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BRUNO BORGES DE CASTILHOS

VARIÁVEIS CLÍNICAS E POLIMORFISMOS NOS GENES DO RANKL/ RANK E OPG E A SUSCETIBILIDADE À REABSORÇÃO RADICULAR APICAL EXTERNA

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Tese apresentada ao Programa de Pós-Graduação em Odontologia Área de concentração em Ortodontia da Pontifícia Universidade Católica do Paraná, como requisito parcial à obtenção do título de Doutor em Odontologia.

Orientadora: Profa. Dra. Paula Cristina Trevilatto
Co-Orientador: Prof. Dr. Cleber Machado de Souza

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Curitiba, 02 de outubro de 2015.

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ARTIGO EM PORTUGUÊS

VARIÁVEIS CLÍNICAS E POLIMORFISMOS NOS GENES DO RANKL/ RANK E OPG E A SUSCETIBILIDADE À REABSORÇÃO RADICULAR APICAL

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RESUMO

Introdução: A identificação de fatores implicados na iniciação e progressão da reabsorção radicular apical externa (RRAE) durante o tratamento ortodôntico tem sido o foco de inúmeros estudos. Os recentes avanços no conhecimento da biologia de células ósseas demonstraram o papel fundamental do Ligante do Receptor Ativador do Fator Nuclear kappa B (RANKL), Receptor Ativador do Fator Nuclear kappa B (RANK) e da Osteoprotegerina (OPG) no sistema de diferenciação e função dos osteoclastos. Polimorfismos nos genes do RANKL/ RANK e OPG têm sido relacionados com condições patológicas, como: osteoporose, artrite reumatoide perda de densidade mineral óssea, câncer e periodontite agressiva. **Objetivo:** Investigar a associação das variáveis ambientais e polimorfismos nos genes do RANKL/ RANK e OPG (*RANKL/ RANK/ OPG*) com a RRAE. **Método:** A amostra deste estudo foi composto de 338 pacientes caucasoides aparentados, de ambos os sexos, média de idade 14,9 anos (8 a 21 anos), com maloclusão Classe II 1ª divisão, que foram submetidos ao tratamento ortodôntico. Radiografias periapicais dos incisivos centrais com as raízes mais longas (dentes de referência) foram tomadas no pré-tratamento e seis meses após o início do tratamento. A análise dos polimorfismos do gene do RANKL/ RANK/ OPG foi realizada pela técnica de PCR em tempo real. Análises univariadas e multivariadas foram realizadas para verificar a associação de variáveis clínicas e genéticas com a RRAE (p<0,05). **Resultados**: O maior comprimento inicial da raiz e a maior idade do paciente (>14 anos) mostraram-se associados à RRAE na análise univariada. Nenhuma associação estatisticamente significante foi encontrada de polimorfismos no gene *RANKL* com a RRAE. No gene *RANK* houve associação do polimorfismo rs12455775 e no gene OPG encontramos associação dos polimorfismos rs3102724, rs2875845, rs1032128 e rs3102728. Após a análise multivariada, as variáveis comprimento inicial da raiz e expansão rápida da maxila, bem como o rs3102724 do gene *OPG* foram associadas com a RRAE. **Conclusão**: As variáveis comprimento inicial da raiz do incisivo central com maior comprimento radicular e a expansão rápida da maxila, bem como o polimorfismo rs3102724 do gene *OPG* foram associadas com a RRAE na população estudada.

Palavras-chave: RANKL, RANK, OPG, movimentação dentária, reabsorção da raiz, maloclusão de Angle Classe II

INTRODUÇÃO

As reabsorções dentárias foram descritas pela primeira vez por Michael Blum, em um livro ilustrado sobre a arte e a ciência da cirurgia odontológica, publicado em 1530 na Alemanha.[1] Mas, em 1927, Ketchan et al. relataram sobre a reabsorção radicular apical externa (RRAE).[2,3] A RRAE situa-se entre os mais comuns e indesejáveis efeitos colaterais do tratamento ortodôntico.[4-11] Estudos têm como objetivo descobrir os diversos fatores relacionados à movimentação ortodôntica e à RRAE, mas essa questão está pouco esclarecida e não é possível prever quem irá desenvolvê-la.[9,12] A frequência da RRAE severa (>3,0mm) durante o tratamento ortodôntico é relatada em 5% a 18% dos casos.[13,14]

Alguns pesquisadores relatam que a aplicação de forças ortodônticas induz um processo local, que possui todas as características da inflamação.[10,11] Essa inflamação é essencial para a movimentação dentária, sendo também o principal componente responsável pelo processo de reabsorção radicular.[10,15] A identificação de fatores implicados na iniciação e progressão da RRAE durante o tratamento ortodôntico tem sido o foco de inúmeros autores. Estes fatores incluem: gênero do paciente,[16] forma radicular,[17] dentes traumatizados previamente ao tratamento ortodôntico,[18] dentes tratados endodonticamente,[19] idade do paciente, estágio de formação radicular no início do tratamento ortodôntico,[16] o tipo do aparelho utilizado,[20] forças aplicadas,[21] duração do tratamento[17] e *background* genético.[6,9,12,22,23]

A grande dificuldade ao avaliar as causas da RRAE é separar as contribuições referentes à genética dos fatores ambientais.[22] Newman et al.[24], foram os primeiros a relatar uma base genética para a RRAE, mas ainda era pouco esclarecida. Harris et al.[9], estimaram o peso relativo do fator hereditariedade através de modelo genético utilizando pares de irmãos. A primeira descrição de um marcador genético que identificava indivíduos mais propensos à RRAE foi relatada por Al Qawasmi et al.[5] Nesse estudo, um polimorfismo no gene da interleucina-1β (*IL1B*$^{+3954}$) foi associado à RRAE durante o tratamento ortodôntico. Mas, ainda assim, não existem marcadores genéticos validados para prever quais pacientes poderão desenvolver RRAE após a movimentação ortodôntica.[25]

Os recentes avanços no conhecimento da biologia de células ósseas demonstram o papel fundamental do receptor ativador do fator nuclear kappa B

(RANK), do ligante do receptor ativador do fator nuclear kappa B (RANKL) e da osteoprotegerina (OPG) no sistema de diferenciação e ativação dos osteoclastos.[26] Entre estes biomarcadores, tanto o RANKL quanto a OPG têm mostrado serem os reguladores-chave da remodelação óssea durante o movimento ortodôntico. O RANKL, produzido por osteoblastos do estroma e células T ativadas, é o fator essencial para a formação, a fusão, a ativação e a sobrevivência de osteoclastos, o que resulta em reabsorção óssea e perda óssea.[27] O RANKL é ativado pelo seu receptor especifico, RANK, localizado em células precursoras osteoclásticas e células dendríticas. Os efeitos do RANKL são neutralizados pela OPG produzida por osteoblastos, células hematopoiéticas e células do sistema imunológico, no qual atua como um receptor solúvel de neutralização, produzindo assim inibição de fases terminais de diferenciação de osteoclastos, supressão da ativação da matriz osteoclástica e a indução de apoptose.[28] Dessa forma, a remodelação óssea é controlada por um equilíbrio entre RANK-RANKL e OPG.

Embora existam muitos estudos experimentais em humanos e animais sobre a associação de RANKL e OPG com remodelação do osso e raiz em algum momento da movimentação ortodôntica, nada se sabe sobre as variações nos níveis desses dois marcadores biológicos através das diferentes fases do tratamento ortodôntico.[29] RANK, RANKL e OPG estão envolvidos em condições fisiopatológicas do metabolismo ósseo. A reabsorção óssea é um importante fator patológico em doenças inflamatórias crônicas, como a periodontite, osteoporose e artrite.[30,31] Um desequilíbrio na proporção RANKL-OPG é fundamental para iniciar a perda óssea associada a essas condições.[32]

Os odontoclastos, responsáveis pela reabsorção de tecidos duros dentais, possuem características morfológicas e funcionais semelhantes aos osteoclastos envolvidos na reabsorção óssea[33], ambos diferenciando a partir de células progenitoras hematopoiéticas da medula óssea e de partilha de várias vias moleculares.[34] No ser humano, a citocina RANKL é o produto de um gene que se encontra no braço longo do cromossomo 13, na região 13q14.[35] O gene *RANKL* é composto por oito exons e introns intermediários e possui uma extensão aproximadamente de 58 kilobases (kb).[36] RANK é uma glicoproteína transmembranar de tipo I, cujo gene está localizado na região do cromossomo 18q22.1, com uma extensão de aproximadamente 80 kilobases e composto por 12 exons e 13 intros. A OPG é codificada por um único gene no cromossomo localizado

na região 8q24, composto por 5 exons e 6 introns.[37]

Polimorfismos, que são alterações genéticas frequentes na população, referem-se à existência de dois ou mais alelos em um determinado *locus* com uma frequência maior do que 1% em uma população.[38] Polimorfismos nos genes do RANKL/ RANK e OPG têm sido relacionados com condições patológicas, como: osteoporose[39,40], artrite reumatoide[30], perda de densidade mineral óssea[41] e periodontite agressiva.[31]

Até o presente momento, nenhum estudo investigou as os polimorfismos do tipo TagSNP no gene do RANKL, RANK e OPG e sua associação com a RRAE.

OBJETIVO GERAL

O objetivo deste estudo foi investigar a associação de variáveis clínicas e polimorfismos nos genes do RANKL, RANK e OPG (*RANKL/ RANK/ OPG*) com a reabsorção radicular apical externa.

