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AAO Foundation Final Report Form
(a/o 5/30/2021)

In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Please prepare a report that addresses the following:

Type of Award: Postdoctoral Fellowship Award

Name(s) of Principal Investigator(s): Ejvis Lamani

Institution: University of Alabama at Birmingham

Title of Project: Genetic Markers in Orthodontics

Period of AAOF Support: NCE to 6/30/2022

Amount of Funding: $100,000

Summary/Abstract

A major opportunity for craniofacial research is the use of advanced techniques to identify the molecular mechanisms responsible for a given disease phenotype. This project investigated a dental anomaly with major adverse clinical outcomes in orthodontics, External Apical Root Resorption (EARR). EARR causes permanent loss of root structures in more than one-third of orthodontic patients. This compromises patient's orthodontic treatment and may jeopardize their overall oral health. Orthodontic patients vary in their susceptibility to EARR with genetic factors accounting for approximately 64% of variations. Furthermore, Short Root Anomaly (SRA), a genetic disorder that presents with compromised crown to root ratios, may increase the risk of root resorption and tooth loss. The causal gene(s) for EARR remain largely unknown. Since EARR is a multifactorial disease, our goal was to evaluated patient- and treatment-related factors associated

with an increased risk of developing this disorder. This study also examines the human genotype-phenotype correlation in EARR patients and identify genetic markers as potential screening tools in diagnosis of patients with EARR.

To evaluate the prevalence and risks associated with root resorption, we have examined 835 orthodontic patients (UAB IRB Protocol Number 160428005). Of these, 195 patients were recruited to participate in our genetic study. We have identified that there are ethnic and racial differences in the prevalence of EARR, with African Americans and Asians displaying more resistance to root resorption than Caucasians and Hispanics. Furthermore, incisor proclination was found to be significantly associated with EARR in Caucasians and Hispanics patients. Treatment time greater than 20 months was also associated with root resorption in Hispanics. In Asians, the Class 3 dental classification as well as the orthodontic treatment that included extraction of teeth increased the risk of EARR. Moreover, SRA was found to increase the risk of patients developing EARR with orthodontic treatment.

Finally, we identified single nucleotide polymorphisms (SNPs) that may play a role in a patient's risk of developing EARR with orthodontic treatment. We found that a SNP in the *SPP1 (OPN)* gene (rs11730582) displayed protection against root resorption in patients expressing the homozygous TT genotype (vs CC or TC+CC). Similarly, the GG genotypes of *IL1A* rs1800587 was protective against root resorption (decreased odd ratios) when compared with GA+AA. On the other hand, a SNP in the *CASP1* gene (rs530537), increased the risk of root resorption when two copies of the C allele were expressed. The AA genotype of another *CASP1* polymorphism (rs580253) had a significant increase in the odds ratio of EARR (vs AG+GG).

Information gleaned from this work may be useful in expanding the molecular understanding of EARR pathogenesis and may be used in the generation of future clinical trials focusing on therapeutic interventions for prevention and management as well as establishing biomarkers for EARR. This will facilitate the orthodontic treatment plan leading to a successful outcome- "Precision Dentistry".

Detailed results and inferences:

1. If the work has been published please attach a pdf of manuscript OR
2. Describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should be included.

Studies and Results

Root resorption due to orthodontic tooth movement may adversely affect the root-crown (R/C) ratios of permanent teeth, especially in patients with SRA, a poorly understood disorder affecting tooth root development. Evaluation of SRA R/C ratios to normal dentition will facilitate diagnosis and orthodontic treatment planning. However, reference values are not available for all ethnic groups. Using panoramic radiographs, we determined R/C ratios of fully developed permanent teeth and their relationship to gender and ethnicity. We analyzed 6,241 teeth from 333 UAB SOD Comprehensive Care Clinic patients age 9-50 years (109 Caucasians, 112 African Americans and 112 Hispanics; 47.4% males and 52.6% females). Only fully developed permanent teeth were included in the study. Patients with craniofacial anomaly, SRA diagnosis, history of trauma, or evidence of prior orthodontic treatment were excluded. Third molars, heavily restored

or worn teeth or radiographs presenting unclear reference points were also excluded. Root lengths and crown heights were measured with modified Lind's method (1).

The R/C ratios of fully developed permanent teeth are shown in Table 1. The mean ratios varied from 1.80-2.21 for the maxillary teeth and 1.83-2.49 for the mandibular teeth. Gender differences were found to be significant ($p<0.005$) only in the lower central incisors (females had lower values than males). We also established ethnic specific R/C reference values for African Americans, Hispanics, and Caucasians (Table 2). Hispanics showed significantly lower ratios as compared to the other two groups in most teeth ($P<0.05$). Significant differences in R/C ratios between African Americans and Caucasians were found in upper lateral incisors, lower central incisors and lower first premolars ($P<0.05$). Our data (published in the *Orthodontics & Craniofacial Research*) shows for the first time that ethnicity is an important factor in R/C ratios of permanent teeth.

Table 1. Mean root to crown ratios between males and females.

