21). The patients were selected between 2005 and 2009 from the Dental Clinics of the Graduate Program in Orthodontics of Profis (Bauru, São Paulo, Brazil), Dental Clinics of the Graduate Program of Thum Institute of Research (Joinville, Santa Catarina, Brazil) and two Private Orthodontic Clinics (Curitiba, Paraná, Brazil). The subjects presented Class II malocclusion, Division 1, orthodontically treated with the use of Edgewise or Straight-Wire techniques. The choice of Angle Class II, Division 1, was due to the fact that it is one of the most frequent malocclusions and requires more biomechanics with longer treatment time than other malocclusions, as well as the fact that it can lead to higher levels of EARR.[30,31] Although the study sample is composed of white subjects only, the white Brazilian population is heterogeneous. Articles have not recommended grouping Brazilians based on ethnicity, color, or geographic origin, because Brazilian individuals classified as black or white have overlapping genotypes due to miscegenation.[32] Patients completed medical and dental questionnaires and signed informed consents after being advised of the nature of the study. This research was approved by the Ethical Committee for Human use at Pontifical Catholic University of Paraná, Paraná, Brazil (protocol number 546/05). Patients could not have: chronic use of antiinflammatory drugs, human immunodeficiency virus infection, immunosuppressive chemotherapy history, any disease that seriously compromises the immune function, pregnancy or lactation, oral trauma, parafunctional behavior, endodontic treatment, extensive carious lesions in the maxillary central incisors, or teeth without complete root formation.

The following methodology was fully reported in the manuscript by Fontana et al.[33] Periapical radiographs of the central incisor teeth with the longer root (reference tooth) were taken before treatment and 6 months after the beginning of treatment. The evaluation method consisted of measuring the root and crown length directly on radiographs (Fig 1). The root apex, the incisal edge, and the cementoenamel junction (CEJ) of each tooth were marked on the x-ray films on a light table. The longitudinal axis of each tooth was constructed from the root apex to the incisal edge following the root canal as accurately as possible. A perpendicular axis was then projected to the longitudinal axis from the mesial to the distal CEJ sides. The crown length was measured from the incisal edge to the projected CEJ, and the root length from the projected CEJ to the root apex (Fig 1). The differences between the 2 measurements indicate the EARR. A correction factor was calculated with the use of the following formula: correction factor = C1/C2 (*C1*, crown length before treatment; *C2*, crown length 6 months after starting treatment). Then EARR was calculated with the use of the following formula: EARR = R1 − (R2 × CF) (*R1*, root length before treatment; *R2*, root length 6 months after treatment start; *CF*, correction factor). EARR was also expressed as a percentage of the original root length: EARR × 100/R1. Any distortions between the pretreatment and follow-up

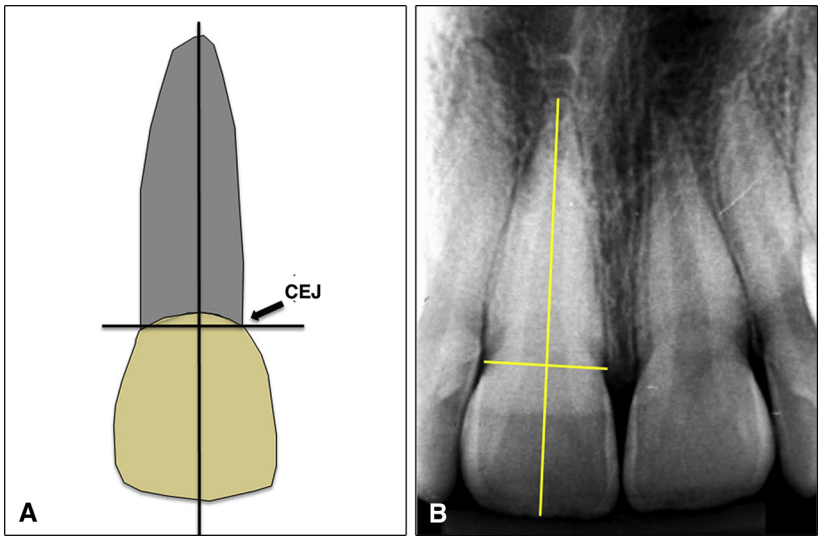

Fig 1. A, Anatomic landmarks to measure the EARR: cementoenamel junction (CEJ). **B,** References to measure the x-ray.

radiographic images were corrected with the use of the crown length registration, assuming crown length to be unchanging over the observation period.[34,35] The EARR was evaluated by 1 examiner (M.L.S.S.N.F). The measurements were made with the use of a fine-tip digital caliper with accuracy up to 0.02 mm (Utustools Professional, Santiago, Chile; Fig 2).

A receiver operating characteristic (ROC) curve was constructed. The ROC curve is applied to identify a cutoff point to the outcome based on the sample data distribution providing the best sensitivity and specificity to the analysis. Sensitivity means the probability of EARR (outcome) being diagnosed if it is really present (to avoid false positives) and specificity means the probability of EARR not being diagnosed if it is really absent (to avoid false negatives), thus allowing the determination of an optimal cutoff point.[36]

According to the ROC curve, the sample was divided into 2 groups: control group, 160 individuals with EARR $\leq$1.43 mm; and test group, 178 individuals with EARR $>$1.43 mm.

The following parameters were evaluated in the orthodontically treated patients: age, sex, initial size of the root of the reference tooth (IR), premolar extraction (PE), use of pendulum appliance, rapid maxillary expansion (RME), and use of elastics.