OBJETIVOS ESPECÍFICOS

- Investigar as variáveis demográficas e clínicas: gênero, idade, comprimento inicial da raiz do incisivo central, extração de pré-molar, uso de aparelho pêndulo, expansão rápida da maxila, uso de elásticos e sua associação com a reabsorção radicular apical externa.
- Analisar a associação de polimorfismos (tagSNPs) no gene *RANKL/ RANK/ OPG* com a reabsorção radicular apical externa.
- Definir os *bins* (blocos gênicos em alto desequilíbrio de ligação) do gene *RANKL*, *RANK* e *OPG* na amostra de pacientes do sul e sudeste do Brasil.

MATERIAL E MÉTODOS

População de estudo

Esse estudo é transversal baseado em populações caso-controle. A amostra deste estudo foi composta de 338 pacientes caucasoides não-aparentados, de ambos os gêneros, média de idade de 14,9 anos (8 a 21 anos), com maloclusão Classe II, 1ª divisão. Os pacientes foram tratados ortodonticamente, por meio das técnicas *Edgewise* ou *Straight-Wire*. A escolha da maloclusão Classe II, 1ª divisão foi devida a este tipo de maloclusão ser uma das mais frequentes e que exige maior tempo de tratamento[42], além do fato de que pode levar a níveis mais elevados de RRAE.[43,44] Os pacientes foram selecionados nos anos de 2008 /2009, dos prontuários das Clínicas Odontológicas do Curso de Pós-Graduação em Ortodontia – Universidade de São Paulo (Bauru-SP), do Curso de Pós-Graduação em Ortodontia do Instituto Thum de Investigação (Joinville-SC) e de duas clínicas privadas de Ortodontia (Curitiba-PR). Embora a amostra do estudo seja composta por caucasóides, a população brasileira branca é heterogênea. Artigos não têm recomendado agrupar brasileiros com base na etnia, cor e origem geográfica, porque os indivíduos brasileiros classificados como brancos ou negros apresentam sobreposição de genótipos, devido à miscigenação.[45]

Os pacientes preencheram questionários com histórico pessoal, médico e odontológico, e assinaram o termo de consentimento livre e esclarecido, após serem avisados da natureza do estudo (aprovado pelo Comitê de Ética em Pesquisa da PUCPR, protocolo n° 546/05). Os pacientes não poderiam ter: uso crônico de anti-inflamatórios, infecção por HIV, história de quimioterapia imunossupressora, qualquer doença que comprometesse gravemente a função imunológica, gravidez ou lactação, trauma oral, comportamento parafuncional observável, tratamento endodôntico e extensas lesões de cárie nos incisivos centrais superiores, e dentes sem completa formação radicular.

Radiografias periapicais dos incisivos centrais com as raízes mais longas (dente referência) foram tomadas no pré-tratamento e seis meses após o início do tratamento. O método de avaliação consistiu na mensuração dos comprimentos de raiz e coroa diretamente nas radiografias (Fig. 1a, b). O ápice da raiz, a borda incisal e a junção amelocementária (JAC) de cada dente foram demarcados nas películas de raios-x sobre um negatoscópio. O eixo longitudinal de cada dente foi determinado

a partir do ápice radicular até a mediana da borda incisal, seguindo o canal radicular com a maior precisão possível. Um eixo perpendicular foi então projetado ao eixo longitudinal do lado mesial ao distal da JAC. O comprimento da coroa foi medido a partir da borda incisal até o eixo perpendicular, e o comprimento da raiz, do eixo perpendicular da JAC até o ápice radicular (Fig. 1a, b). A diferença resultante entre as medidas de pré-tratamento e seis meses após o início do tratamento pode indicar a presença de RRAE. Um fator de correção (FC) foi calculado: FC = C1/C2 (C1 é o comprimento da coroa no pré-tratamento, C2 é o comprimento da coroa 6 meses após o início do tratamento). Então, a RRAE foi calculada utilizando a seguinte fórmula: R1- RRAE = (R2xFC); R1 é o comprimento da raiz no pré-tratamento e R2 é o comprimento da raiz de 6 meses após o início do tratamento. RRAE também foi expressa como uma porcentagem do comprimento da raiz original: RRAE x 100/R1.

Qualquer distorção entre o pré-tratamento e a imagem radiográfica de acompanhamento foi corrigido utilizando o registro do comprimento da coroa, assumindo que o comprimento da coroa foi imutável durante o período de observação.[46,47] A RRAE foi avaliada por um único examinador (MLSSNF). As radiografias foram examinadas sobre um negatoscópio e as medições foram feitas com a ponta do paquímetro digital com precisão de até 0,02 mm (UYUSTOOLS Professional, Paquímetro Digital Eletrônico, EUA) (Fig. 2).

O valor de 1,43 mm foi estabelecido por meio da construção de uma curva de características de operação do receptor (ROC), com base na distribuição dos valores de RRAE da própria amostra. Dessa forma, a amostra foi dividida em dois grupos:

Grupo 1: 160 indivíduos sem RRAE e com RRAE ≤ 1,43 milímetros.
Grupo 2: 178 indivíduos com RRAE > 1,43 milímetros.

Parâmetros clínicos

Os seguintes parâmetros foram avaliados: gênero, idade, comprimento inicial da raiz do dente de referência (RI), extração de pré-molar (EP), uso de aparelho pêndulo, expansão rápida da maxila (ERM) e uso de elásticos (EX).

Coleta e purificação do DNA

As células foram obtidas por meio de um bochecho com solução de glicose a 3% durante um minuto e raspagem da mucosa bucal com uma espátula esterilizada.[48] O DNA foi extraído a partir de células epiteliais bucais com acetato de amônio a 10 M e EDTA 1 mM.[49]

Análise de polimorfismos nos genes RANKL / RANK / OPG

Os tagSNPs dos genes *RANKL, RANK e OPG* foram selecionados, de acordo com a informação disponível no site *International HapMap Project, phase III/Rel#2* (http//www.hapmap.org, 2012). Todos os marcadores selecionados (42) apresentaram frequência alélica mínima (FAM) de 0,05 na população CEU (residentes de Utah com ascendência europeia do norte e oeste). O parâmetro de corte para definir DL entre dois marcadores foi r^2>0,8. Seguindo estes critérios, os seguintes tagSNPs foram selecionados:

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.

OPG: rs11573938, rs3102724, rs11573884, rs2875845, rs1032128, rs3134057, rs1485289, rs3134060, RS3102728, rs11573856, rs7010267, rs11573901

Os pacientes foram genotipados para os tagSNPs por meio da técnica de PCR em tempo real (Applied Biosystems 7500 Real-Time PCR System), com o uso da tecnologia TaqMan™ (Applied Biosystems).[50] Foi utilizado controle negativo em todas as genotipagens realizadas.

Análise estatística

Variáveis categóricas foram expressas por frequências e porcentagens. Ajustes da curva ROC foram feitas para RRAE, idade e comprimento inicial radicular, com o objetivo de determinar pontos de corte associados à RRAE. As comparações entre os grupos em relação às variáveis categóricas dicotômicas foram comparadas utilizando-se o teste do Qui-quadrado de Pearson ou exato de Fisher, quando indicado. Para as variáveis categóricas dicotômicas que apresentaram diferença

estatisticamente significante foi aplicado teste de *Odds Ratio* para estimativa de risco. A regressão logística foi utilizada para o modelo genético aditivo. As análises foram realizadas com o software estatístico IBM® SPSS® 21. Resultados com valor de *p* abaixo de 0,05 foram considerados significativos. Exclusivamente para as variáveis genéticas, foi utilizado o Haploview® 4.2 para estimar o equilíbrio de Hardy-Weinberg e o desequilíbrio de ligação entre os tagSNPs testados.

Para a análise multivariada, foi utilizado o modelo de regressão logística de múltiplos passos de Wald, com valor de corte de 0,05. O modelo incluiu as variáveis independentes que apresentaram valores de $p<0,20$ na análise univariada. Quando o tagSNP apresentou mais de um valor de *p* abaixo de 0,05, foi selecionado o modelo genético com menor valor de *p*.

RESULTADOS

Parâmetros clínicos

Todos os pacientes foram elegíveis. Não houve diferenças estatisticamente significativas observadas entre os grupos em relação ao gênero, uso de aparelho de pêndulo, uso de elásticos, expansão rápida da maxila e extração de pré-molar. Diferença estatisticamente significante foi encontrada entre os grupos em relação ao comprimento inicial da raiz do incisivo central (p=0,001) e a idade dos pacientes foram associados à RRAE (p=0,030) (Tabela I).

Análise de polimorfismos nos genes RANKL/ RANK/ OPG

A distribuição de genótipos do *RANKL* está em equilíbrio de Hardy-Weinberg, o que mostra que a genotipagem está confiável e as frequências genotípicas esperadas (estimadas pelo binômio de Newton) estão de acordo com as frequências genotípicas observadas. Nenhuma diferença estatisticamente significante foi encontrada na frequência genotípica dos polimorfismos do gene *RANKL* entre os grupos (Tabela II), ou seja, não houve associação de nenhum polimorfismo com a RRAE, nem no sentido de suscetibilidade (predisposição) nem no sentido de proteção. O mapa de desequilíbrio de ligação para a população estudada pode ser observado na figura 3 (a,b,c).

A distribuição de genótipos do *RANK* está em equilíbrio de Hardy-Weinberg. Houve aumento da frequência do alelo T do rs12455775 no grupo com RRAE (modelo recessivo G p=0.006) do gene *RANK* (Tabela II), ou seja, houve associação do polimorfismo (G/T intron 2) com a RRAE.