Teeth	Gender	N	Mean	SD	95% CI	P value	FDR
8,9	F	329	1.80	0.26	1.77-1.82	0.1098	0.3074
	M	293	1.84	0.28	1.80-1.87		
7,10	F	286	2.02	0.30	1.99-2.06	0.0749	0.2706
	M	252	2.09	0.35	2.04-2.13		
6,11	F	242	2.21	0.36	2.16-2.25	0.0557	0.2706
	M	190	2.12	0.36	2.07-2.18		
5,12	F	152	2.13	0.35	2.08-2.19	0.0773	0.2706
	M	140	2.05	0.31	2.00-2.11		
4,13	F	200	2.15	0.38	2.10-2.21	0.9929	0.9929
	M	190	2.15	0.39	2.09-2.20		
3,14	F	212	2.04	0.32	2.00-2.08	0.3006	0.5793
	M	174	1.98	0.33	1.93-2.03		
2,15	F	231	2.11	0.32	2.07-2.15	0.5673	0.722
	M	192	2.09	0.33	2.04-2.14		
24,25	F	290	1.83	0.30	1.80-1.87	0.0040*	0.056
	M	224	1.94	0.34	1.89-1.98		
23,26	F	279	1.97	0.33	1.93-2.01	0.4381	0.6133
	M	217	1.99	0.34	1.95-2.04		
22,27	F	240	2.17	0.40	2.12-2.22	0.2641	0.5793
	M	181	2.21	0.40	2.16-2.27		
21,28	F	274	2.35	0.39	2.31-2.40	0.3724	0.5793
	M	220	2.30	0.44	2.24-2.36		
20,29	F	247	2.49	0.39	2.44-2.53	0.7127	0.8315
	M	216	2.49	0.41	2.43-2.54		
19,30	F	193	2.19	0.24	2.16-2.23	0.9684	0.9929
	M	164	2.20	0.34	2.15-2.26		
18,31	F	216	2.10	0.32	2.06-2.14	0.3659	0.5793
	M	197	2.07	0.31	2.03-2.12		

*P<0.05. F: female; M: male; SD: standard deviations; CI: confidence intervals; FDR: false discovery rate.

Table 2. The effect of ethnicity in root to crown ratios.

Teeth	African American	Hispanic	Caucasian	F test	P Values

	Mean	SD	Mean	SD	Mean	SD		FDR	AA vs H	AA vs C	H vs C
8,9	1.83	0.25	1.76	0.26	1.86	0.29	0.0051*	0.0143	0.0215*	0.3901	0.0017*
7,10	2.01	0.28	2.00	0.30	2.17	0.39	<.0001*	0.0005	0.7423	<.0001*	<.0001*
6,11	2.22	0.35	2.09	0.38	2.18	0.35	0.0321*	0.056	0.0093*	0.4459	0.1014
5,12	2.17	0.35	2.03	0.30	2.07	0.33	0.0175*	0.0368	0.0053*	0.0774	0.3212
4,13	2.17	0.38	2.14	0.39	2.13	0.39	0.7203	0.7757			
3,14	2.00	0.35	2.08	0.33	1.95	0.26	0.0360*	0.056	0.0586	0.4057	0.0149*
2,15	2.11	0.31	2.13	0.37	2.06	0.28	0.3947	0.4605			
24,25	1.88	0.29	1.79	0.33	1.98	0.34	0.0003*	0.0011	0.0250*	0.0303*	<.0001*
23,26	1.98	0.31	1.93	0.34	2.04	0.36	0.0442*	0.0619	0.1887	0.1629	0.0127*
22,27	2.17	0.38	2.15	0.38	2.26	0.45	0.0857	0.1091			
21,28	2.37	0.38	2.20	0.40	2.48	0.43	<.0001*	0.0005	0.0013*	0.0226*	<.0001*
20,29	2.52	0.35	2.37	0.43	2.61	0.38	<.0001*	0.0005	0.0058*	0.0807	<.0001*
19,30	2.20	0.30	2.18	0.24	2.21	0.32	0.8777	0.8777			
18,31	2.09	0.29	2.02	0.33	2.17	0.31	0.0184	0.0368			

*P<0.05. SD: standard deviations; AA: African American; H: Hispanic; C: Caucasian; FDR: false discovery rate.

The prevalence of SRA varies with ethnicity (1-3). While in individuals of European ancestry SRA prevalence is low (2.4-2.7%), in Asian populations it has been reported up to 10% and may be even higher in Hispanics. However, to date there is no data looking at this disorder in the African American population. With the Diversity Index in the U.S. increasing from 54.6 in 2000 to 60.6 in 2010 and the Hispanic and African American populations growing by 43% and 12.3%, respectively, a better understanding of this dental anomaly in these populations is needed to aid the diagnosis and management of the condition (4).

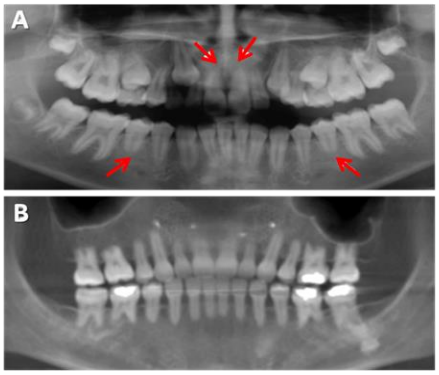

Fig. 1. Panoramic radiographs from a patient with (A) localized SRA (arrows) and (B) generalized SRA

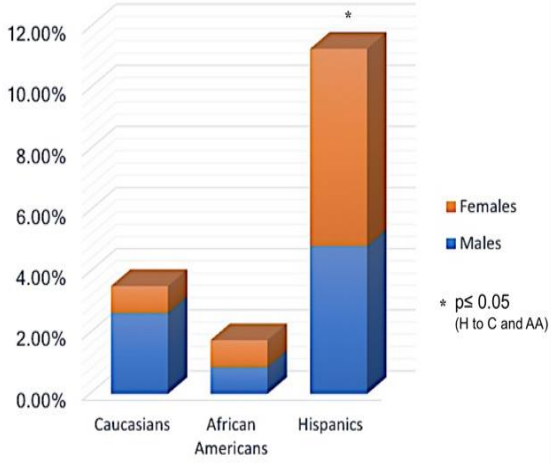

Fig. 2. Prevalence of SRA among males and females in a healthy Alabama population

