REVIEW ARTICLE

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Pathways in external apical root resorption associated with orthodontia

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Structured Abstract

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To review studies investigating if genetic factors play a role in external apical root resorption (EARR) during orthodontic treatment. Heritability estimation in human sib-pairs, comparison of multiple inbred mouse strains, human sib-pair linkage and parents-child trio association studies, and two gene (*Il-1b*, and *P2rx7*) knock out mouse models. Heritability for EARR of the maxillary central incisors concurrent with orthodontic treatment is 0.8. DBA/2J, BALB/cJ, and 129P3/J inbred mouse strains are highly susceptible ($p < .05$) to histological root resorption (RR) associated with orthodontic force (RRAOF), whereas A/J, C57BL/6J and SJL/J mice are resistant. Non-parametric sibling pair linkage analysis identified evidence of linkage (LOD = 2.5; $p = 0.02$) of EARR with microsatellite *D18S64* (tightly linked to *TNFRSF11A*, also known as RANK). There is significant linkage disequilibrium of *IL-1B* ($p = 0.0003$), and *OPG* ($p = 0.003$) with EARR. RRAOF increases in *Il1b* KO ($p \leq 0.013$), and increases in *P2rx7* KO ($p < 0.02$) mice compared to wild-type. Genetic factors play a marked role in EARR concurrent with orthodontic force, accounting for one-half to two-thirds of the variation. Two pathways for this may involve: 1) activation control of osteoclasts through the ATP/P2XR7/IL-1B inflammation modulation pathway; and 2) RANK/RANKL/OPG osteoclast activation control. Histological RR occurs and is typically healed. If resorption outpaces healing, then EARR develops. Normal and parafunctional forces, as well as orthodontic forces, may add to or interact with the individual's susceptibility to pass the threshold of developing EARR.

Key words: interleukin-1beta; orthodontics; osteoprotegerin; P2XR7; root resorption

Introduction

Root resorption detectable histologically can be a preliminary step toward external apical root resorption (EARR) that is permanent and detectable radiographically. It is believed that when root resorption (RR) exceeds the reparative capacity of cementum, EARR ensues. Exposure of dentin increases the likelihood of osteoclastic attack and EARR, particularly if the tooth is subjected to forces from alternating directions in a parafunctional manner (1).

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From 7% to 13% of individuals who have not had orthodontic treatment show some EARR (2, 3), presumably at least in part as a function of occlusal forces. There is an association of EARR in those who have not received orthodontic treatment with missing teeth, increased periodontal probing depths, and reduced crestal bone heights (3). Individuals with bruxism, chronic nail biting, and anterior open bites with concomitant tongue thrust may also show an increased extent of EARR before orthodontic treatment (4). Dental trauma, especially with re-implantation of an avulsed tooth, is also associated with increased EARR (5).

External apical RR is also increased as a pathologic consequence of orthodontic mechanical loading in some patients (6, 7). The amount of orthodontic movement is positively associated with the resulting extent of EARR (8–10). Orthodontic tooth movement, or 'biomechanics', has been found to account for approximately one-tenth to one-third of the total variation in EARR (11–13).

Owman-Moll and coworkers showed that individual variation overshadowed the force magnitude and the force type in defining the susceptibility to histological RR associated with orthodontic force (14). Individual variations were considerable regarding both extension and depth of histoloigcal RR within individuals, and these were not correlated to the magnitude of tooth movement achieved (15).

There is considerable individual variation in EARR associated with orthodontic treatment, indicating an individual predisposition and multifactorial etiology (16–21). Heritability estimates have shown approximately half of EARR variation concurrent with orthodontia, and almost two-thirds of maxillary central incisor EARR specifically, can be attributed to genetic variation (21, 22). A retrospective twin study on EARR found evidence for both a genetic and environmental factors influencing EARR (23). In addition, studies in a panel of different inbred mice also supported a genetic component involving multiple genes in histological RR (24, 25).