A distribuição de genótipos do *OPG* está em equilíbrio de Hardy-Weinberg. Neste gene, três polimorfismos tagSNPs não foram amplificados, possivelmente por desenho inapropriado dos *primers*, sendo os rs11573856, rs7010267, rs11573901 desconsiderados da análise. Houve aumento da frequência do alelo A do rs3102724 A/G intron no grupo com RRAE (modelo aditivo p=0.002 e dominante A p=0.001), aumento da frequência do alelo G do rs2875845 G/A - intron (modelo aditivo p=0.027 e dominante G p =0.042), aumento da frequência do alelo A do rs1032128 A/G - intron (modelo recessivo A p=0.019) e aumento da frequência do alelo C do rs3102728 C/T – intron (modelo dominante C p=0.014) do gene *OPG* (Tabela II), ou seja, houve associação de polimorfismos no gene *OPG* com a RRAE.

Análise Multivariada

Para a análise multivariada, foram consideradas as variáveis com valor de $p<0,200$ (idade: $p=0,030$, EP: $p=0,086$, RI: $p=0,001$, ERM: $p=0,177$, PENDEX: $p=0,134$). No gene do RANKL, rs1038434 modelo recessivo para o alelo T: $p=0,198$, rs931273 modelo aditivo: $p=0,108$. No gene do RANK, rs4485469 modelo dominante para o alelo A: $p=0,146$, rs8086340 modelo aditivo: $p=0,105$, rs17069845 modelo dominante para o alelo T: $p=0103$, rs12455775 modelo recessivo para o alelo T: $p=0,006$, rs7236060 modelo recessivo para o alelo A: $p= 0,085$, rs9951012 modelo dominante para o alelo G: $p=0,148$, rs12970081 modelo recessivo para o alelo A: $p=0,110$, rs17069898 modelo dominante para o alelo A: $p=0,160$, rs4426449 modelo dominante para o alelo C: $p=0,111$. Para o gene da OPG, rs3102724 modelo dominante para o alelo A: $p=0,001$, rs2875845 modelo aditivo: $p=0,027$, rs1032128 modelo recessivo para o alelo G: $p=0,019$, rs3134057 modelo dominante para o alelo G: $p=0,074$, rs3102728 modelo dominante para o alelo C: $p=0,014$.

Após a análise multivariada, as variáveis RI: $p=0,001$, polimorfismo *RANKL* rs12455775: $p=0,012$ e polimorfismo *OPG* rs3102724 $p=0,001$ mantiveram-se associadas com RRAE, adicionalmente, a variável ERM associou-se com RRAE $p=0,030$.

DISCUSSÃO

Sabe-se que a RRAE é um efeito secundário indesejável no tratamento ortodôntico e que, na maioria dos casos, ela ocorrerá de forma leve, não tendo significado clínico.[51] No entanto, a RRAE apresenta um aspecto clinicamente importante quando 1 a 2 mm (¼) do comprimento da raiz são perdidos.[52] Casos graves são considerados quando a reabsorção atinge mais que ¼ da raiz do dente (> 3,0 mm) e ocorrem em apenas 1 a 5 % dos pacientes.[53]

A etiologia da RRAE é complexa e vários fatores mecânicos e biológicos podem contribuir para sua ocorrência.[54] Fatores isolados ou associados podem contribuir para o desenvolvimento da RRAE, como idade do paciente, tipo do aparelho ortodôntico, magnitude e duração da força, direção do movimento dentário[55] e *background* genético.[9]

Neste estudo, o comprimento inicial da raiz do incisivo central superior (dente referência) e a idade do paciente foram associados com a RRAE, nas análises univariada e multivariada. A associação do comprimento inicial da raiz dental com a RRAE pode ser explicada pelo fato de o dente com maior raiz provavelmente apresentar maior deslocamento tanto em relação à movimentação apical quanto ao torque.[56] No entanto, a RRAE pode ser mais preocupante em raízes mais curtas que em raízes médias ou longas.[56-58] Foi observada relação entre maior RRAE e pacientes mais velhos.[20,59] Fatores como o ligamento periodontal se tornar mais estreito e menos vascularizado, o osso alveolar se adensar e ser menos vascularizado, e o cemento mais espesso com a idade aumentam o risco à reabsorção radicular.[60,61]

A associação entre extrações de pré-molares e RRAE em pacientes tratados ortodonticamente tem sido relatada em alguns estudos.[4,44,47] Isto pode ser explicado por serem os incisivos os dentes que recebem maior força e movimentação na retração anterior ortodôntica. Pacientes estão mais propenso à RRAE, após as fases de alinhamento, nivelamento e retração anterior, durante as quais ocorrerá maior movimentação dos incisivos para o fechamento dos espaços.[44] Talvez por essa razão, nosso trabalho não encontrou associação da extração de pré-molares com a RRAE, assim como outros autores.[8,59] Como sugestão, o acompanhamento radiográfico do paciente por um período mais longo torna-se desejável, pois em muitos casos a exodontia será realizada após os seis meses de tratamento.

O uso de elásticos de Classe II não se mostrou diferente entre os grupos em relação à RRAE. No entanto, alguns autores[46,58] relataram que o uso de elásticos Classe II consiste em um fator de risco, por ser uma força relativamente intensa e, algumas vezes, intermitente. Assim, poderia constituir maior risco, principalmente no dente de apoio anterior.

Assim como Brezniak et al.[10,60] relataram, não houve diferença entre pacientes quanto ao gênero, o que também foi observado por outros autores[57,59], especialmente em estudos com amostras maiores. No entanto, encontram-se na literatura autores evidenciando maior presença de RRAE tanto em homens[19,62] quanto nas mulheres[24,63], sendo ainda pouco conclusivo afirmar se e qual gênero aumenta a predisposição à RRAE.

Os pacientes dessa amostra que utilizaram o aparelho de expansão rápida da maxila tipo Haas também utilizaram o aparelho pêndulo, sendo que os incisivos não apresentaram maior RRAE durante a ERM e uso do aparelho pêndulo. Estudos em seres humanos têm focado em molares e pré-molares em associação com a RRAE.[64] No entanto, após a análise multivariada, ERM associou-se com a RRAE. Esta associação significativa pode ser explicada pelo fato de que incisivos superiores são os dentes mais próximos da sutura intermaxilar e pelo alto nível de maturação óssea dessa sutura em desenvolvimento. Dessa forma, os incisivos centrais superiores devem ser avaliados em novos estudos, como dente de referência.[65]

O uso do raio-x tem sido a melhor maneira em custo e benefício para diagnosticar a presença de RRAE[47] e o método com radiografias periapicais é o melhor para estudos clínicos de RRAE, por isso tem sido utilizado pela grande maioria dos autores.[2,9,16,18,24,46,66-68] Radiografias periapicais são muito superiores às panorâmicas, oclusais e teleradiografia de perfil para o estudo das raízes dentárias, pois com essa técnica ocorre menor exposição do paciente à radiação, menor distorção e menor sobreposição de imagens.[67] No entanto, ela apresenta pontos negativos, como vista restrita, visão bidimensional, dificuldade na padronização da técnica, além de ser um método estático que não permite afirmar a dinâmica da reabsorção (se em curso ou encerrada).[12,69] Acredita-se que a tomografia computadoriza é a melhor técnica para visualizar a RRAE, mas seu alto custo e dificuldade para realizar, tornam ainda esse método pouco utilizado.[70]

É sugerido que pacientes suscetíveis à RRAE possam ser identificados já no início do tratamento, através do raio-x periapical, realizado após os primeiros 6 meses de tratamento.[71,72] A presença de RRAE no início do tratamento (ou até mesmo antes deste) pode ser um preditor de maior risco de reabsorção durante o tratamento.[73,74] Segundo Artun et al.[74], as chances de apresentar um incisivo com mais de 5,0 mm de RRAE ao final do tratamento é três vezes maior quando aos 6 meses de tratamento o paciente apresenta um incisivo com mais de 1,0 mm de RRAE e 15 vezes maior quando apresenta 2,0 mm de RRAE. Caso a RRAE grave (>3,0mm) seja descoberta, o cirurgião-dentista deve informar ao seu paciente e o tratamento ativo deve ser interrompido durante 3-4 meses e, após isso, o acompanhamento radiográfico deve ser realizado.[71]

A descoberta de marcadores genéticos pode ajudar a identificar pacientes com maior risco à RRAE antes de iniciar o tratamento.[75] Nesse contexto, Newman et al.[24], em 1975, foram os primeiros a propor formalmente uma base genética para a RRAE. Mais tarde, Al-Qawasmi et al.[5] evidenciaram a associação de polimorfismos no gene da IL1-β com RRAE em um estudo envolvendo 35 famílias. Ademais, uma região do cromossomo 18 (TNFRSF11A) mostrou-se ligada com a RRAE.[6] Mais recentemente, outra pesquisa identificou uma associação entre RRAE e alelos do gene da IL1-β.[76] Também, o genótipo TT de um SNP no gene da IL-1α associou-se à RRAE em uma população americana[12] e um polimorfismo no gene do receptor da vitamina D (VDR) foi fracamente associado com a RRAE em uma população brasileira.[68] No entanto, ainda são escassos os estudos tentando definir marcadores de risco genético (suscetibilidade/predisposição) à RRAE e esses ainda são pouco preditivos.