Cells were obtained with the use of a 3% glucose solution mouthwash for 1 minute and scraping the oral mucosa with a sterile spatula.[37] DNA was extracted from epithelial oral cells with the use of ammonium acetate (10 mol/L) and EDTA (1 mmol/L).[38]

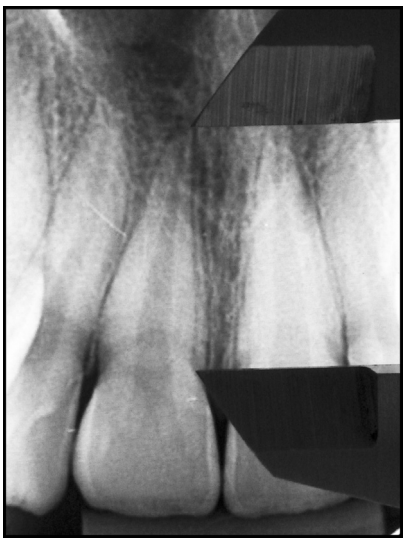

Fig 2. Measurements of the electronic digital caliper on film x-ray.

The tag single-nucleotide polymorphisms (SNPs) of RANKL, RANK, and OPG genes were selected according to the information available on the site International HapMap Project, phase III/Rel#2 (www.hapmap.org; 2012). All 42 selected markers had a minimum allele frequency of 0.05 in the "CEU" population (Utah residents with northern and western European ancestry). The cutoff parameter to define linkage disequilibrium (LD) between 2 markers was $r^2 > 0.8$. LD is when

different SNP alleles of the same gene have a great chance to be inherited together. The degree of chance is given by r^2.

Following these criteria, the following tag SNPs were selected: *RANKL:* rs1038434, rs3742257, rs931273, rs12585229; *RANK:* rs7233197, rs4941125, rs4485469, rs4941129, rs7237982, rs8086340, rs17069845, rs12956925, rs17720953, rs4500848, rs12455775, rs3826620, rs7236060, rs9951012, rs6567272, rs4524034, rs12970081, rs8083511, rs8099222, rs7239667, rs17069898, rs17069902, rs8089829, rs17069904, rs12959396, rs4426449; and *OPG:* rs11573938, rs3102724, rs11573884, rs2875845, rs1032128, rs3134057, rs1485289, rs3134060, RS3102728, rs11573856, rs7010267, rs11573901.

Patients were genotyped for tag SNPs by means of real-time polymerase chain reaction (PCR; Applied Biosystems 7500 Real-Time PCR System) using the TaqMan technology (Applied Biosystems).[39] A negative control was used in all genotyping performed.

Statistical analysis

All of the 7 categoric variables were expressed as frequencies and percentages: age, sex, IR, PE, use of pendulum appliance, RME, and use of elastics. Age and IR had cutoff points as defined by ROC curves.[33] Comparisons between the groups regarding the dichotomous categoric variables were made with the use of the chi-square test or the Fisher exact test when indicated. Odds ratio test was used considerig dichotomous categoric variables with statistically significant difference for risk assessment. Logistic regression was used for the additive genetic model. The analysis was performed with the use of SPSS statistical software IBM 21. Results with P VALUES <0.05 were considered significant. For genetic variables, Haploview 4.2 was used to estimate the Hardy-Weinberg equilibrium and LD between tag SNPs tested.

For the multivariate analysis, the Wald logistic regression model of multiple steps was used, with a 0.05 cutoff value. The model included independent variables with P values <0.20 in the univariate analysis. When more than 1 tag SNP model had a P value <0.05, the genetic model with the lowest P value was selected.

RESULTS

There were no statistically significant differences between the groups regarding sex, use of pendulum appliance, RME, and premolar extraction. A statistically significant difference was found between the groups regarding the initial length of the central incisor root ($P = 0.001$) and patient age ($P = 0.030$; Table I).

The distribution of *RANKL*, *RANK*, *OPG* genotypes was in Hardy-Weinberg equilibrium in the control group. The Hardy-Weinberg equilibrium is calculated by means of a formula that investigates if the expected allele frequencies are consistent with those observed in the sample studied. It means that the allele frequencies will remain constant for generations.

No statistically significant differences were found in genotypic frequency of polymorphisms in the RANKL gene between the groups (Table II). The LD map for this gene is shown in Figure 3.

For the RANK gene, there was a statitically significant increase in the frequency of allele T of rs12455775 in the group with EARR (recessive model for allele G; $P = 0.006$; Table II). However, the low frequency of the G allele may not allow a reliable result.

Three tag SNPs were not amplified (the PCR reaction did not work, so it was not possible to visualize the genetic results), possibly owing to inappropriate design of primers, so rs11573856, rs7010267, and rs11573901 were disregarded from the analysis. We observed an increase in the frequency of allele A of rs3102724 (additive model: $P = 0.002$; dominant A: $P = 0.001$) in the EARR group. Also, we found an increased frequency of the G allele of rs2875845 (additive model: $P = 0.027$; dominant G: $P = 0.042$) in the EARR group. Moreover, an increased frequency of allele A of rs1032128 (recessive model A: $P = 0.019$) was found in the EARR group. Finally, an increased frequency of C allele of rs3102728 (dominant model C: $P = 0.014$) also was found in the EARR group (Table II).

The clinical variables that presented $P <0.200$ in the univariate analysis were age ($P = 0.030$), PE ($P = 0.086$), IR ($P = 0.001$), RME ($P = 0.177$), and use of pendulum appliance ($P = 0.134$). They were included in the multivariate analysis.

The genetic variables included in the multivariate analysis were: *RANKL:* rs1038434 recessive model for the C allele ($P = 0.198$) and rs931273 additive model ($P = 0.108$); *RANK:* rs4485469 dominant model for the A allele ($P = 0.146$), rs8086340 additive model ($P = 0.105$), rs17069845 dominant model for the T allele ($P = 0.103$), rs12455775 recessive model for the G allele ($P = 0.006$), rs7236060 recessive model for the allele A ($P = 0.085$), rs9951012 dominant model for the G allele ($P = 0.148$), rs12970081 recessive model for the G allele ($P = 0.110$), rs17069898 dominant model for the A allele ($P = 0.160$), and rs4426449 dominant model for the C allele ($P = 0.111$); and *OPG:* rs3102724 dominant model for A allele ($P = 0.001$), rs2875845 additive model ($P = 0.027$), rs1032128

Table I. Results for univariate analysis, considering the clinical variables for individuals with and without EARR