To address the prevalence of SRA, a total of 353 patient radiographs (113 Caucasians, 114 African Americans and 126 Hispanics; 48% males and 52% females) were analyzed. SRA diagnosis was based on a root to crown (R/C) ratio ≤1.1 of affected teeth. Maxillary incisors were the most commonly affected teeth followed by maxillary or mandibular premolars (Fig. 1). Although, no gender differences in the SRA prevalence were identified in our study, the SRA distribution varied from 1.75% in African Americans to 3.54% in Caucasians and 11.29% in Hispanics (Fig. 2). Our data confirms that ethnicity is an important factor in SRA prevalence.

Since SRA is suggested to be a risk factor for root resorption, we examined 224 records from patients (58 Caucasians, 80 African Americans and 86 Hispanics) that had completed orthodontic treatment in our clinic. EARR was recorded when greater than 2mm of root structure was lost. Our study shows that EARR was evident in 46.4% of orthodontic patients (37.5% in African Americans, 56.9% in Caucasians and 47.7% in Hispanics, Fig. 3), much higher than the average 30% reported due to orthodontic treatment (5, 6). We also found that SRA patients had a relative risk of 2.33 times greater for developing EARR (1.83 in Caucasians, 2.72 in African Americans and 2.78 in Hispanics). **These data indicate: 1) SRA is more prevalent than initially reported; and 2) shows a high predisposition for root resorption.**

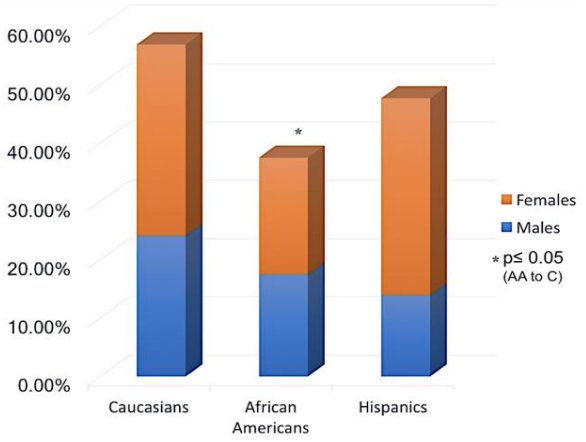

Fig. 3. Prevalence of EARR in orthodontic patients

Since EARR is a multifactorial disease we evaluated patient- and treatment-related factors associated with an increased risk of developing this disorder. We examined record of 73 Caucasian patients who had completed orthodontic treatment within 30 months and found no significant correlations between EARR and treatment related factors; although, treatment time >26 months approached significance (p=0.052) (Table 3). Of the patients related factors significant association with development of EARR was only seen with maxillary incisor proclination >108° (p=0.000032).

Table 3. Association of patient and treatment related factors with EARR in Caucasians

	EARR	No EARR	P Value
Dental Classification			0.75
Class I	26	18	
Class II	9	7	
Class III	9	4	
Skeletal Classification			0.82
Class I	18	14	
Class II	17	10	
Class III	9	5	
ANB			0.187871
0-3 degrees	20.0	16.0	
< 0 or >3 degrees	24.0	13.0	
U1-SN			* 0.000032
<108 degrees	13	23	
>108 degrees	31	6	
IMPA			0.53
<100 degrees	40.0	25.0	
>100 degrees	4.0	4.0	
Overjet correction			0.81
<5 mm	30	19	
>5 mm	14	10	
Treatment Protocol			0.18
Extraction	10	3	
Non-extraction	34	26	
Treatment time			0.052
<26.61 months	19.0	13.0	
>26.61 months	25.0	16.0	

We also examined the ethnic differences in the association of EARR with patient- and treatment-related factors. Our study of 336 African American orthodontic patients found a prevalence of moderate (at least 20%) and severe (at least 50%) EARR to be 29.8% and 0.3%, respectively. This differs from the initial pilot study which used the 2mm mark of root resorption (Fig. 3) and reported a prevalence of 37.5%. In this ethnicity, the associations between the patient specific and treatment specific variables and moderate EARR were not statistically significant (Table 4).

Table 4. Association of patient and treatment related factors with EARR in African Americans

	EARR	No EARR	P-value
Gender			0.31
Female	55 (27%)	144 (73%)	
Male	45 (33%)	92 (67%)	
Age			0.72
<10	6 (43%)	8 (57%)	
11-20	75 (29%)	182 (71%)	
21-30	10 (31%)	22 (69%)	
>31	9 (27%)	24 (73%)	
Dental Classification			0.78
Class 1	63 (30%)	146 (70%)	
Class 2	28 (28%)	73 (72%)	
Class 3	9 (35%)	17 (65%)	
Skeletal Classification			0.84
Class 1	24 (32%)	52 (68%)	
Class 2	69 (29%)	170 (71%)	
Class 3	7 (33%)	14 (67%)	
ANB			0.54
<0	6 (38%)	10 (62%)	
0-5	52 (32%)	113 (68%)	
>5	42 (27%)	113 (73%)	
OJ			0.81
<0	3 (30%)	7 (70%)	
0-4	53 (31%)	116 (69%)	
>4	44 (28%)	113 (72%)	
U1-SN			0.37
<100	6 (22%)	21 (78%)	
100-115	56 (28%)	142 (72%)	
>115	38 (34%)	73 (66%)	
IMPA			0.45
<90	14 (30%)	33 (70%)	
90-100	40 (26%)	111 (74%)	
>100	46 (33%)	92 (67%)	
Treatment Type			0.18
Extraction	27 (36%)	48 (64%)	
Non-extraction	73 (22%)	188 (78%)	
Treatment Time			0.34
<19	6 (19%)	26 (81%)	
19-30	58 (32%)	126 (68%)	
>30	36 (30%)	84 (70%)	