A descoberta do sistema RANKL / RANK / OPG, em meados dos anos 1990, levou a grandes avanços na compreensão da reabsorção óssea. Era conhecido por muitos anos antes desta descoberta que células osteoblásticas do estroma regulavam a formação de osteoclastos.[26] RANKL / RANK regulam a diferenciação e ativação de osteoclastos na remodelação óssea normal e a reabsorção óssea sob uma variedade de condições patológicas, caracterizadas pelo aumento da remodelação óssea. A OPG protege o osso da excessiva reabsorção a partir da ligação com RANKL, assim impedindo-a de se ligar a RANK. Desse modo, a concentração relativa de RANKL e OPG no osso se torna um fator determinante.[26,27,77] Estudos também revelaram novas funções dessa tríade em

outras doenças, sugerindo que, em resposta às forças mecânicas, osteócitos regulam o recrutamento de osteoclastos para a reabsorção óssea, induzindo a expressão de RANKL por células osteoblásticas no micro-ambiente local.[78-80]

Polimorfismos de nucleotídeos únicos (SNPs) são as formas mais comuns de variação do DNA no genoma humano. Recentemente, vários estudos buscam uma abordagem genética que se utiliza de SNPs em desequilíbrio de ligação (DL). Dessa forma, não seria necessário genotipar todos os SNPs de um determinado gene, mas SNPs "alvos" (tagSNP), que capturam toda a informação do gene em termos de variabilidade. Essa estratégia destina-se a capturar o máximo de informação sobre a variabilidade de um gene, com a investigação de menos SNPs, reduzindo custos e tempo de genotipagem.[81]

Até o momento, na Odontologia, polimorfismos tagSNP no gene da RANK/ OPG/ RANKL foram somente associados com a doença periodontal.[80] No entanto, em nosso conhecimento, este é o primeiro estudo a investigar a associação de polimorfismos em genes do sistema RANK / RANKL / OPG com a susceptibilidade à RRAE em pacientes tratados ortodonticamente.

Polimorfismos no gene do RANKL têm sido associados com a densidade mineral óssea, remodelação óssea e doenças onde a perda óssea é um sinal crucial.[82,83] Observou-se que não houve associação de polimorfismos no gene do RANKL com à RRAE, embora a literatura apresente estudos que encontraram polimorfismos no gene do RANKL associados a doenças ósseas.[39,84]

RANK, localizado em células precursoras osteoclásticas e células dendríticas, é responsável pela ativação dos osteoclastos.[26] Polimorfismos no gene do RANK têm sido associados com casos de câncer de esôfago[85], artrite reumatóide[86] e outras doenças.[87] Outros autores procuraram associar polimorfismos no gene do RANK com a RRAE[65], mas não encontraram nenhuma associação. Em nosso estudo, que contemplou a análise de todo o gene com 26 polimorfismos tagSNPs, encontrou-se associação com o polimorfismo, sendo o alelo T o de maior risco para a doença. Na análise multivariada o rs12455775 manteve-se associado com a RRAE. No entanto, mesmo com a associação sendo mantida após a análise multivariada, a baixa freqüência do alelo mais raro pode indicar que a replicação com uma amostra maior, é necessária. Al-Qawasmi et al.[6] relataram a associação, por meio de um estudo de ligação, da RRAE com o *locus* do gene *RANK*, em incisivo central superior em

pacientes Classe I. Esse estudo sugere que o gene do RANK é um marcador genético candidato para a predisposição à RRAE durante o tratamento ortodôntico.

A principal ação biológica da OPG é a inibição da diferenciação dos osteoclastos, a inibição da reabsorção de osteoclastos e a estimulação da apoptose de osteoclastos.[88] Em relação aos polimorfismos do gene da OPG, foram associados com várias doenças, como a perimplantite[89], câncer de mama[90], osteoporose.[91] Neste estudo, houve associação dos polimorfismos rs3102724, rs2875845, rs1032128 e rs3102728 com a RRAE, sugerindo forte associação do gene da OPG com a RRAE, sendo o primeiro trabalho a encontrar tal associação. O rs1032128 foi estudado no trabalho de Hsu et al.[92], no qual os autores encontraram associação do gene *OPG* com a densidade mineral óssea na espinha lombar. O mesmo polimorfismo também foi associado com menor espessura cortical no osso rádio do antebraço.[93] Em outro trabalho, o rs2875845 não foi associado a níveis de biomarcadores inflamatórios sistêmicos.[94] Roshandel et al.[93] observaram que o polimorfismo no rs3102724 foi associado com menor densidade mineral óssea na parte distal do osso rádio. Esses resultados mostram a influência de polimorfismos no sistema RANKL/ RANK/ OPG no controle do metabolismo ósseo, devendo ser foco de novos estudos. Após a análise multivariada, o rs3102724 manteve-se associado com a RRAE, mostrando sua influência sobre o processo da RRAE.

No futuro esse gene poderia ser sequenciado em pacientes com fenótipo extremo, ou seja, com uma RRAE severa em pouco tempo de tratamento ortodôntico.

Os polimorfismos rs931273 e rs12585229 do gene *RANKL*, rs7239667 e rs17069898 do gene *RANK,* rs3102724 e rs3134057 do gene *OPG*, estão em alto desequilíbrio de ligação na população brasileira estudada, o que significa que, em futuros estudos investigando os genes *RANKL, RANK, OPG* e sua associação com doenças e outras condições fisiopatológicas, apenas um dos dois SNPs acima mencionados será necessário para a investigação do gene completo.

Mais estudos são necessários, incluindo os polimorfismos do gene da OPG que não amplificaram, em amostras mais amplas e para a elucidação da participação de variações desses genes no complexo processo de RRAE.

CONCLUSÃO

Observou-se que o maior comprimento inicial da raiz do incisivo central superior e a expansão rápida da maxila foram associados com a reabsorção radicular apical externa na população estudada. Com relação à análise de polimorfismos nos genes *RANKL*, *RANK* e *OPG*, apenas o rs3102724 do gene da *OPG* esteve associado a reabsorção radicular.

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TABELAS

Tabela I Resultados para análises univariadas, considerando as variáveis clínicas para os indivíduos com e sem RRAE.

Dados dos Pacientes	Grupo 1[1] (n = 160)		Grupo 2[2] (n = 178)		Univariada	
					valor de *p*	OR (CI 95%)
	N	%	N	%		
Raiz inicial (mm)[b]						
<30	135	84,4	123	69,1	0,001	2,41 (1,42-4,11)
>30	25	15,6	55	30,9		
Idade (anos)[a]						
< 14 anos	79	49,4	67	37,6	0,030	1,61 (1,05-2,49)
> 14 anos	81	50,6	111	62,4		
EP						
Não	143	89,4	147	82,6	0,086	1,77 (0,94-3,34)
Sim	17	10,6	31	17,4		
Elásticos						
Não	133	83,1	143	80,3	0,574	1,20 (,.69-2,10)
Sim	27	16,9	35	19,7		
Sexo						
Masculino	74	46,3	80	44,9	0,810	1,054 (0,68-1,61)
Feminino	86	53,8	98	55,1		
Pêndulo						
Não	113	70,6	139	78,1	0,134	0.67 (0,41-1,10)
Sim	47	29,4	39	45,3		
ERM						
Não	130	81,3	155	87,1	0,177	0,64 (0,35-1,16)
Sim	30	18,8	23	12,9		

[a] Ponto de corte (14 anos) sugerido pela curva ROC (0.574, p=0.017).

[b] Ponto de corte(30 mm) sugerido pela curva ROC (0.620, p<0.001)

EP- Extração de pré- molar

ERM - Expansão rápida da maxila

Tabela II. Os resultados das análises univariadas de *RANKL/ RANK/ OPG* tagSNPs entre os grupos controle (160) e de estudo (178).

Gene	tag SNP	Variation [1/2]	% de genotyping	Genetic Model	Groups	Genotype (%) Homozygous 1	Heterozygous	Homozygous 2	Univariate *p*-value	OR (CI 95%)
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 (0.70-177)
					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)		
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[1]	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	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	.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)		
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	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	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	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)			
RANK	rs17069904	[G/A]	95,6	Additive	Control	115 (73.7)	37 (23.7)	4 (2.6)	0.504	
					Study	126,2 (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	126,2 (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)		25 (16.1)	0.111	1.72 (0,88-3.36)
					Study	143 (89.9)		16 (10.1)		
				Recessive[1]	Control	67 (43.2)	88 (56.8)		0.714	1.08 (0.70-1.70)
					Study	72 (45.3)	87 (54.7)			

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[1]	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)		
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	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)		

FIGURAS

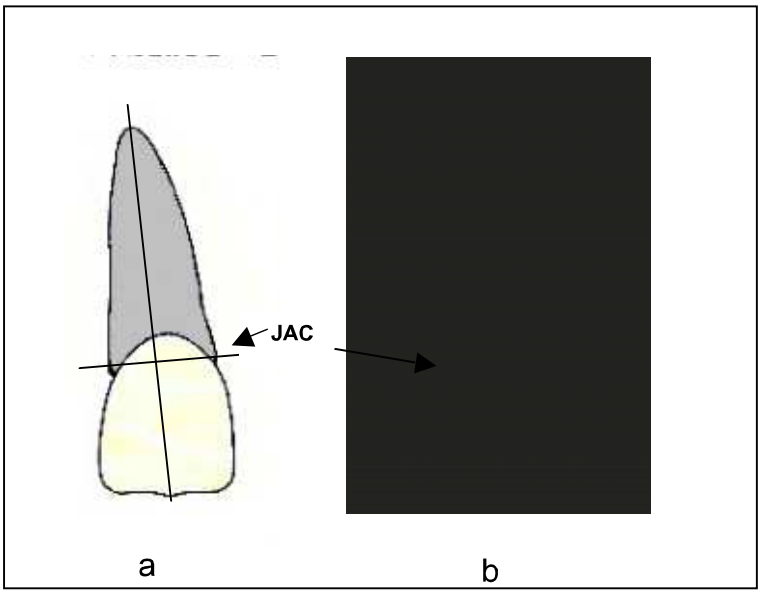
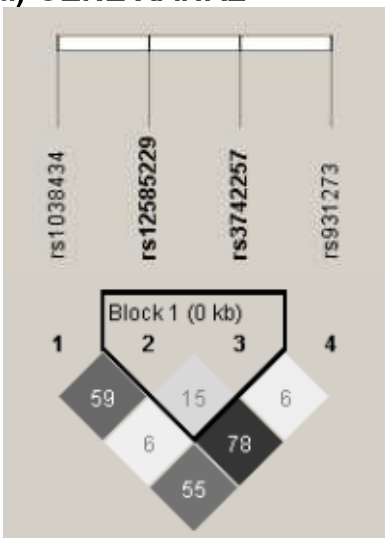