	Control group (n = 160)		*Study group (n = 178)*		*Univariate analysis*	
Patient data	*n*	*%*	*n*	*%*	*P*	*OR (95% CI)*
Initial root length*					0.001	2.41 (1.42-4.11)
<30 mm	135	84.4	123	69.1		
>30 mm	25	15.6	55	30.9		
Age (y)*					0.030	1.61 (1.05-2.49)
<14 y	79	49.4	67	37.6		
>14 y	81	50.6	111	62.4		
Premolar extraction					0.086	1.77 (0.94-3.34)
No	143	89.4	147	82.6		
Yes	17	10.6	31	17.4		
Elastics					0.574	1.20 (0.69-2.10)
No	133	83.1	143	80.3		
Yes	27	16.9	35	19.7		
Sex					0.810	1.054 (0.68-1.61)
Male	74	46.3	80	44.9		
Female	86	53.8	98	55.1		
Pendulum					0.134	0.67 (0.41-1.10)
No	113	70.6	139	78.1		
Yes	47	29.4	39	45.3		
Rapid maxillary expansion					0.177	0.64 (0.35-1.16)
No	130	81.3	155	87.1		
Yes	30	18.8	23	12.9		

*Cutoff points suggested by ROC curves.

recessive model allele A ($P = 0.019$), rs3134057 dominant model for the G allele ($P = 0.074$), and rs3102728 dominant model for the C allele ($P = 0.014$).

After multivariate analysis, IR ($P = 0.001$) and the genetic polymorphisms *RANKL* rs12455775 ($P = 0.012$) and *OPG* rs3102724 ($P = 0.001$) remained associated with EARR. In addition, RME was associated with EARR ($P = 0.030$).

DISCUSSION

It is known that EARR is an undesirable side-effect in orthodontic treatment that, in most cases, has no clinical significance.[40] However, EARR is clinically important when 1-2 mm of the root length is lost.[41] Severe cases are considered when resorption reaches >5.0 mm of the tooth root length and occur in only 1%-5% of patients.[42]

The etiology of EARR is complex, and various mechanical and biological factors may contribute to its occurrence.[43] Isolated or associated factors may contribute to the development of EARR, such as age, type of orthodontic appliance, magnitude and duration of the force, direction of tooth movement, and genetic background.[3]

In the present study, the initial length of the upper central incisor root (reference tooth) and patient age were associated with EARR. Teeth with the longest roots are more prone to pressure in the apical region due to torque caused by a greater displacement.[44] However, EARR might be more harmful in shorter roots than in medium or long roots.[44-46]

EARR has been more related to older patients.[14,47] Those patients usually present narrower and less vascularized periodontal ligament, thicker and less vascularized alveolar bone, and thicker cementum, increasing risk of root resorption.[48,49]

The association between EARR and premolar extraction in patients treated orthodontically has been reported in some studies.[31,35,50] The incisors tend to suffer greater strength and movement during orthodontic retraction. Patients are more prone to EARR after alignment, leveling, and retraction, during which there will be more movement of the incisors to close spaces. It may be for this reason that we failed to find an association of premolar extractions with EARR, which is in accordance with other studies.[2,47] A radiographic monitoring of the patient for a longer period becomes desirable in many cases when the extraction will be performed after 6 months of treatment.

The use of Class II elastics was not different between the groups regarding EARR. However, some authors reported that the use Class II elastics represents a risk factor for EARR,[34,46] because it is a relatively strong force and, sometimes, inconstant.

Table II. Results of univariate analyses of *RANKL*, *RANK*, and *OPG* tag SNPs between control (n = 160) and study (n = 178) groups