*P<.05

Similarly, in examining the 129 Hispanic patients, root resorption was recorded when 20% or more of root structure loss was measured on final radiographs. The overall EARR prevalence in this patient pool was 37.21% (Table 5). Treatment time greater than or equal to 20 months was statistically significant with a p-value of 0.03 (Table 6). Of the patient related factors significant association with development of EARR was seen with maxillary incisor proclination (p=0.005). Sex also proved to be statistically significant in correlation with EARR (p=0.04).

Table 5: EARR Prevalence in Hispanics

EARR	Frequency	Percentage
<20%	81	62.79%
≥20%	48	37.21%
<33%	115	89.15%
≥33%	14	10.85%
<50%	127	98.45%
≥50%	2	1.55%

Table 6: Association of EARR to Patient and Treatment Related Factors in Hispanics

	EARR	No EARR	P-Value
Sex			0.04
Female	24 (18.6%)	55 (42.6%)	
Male	24 (18.6%)	26 (20.2%)	
Age			0.41
<10	1 (0.8%)	6 (4.7%)	
10-18	39 (30.2%)	64 (49.6%)	
>18	8 (6.2%)	11 (8.5%)	
Dental Classification			0.5
Class I	21 (16.3%)	44 (34.1%)	
Class II	21 (16.3%)	28 (21.7%)	
Class III	6 (4.7%)	9 (7%)	
ANB			0.86
<3	20 (15.5%)	35 (27.1%)	
≥3	28 (21.7%)	46 (35.7%)	
Overjet			0.07
<0	3 (2.3%)	5 (3.9%)	
0-4	18 (13.9%)	47 (36.4%)	
>4	27 (20.9%)	29 (22.5%)	
U1-SN			0.005
<109	15 (11.6%)	57 (44.2%)	
≥109	25 (19.4%)	32 (24.8%)	
IMPA			0.08
<102	44 (34.1%)	65 (50.4%)	
≥102	4 (3.1%)	16 (12.4%)	
Treatment Type			0.77
Extraction	26 (20.2%)	46 (35.7%)	
Non-extraction	22 (17.1%)	35 (27.1%)	
Treatment Time			0.03
<20	6 (4.7%)	24 (18.6%)	
≥20	42 (32.6%)	57 (44.2%)	

We also examined EARR in 137 Asian patients and found that moderate and severe root resorptions occurred in 22% and 0.7% of patients, respectively. Related to EARR association with patient and treatment factors, we identified that Class III dental classification and extraction treatment significantly affect root resorption in this orthodontic patient group (Table 7 and 8).

Table 7: Association of EARR to Treatment Related Factors in Asians

Treatment Factors	No EARR	EARR	P-Value
Treatment time			0.70
< 20	32 (82%)	7 (18%)	
20-30	51 (76.1%)	16 (23.9%)	
> 30	23 (74.1%)	8 (25.9%)	
Treatment type			0.027
Ext	29 (65.9%)	15 (34.1%)	
Non-Ext	77 (82.8%)	16 (17.2%)	

Table 8: Association of EARR to Patient Related Factors in Asians

Patient -Related Factors	No EARR	EARR	P-Value
Gender			0.97
Male	38 (77.5%)	11 (22.5%)	
Female	68 (77.3%)	20 (14.6%)	
Age			0.42
< 11	4 (57.2%)	3 (42.8%)	
11-20	74 (78.7%)	20 (21.3%)	
> 20	28 (77.7%)	8 (22.3%)	
Dental classification			0.035
Class 1	44 (81.5%)	10 (18.5%)	
Class 2	55 (79.7%)	14 (20.3%)	
Class 3	7 (50%)	7 (50%)	
Skeletal classification			0.097
Class 1	40 (76.9%)	12 (23.1%)	
Class 2	54 (83%)	11 (16%)	
Class 3	12 (60%)	8 (40%)	
ANB			0.15
< 0	11 (61.1%)	7 (38.9%)	
0-5	71 (78%)	20 (22%)	

> 5	24 (85.7%)	4 (14.3%)	
U1-SN			0.059
< 100	10 (62.5%)	6 (37.5%)	
100-115	69 (84.1%)	13 (15.9%)	
> 115	27 (69.3%)	12 (30.7%)	
OJ			0.93
< 0	5 (71.4%)	2 (28.6%)	
0-4	53 (77.9%)	15 (22.1%)	
>4	47 (77%)	14 (23%)	
IMPA			0.40
< 90	22 (78.5%)	6 (21.4%)	
90-100	62 (80.5%)	15 (19.5%)	
>100	22 (68.7%)	10 (31.3%)	

Our work also includes studying the genetics of SRA and EARR. Genetic variants or single nucleotide polymorphism (SNPs) have been suggested as major determinants of oral diseases (e.g. periodontitis)(7). Previously, we have identified a NFIC mutation associated with incomplete or absent roots in autosomal recessive Radicular Dental Dysplasia (AR RDD) patients, representing the extreme end of SRA. This mutation is a rare single-nucleotide polymorphism (SNP) in the general population with a frequency of ~1%. To date, more than 5000 variants have been reported in dbSNP/Humsavar and Database of Genomic Variants for NFI-C.