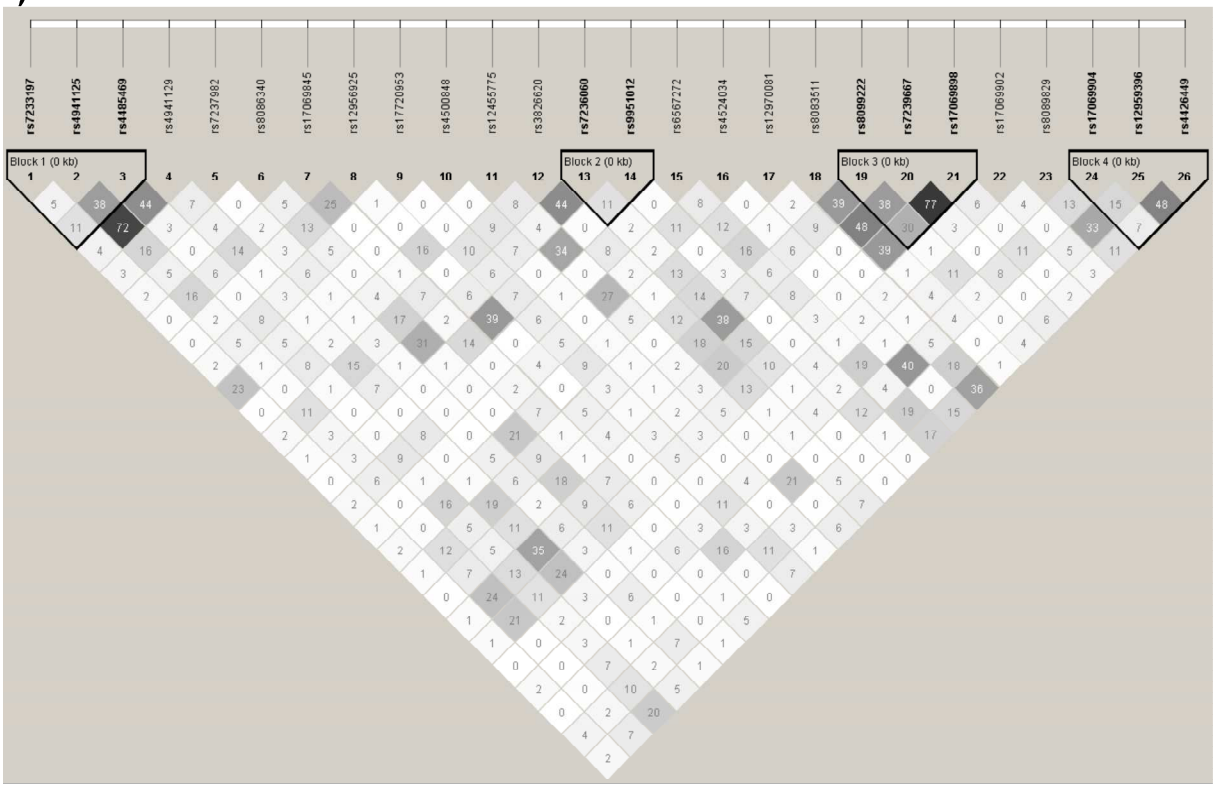
Fig 1. (a) Referências anatômicas para mensurar a RRAE: Junção amelocementária (JAC). (b) Referências para mensuração no raio-x.

Fig 2. Medidas realizadas com o paquímetro digital eletrônico sobre a película de raio-x.

a) GENE *RANKL*



b) GENE *RANK*



c) GENE *OPG*

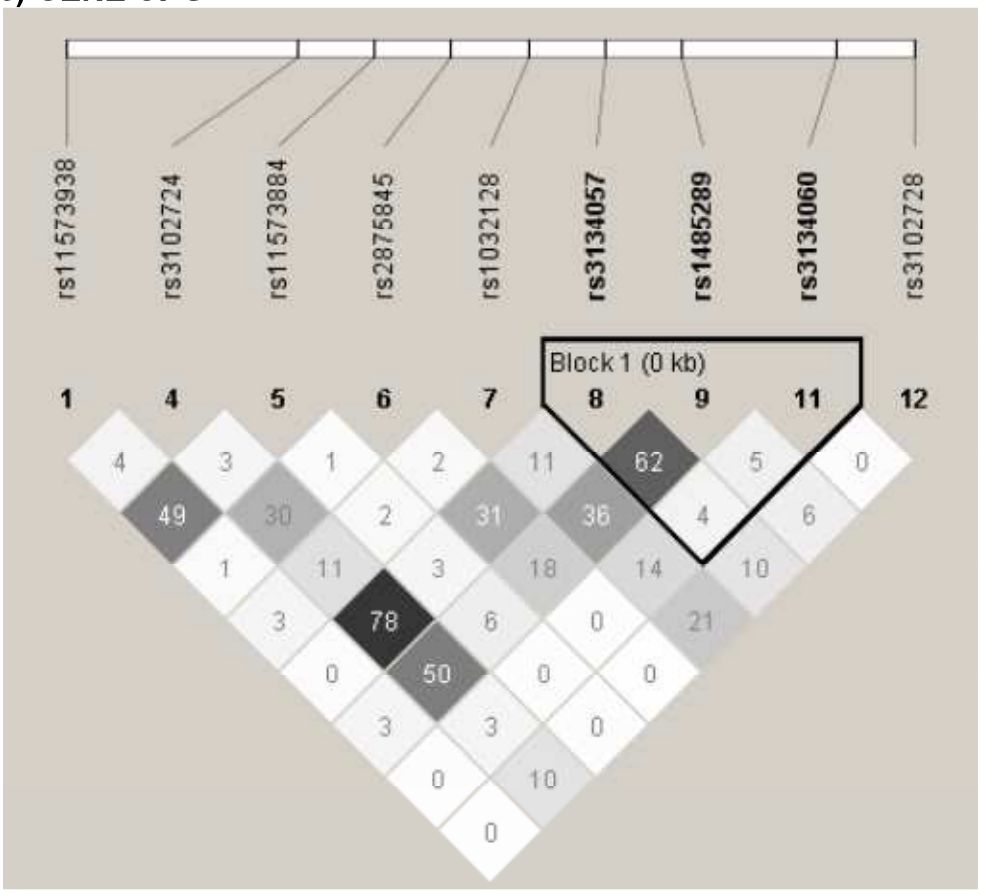


Fig 3. Análise do desequilíbrio de ligação (DL) entre os SNPs no gene do RANKL/ RANK e OPG. O número dentro de quadrados indica a proporção de % em DL. A intensidade da cor dentro dos quadrados reflete o DL entre dois locus, a intensidade mais escura representa o maior DL entre os SNPs

BRUNO BORGES DE CASTILHOS

VARIABLE CLINICAL AND *RANKL* / *RANK* / *OPG* GENE POLYMORPHISMS AND SUSCEPTIBILITY TO EXTERNAL APICAL ROOT RESORPTION

CURITIBA
2015

SUMMARY

ABSTRACT

Introduction: The identification of factors involved in the initiation and progression of external apical root resorption (EARR) during orthodontic treatment has been the focus of many studies. The recent advances in the understanding of bone cell biology have demonstrated the key role of Factor Receptor Activator Nuclear kappa B (RANK), ligant of the receptor activator of nuclear factor kappa B (RANKL), and osteoprotegerin (OPG) in the differentiation and function of osteoclasts and consequently in the bone remodeling. Polymorphisms are variants in genes which can impact on the amount and/or function of the expressed coded proteins. Polymorphisms in genes *RANKL* / *RANK* and *OPG* have been related with pathological conditions, such as osteoporosis, rheumatoid arthritis, loss of bone mineral density, and aggressive periodontitis. Objective: Investigate the association of clinical variables and polymorphisms in genes of the RANKL / RANK and OPG (*RANKL / RANK / OPG*) with external apical root resorption. Method: The sample was composed of 338 Brazilian unrelated patients of both genders, average age 14.9 years (8-21 years) with Class II malocclusion 1st division, orthodontically treated. Periapical radiographs of the central incisor teeth with the longer roots (reference teeth) were taken before treatment and six months after starting treatment. DNA was extracted from buccal epithelial cells with 10 M ammonium acetate and 1 mM EDTA. The analysis of gene polymorphisms in the *RANKL / RANK / OPG* was performed by real time PCR. Univariate and multivariate analyzes were performed to verify the association of clinical and genetic variables with EARR (p<0.05). Results: The greater initial root length and patient age were associated with EARR. Considering the study of polymorphisms of *RANKL* gene, no significant association was found of genetic polymorphisms with EARR. For *RANK* gene polymorphism, only rs12455775 was associated with EARR. Regarding *OPG* gene polymorphisms it was found an association of rs3102724, rs2875845, rs1032128 and rs3102728 with EARR. After multivariate analysis, the variables initial root lengh and the rapid maxillary expansion, as well as rs3102724 of the *OPG* gene were associated with EARR. Conclusion: Longer roots of upper central incisor 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.

Keywords: RANKL, RANK, OPG, tooth movement, external apical root resorption.

