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Are mTOR and ER stress pathway genes associated with oral and bone diseases?

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Abstract

The purpose of this cohort study was to identify associations between combined oral and bone disease phenotypes and genes present in cell regulatory pathways. The studied pathways play important roles in cellular growth, proliferation, differentiation and homeostasis. DNA samples extracted from whole saliva of 3,912 individuals were genotyped and these data analyzed according to dental caries experience, periapical lesions, periodontitis, osteoporosis, or temporomandibular joint discomfort. Samples were obtained from the Dental Registry and DNA Repository project at the University of Pittsburgh. Twenty-seven polymorphisms in eight genes related to mTOR or ER stress pathways were selected for genotyping. Allele frequencies and Hardy–Weinberg equilibrium were calculated. Analyses were performed comparing genotypes between affected and unaffected individuals for each phenotype, as well as for the associated phenotypes combined. For all analyses, we used the software PLINK with an alpha of 0.002. Borderline associations with multiple variants of several genes were found suggesting that both pathways may be involved in the susceptibility to multiple conditions affecting the oral cavity and bones. When combining patients that had concomitant dental caries, periodontitis, and periapical pathology, several markers in *RHEB* showed statistically significant association. Multiple conditions affecting bone and teeth (i.e. dental caries, periodontitis, periapical lesion formation and osteoporosis) appear to share similar underlying genetic etiological factors, which allow us to hypothesize that instead of individually, they should be studied in conjunction in human populations.

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Declaration of Interests:

The authors certify that they have NO conflict of interests.

Keywords

Dental Caries; Periodontitis; Endodontics; Osteoporosis; Endoplasmic Reticulum Stress; mTOR signalling

Introduction

Poor oral health continues to be a reason for developing health policies that protect those most susceptible in populations and enhance preventive strategies. Since the most common of these conditions (i.e. dental caries and periodontitis) are bacteria-mediated affections, water fluoridation, and oral hygiene-based preventive strategies are justified. Furthermore, individuals affected by these common conditions commonly develop additional health problems. Symptoms for these distinct conditions can be similar, such as developing discomfort, pain, and inflammation. Overtime, the chronic character of dental caries and periodontitis creating a chronic inflammatory state can lead to inadequate amino acid supply, which lead to reduced protein synthesis and degradation [Mercier et al., 2002]. With that concept in mind, we believe that certain pathways will be associated with multiple conditions, for instance, pathways related to protein synthesis.

Mammalian target of rapamycin (*mTOR*) signaling is a master regulator of protein synthesis. Tuberous sclerosis 1 and 2 (*TSC1* and *TSC2*) are two tumor suppressor genes that when activated repress *mTOR* function. When *TSC1* and *TSC2* are defective, like in the tuberous sclerosis complex, these two genes lose their function, leading to hypermineralization of bones. *MTOR* signaling is comprised of two complexes – *mTORC1* and *mTORC2*, the first is a center regulator for protein synthesis and the second is a center regulator for the serine/threonine kinase (AKT) in the cell. *RHEB* (Ras homolog enriched in brain) is a positive regulator for both *mTORC1* and *mTORC2*, but its function is repressed by *TSC1* and *TSC2* [Brown et al., 1994]. Activation of the *mTOR* signaling by alcohol use can inhibit odontogenic differentiation of human dental pulp cells [Qin et al., 2017]. When *mTOR* signaling is inhibited, there is an alleviation of the inflammatory response to periodontal pathogens such as *Porphyromonas gingivalis* [Xia et al., 2017], what leads us to believe that variation in *mTOR* signaling genes will play a role in the periodontal disease outcomes.

The other genes that we looked for association in this present study are genes that influence the endoplasmic reticulum stress (ER stress). ER stress is a phenomenon that occurs when signals emanating from the endoplasmic reticulum induce a transcriptional program that enables cells to survive the stress generated by an elevated amount of protein synthesized [Schonthal, 2012]. When affected cells sense there is ER stress, three signal pathways can be activated: ER transmembrane inositol-requiring enzyme 1 (*ERN1*) α and β, protein kinase-like ER kinase [Reimold et al.], and activating transcription factor 6 (*ATF6*). Collectively, these pathways can restore intracellular homeostasis. This coordinated response, the unfolded protein response (UPR), facilitates the folding, processing, export, and degradation of proteins emanating from the endoplasmic reticulum during stressed conditions [Ron and Walter, 2007]. *ERN1* is an endoplasmic reticulum membrane domain, which has a dual function of making apoptosis and serving as an endoribonucleous. *ERN1* splices a 26-base

pair sequence of *Xbp1* [Yoshida et al., 2001]. *Xbp1* can control many genes that are involved in the biogenesis of ER as well as protein folding. In addition, *Xbp1* expression was also demonstrated to play a role in ameloblasts endoplasmic reticulum volume during enamel formation [Tsuchiya et al., 2008]. This pathway is essential to maintain intracellular homeostasis. The conditions we are studying here are complex with variable levels of genetic influences that modulate both the development of underlying affected structures and active protein synthesis, including folding and transport, which mechanisms are not well understood in regards to pathogenesis of each disease.

In this present study, we tested the hypothesis that variations in genes belonging to the *mTOR* and ER stress pathways may play a role in oral/bone diseases such as dental caries, periodontitis, periapical pathology, osteoporosis, and temporomandibular joint discomfort. We believe that genetic variation in the selected genes will disrupt protein synthesis, affect bone metabolism, and influence chronic inflammatory states.