For mutational analysis, DNA from patient saliva was amplified with NFI-C primers and bidirectional Sanger sequencing was used to identify potential mutations in SRA patients. Our analyses have identified an NFI-C SNP present in SRA patients but not in the unaffected family members (Fig. 4). This is an A to G polymorphism in the intron between exons 8 and 9 of the NFI-C gene. However, more data is needed for linking this SNP to the SRA phenotype. We will continue screening of this transcription factor, which as the root master gene is a strong candidate for potential SNPs associated with SRA and EARR.

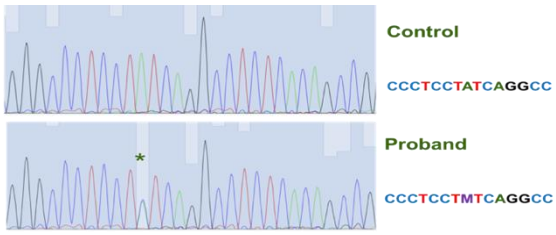

Fig. 4. Mutational analysis of NFI-C gene in SRA patients. * rs773349786; M= C/A

We have also examined the cellular response to mechanical forces using a well-established *in vitro* periodontal ligament (PDL) model system that mimics compressive forces of orthodontic tooth movement (8, 9). This PDL model system is used to study cellular response to mechanical forces. Our data shows the RDD NFI-C mutation affects the PDL's response to mechanical load over 24 hrs. Changes in RANKL expression (in a force-dependent manner) were seen in the RDD PDL cells as compared to control PDL cells (Fig. 5). These studies suggest that loading weights of 2 g/cm^2 may be optimal for this *in vitro* system. This data correlates with published data demonstrating maximum genes expression at this mechanical force in compressed PDL cells (10). Furthermore, these experimental conditions were used to examine the influence of static pressure on the root resorption-signaling pathway of SRA PDL cells recently established from extracted premolars of an SRA patient (Fig. 6). The SRA cells showed no significant differences in RANKL and OPG expression during the 24hrs pressure gradient. Similar results were observed by Western blotting. However, ELISA found significantly higher amount of secreted OPG protein in SRA cells placed under constant pressure (Figure 7).

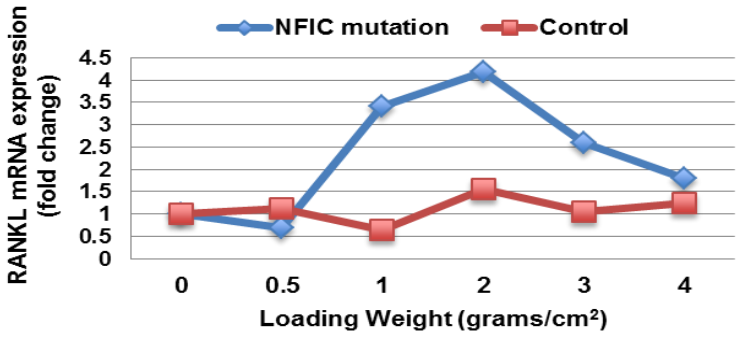

Fig. 5. The Effect of mechanical stress on RANKL expression in PDL cells over 24 hrs

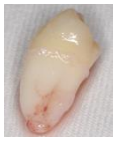

Fig. 6. Mandibular premolar extracted for orthodontic treatment from a patient diagnosed with SRA

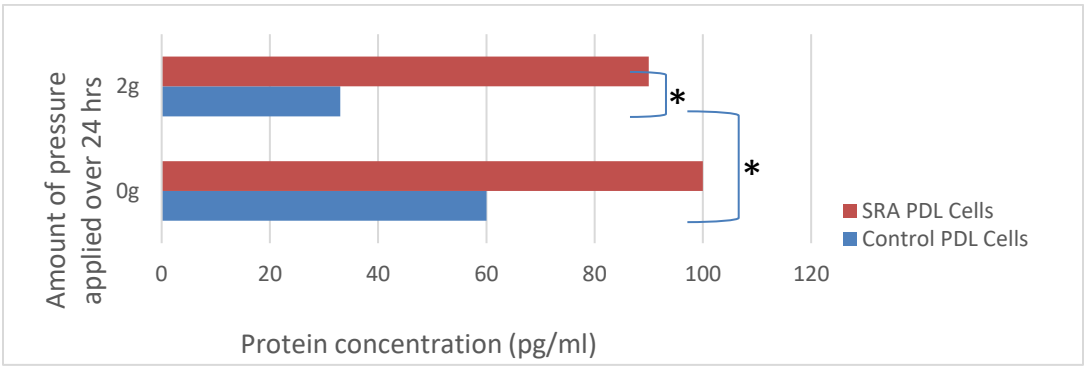

Fig. 7. Quantification of secreted OPG protein in SRA and control PDL cells. *p<0.05

Furthermore, in order to evaluate the association of single nucleotide polymorphisms with the risk of EARR, we investigated the genotype distribution of 17 SNPs (from 11 genes, Table 9) in 195 orthodontic patients. We also examined how other selected patient-related (ethnicity, sex, age, dental and skeletal classification, ANB, U1-SN and overjet) as well as treatment-related factors (treatment time and treatment type, i.e. extraction vs. non-extraction) contribute to root resorption in these patients. EARR diagnosis was recorded when at least 20% of the root length was lost on any of the four maxillary incisors.