INTRODUCTION

Root resorption was first described by Michael Blum, in 1530, in Germany.[1] In 1927, Ketchan et al. started clinical reporting on the external apical root resorption (EARR).[2,3] Today, it is well known that EARR is among the most common and undesirable side effects of orthodontic treatment.[4-11] Several studies have been designed to discover the etiological factors related to EARR, but so far the issue is still unclear and it is difficult to predict who will develop it.[9,12] The frequency of severe EARR during orthodontic treatment is reported to range from 5% to 18% of cases.[13,14]

The application of orthodontic forces induces a local process, with inflammatory characteristics.[10,11] This inflammation is essential for tooth movement, also being the main component responsible for the radicular resorption process.[10,15] 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: gender, radicular anatomy[17], trauma[18], endodontic treatment[19], age, stage of root formation at the beginning of orthodontic treatment[16], type of device[20], forces applied, treatment duration and genetic background.[6,9,12,22,23]

The difficulty in assessing the causes of EARR is to separate the contribution related to the genetics and to the environment.[22]

Newman et al.[24] were the first to report a genetic basis for EARR. Harris et al.[9] 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.[5] In this study, a polymorphism in the interleukin-1β gene (*IL1B* +3954) was associated with EARR during orthodontic treatment. But so far, there are no validated genetic markers to predict which patients will develop EARR after orthodontic treatment.[25]

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.[26] 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.[27] RANKL is activated by its specific receptor, RANK, located on osteoclast precursors and dendritic cells. The effects of RANKL are counteracted by OPG, that is a soluble receptor produced by osteoblasts, hematopoietic and immune cells, which acts inhibiting osteoclast differentiation and inducing apoptosis.[28] Consequently, bone remodeling is dependent of a balance in the RANK/RANKL/OPG system.

Odontoclasts are morphologically similar to osteoclasts both differentiating from hematopoietic progenitor cells of the bone marrow, which share multiple molecular pathways and involved in bone resorption[25].[34] In humans, the cytokine RANKL is coded by a gene located in the long arm of chromosome 13 in the region 13q14.[35] *RANKL* gene is composed of eight exons with about 58 kilobases (kb) .[36] *RANK* is a transmembrane glycoprotein of type I, whose gene is located on chromosome 18q22.1 region, with an extension of about 80 kilobases and composed by exons 12. *OPG* is encoded by a single gene located on chromosome 8q24 region composed of exons 5 and 6 introns.[37]

Polymorphisms refer to the existence of two or more alleles at a particular locus with a frequency greater than 1% in a population.[38] Polymorphisms in *RANKL*/*RANK*/*OPG* genes have been related to pathological conditions, such as osteoporosis[39,40], rheumatoid arthritis[22], mineral density bone loss[33] and agressive periodontitis.[23]

To date, there are no studies investigating the association of polymorphisms in those genes with EARR. Thus, the aim of this study was to investigate the association of clinical variables and polymorphisms in *RANKL*, *RANK* and *OPG* genes with external apical root resorption.

MATERIAL AND METHODS

The sample was composed of 338 unrelated Caucasian patients of both genders, mean age 14.9 years (8-21 years) with Class II malocclusion, division 1. Patients were orthodontically treated, with Edgewise or Straight-Wire techniques. The choice of Angle Class II, division 1, was due to the fact that type of malocclusion is one of the most frequent techniques and requires longer treatment[42], besides the fact that it can lead to higher levels of EARR.[43,44] Patients were selected from the medical records of the Dental Clinics of the Graduate Program in Orthodontics - University of São Paulo (Bauru-SP), Course of Graduate Studies in Orthodontics - Thum Research Institute (Joinville-SC) and two private orthodontic clinics (Curitiba-PR) during the period of 2008 to 2009. Although the study sample is comprised of Caucasians, the white Brazilian population is heterogeneous. Articles have not recommended grouping Brazilians based on ethnicity, color or geographical origin, because Brazilian individuals classified as black or white have overlapping genotypes due to miscegenation.[45]

Patients completed medical and dental questionnaires and signed an informed consent, after being advised of the nature of the study (approved by the Research Ethics Committee of PUCPR, Protocol No. 546/05). Patients could not have: chronic use of anti-inflammatory drugs, HIV infection, immunosuppressive chemotherapy history, any disease that seriously compromise the immune function, pregnancy or lactation, oral trauma, parafuncional behavior, endodontic treatment, extensive carious lesions in maxillary central incisors and teeth without complete root formation.

Periapical radiographs of the central incisor teeth with the longer root (reference tooth) were taken before treatment and six months after the beginning of treatment. The evaluation method consisted of measuring the root and crown length directly on radiographs (Fig. 1a, b). 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 cementoenamel junction sides. The crown length was measured from the incisal edge to the projected cementoenamel junction, and the root length from the projected cementoenamel

junction to the root apex (Fig. 1a, b). The differences between the 2 measurements indicate the EARR. A correction factor was calculated by using the following formula: correction factor = C1/C2 (C1, crown length before treatment; C2, crown length six months after starting treatment). Then EARR was calculated with the following formula: EARR = R1 – (R2 x CF) (R1, root length before treatment; R2, root length six months after treatment start; CF, correction factor). EARR was also expressed as a percentage of the original root length: EARR x 100/R1. Any distortions between the pretreatment and follow-up radiographic images were corrected by using the crown length registration, assuming crown length to be unchanging over the observation period.[38,39] The EARR was evaluated by one examiner (M.L.S.S.N.F). The measurements were made with a fine-tip digital caliper with accuracy up to 0.02 mm (electronic digital vernier caliper; Utustools Professional, Santiago, Chile) (Fig. 2).

The receiver operating characteristic (ROC) curve was constructed to verify the cutoff point based on the sample data distribution for EARR, and the value of 1.43 mm was obtained (ML citAR). The sample was divided into two groups:

Group 1: 160 individuals with EARR ≤ *1.43 mm.*

Group 2: 178 individuals with EARR > *1.43 mm.*

Clinical parameters

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

Collection and DNA purification

Cells were obtained by a mouthwash with 3% glucose solution for 1 minute and by scraping the oral mucosa with a sterile spatula.[48] DNA was extracted from epithelial oral cells with ammonium acetate (10 mol/L) and EDTA (1 mmol/L).[49]

Analysis of polymorphisms in RANKL / RANK / OPG genes

The tagSNPs of *RANKL*, *RANK* and *OPG* genes were selected, according to the information available on the site International HapMap Project, phase III / Rel # 2 (http//www.hapmap.org, 2012). All selected markers (42) had a minimum allele frequency (MAF) of 0.05 in the CEU population (Utah residents with northern and

western European ancestry). Cutting parameter to define DL between two markers was r2> 0.8. Following these criteria, the following tagSNPs 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.

OPG: rs11573938, rs3102724, rs11573884, rs2875845, rs1032128, rs3134057, rs1485289, rs3134060, RS3102728, rs11573856, rs7010267, rs11573901

Patients were genotyped for tagSNPs by real time PCR (Applied Biosystems 7500 Real-Time PCR System) using the TaqMan™ technology (Applied Biosystems).[50] Always using a negative control in all genotyping performed .

Statistical analysis

Categorical variables were expressed as frequencies and percentages. Adjustments were made to ROC curve for EARR, age and initial root length, with the aim of determining cutting points associated with EARR.[68] Comparisons between the groups regarding the dichotomous categorical variables were compared using the chi-square test or Fisher's exact test when indicated. For dichotomous categorical variables with statistically significant difference was applied Odds Ratio test for risk assessment. Logistic regression was used for the additive genetic model. The analyzes were performed with SPSS statistical software IBM® 21. Results with *p*-value lower than 0.05 were considered significant. For genetic variables, Haploview® 4.2 was used to estimate the Hardy-Weinberg and linkage disequilibrium between tagSNPs tested.

For the multivariate analysis, the logistic regression model of multiple steps Wald 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 one tagSNP model had a *p* value below 0.05 it was selected the genetic model with the lowest *p*-value.

RESULTS

Clinical Parameters

There were no statistically significant differences between the groups in relation to gender, use of pendulum apparatus, rapid maxillary expansion and pre-molar extraction. A statistically significant difference was found between the groups in relation to the initial length of the central incisor root (p=0.001) and patient age with (p=0.030) (Table I).

Analysis polymorphisms in RANKL/RANK/OPG genes

The distribution of *RANKL* genotypes was in Hardy-Weinberg equilibrium. No statistically significant differences were found in genotypic frequency of polymorphisms in *RANKL* gene between the groups (Table II). The linkage disequilibrium map for this gene can be seen in figure 3 (a,b,c).

The distribution of *RANK* genotypes was in Hardy-Weinberg equilibrium. 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.

The distribution of *OPG* genotypes was in Hardy-Weinberg equilibrium. Three polymorphisms tagSNPs were not amplified, possibly by inappropriate design of primers, thus rs11573856, rs7010267, rs11573901 were disregarded from the analysis. 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, it was found an increased frequency of the G allele of rs2875845 (additive model, p=0.027; dominant G, p=0.042) in the EARR group. Morover, an increased frequency of allele A of rs1032128 (recessive model A, p=0.019) in the EARR group. Finally, an increased frequency of C allele of rs3102728 (dominant model C, p=0.014) in the EARR group (Table II).

Multivariate Analysis

The clinical variables which presented p<0.200 were: age (p=0.030), PE (p=0.086), RI (p=0.001), RPE (p=0.177), pendex (p=0.134).

The genetic variables were: In the *RANKL* gene, rs1038434 recessive model for the C allele (p=0.198), rs931273 additive model (p=0.108). In the *RANK* gene, 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 for the allele A (*p*=0.085), rs9951012 dominant model for the G allele (*p*=0.148), rs12970081 recessive for the G allele model (*p*=0.110), rs17069898 dominant model for the A allele (*p*=0.160), rs4426449 dominant model for the C allele (*p*=0.111). For OPG gene, a dominant model for A allele rs3102724 (*p*=0.001), for the additive model rs2875845 (*p*=0.027), recessive model allele A rs1032128 (*p*=0.019), rs3134057 dominant model for the G allele (*p*=0.074), rs3102728 dominant model for the C allele (*p*=0.014).