While there is a relationship between orthodontic force and RR, it is against the backdrop of previously undefined individual susceptibility. Since mechanical forces and other environmental factors do not adequately explain the variation seen among individual expressions of EARR, interest has increased on genetic factors influencing the susceptibility to EARR. The reaction to orthodontic force, including rate of tooth movement, can differ depending on the individual's genetic background (1, 26).

ATP/P2XR7/IL-1B pathway

Clinical assocaition of IL-1B with EARR concurrent with orthodontia

In 2003, Al-Qawasmi et al. identified an IL-1B (+3953/+3954, rs1143634) polymorphism in orthodontically treated individuals as having a role in the genetic influence on EARR. The polymorphism variation was found to account for 15% of the variation in EARR in that sample. Persons in their sample homozygous for the IL-1B allele 1 had a 5.6 fold (95% CI 1.9–21.2) increased risk of EARR greater than 2 mm as compared with those who are not homozygous for the IL-1 beta allele 1. Data indicate that allele 1 at the IL-1B gene, known to decrease the production of IL-1 cytokine *in vivo* (27, 28), significantly increases the risk of EARR (29).

Il1b knockout mouse model

A murine model in which RR is induced by orthodontic force was applied to interleukin-1b knockout (*Il1b*−/−) mice to further investigate the role of interleukin 1-b in RR (30). Thirty-three male mice of the wild-type strain (C57BL/6J,+/+) and the *Il1b* knockout (B6.129-IL-1B^{tmChaplin}) strain (31) obtained from David Chaplin (University of Alabama at Birmingham, Birmingham, AL, USA) were divided into control or treatment groups. A red Elgiloy® cobalt steel alloy 0.0058 × 0.022 inch open coil spring (Rocky Mountain Orthodontics, Denver, CO, USA) was used to apply the orthodontic force as described previously (24). The number of control (C) or treated (T) mice per group were as follows: wild-type (C = 7, T = 8), and knockout (C = 8, T = 10). Both the control and treated animals were fed a diet of finely milled mouse chow *ad libitum* to minimize discomfort and appliance distortion in the treated mice. The Wilcoxon rank-sum non-parametric test was used to evaluate differences in the mean root resorption (MRR), root resorption attributable to orthodontic force (RRAOF = force value-baseline value) and tartrate resistant acid phosphatase (TRAP) measures associated

with *Il1b* gene status, sex, or treatment status. Assuming $\alpha = 0.05$ and using a Bonferroni correction to adjust for four distinct non-parametric tests for each phenotypic hypotheses yielded an adjusted significance level (α^*) of 0.0127. Finally, the Spearman correlation coefficient was computed for TRAP and RRAOF to quantify the association between these two variables ($\alpha = 0.05$).

Nine days into the experiment, the mice in all groups were euthanized. Immediately after that the maxillae were removed, fixed, and demineralized. Paraffin embedded specimens were cut into parasagittal sections of 5 μm thickness. Evaluation of RR on the mesial aspect of the mesial root of the maxillary first molar on eight H&E stained sections was analyzed using light microscopy at X100 magnification as described elsewhere (32). Four additional sections also selected randomly were stained for TRAP and evaluated as before (24).

There was no significant difference in MRR ($p = 0.64$) between the untreated wild-type and untreated knockout mice. There was a significant difference in both the wild-type and knockout animals with treatment compared to their respective controls. There was also a significant difference between the treated wild-type and the treated knockout mice, with the knockout mice having approximately three times the percent MRR of the treated wild-type mice (Fig. 1). Thus, the absence of IL-1β cytokine, a typical mediator of the inflammatory response, increased orthodontically-induced RR. Interestingly, this effect was not mediated by significant changes in the number of TRAP positive cells near the root surface.