Gene	Tag SNP	Variation (1/2)	Sample genotyped (%)	Genetic model	Group	Genotype (%)			Univariate	
						Homozygous 1	Heterozygous	Homozygous 2	P	OR (95% CI)
RANKL	rs1038434	C/T	95.3	Additive	Control	84 (53.9)	60 (38.2)	13 (8.3)	0.254	
					Study	100 (60.6)	53 (32.1)	12 (7.3)		
				Dominant 1	Control	144 (91.7)		13 (8.3)	0.950	1.15 (0.51-2.60)
					Study	153 (92.7)		12 (7.3)		
				Recessive 1	Control	84 (53.9)	73 (46.5)		0.198	1.34 (0.86-2.08)
					Study	100 (60.6)	65 (39.4)			
RANKL	rs12585229	C/T	98.8	Additive	Control	102 (64.2)	48 (30.2)	9 (5.7)	0.213	
					Study	122 (69.7)	47 (26.9)	6 (3.4)		
				Dominant 1	Control	150 (94.3)		9 (5.7)	0.325	1.69 (0.59-4.86)
					Study	169 (96.6)		6 (3.4)		
				Recessive 1	Control	102 (64.2)	57 (35.8)		0.280	1.286 (0.814-2.03)
					Study	122 (69.7)	53 (30.3)			
RANKL	rs3742257	C/T	96.4	Additive	Control	52 (33.5)	67 (43.2)	36 (23.3)	0.642	
					Study	55 (32.2)	85 (49.7)	31 (18.1)		
				Dominant 1	Control	119 (76.8)		36 (23.3)	0.255	1.37 (0.80-2.34)
					Study	140 (81.9)		31 (18.1)		
				Recessive 1	Control	52 (33.5)	103 (66.5)		0.790	0.93 (0.59-1.49)
					Study	55 (32.2)	116 (67.8)			
RANKL	rs931273	C/T	97.3	Additive	Control	92 (58.6)	54 (34.4)	11 (7.0)	0.108	
					Study	114 (66.3)	51 (29.7)	7 (4.1)		
				Dominant 1	Control	146 (93.0)		11 (7.0)	0.242	1.78 (0.67-4.70)
					Study	155 (95.9)		7 (4.1)		
				Recessive 1	Control	92 (58.6)	65 (41.4)		0.150	0.84 (0.89-2.17)
					Study	114 (66.3)	58 (33.7)			
RANK	rsrs7233197	T/C	92.3	Additive	Control	1 (0.7)	28 (19.3)	116 (80.0)	0.643	
					Study	0 (0)	31 (18.6)	136 (81.4)		
				Dominant 1	Control	29 (20.0)		116 (80.0)	0.748	0.912 (0.52-1.60)
					Study	31 (18.6)		136 (81.4)		
				Recessive 1	Control	1 (0.7)	144 (99.3)		0.465	0.463 (0.41-0.52)
					Study	0 (0)	167 (100)			
RANK	rs4941125	A/G	96.4	Additive	Control	71 (45.5)	65 (41.7)	20 (12.8)	0.912	
					Study	77 (45.3)	73 (42.9)	20 (11.8)		
				Dominant 1	Control	136 (87.2)		20 (12.8)	0.772	1.03 (0.57-2.14)
					Study	150 (88.2)		20 (11.8)		
				Recessive 1	Control	71 (45.5)	85 (54.5)		0.968	0.99 (0.64-1.53)
					Study	77 (45.3)	93 (54.7)			
RANK	rs4485469	A/G	84.9	Additive	Control	45 (33.6)	54 (40.3)	35 (26.1)	0.349	
					Study	53 (34.6)	71 (46.4)	29 (19.0)		
				Dominant 1	Control	99 (73.9)		35 (26.1)	0.146	1.51 (0.86-2.64)
					Study	124 (81.0)		29 (19.0)		
				Recessive 1	Control	45 (33.6)	89 (66.4)		0.850	1.05 (0.64-1.71)
					Study	53 (34.6)	100 (65.4)			
RANK	rs4941129	T/C	87.9	Additive	Control	57 (41.0)	62 (44.6)	20 (14.4)	0.759	
					Study	69 (43.7)	66 (41.8)	23 (14.6)		
				Dominant 1	Control	119 (85.6)		20 (14.4)	0.967	0.99 (0.51-1.89)
					Study	135 (85.4)		23 (14.6)		
				Recessive 1	Control	57 (41.0)	82 (59.0)		0.643	1.11 (0.70-1.77)
					Study	69 (43.7)	89 (56.3)			
RANK	rs7237982	A/G	96.2	Additive	Control	93 (60.0)	51 (32.9)	11 (7.1)	0.795	
					Study	105 (61.8)	53 (31.2)	12 (7.1)		
				Dominant 1	Control	144 (92.9)		11 (7.1)	0.989	1.00 (0.43-2.35)
					Study	158 (92.9)		12 (7.1)		
				Recessive 1	Control	93 (60.0)	62 (40.0)		0.745	1.08 (0.69-1.68)
					Study	105 (61.8)	65 (38.2)			

Table II. Continued

Gene	Tag SNP	Variation (1/2)	Sample genotyped (%)	Genetic model	Group	Genotype (%)			Univariate	
						Homozygous 1	Heterozygous	Homozygous 2	P	OR (95% CI)
RANK	rs8086340	C/G	96.4	Additive	Control	16 (10.3)	80 (51.6)	59 (38.1)	0.105	
					Study	28 (16.4)	88 (51.5)	55 (32.2)		
				Dominant 1	Control	96 (61.9)		59 (38.1)	0.265	1.30 (0.82-2.04)
					Study	116 (67.8)		55 (32.2)		
				Recessive 1	Control	16 (10.3)	139 (89.7)		0.110	1.70 (0.88-3.28)
					Study	28 (16.4)	143 (83.6)			
RANK	rs17069845	T/C	94.4	Additive	Control	128 (85.3)	17 (11.3)	5 (3.3)	0.669	
					Study	143 (84.6)	25 (14.8)	1 (0.6)		
				Dominant 1	Control	145 (96.7)		5 (3.3)	0.103	5.79 (0.67-50.16)
					Study	168 (99.4)		1 (0.6)		
				Recessive 1	Control	128 (85.3)	22 (14.7)		0.858	0.94 (0.51-1.75)
					Study	143 (84.6)	26 (15.4)			
RANK	rs12956925	G/A	96.7	Additive	Control	91 (58.7)	54 (34.8)	10 (6.5)	0.469	
					Study	104 (60.5)	62 (36.0)	6 (3.5)		
				Dominant 1	Control	145 (93.5)		10 (6.5)	0.305	1.91 (0.68-5.37)
					Study	166 (96.5)		6 (3.5)		
				Recessive 1	Control	91 (58.7)	64 (41.3)		0.747	1.08 (0.69-1.68)
					Study	104 (60.5)	68 (39.5)			
RANK	rs17720953	A/G	96.7	Additive	Control	3 (1.9)	53 (34.2)	99 (63.9)	0.804	
					Study	5 (2.9)	58 (33.7)	109 (63.4)		
				Dominant 1	Control	56 (36.1)		99 (63.9)	0.925	1.02 (0.65-1.60)
					Study	63 (36.6)		109 (63.4)		
				Recessive 1	Control	3 (1.9)	152 (98.1)		0.726	1.52 (0.36-6.45)
					Study	5 (2.9)	167 (97.1)			
RANK	rs4500848	T/C	97.6	Additive	Control	1 (0.6)	15 (9.5)	142 (89.9)	0.365	
					Study	4 (2.3)	17 (9.9)	151 (87.8)		
				Dominant 1	Control	16 (10.1)		142 (89.9)	0.549	1.24 (0.62-2.46)
					Study	21 (12.2)		151 (87.8)		
				Recessive 2	Control	1 (0.6)	157 (99.4)		0.373	3.73 (0.41-33.80)
					Study	4 (2.3)	168 (97.7)			
RANK	rs12455775	G/T	81.1	Additive	Control	11 (9.2)	21 (17.6)	87 (73.1)	0.782	
					Study	3 (1.9)	47 (30.3)	105 (67.7)		
				Dominant 1	Control	32 (26.9)		87 (73.1)	0.336	1.30 (0.76-2.19)
					Study	50 (32.3)		105 (67.7)		
				Recessive 1	Control	11 (9.2)	108 (90.8)		0.006	0.194 (0.05-0.71)
					Study	3 (1.9)	152 (98.1)			
RANK	rs3826620	T/G	97.0	Additive	Control	17 (11.0)	64 (41.3)	74 (47.7)	0.841	
					Study	22 (12.7)	68 (39.3)	83 (48.0)		
				Dominant 1	Control	81 (52.3)		74 (47.7)	0.966	0.991 (0.64-1.53)
					Study	90 (52.0)		83 (48.0)		
				Recessive 1	Control	17 (11.0)	138 (89.0)		0.625	1.18 (0.603-2.32)
					Study	22 (12.7)	151 (87.3)			
RANK	rs7236060	A/G	89.3	Additive	Control	73 (51.4)	59 (41.5)	10 (7.0)	0.459	
					Study	98 (61.3)	44 (27.5)	18 (11.3)		
				Dominant 1	Control	132 (93.0)		10 (7.0)	0.208	0.60 (0.27-1.34)
					Study	142 (88.8)		18 (11.3)		
				Recessive 1	Control	73 (51.4)	69 (48.6)		0.085	1.49 (0.94-2.36)
					Study	98 (61.3)	62 (38.8)			
RANK	rs9951012	G/A	96.7	Additive	Control	74 (48.1)	68 (44.2)	12 (7.8)	0.158	
					Study	93 (53.8)	73 (42.2)	7 (4.0)		
				Dominant 1	Control	142 (92.2)		12 (7.8)	0.148	2.00 (0.77-5.23)
					Study	166 (96.0)		7 (4.0)		
				Recessive 1	Control	74 (48.1)	80 (51.9)		0.303	1.25 (0.81-1.94)
					Study	93 (53.8)	80 (46.2)			