After multivariate analysis, RI (*p*=0.001) and the genetic polymorphisms *RANKL* rs12455775 (*p*=0.012) and *OPG* rs3102724 (*p*=0.001) remained associated with EARR. Additionally, RPE was associated with EARR (*p*=0.030).

DISCUSSION

It is known that EARR is an undesirable side effect in orthodontic treatment and that, in most cases, has no clinical significance.[51] However, EARR is clinically important when 1 to 2 mm (¼) of the root length are lost.[52] Severe cases are considered when resorption reaches more than ¼ of the tooth root length (> 5.0 mm) and occur in only 1 to 5% of patients.[53]

The etiology of EARR is complex and various mechanical and biological factors may contribute to its occurrence.[54] 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[55] and genetic background.[9]

In this study, the initial length of the upper central incisor root (reference tooth) and patient age were associated with EARR. Teeth with the longest root are more prone to press apical region to due to torque caused by a bigger displacement.[56] However, EARR might be more harmful in shorter roots than in medium or long roots.[56-58] EARR has been more related to older patients.[20,59] Those patients usually present narrower and less vascularized periodontal ligament, thicken and less vascularized alveolar bone, and thicker cementum increasing risk of root resorption.[60, 61] The association between EARR and premolar extraction in patients treated orthodontically has been reported in some studies.[4,44,47] 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. Maybe for this reason, in this study we failed to find an association of premoler extractions with EARR, in accordance with other studies.[8,59] A radiographic monitoring of the patient for a longer period becomes desirable once in many cases the extraction will be performed after six 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 to EARR [46,58], because it is a relatively strong force and, sometimes, inconstant. In this study, we did not observe differences in relation to gender. This result is consistent with other findings.[57,59,60] On the other hand, other studies show more EARR in men[19,62] and others in women[24,63], being inconclusive. Patients in this sample who used the maxillary rapid expansion

apparatus of the type Hass also used the pendulum appliance. After the multivariate analysis, RPE was associated with EARR. Studies using RPE in humans have focused on the association analysis of molars and premolars with EARR.[64] In this study, the association may be explained by the fact that the upper incisors are closest to the intermaxillary suture in development. Thus, upper central incisors should be evaluated in further studies as the reference tooth.[65]

The use of x-ray has been the best cost-and-benefit way to diagnose the presence of EARR[47], then it has been used by most authors.[2,9,16,18,24,46,66-68] Periapical radiographs are better than panoramic, occlusal and lateral ones for the study of tooth roots, because this technique present less radiation, less distortion and less overlapping images.[67] However, it has a restricted view, difficulty in standardization, as well as it is a static method and does not predict resorption outcome.[12,69] Today it is believed that the CT scan is the best technique to obseve EARR, but it is expensive and difficult to performe, thus rarely used. It is suggested that patients susceptible to EARR can be detected by periapical x-ray during the first 6 months of treatment.[71,72] The presence of EARR at the beginning of treatment (or even before this) might be a predictor of increased risk to EARR.[73,74] According Artun et al.[74], the chances of presenting an incisive with more than 5.0 mm EARR at the end of treatment is three times higher when the patient has an incisive over 1.0 EARR mm at the 6 months of treatment and 15 times higher when presenting EARR 2.0 mm. If severe EARR (> 3.0 mm) is discovered, the dentist must inform his patient and the treatment should be discontinued for 3-4 months, being adviced the radiographic monitoring.[71]

The discovery of genetic markers may help identify patients at higher risk for EARR before starting treatment. [75] In this context, Newman et al.[24], in 1975, were the first to propose a formal genetic basis for EARR. Al-Qawasmi et al.[5] 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.[6] More recently, other research has identified an association between alleles of EARR and IL-1β.[76] Also, the TT genotype in IL-1α gene was associated with EARR in an American population[12]. Also, polymorphisms in the vitamin D receptor gene (VDR) were weakly associated with EARR in a Brazilian population.[68] However, there are few studies attempting to define genetic risk markers (susceptibility / predisposition) to EARR and these are poorly predictive.

The discovery of the RANKL / RANK / OPG system in the mid-1990s led to major advances in the understanding of bone resorption. It was known for many years prior to this discovery that osteoblastic stromal cells regulated formation of osteoclasts.[26] RANKL / RANK regulate the differentiation and activation of osteoclasts in normal bone remodeling and bone resorption in a variety of pathological conditions characterized by increased bone remodeling. OPG protects bone resorption excessive from the connection with RANKL, thereby preventing it from binding to RANK. The relative concentration of RANKL and OPG in bone becomes a determinant factor.[26,27,77] Studies also revealed new functions of this triad in other diseases, 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 cells. [78-80]

Single nucleotide polymorphisms (SNPs) are the most common forms of DNA variation in the human genome. Recently, several studies seek a genetic approach using SNPs in linkage disequilibrium (LD). Thereby, it is not necessary to genotype SNPs all of a particular gene, but SNPs "targets" (tagSNP) that capture all information in terms of gene variability. This strategy is intended to capture as much information about the variability of a gene with less SNPs, reducing costs and genotyping time. [81]

So far in dentistry, tagSNP polymorphisms in the gene of RANK / OPG / RANKL were only associated with periodontal disease.[80] However, to our knowledge, this is the first study to investigate the association of polymorphisms in genes of the system RANK / RANKL / OPG with susceptibility to EARR in orthodontic treated patients.

RANKL is a regulatory cytokine for differentiation and activation of osteoclasts, whose expression is regulated by various hormones and cytokines, resulting in bone resorption. The *RANKL* gene polymorphisms have been associated with bone mineral density and bone remodeling diseases where bone loss is a major sign.[82,83] It was observed no association of polymorphisms in the gene of the RANKL with EARR. However, the literature presents many studies that found an association of *RANKL* gene polymorphisms with bone diseases.[39,84]

RANK, located in osteoclastic precursor and dendritic cells, is responsible for the activation of osteoclasts.[26] Polymorphisms in the *RANK* gene have been associated with cases of esophageal cancer[85], rheumatoid arthritis[86] and other

diseases.[87] Other authors sought to associate polymorphisms in the *RANK* gene with EARR[65], but found no association. In our study, which included the analysis of the polymorphisms representing the entire gene, it was found an association of the polymorphism rs12455775 – intron with EARR, being the T the risk allele for EARR. However, even if the association was maintained after the multivariate analysis, the low frequency of the most rare allele might indicate that replication with a greater sample is mandatory. Al-Qawasmi et al.[6] reported the association, through a linkage study, between EARR and *RANK* gene locus in maxillary central incisor in patients Class I. This study suggests that the *RANK* gene is a candidate gene to the predisposition to EARR during orthodontic treatment.

The main biological activity of OPG is to inhibit osteoclast differentiation, the inhibition of osteoclasts resorption and stimulation of osteoclast apoptosis.[88] In relation to the polymorphisms of the OPG gene have been associated with several diseases, such as periimplantitis[89], breast cancer[90], osteoporosis.[91] In this study, there was an association of polymorphisms rs3102724, rs2875845, rs1032128 and rs3102728 with EARR, suggesting a strong association of OPG gene with EARR, the first study to find such an association. The rs1032128 was studied by Hsu et al.[92], whom found an association of *OPG* gene with bone mineral density in the lumbar spine. The same polymorphism was also associated with low bone cortical thickness in radial forearm. In another study, the rs2875845 was not associated with levels of systemic inflammatory biomarkers.[94] Roshandel et al.[93] observed that the polymorphism rs3102724 was associated with lower density bone mineral in the distal part of the radius bone. These findings show the influence of polymorphisms in RANKL / RANK / OPG system in the regulation of bone metabolism and 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, with a severe EARR orthodontic treatment in a short time.

In relation to linkage disequilibrium, polymorphisms rs931273 and rs12585229 in the *RANKL* gene, rs7239667 and rs17069898 in the *RANK* gene, rs3102724 and rs3134057 of the *OPG* gene are in high LD in the population studied, which means that, in future studies investigating the genes *RANKL*, *RANK*, *OPG*, only one of the two aforementioned SNPs for each gene will be necessary to reach the complete gene information.

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

In concusion, it was observed that the initial length of the root of maxillary central incisor and the rapid maxillary expansion were associated with external apical root resorption in this population. Regarding the analysis of polymorphisms in the genes *RANKL*, *RANK* and *OPG*, rs3102724 was suggested as a new marker of EARR susceptibility.

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TABLES

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

Patients Data	Control Group (n = 160)		Study Group (n = 178)		Univariate	
					p- value	OR (CI 95%)
	N	%	N	%		
Initial Root(mm)						
≤30	135	84,4	123	69,1	**0.001**	2.41 (1.42-4.11)
>30	25	15,6	55	30,9		
Age(years)						
≤ 14 years	79	49,4	67	37,6	**0.030**	1.61 (1.05-2.49)
> 14 years	81	50.6	111	62,4		
PE						
No	143	89,4	147	82,6	0.086	1.77 (0.94-3,34)
Yes	17	10,6	31	17,4		
Elastics						
No	133	83,1	143	80,3	0.574	1.20 (0.69-2,10)
Yes	27	16,9	35	19,7		
Gender						
Male	74	46,3	80	44,9	0.810	1.054 (0.68-1.61)
Female	86	53,8	98	55,1		
Pendulum						
No	113	70,6	139	78,1	0.134	0.67 (0.41-1.10)
Yes	47	29,4	39	45,3		
RPE						
No	130	81,3	155	87,1	0.177	0.64 (0.35-1.16)
Yes	30	18,8	23	12,9		

[a] Cutting point (14 years) suggested by ROC curve

[b] Cut-off point (30 mm) sugestedo by ROC curve

PE - Premolar Extraction

RPE - Rapidal Palatal Expansion

Table II. The results of univariate analyses of *RANKL* / *RANK* / *OPG* tagSNPs between the control (160) and study (178) group.