P2rx7 (*P2x7r*) knockout mouse model

Viecilli, Katona et al. refined the murine model to bring the force (and the resulting stress) applied to a clinically relevant level, and to evaluate the effect of force and RR using histology, and in three dimensions finite element modeling and μCT imaging, to investigate the effect of orthodontic force in the mouse in which the gene (*P2rx7*) for purinergic receptor P2X, ligand-gated ion channel, seven has been inactivated (33–35). Similar to the findings in the *Il1b* knockout mouse, there was no difference at baseline between the wild-type and knockout mice RR, while the application of force resulted in a significant increase in wild-type RR, and in addition a significant ($p < 0.02$) increase in RR in the knockout mice with force applied over the force applied wild-type mice.

Recently the role of ATP and its cognate receptors, including purinergic receptor P2X, ligand-gated ion channel, seven, in the inflammatory process has been shown to involve the metabolism of apoptic and necrotic tissue (36). Following mechanical trauma damaged cells release ATP that leads to the activation of the receptor on the cell surface of macrophages and some other cell types, which in turn releases interleukin-1 cytokines. The released cytokines affect non-bone marrow derived cells, which, in turn, release chemoattractants for neutrophils and lymphocytes (37). The neutrophils can act quickly to eliminate apoptotic cells and prevent further necrosis. A failure or decrease in this process results in an attenuated acute inflammatory response that may not resolve, resulting in an

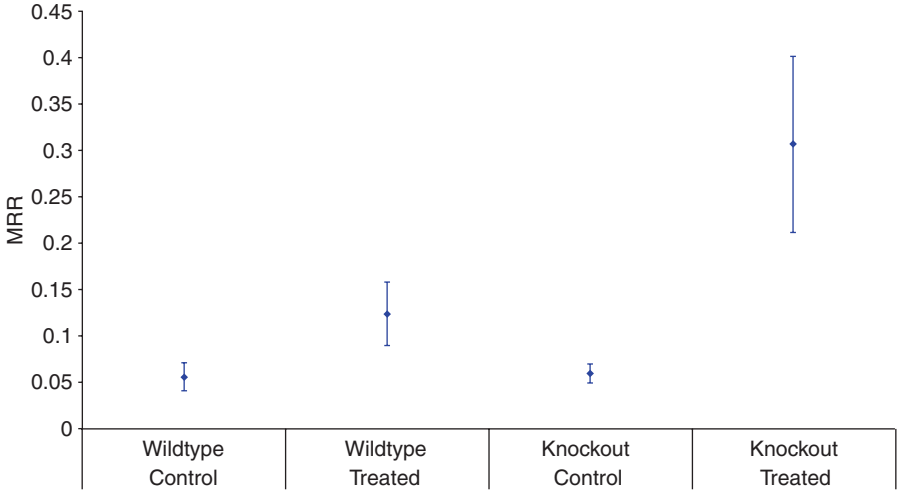

Fig. 1. Root resorption attributed to orthodontic force (RRAOF) for the two groups of treated mice. Each point represents mean RRAOF and the vertical bars represents one standard deviation above and below mean value.

overwhelming chronic response with generalized tissue damage (36, 38).

Macrophages from *P2rx7* knockout mice are unable to respond to extracellular ATP. *P2rx7* knockout mice primed with lipopolysaccharides and challenged with ATP *in vivo* failed to generate significant levels of interleukin-1 beta (39). The finding that RR increases with force in both *Il1b* and *P2rx7* knockout mice further strengthens the evidence that interleukin-1 beta may in some cases play a role in EARR, and indicates that variation in purinergic receptor P2X, ligand-gated ion channel, 7, as well as other proteins involved in the maturation and release of interleukin-1 beta, could also be a factor. The observation that the RR in both the *Il1b* and *P2rx7* knockout mice is not statistically significant from that seen in their respective wild-type mice before orthodontic force is applied, but does significantly increase with orthodontic force, provides evidence for an interaction between genotype and environmental factors that influence RR.