Table II. Continued

Gene	Tag SNP	Variation (1/2)	Sample genotyped (%)	Genetic model	Group	Genotype (%)			Univariate	
						Homozygous 1	Heterozygous	Homozygous 2	P	OR (95% CI)
RANK	rs6567272	C/T	96.2	Additive	Control	85 (54.5)	70 (44.9)	1 (0.6)	0.339	
					Study	100 (59.2)	69 (40.8)	0 (0.0)		
				Dominant 1	Control	155 (99.4)		1 (0.6)	0.480	2.05 (1.86-2.34)
					Study	169 (100.0)		0 (0.0)		
				Recessive 1	Control	85 (54.5)	71 (45.5)		0.394	1.21 (0.78-1.88)
					Study	100 (59.2)	69 (40.8)			
RANK	rs4524034	A/G	96.2	Additive	Control	89 (57.8)	52 (33.8)	13 (8.4)	0.578	
					Study	101 (59.1)	60 (35.1)	10 (5.8)		
				Dominant 1	Control	141 (91.6)		13 (8.4)	0.363	1.48 (0.63-3.50)
					Study	161 (94.2)		10 (5.8)		
				Recessive 1	Control	89 (57.8)	65 (42.2)		0.816	1.05 (0.68-1.64)
					Study	101 (59.1)	70 (40.9)			
RANK	rs12970081	G/A	97.3	Additive	Control	71 (45.5)	69 (44.2)	16 (10.3)	0.152	
					Study	94 (54.3)	64 (37.0)	15 (8.7)		
				Dominant 1	Control	140 (89.7)		16 (10.3)	0.623	1.20 (0.57-2.52)
					Study	158 (91.3)		15 (8.7)		
				Recessive 1	Control	71 (45.5)	85 (54.5)		0.110	1.42 (0.92-2.20)
					Study	94 (54.3)	79 (45.7)			
RANK	rs8083511	C/A	97.0	Additive	Control	12 (7.7)	53 (34.2)	90 (58.1)	0.747	
					Study	18 (10.4)	54 (31.2)	101 (58.4)		
				Dominant 1	Control	65 (41.9)		90 (58.1)	0.954	0.99 (0.63-1.53)
					Study	72 (41.6)		101 (58.4)		
				Recessive 1	Control	12 (7.7)	143 (92.3)		0.404	1.38 (0.64-2.97)
					Study	18 (10.4)	155 (89.6)			
RANK	rs8099222	C/T	94.7	Additive	Control	13 (8.6)	38 (25.0)	101 (66.4)	0.729	
					Study	16 (9.5)	43 (25.6)	109 (64.9)		
				Dominant 1	Control	51 (33.6.8)		101 (66.4)	0.768	1.07 (0.67-1.70)
					Study	59 (35.1)		109 (64.9)		
				Recessive 1	Control	13 (8.6)	139 (91.4)		0.762	1.12 (0.52-2.42)
					Study	16 (9.5)	152 (90.5)			
RANK	rs7239667	G/C	97.0	Additive	Control	62 (39.7)	60 (38.5)	34 (21.8)	0.317	
					Study	73 (42.4)	71 (41.3)	28 (16.3)		
				Dominant 1	Control	122 (78.2)		34 (21.8)	0.203	1.43 (0.82-2.50)
					Study	144 (83.3)		28 (16.3)		
				Recessive 1	Control	62 (39.7)	94 (60.3)		0.620	1.11 (0.71-1.73)
					Study	73 (42.4)	99 (57.6)			
RANK	rs17069898	A/G	97.0	Additive	Control	58 (37.2)	59 (37.8)	39 (25.0)	0.669	
					Study	59 (34.3)	81 (47.1)	32 (18.6)		
				Dominant 1	Control	117 (75.0)		39 (25.0)	0.160	1.46 (0.86-2.47)
					Study	140 (81.4)		32 (18.6)		
				Recessive 1	Control	58 (37.2)	98 (62.8)		0.587	0.88 (0.56-1.39)
					Study	59 (34.3)	113 (65.7)			
RANK	rs17069902	C/T	97.9	Additive	Control	142 (89.9)	14 (8.9)	2 (1.3)	0.913	
					Study	155 (89.6)	17 (9.8)	1 (0.6)		
				Dominant 1	Control	156 (98.7)		2 (1.3)	0.608	2.20 (0.19-24.56)
					Study	172 (99.4)		1 (0.6)		
				Recessive 1	Control	142 (89.9)	16 (10.1)		0.934	0.97 (0.48-1.97)
					Study	155 (89.6)	18 (10.4)			
RANK	rs8089829	G/A	97.3	Additive	Control	48 (30.4)	63 (39.9)	47 (29.7)	0.894	
					Study	49 (28.7)	76 (44.4)	46 (26.9)		
				Dominant 1	Control	111 (70.3)		47 (29.7)	0.567	1.15 (0.71-1.86)
					Study	125 (73.1)		46 (26.9)		
				Recessive 1	Control	48 (30.4)	110 (69.6)		0.732	0.92 (0.57-1.48)
					Study	49 (28.7)	122 (71.3)			