Gene	tag SNP	Variation [1/2]	% de genotyping	Genetic Model	Groups	Genotype (%) Homozygous 1	Heterozygous	Homozygous 2	Univariate *p*-value	Univariate OR (CI 95%)
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 (0.70-177)
					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)			
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[1]	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	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	.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)			
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	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	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	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)			
RANK	rs17069904	[G/A]	95,6	Additive	Control	115 (73.7)	37 (23.7)	4 (2.6)	0.504	
					Study	126,2 (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	126,2 (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)		25 (16.1)	0.111	1.72 (0,88-3.36)
					Study	143 (89.9)		16 (10.1)		
				Recessive[1]	Control	67 (43.2)	88 (56.8)		0.714	1.08 (0.70-1.70)
					Study	72 (45.3)	87 (54.7)			

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[1]	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)		
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	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)		

FIGURES

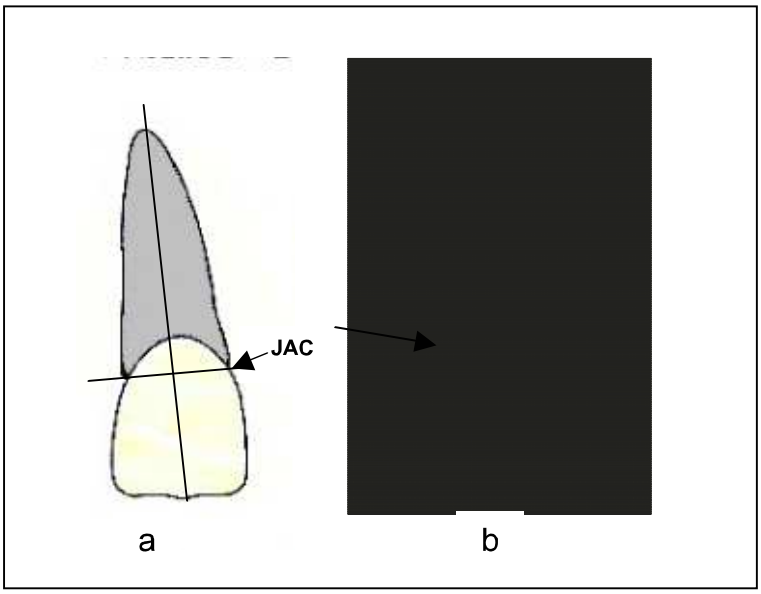
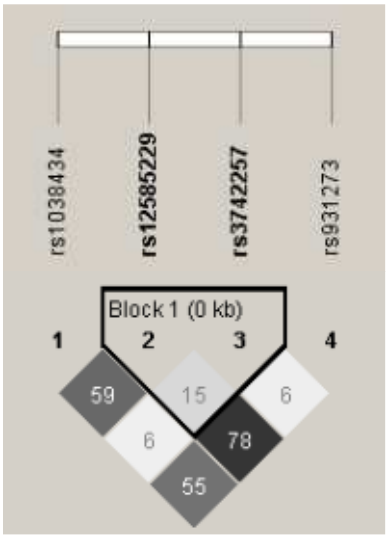


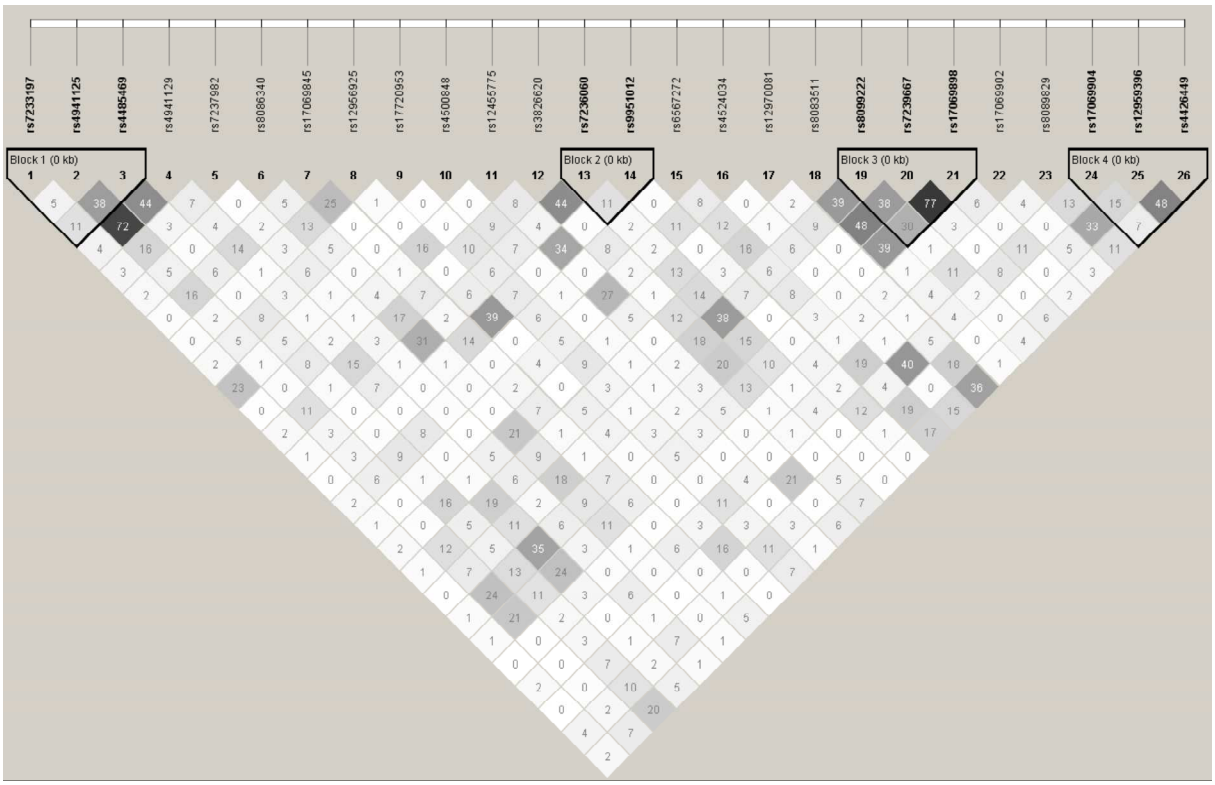
Figure 1. (a) Anatomical landmarks to measure the RRAE: cementoenamel junction (CEJ). (b) References to measure the x-ray.

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

a) Gene *RANKL*



b) Gene *RANK*



c) Gene *OPG*

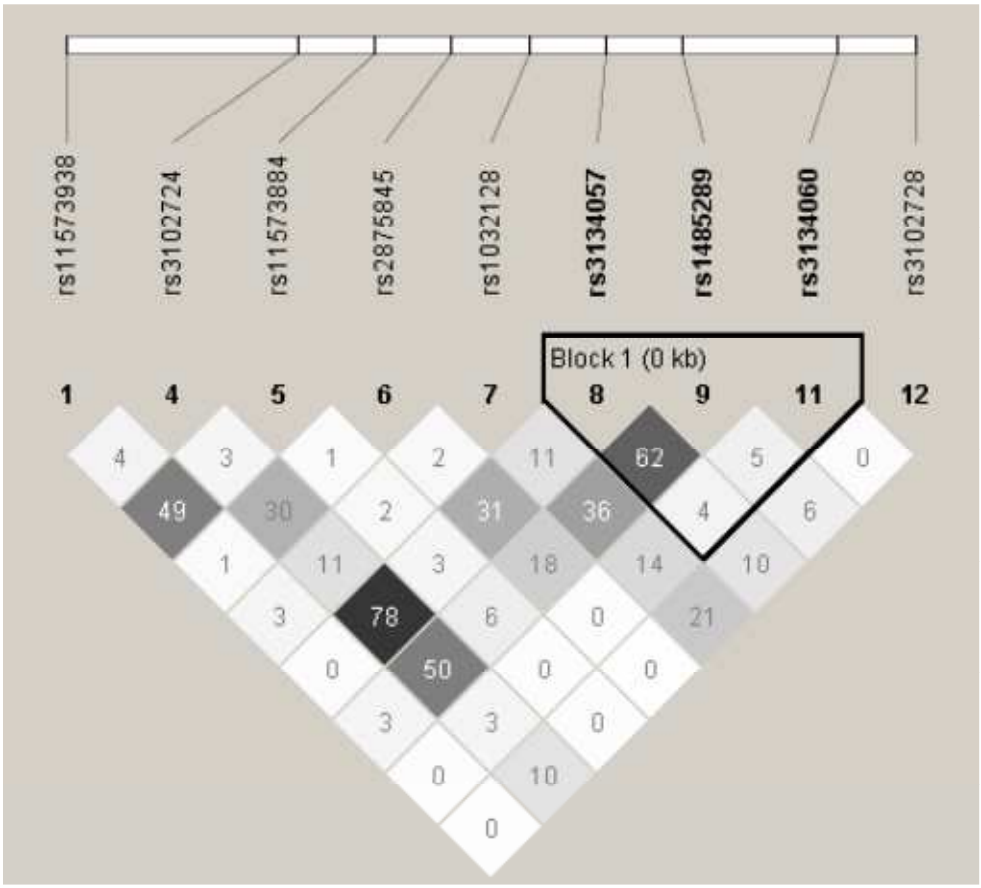


Figure 3. Analysis of linkage disequilibrium (LD) between the SNPs in the genes *RANKL* / *RANK* and *OPG*. The number within square indicates the LD ratio in %.