RANK/RANKL/OPG pathway

Osteoblasts and stromal stem cells express receptor activator of NF-κ B ligand (RANKL), which binds to its receptor activator of nuclear factor-κ B (RANK), on the surface of osteoclasts and their precursors. This regulates the differentiation of precursors into multinucleated osteoclasts and osteoclast activation and survival both normally and in most pathologic conditions associated with increased bone resorption. Osteoprotegerin (OPG, coded for by the *TNFRSF11B* gene) is secreted by osteoblasts and osteogenic stromal stem cells and protects from excessive bone resorption by binding to RANKL and preventing it from interacting with RANK (40).

Familial expansile osteolysis (FEO, OMIM no. 174810) is a rare, autosomal dominant bone disorder characterized by osteolytic lesions, which develop usually in the long bones during early adulthood, and can also result in spontaneous resorption of teeth and loss of the dentition. FEO is caused by mutations in the *TNFRSF11A* gene that encodes RANK (41). Based upon this condition and the importance of the RANK/RANKL/OPG pathway in the control of osteoclast activation, *TNFRSF11A* (RANK) is a candidate gene for EARR.

D18S64, which is tightly linked to TNFRSF11A (RANK)

Non-parametric sibling pair linkage analysis identified evidence of linkage (LOD = 2.5; $p = 0.02$) of EARR affecting the maxillary central incisor with the microsatellite marker *D18S64*, which is tightly linked to *TNFRSF11A* (RANK). This indicates that the *TNFRSF11A* locus, or another tightly linked gene, is associated with EARR. This was the first genetic data in a clinical setting to suggest that the RANK/RANKL/OPG pathway may be a factor in some patients with EARR (42).

TNFRSF11B (Osteoprotegerin)

Osteoprotegerin knockout mice subjected to orthodontic forces have a significant increase in osteoclasts and lower bone mineral density (43, 44). Increases in OPG production inhibit orthodontic tooth movement in rats with a decrease in osteoclasts (45, 46). In addition, OPG is associated with physiologic and pathologic RR (47, 48). More evidence of the RANK/RANKL/OPG pathway being involved with some cases of EARR comes from a clinical study involving the *TNFRSF11B* (OPG) gene (49).

This study evaluated the association between the single nucleotide polymorphism (SNP) rs2073618 of the *TNFRSF11B* (OPG) gene and EARR in orthodontically treated patients. This *TNFRSF11B* SNP is located in the first of five exons, changing the nucleotide (G $\rightarrow$ C) at position 1181 in the third codon, resulting in the third amino acid changing from lysine (Lys) to asparagine (Asn). This SNP is a factor in bone mineral density and the susceptibility to Paget's disease of bone (50, 51). Indiana University Institutional Review Board review approved informed consent was obtained from all participants. A total of 135 Caucasian subjects were studied.

Radiographic evaluation of EARR took place by measuring the maxillary central incisors. Pre- and post-treatment occlusal radiographs were scanned and measurements made using Adobe Photoshop CS Version 8.0 (Adobe, Seattle, WA, USA). The rule-of-three formula was used to quantify EARR using the median CEJ (11, 52).

To assess the reproducibility and method error of the radiographic measurements, double measurements were made 2 months apart on 24 randomly selected occlusal radiographs. The error for EARR calculations

on the occlusal radiographs was 0.18 mm using the equation $S_x = \sqrt{(\sum D^2/2N)}$, where S_x is the error, D is the difference between the double measurement, and N is the number of double measurements (53).

For DNA analysis the inside of the cheek was scraped 10 times with two sterile nylon bristle brushes. The cells obtained underwent DNA isolation with the Puregene method (Gentra Systems, Minneapolis, MN, USA) and were stored at −80°C.

Automated polymerase chain reaction and allelic discrimination using the 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) and Taqman® polymerase probes and primers determined genotypes (G,G; G,C; or C,C).

All analyzes used the greater of the EARR measurements for the two incisors. Stepwise linear regression analysis was employed using treatment time, overjet, overbite, Angle molar classification and sex to identify significant covariates with EARR. A p-value of 0.10 or less was required for retention in the model. Significant covariates were used in all subsequent analyzes. Analyzes were performed categorizing EARR measurements as either affected (EARR ≥ 2 mm) or unaffected (EARR < 2 mm).