Table II. Continued

Gene	Tag SNP	Variation (1/2)	Sample genotyped (%)	Genetic model	Group	Genotype (%)			Univariate	
						Homozygous 1	Heterozygous	Homozygous 2	P	OR (95% CI)
RANK	rs17069904	G/A	95.6	Additive	Control	115 (73.7)	37 (23.7)	4 (2.6)	0.504	
					Study	126 (77.2)	34 (20.4)	4 (2.4)		
				Dominant 1	Control	152 (97.4)		4 (2.6)	1.000	1.07 (0.26-4.36)
					Study	163 (97.6)		4 (2.4)		
				Recessive 1	Control	115 (73.7)	41 (26.3)		0.461	1.21 (0.73-2.01)
					Study	126 (77.2)	38 (22.8)			
RANK	rs12959396	T/G	97.3	Additive	Control	38 (24.2)	77 (49.0)	42 (26.8)	0.281	
					Study	46 (26.7)	90 (52.3)	36 (20.9)		
				Dominant 1	Control	115 (73.2)		42 (26.8)	0.215	1.38 (0.83-2.30)
					Study	136 (79.1)		36 (20.9)		
				Recessive 1	Control	38 (24.2)	119 (75.8)		0.598	1.14 (0.69-1.88)
					Study	46 (26.7)	126 (73.3)			
RANK	rs4426449	C/T	92.9	Additive	Control	67 (43.2)	63 (40.6)	25 (16.1)	0.297	
					Study	72 (45.3)	71 (44.7)	16 (10.1)		
				Dominant 1	Control	130 (83.9)	88 (56.8)	25 (16.1)	0.111	1.72 (0.88-3.36)
					Study	143 (89.9)	87 (54.7)	16 (10.1)		
				Recessive 1	Control	67 (43.2)			0.714	1.08 (0.70-1.70)
					Study	72 (45.3)				
OPG	rs11573938	C/T	95.0	Additive	Control	129 (83.8)	23 (14.9)	2 (1.2)	0.460	
					Study	143 (85.6)	24 (14.4)	0 (0.0)		
				Dominant 1	Control	152 (98.7)		2 (1.2)	0.229	2.10 (1.87-2.35)
					Study	167 (100.0)		0 (0.0)		
				Recessive 1	Control	129 (83.8)	25 (16.2)		0.643	1.15 (0.63-2.12)
					Study	143 (85.6)	24 (14.4)			
OPG	rs3102724	A/G	82.2	Additive	Control	13 (10.3)	39 (31.0)	74 (58.7)	0.002	
					Study	26 (17.1)	68 (44.7)	58 (38.2)		
				Dominant 1	Control	52 (41.3)		74 (58.7)	0.001	2.30 (1.42-3.73)
					Study	94 (61.8)		58 (38.2)		
				Recessive 1	Control	13 (10.3)	113 (89.7)		0.105	1.79 (0.88-3.66)
					Study	26 (17.1)	126 (82.9)			
OPG	rs11573884	C/G	93.5	Additive	Control	0 (0.0)	20 (13.3)	130 (86.7)	0.984	
					Study	1 (0.6)	20 (12.0)	145 (87.3)		
				Dominant 2	Control	20 (13.3)		130 (86.7)	0.857	0.94 (0.49-1.81)
					Study	21 (12.7)		145 (87.3)		
				Recessive 1	Control	0 (0.0)	150 (100.0)		1.000	0.52 (0.47-0.58)
					Study	1 (0.6)	165 (99.4)			
OPG	rs2875845	G/A	91.1	Additive	Control	2 (1.4)	34 (23.9)	106 (74.6)	0.027	
					Study	7 (4.2)	53 (31.9)	106 (63.9)		
				Dominant 1	Control	36 (25.4)		106 (74.6)	0.042	1.67 (1.02-2.73)
					Study	60 (36.1)		106 (63.9)		
				Recessive 1	Control	2 (1.4)	140 (98.6)		0.185	3.08 (0.63-15.07)
					Study	7 (4.2)	159 (95.8)			
OPG	rs1032128	A/G	94.7	Additive	Control	7 (4.6)	66 (58.9)	79 (52.0)	0.430	
					Study	20 (11.9)	58 (34.5)	90 (53.6)		
				Dominant 1	Control	73 (48.0)		79 (52.0)	0.775	0.94 (0.60-1.45)
					Study	78 (46.4)		90 (53.6)		
				Recessive 1	Control	7 (4.6)	145 (95.4)		0.019	2.80 (1.15-6.82)
					Study	20 (11.9)	148 (88.1)			
OPG	rs3134057	G/A	91.1	Additive	Control	21 (14.3)	66 (44.9)	60 (40.8)	0.157	
					Study	25 (15.5)	86 (53.4)	50 (31.1)		
				Dominant 1	Control	87 (59.2)		60 (40.8)	0.074	1.53 (0.96-2.44)
					Study	111 (68.9)		50 (31.1)		
				Recessive 1	Control	21 (14.3)	126 (85.7)		0.760	1.10 (0.59-2.07)
					Study	25 (15.5)	136 (84.5)			