TABLE 9: The SNPs Genotyped in EARR and Control Orthodontic Patients

SNP	Gene	Polymorphism
rs1800587	IL1A	G/A
rs1143634	IL1B	G/A
rs419598	IL1RN	C/T
rs1800796	IL6	C/G
rs731236	VDR	A/G
rs3102735	TNFRSF11B (OPG)	C/T
rs2073618	TNFRSF11B (OPG)	G/C
rs9138	SPP1 (OPN)	A/C
rs11730582	SPP1 (OPN)	T/C
rs1805034	TNFRSF11A (RANK)	C/T
rs8086340	TNFRSF11A (RANK)	C/G
rs1718119	P2RX7	A/G
rs2230912	P2RX7	A/G
rs1059703	IRAK1	A/G
rs530537	CASP1	C/T
rs580253	CASP1	A/G
rs554344	CASP1	C/G

Of the 195 patients recruited for SNP genotyping, 53 were diagnosed with EARR and 142 were used as controls (Table 10). The racial/ethnic groups identified within our sample included Caucasians (74%), African Americans (16%), Hispanics (6%), and Asians and South Asians (4%). We found no statistically significant association between ethnicity and EARR diagnosis; however, the majority of our sample was comprised of Caucasians and there was not a good representation of the other ethnicities. We also found that more females developed root resorption (58.5% in EARR vs 54.2% in controls) compared to males (41.5% in EARR vs 45.8% in controls). As can be seen in Table 10, there were also no statistically significant associations between EARR and other patient-related factors such as age, overall dental and skeletal classification, ANB, U1-SN, and overjet. On the other hand, while treatment time was not significantly correlated to risk of EARR in our sample, the extraction treatment type displayed significant association with root resorption. Looking at the odds ratios (Table 11), not only did we observed that patients who had upper extraction as part of the treatment had an increase of 7.05 times in the odds ratios of developing root resorption (compared to non-extraction or upper/lower extractions), but we also identified that in comparison to dental class I patients, those with a class II dental classification had 2.02 times the odds ratio of root resorption. Moreover, even though not statistically significant, the trend was that for every year increase in age, the odds to obtain EARR disorder decreased by 3% and for every month increase in treatment time, the odds to obtain EARR disorder increased by 2%.

Table 10: Patient- and Treatment Related Characteristics

Variable	EARR (N=53)	Controls (N=142)	P-value
Ethnicity, n(%)			0.45
Caucasian	43 (81.1%)	101 (71.1%)	
African American	6 (11.3%)	25 (17.6%)	
Hispanic	3 (5.7%)	8 (5.6%)	
Asian and South Asian	1 (1.9%)	8 (5.6%)	
Sex, n(%)			0.59
Female	31 (58.5%)	77 (54.2%)	
Male	22 (41.5%)	65 (45.8%)	
Age(Y)	14.6 (5.6)	16.8 (9.8)	0.06
Dental Classification			0.07
Class I	18 (34.0%)	74 (52.1%)	
Class II	29 (54.7%)	59 (41.6%)	
Class III	6 (11.3%)	9 (6.3%)	
Skeletal Classification			0.16
Class I	23 (43.4%)	75 (52.8%)	
Class II	24 (45.3%)	61 (43.0%)	
Class III	6 (11.3%)	6 (4.2%)	
ANB	2.3 (3.4)	2.7 (2.7)	0.52
U1-SN	106.8 (15.7)	106.4 (9.1)	0.87
OJ	4.4 (3.5)	4.3 (2.2)	0.81
Extractions, n(%)			0.006
No	42 (79.3%)	128 (90.1%)	
Upper (2PMs)	7 (13.2%)	3 (2.1%)	
Upper/Lower (4 PMs)	4 (7.6%)	11 (7.8%)	
Treatment time(m)	19.8 (5.8)	19.0 (7.0)	0.44

Table 11: Odd Ratios of Specific Treatment and Patient Related Factors Associated with EARR

Variable	Odds ratio	95% confidence interval		P-value
Patient-related factors				
Dental classification				0.07
II vs I	2.02	1.02	3.99	0.04
III vs I	2.74	0.86	8.69	0.09
Skeletal				0.16
II vs I	1.28	0.66	2.49	0.46
III vs I	3.26	0.96	11.09	0.06
OJ	1.02	0.90	1.15	0.77
ANB	0.96	0.86	1.07	0.47
U1-SN	1.003	0.97	1.03	0.83
age	0.97	0.92	1.01	0.14
Treatment-related factors				
Extractions				0.006
2 vs (2PMs vs No or 4PMs)	7.05	1.75	28.39	
Treatment time	1.02	0.97	1.07	0.44

Next, when analyzing the genotype distribution of 17 SNPs from 11 gene, we identified one SNP from the CASP1 (rs530537) gene to be significantly associated with EARR (Table 12). Furthermore, as seen in Table 13, the CC genotype of this SNP displayed significantly higher odds ratios of association with root resorption (compared to CT, TT, or CT+TT). Moreover, the AA genotype of another CASP1 polymorphism (rs580253) had an increase of 2.19 times in the odds ratio of EARR versus AG+GG. We also observed that the GG and the TT genotypes of IL1A rs1800587 and SPP1 (OPN) rs11730582 polymorphisms, respectively, were protective against root resorption (decreased odd ratios) when compared with GA+AA and CC or TC+CC, respectively. On the other hand, the multiple regression analysis found no interactions between SNPs and the other variables with one exception: IL1A rs1800587 (G/A) interacts with skeletal classification (p=0.04). Specifically, the EARR odds ratio for GG vs GA+AA in skeletal class I patients is 0.22 with 95% confidence interval (0.07, 0.65).