The mean age of the patients pre-treatment was 14.6 years (±6.9 SD). The average interval between pre-treatment and post-treatment radiographs (treatment time) was 1.6 years (±0.5 SD). Based on Hardy–Weinberg equilibrium the expected and observed counts for OPG genotypes were not significantly different using the chi-squared test ($\chi^2_1 = 0.043$; $p = 0.84$).

Regression analysis indicated that sex, overjet, overbite, and molar (Angle) classification were poor predictors for EARR. Therefore these covariates were excluded from the linear regression test based on $p > 0.10$. The length of treatment ($p = 0.009$) variable was used in subsequent statistical models, and indicated that increases in length of treatment resulted in increased EARR.

Logistic regression of the three genotype groups found the odds ratio was 1.9 with a 95% confidence interval of (1.1, 3.3). That is, for each copy of allele C a person was 1.9 times more likely to be affected. Combining the two least affected genotype groups, the odds ratio was 2.8 with a 95% confidence interval of (1.2, 6.7) indicating a person with a C,C genotype is 2.8 times more likely to be affected than a person with a G,G or G,C genotype. Both the three and two group logistic regression analyzes obtained a statistically significant effect for the OPG genotype (both $p = 0.02$).

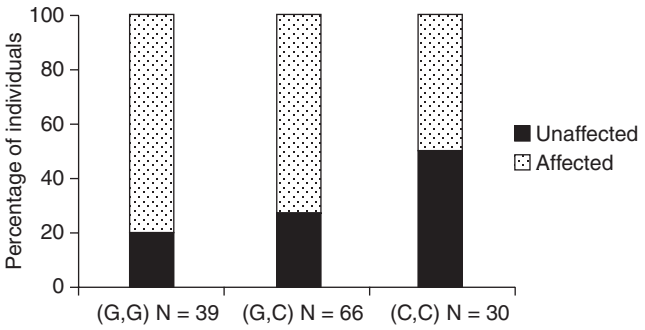

Fig. 2. The percentage of individuals affected (external apical root resorption ≥ 2 mm in at least one maxillary central incisor) by *TNFRSF11B* Osteoprotegerin (OPG) single nucleotide polymorphism rs2073618 genotype. There is a significant association between affection status and the OPG genotype. ($\chi^2_1 = 8.5339$; $p = 0.003$).

The frequencies for affection status by genotype are in shown in Fig. 2. Using the three group OPG genotypes, a chi-square test yielded ($\chi^2_1 = 8.5339$; $p = 0.003$); indicating a significant association between affection status and the OPG genotype. Similar results were obtained for the two group genotype test ($\chi^2_1 = 8.3680$; $p = 0.004$). The data indicates the G1181C OPG polymorphism accounts for approximately 8% of total EARR variation in the sample.

Conclusions

Genetic factors play a marked role in EARR concurrent with orthodontic force, accounting for one-half to two-thirds of the variation. Two pathways for this may involve: 1) activation control of osteoclasts through the ATP/P2XR7/IL-1B inflammation modulation pathway; and 2) RANK/RANKL/OPG osteoclast activation control pathway. Further research into the association of orthodontic treatment and genetic variation, particularly in the genes that code for proteins involved in the ATP/P2XR7/IL-1B and RANK/RANKL/OPG pathways are likely to further clarify the genetic factors associated with EARR concurrent with orthodontic treatment.

Clinical relevance

External apical root resorption can happen with or without orthodontic treatment. The increased incidence in orthodontic patients can result in the orthodontist

being blamed for its occurrence, presumably because of too great a force being placed on the teeth, and or moving the teeth from trabecular bone into more dense cancellous bone. It had been recognized that some patients were more susceptible to EARR than others, and that sometimes this tendency 'ran in families'. Research in this area indicates that the patient's genetic makeup has a substantial influence on EARR, indicating that it is a complex trait.

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