Table II. Continued

Gene	Tag SNP	Variation (1/2)	Sample genotyped (%)	Genetic model	Group	Genotype (%)			Univariate	
						Homozygous 1	Heterozygous	Homozygous 2	P	OR (95% CI)
OPG	rs1485289	A/G	91.1	Additive	Control	35 (24.3)	73 (50.7)	36 (25.0)	0.405	
					Study	48 (29.3)	78 (47.6)	38 (23.2)		
				Dominant 1	Control	108 (75.0)		36 (25.0)	0.708	1.10 (0.65-1.90)
					Study	126 (76.8)		38 (23.2)		
				Recessive 1	Control	35 (24.3)	109 (75.7)		0.327	1.29 (0.77-2.14)
					Study	48 (29.3)	116 (70.7)			
OPG	rs3134060	A/G	95.0	Additive	Control	128 (84.2)	24 (15.8)	0 (0.0)	0.501	
					Study	149 (88.2)	18 (10.7)	2 (1.2)		
				Dominant 1	Control	152 (100.0)		0 (0.0)	0.500	1.91 (1.72-2.12)
					Study	167 (98.8)		2 (1.2)		
				Recessive 1	Control	128 (84.2)	24 (15.8)		0.304	1.40 (0.74-2.64)
					Study	149 (88.2)	20 (11.8)			
OPG	RS3102728	C/T	88.8	Additive	Control	8 (5.6)	10 (7.0)	125 (87.4)	0.129	
					Study	6 (3.8)	31 (19.7)	120 (76.4)		
				Dominant 1	Control	18 (12.6)		125 (87.4)	0.014	2.14 (1.15-3.97)
					Study	37 (23.6)		120 (76.4)		
				Recessive 1	Control	8 (5.6)	135 (94.4)		0.467	0.67 (0.23-1.98)
					Study	6 (3.8)	151 (96.2)			

We did not observe differences regarding sex, which was consistent with some previous findings.[45,47] On the other hand, other studies showed more EARR in men[12,51] and others in women.[17,52]

Patients who used the RME apparatus of the Haas type also used the pendulum appliance. After the multivariate analysis, RME was associated with EARR. Studies using RME in humans have focused on the association analysis of molars and premolars with EARR.[53] In this study, the association may be explained by the fact that the upper incisors are closest to the intermaxillary suture in development. Therefore, upper central incisors should be evaluated in further studies as the reference tooth.[54]

The use of x-ray has been the best cost-benefit way to diagnose the presence of EARR[35] and therefore used by most authors.[17,34,55] Periapical radiographs are better than panoramic, occlusal, and lateral ones for the study of tooth roots, because this technique presents less radiation, less distortion, and less overlapping of images.[55] However, it has a restricted view and difficulty in standardization, and it is a static method and does not predict resorption outcome.[4,56] Today, it is thought that computed tomography is the best technique to observe EARR, but it is expensive and difficult to perform, and therefore rarely used. It is suggested that patients susceptible to EARR can be detected by periapical x-ray during the first 6 months of treatment.[57] The presence of resorption at the beginning of treatment (or even before) might be a predictor of increased risk to EARR.[58] According to Artun et al,[58] the chances of presenting an incisor with >5.0 mm EARR at the end of treatment is 3 times higher when the patient has >1.0 mm EARR after the 6 months of treatment and 15 times higher when presenting 2.0 mm EARR. If >3.0 mm EARR is identified, the dentist must inform the patient and treatment should be discontinued for 3-4 months, with radiographic monitoring advised.[59]

The use of genetic markers may help to identify patients at higher risk for EARR before starting treatment.[60] In this context, Newman et al,[17] in 1975, were the first to propose a formal genetic basis for EARR. Al-Qawasmi et al[1] showed the association of polymorphisms in the IL-1β gene with EARR in a study involving 35 families. Moreover, a region of chromosome 18 (TNFRSF11A) proved to be linked to EARR.[61] More recently, other research has identified an association between alleles of IL-1β and EARR.[62] Also, the TT genotype in the IL-1α gene was associated with EARR in an American population.[4] And polymorphisms in the vitamin D receptor gene were weakly associated with EARR in a Brazilian population.[33] However, there are few studies attempting to define genetic risk markers (susceptibility/predisposition) to EARR, and they are poorly predictive.

The balance of the relative concentration of RANKL, RANK, and OPG in bone becomes a determinant factor.[18,19,63] Studies have revealed new functions of this triad in other diseases as well, suggesting that, in response to mechanical forces, osteocytes regulate the recruitment of osteoclasts to the site of bone resorption induced by RANKL expression in the osteoblastic