Table 12: Distribution of SNP Genotypes in EARR and Control Patients

SNP	EARR (N, %)			Controls (, %)			p-value
IL1A: rs1800587	GG 21 (39.6%)	GA 26 (49.1%)	AA 6 (11.3%)	GG 79 (55.6%)	GA 53 (37.3%)	AA 10 (7.1%)	0.13
IL1B: rs1143634	GG 28 (52.8%)	GA 21 (39.6%)	AA 4 (7.6%)	GG 91 (64.1%)	GA 41 (28.9%)	AA 10 (7%)	0.33
IL1RN: rs419598	CC 6 (11.3%)	CT 12 (22.7%)	TT 35 (66%)	CC 9 (6.3%)	CT 53 (37.3%)	TT 80 (56.4%)	0.11
IL6: rs1800796	CC 5 (3.5%)	CG 20 (14.1%)	GG 117 (82.4%)	CC 1 (1.9%)	CG 7 (13.2%)	GG 45 (84.9%)	0.83
VDR: rs731236	AA 53 (37.3%)	AG 71 (50%)	GG 18 (12.7%)	AA 22 (41.5%)	AG 25 (47.2%)	GG 6 (11.3%)	0.86
TNFRSF11B (OPG): rs3102735	CC 5 (3.5%)	CT 39 (27.5%)	TT 98 (69%)	CC 2 (3.8%)	CT 17 (32.1%)	TT 34 (64.1%)	0.81
TNFRSF11B (OPG): rs2073618	GG 31 (21.8%)	GC 69 (48.6%)	CC 42 (29.6%)	GG 7 (13.2%)	GC 29 54.7%)	CC 17 (32.1%)	0.40
SPP1 (OPN): rs9138	AA 73 (51.4%)	AC 62 (43.7%)	CC 7 (4.9%)	AA 27 (51%)	AC 21 (39.6%)	CC 5 (9.4%)	0.49
SPP1 (OPN): rs11730582	TT 52 (36.6%)	TC 64 (45.1%)	CC 26 (18.3%)	TT 11 20.8%)	TC 27 (50.9%)	CC 15 (28.3%)	0.08
TNFRSF11A (RANK): rs1805034	CC 23 (16.2%)	CT 71 (50%)	TT 48 (33.8%)	CC 14 (26.4%)	CT 24 (45.3%)	TT 15 (28.3%)	0.27
TNFRSF11A (RANK): rs8086340	CC 23 (16.2%)	CG 79 (55.6%)	GG 40 (28.2%)	CC 10 (18.9%)	CG 26 (49%)	GG 17 (32.1%)	0.72
P2RX7: rs1718119	AA 13 (24.5%)	AG 21 (39.6%)	GG 19 (35.8%)	AA 28 (19.7%)	AG 59 (41.6%)	GG 55 (38.7%)	0.76

P2RX7: rs2230912	AA 34 (64.2%)	AG 16 (30.2%)	GG 3 (5.6%)	AA 103 (72.6%)	AG 33 (23.2%)	GG 6 (4.2%)	0.56
IRAK1: rs1059703	AA 36 (67.9%)	AG 10 (18.9%)	GG 7 (13.2%)	AA 90 (63.4%)	AG 29 (20.4%)	GG 23 (16.2%)	0.82
CASP1: rs530537	CC 21 (39.6%)	CT 19 (35.9%)	TT 13 (24.5%)	CC 25 (17.6%)	CT 79 (55.6%)	TT 38 (26.8%)	0.004
CASP1: rs580253	AA 14 (26.4%)	AG 5 (9.4%)	GG 34 (64.2%)	AA 20 (14.1%)	AG 21 (14.8%)	GG 101 (71.1%)	0.11
CASP1: rs554344	CC 1 (1.9%)	CG 19 (35.8%)	GG 33 (62.3%)	CC 5 (3.5%)	CG 38 (26.8%)	GG 99 (69.7%)	0.47

Table 13: Odd Ratios of Specific SNPs Associated with EARR

Variable	Odds ratio	95% confidence interval		P-value
IL1A: rs1800587				
GG vs GA	0.54	0.28	1.06	0.07
GG vs GA+AA	0.52	0.28	0.995	0.048
IL1RN: rs419598				
CC vs CT	2.94	0.88	9.86	0.08
TT vs CT	1.93	0.92	4.06	0.08
CC+TT vs CT	2.04	0.98	4.21	0.06
SPP1 (OPN): rs11730582				
TT vs TC	0.50	0.23	1.11	0.09
TT vs CC	0.37	0.15	0.91	0.03
TT vs TC+CC	0.45	0.22	0.96	0.04
CC vs TC+TT	1.76	0.85	3.67	0.13
CASP1: s530537				
CC vs CT	3.49	1.62	7.52	0.001
CC vs TT	2.46	1.04	5.78	0.04
CC + TT vs CT	3.07	1.53	6.18	0.002
CC vs CT+TT	2.24	1.17	4.31	0.015
CASP1: rs580253				
AA vs GG	2.08	0.95	4.56	0.07
AA vs AG+GG	2.19	1.01	4.74	0.047

To our knowledge, this is the first study to demonstrate that a CASP1 polymorphisms significantly associates with risk of EARR. Future studies will be needed to further validate these results. We will continue to recruit orthodontic patients to increase the power of these studies. Special efforts should be made in the future to address the underrepresented racial/ethnic groups and evaluate any inherent differences in the allelic distribution among these populations.

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<u>Respond to the following questions</u>:

1. Were the original, specific aims of the proposal realized?

   As described in the studies and results, we have identified ethnic differences in the root to crown ratios in permanent dentition and the SRA disease prevalence, as well as the risk of SRA patients to developing root resorption with orthodontic treatment. Furthermore, we have established factors that increase the risk of patients to develop root resorption with orthodontic treatment. Finally, we have identified SNPs that significantly associate with EARR.

2. Were the results published?
   a. If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers
      1. Wang, J., Rousso, C., Christensen, B.I., Li, P., Kau, C.H., MacDougall, M., Lamani, E. (2019) Ethnic Differences of Root to Crown Ratios in the Permanent Dentition. Orthod Craniofac Res. 22(2):99-104.
   b. Was AAOF support acknowledged?
      Yes
   c. If not, are there plans to publish? If not, why not?
      The second manuscript has been submitted to the *European Journal of Orthodontics*.

A third manuscript is in preparation of the *Orthodontics and Craniofacial Research*. AAOF has been acknowledged.

3. Have the results of this proposal been presented?
    a. If so, list titles, author or co-authors of these presentation/s, year and locations
        1. **Lamani, E**., Mamaeva, O., MacDougall, M. NFI-C Regulation of Genes Involved in Root Elongation and Resorption. Oral presentation, 2017 IADR/AARD Meeting
        2. Rousso, C., Lin, C.P., MacDougall, M., **Lamani, E**. Ethnic Differences of Root-Crown Ratios in the Permanent Dentition. Poster presentation, 2017 IADR/AARD Meeting
        3. **Lamani, E**., Browne, C., Kau, CH. External Apical Root Resorption in Orthodontic Patients. Poster Presentation, 2018 AADR Meeting, Fort Lauderdale, FL.
        4. Christensen, B., Rousso, C., Lin, C.P., MacDougall, M., **Lamani, E**. Ethnic Differences in the Prevalence of Short Root Anomaly. Poster Presentation, 2018 AADR Meeting, Fort Lauderdale, FL.
        5. Wang, J., Rousso, C., Christensen, B.I., Li, P., Kau, C.H., MacDougall, M., **Lamani, E.** (2019) Ethnic Differences of Root to Crown Ratios in the Permanent Dentition. Orthod Craniofac Res. 22(2):99-104.
        6. Baghaei, N., Christensen, B., Macdougall, M., **Lamani, E**. Investigation of Short Root Anomaly and Root Resorption. Poster Presentation, 2019, NCUR Meeting, Kennesaw, GA.
        7. **Lamani, E**., Serra, R., Korf, B., MacDougall, M. Short Root Anomaly in Hispanic Families. Oral Presentation, 2019. IADR Meeting, Vancouver CA.
        8. Lamani, E., Litchfield, F., Kau, C. External Apical Root Resorption in African American Orthodontic Patients. Oral Presentation, 2020. IADR Meeting, Washington, DC.
        9. Lamani, E. Risk Factors Contributing to External Apical Root Resorption in Orthodontic Patients. (2020) American Association of Orthodontics Annual Meeting Invited Speaker. Atlanta, GA.
        10. Lamani, E., Litchfield, F., Kau, C. Analysis of Risk Factors Associated with External Apical Root Resorption. Oral Presentation, 2021 IADR Meeting.
        11. Topkara, A., Aswad, N., Lamani, E. External Apical Root Resorption after Orthodontic Treatment in Asian Patients. Poster Presentation, 2021 IADR Meeting.
        12. Lamani, E. Genes Associated with Root Resorption. Invited Speaker for the 2022 COAST Meeting scheduled for Nov 6-9th.
    b. Was AAOF support acknowledged?
        Yes
    c. If not, are there plans to do so? If not, why not?

4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?

    The AAOF funding has not only allowed me protected research time to pursue the studies related to root resorption, but also to grow as an orthodontic clinician scientist. I was able to complete the "Genetics and Genomics in Clinical Research", "Laboratory Methods", "Rigor, Reproducibility & Transparency", "Responsible Conduct of Research", "Mentoring Case Discussions Series", "Clinical Investigator Training Program", "ADEA Emerging Academic Leaders Program" and the ABO clinical exam. I have attended various scientific

meetings such as COAST, AAO, and AADR/IADR. I have received a UAB pilot grant and have submitted an NIH R03 grant.

My career goal is to become an NIDCR funded independent orthodontist-scientist. My research goal is to develop a translational research program to explore the mechanisms regulating dental disorders that could translate into implementation of diagnostic biomarkers and targeted therapies. My current research focuses to elucidate cellular signaling involved in the developmental and resorptive processes of the dentition in orthodontic patients. In order to achieve this, I will continue to build relationships and grow my network of collaborators by participating at scientific meetings. I believe, through collaborative work, I will succeed in obtaining further funding from AAOF (Biomedical Award) and NIDCR (R01 Grant).

Accounting for Project; (i.e.), any leftover funds, etc.

All the funds provided ($100,000) were used during the course of the grant.