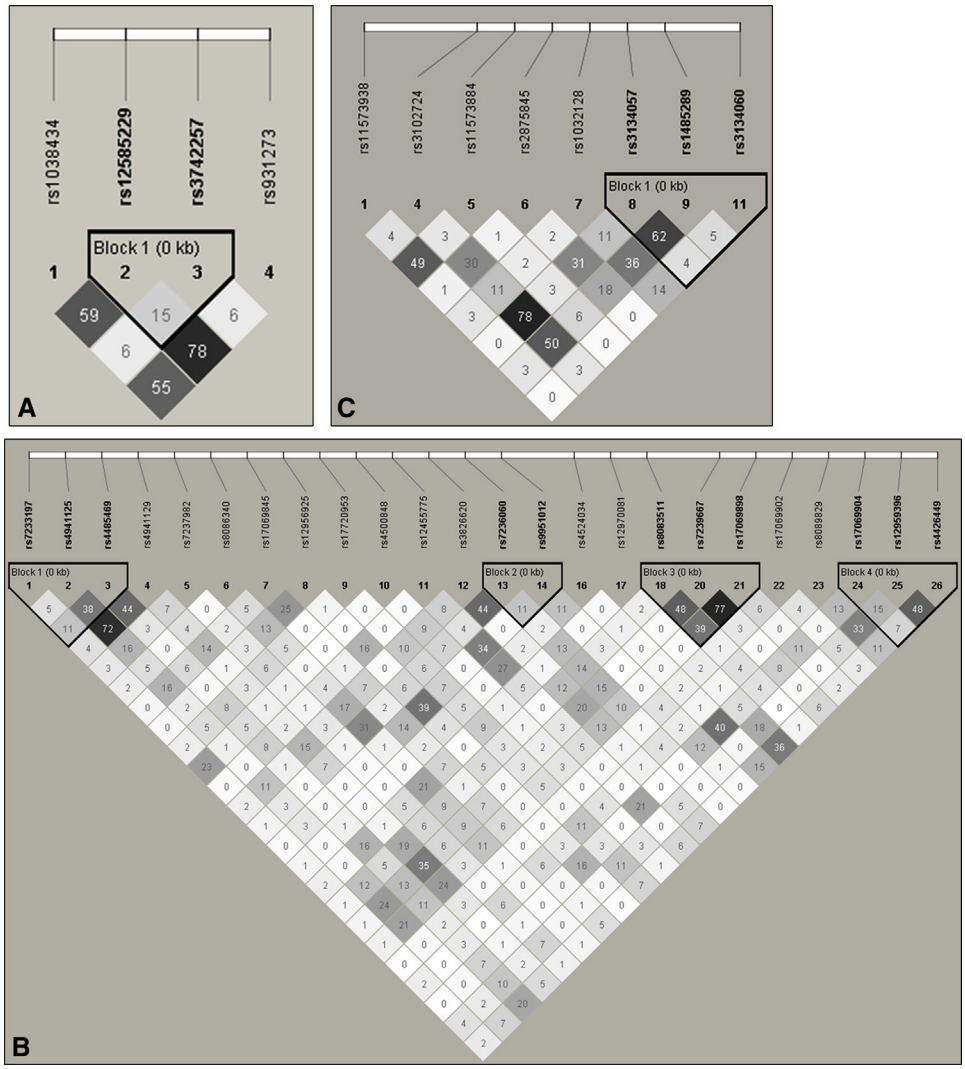

Fig 3. Analysis of linkage disequilibrium between the SNPs in the genes *RANKL* **(A)**, *RANK* **(B)**, and *OPG* **(C)**. The numbers within the squares indicate the LD ratios (%).

cells.[64,65] Pathogenic mechanisms of root resorption seem to be quite similar to those of osteoclastic bone resorption.[66,67] The periodontal ligament environment subjected to orthodontic forces during apical root resorption presents changes in the levels of OPG and RANKL,[68] leading to increased root clastogenesis.

SNPs are the most common forms of DNA variation in the human genome. Recently, several studies have sought a genetic approach with the use of SNPs in LD. Thereby, it is not necessary to genotype all SNPs of a particular gene, but SNP "targets" (tag SNPs) that capture all of the information regarding gene variability. This strategy is intended to capture as much information about the variability of a gene with the use of fewer SNPs, reducing costs and genotyping time.[69]

So far in dentistry, tag SNP polymorphisms in the RANK, OPG, and RANKL genes were associated only with periodontal disease.[64] However, to our knowledge, this is the first study to investigate the association of polymorphisms in the 3 genes of the RANK/RANKL/OPG system with susceptibility to EARR in orthodontically treated patients. Furthermore, we carried out the complete physical mapping of the 3 genes. The RANKL gene polymorphisms have been associated with bone mineral density and bone remodeling diseases where bone loss is a major sign.[70,71] We observed no association of polymorphisms in the RANKL gene with EARR. However, the literature presents many studies that found an association of *RANKL* polymorphisms with bone diseases.[25,72]

Polymorphisms in the RANK gene have been associated with cases of esophageal cancer,[73] rheumatoid arthritis,[74] and other diseases.[75] Other authors sought to associate polymorphisms in the RANK gene with EARR,[54] but found no association. In the present study, which included analysis of polymorphisms representing the entire gene, we found an association of the polymorphism rs12455775 with EARR, with T being the risk allele for EARR. However, even though the association was maintained after the multivariate analysis, the low frequency of the rarest allele might indicate that replication with a greater sample is mandatory. Al-Qawasmi et al[61] reported the association, through a linkage study, between EARR and RANK gene locus in maxillary central incisor in Class I patients. This study suggests that *RANK* is a candidate gene to the predisposition to EARR during orthodontic treatment.

Polymorphisms of the OPG gene have been associated with several diseases, such as periimplantitis,[76] breast cancer,[77] osteoporosis.[78] There was an association of polymorphisms rs3102724, rs2875845, rs1032128, and rs3102728 with EARR, suggesting a strong association of *OPG* with EARR. The rs1032128 SNP was studied by Hsu et al,[79] who found an association of *OPG* with bone mineral density in the lumbar spine. The same polymorphism was also associated with low bone cortical thickness in the radial forearm. In another study, the rs2875845 SNP was not associated with levels of systemic inflammatory biomarkers.[80] Roshandel et al[81] observed that rs3102724 was associated with lower density bone mineral in the distal part of the radius bone. The influence of polymorphisms in the RANKL/RANK/OPG system in the regulation of bone metabolism should be the focus of further studies. After multivariate analysis, rs3102724 remained associated with EARR, showing its influence on the process of EARR. In the future, this gene could be sequenced in patients with extreme phenotypes (severe EARR) for further understanding about the contribution of OPG in the process of resorption.

In relation to the LD map, polymorphisms rs931273 and rs12585229 in *RANKL*, rs7239667 and rs17069898 in *RANK*, and rs3102724 and rs3134057 in *OPG* were in high LD in the population studied, which means that in future studies investigating the genes *RANKL*, *RANK*, *OPG*, only 1 of the 2 aforementioned SNPs for each gene would be necessary to reach the complete gene information.

More studies are needed, including the *OPG* polymorphisms not amplified in this study, with the use of larger samples to elucidate the involvement of these genes in the complex process of EARR. However, some clear signs of association were observed, especially in the OPG gene.

In conclusion, after the multivariate analysis, it was observed that the initial length of the root of maxillary central incisor and RME were associated with EARR in the study population. Regarding the analysis of polymorphisms in the genes *RANKL*, *RANK*, and *OPG*, several SNPs in *RANK* and *OPG* were associated with EARR, but only the association of the rs3102724 in *OPG* remained after multivariate analysis, suggesting that this polymorphism may be proposed as a new marker for EARR susceptibility.

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