Materials and Methods

We selected 27 markers in eight genes of the *mTOR* and ER stress pathways to be tested in five oral/bone disease phenotypes (dental caries, periapical lesions due to deep caries lesions in dentin, periodontitis, osteoporosis, and temporomandibular joint symptoms), described in detail below. In this cohort study, a dataset consisting of DNA samples from 3,912 unrelated subjects who sought treatment at the University of Pittsburgh dental clinics was utilized. Individual samples and clinical history were obtained through the Dental Registry and DNA Repository of the School of Dental Medicine, University of Pittsburgh. Subjects' mean age was 40.9 ± 19.3 years (ranging from 6 to 92 years-old). This project has the approval of the University of Pittsburgh Institutional Review Board (IRB # 0606091). Written informed consent documents were obtained from all subjects. Age appropriate assent documents were used for children between 6 and 14 years and signed informed consent documents were obtained from the parents. For all comparisons described below, there were no significant differences in the distribution of ages and the frequency of Whites and Blacks between the two comparison groups (data not shown). This manuscript follows the STROBE guidelines for reporting observational studies.

Phenotypes and Sample Selection

One of the authors (M.B.) carried out the extraction of clinical data after being calibrated by an experienced specialist (A.R.V.). The clinical data included the complete oral conditions and treatments present in the database for each of the patients seeking care between September 2006 and January 2013. The intra-examiner agreement was assessed by a second extraction of clinical data in 10% of the sample after 2 weeks, with a kappa of 1.0. Since each phenotype studied is recovered from a registry of clinical information derived from the dental clinics of the University of Pittsburgh, calculating inter-or intra-examiner agreement is not possible. All phenotypes are recorded following the same guidelines by students in training under the supervision of experienced dental professionals who are calibrated annually.

Dental Caries—We selected 1,481 samples (715 males and 766 females) to evaluate the dental caries phenotype.The presence and severity of dental caries was taken into consideration and we used the DMFT (decayed, missing due to caries and filled teeth) score to assign individuals to one of the comparison groups. The population was classified as either having 'less severe' (N=553) or 'more severe' (N=853) caries experience, based on DMFT/dmft distribution (DMFT/dmft mean and standard deviation) and subject's age. The mean DMFT score was 15.9 with a standard deviation of 8.7 and ranged from 0 to 28. The criteria used here for classification of caries experience took age into consideration, since it is expected that caries experience will increase in the general population with age [Liss et al., 1982]. The water in the Pittsburgh area is artificially fluoridated. Table 1 describes the criteria for defining individuals with higher or lower caries experience. This study sample was previously described [Kuchler et al., 2014].

Power calculations [Purcell et al., 2003], assuming that our marker alleles were in complete linkage disequilibrium with the genetic variant contributing to caries susceptibility, and that the chance of having a distinct (very low or very high) caries susceptibility increased two and a half-fold when having one copy of the caries susceptibility allele, suggested a 92% power to detect a possible association with our sample size. This procedure was for a marker B in linkage disequilibrium with our test locus A. Other parameters specified in the calculations were the high-risk allele frequency for the allele A (set at 0.1); the disease prevalence in the general population [set at 0.5, corresponding to the approximate frequency of caries-free individuals or highly affected individuals (DMFT=20)], and the genotype risks for the Aa and AA genotypes relative to the baseline aa genotype risk. We used most of the same power parameters for all five phenotypes (dental caries, periodontitis, periapical pathology, osteoporosis, and temporomandibular joint discomfort).

Periapical Lesions Due to Deep Caries Lesions in Dentin—Sixteen hundred radiographic records were screened for subjects with deep carious lesions in dentin with or without periapical lesions (≥3mm in diameter). The criteria used to select the affected group was the presence of both deep carious lesions and periapical lesions (110 individuals, 57 males and 53 females with an average age of 57 years and a standard deviation of 10 years) and for comparison, we selected a group in which they had the presence of deep carious lesions but absence of periapical lesions (158 individuals, 65 males and 93 females with an average age of 58 years and a standard deviation of 8 years). This cohort of a total of 268 samples has been previously reported [Menezes-Silva et al., 2012]. We estimated to have 85% power to detect an association with the studied sample size. The disease prevalence in the general population was set at 0.5, corresponding to the approximate frequency of deep caries lesions and absence of periapical lesions in the study sample.

Periodontitis—Individuals were considered affected if presenting at least three teeth exhibiting sites of clinical attachment loss equal or greater to 5 mm in two different quadrants (61 individuals, 22 males and 39 females). For comparison, we selected individuals showing absence of clinical attachment loss and no sites with probing depth greater than 3 mm (325 individuals, 144 males and 181 females) totalizing 386 samples selected for genotyping (average age was 50 years with a standard deviation of 8 years). This

sample has been previously described [Letra et al., 2012]. Considering a prevalence of periodontitis of 60%, a power of 80% was estimated for this phenotype in our study sample.

Osteoporosis—Twenty-two cases of osteoporosis/osteopenia were identified (20 females and 2 males, mean age of 62 years), and 553 unaffected individuals older than 50 years of age (221 men, 332 women, mean age of 68 years) were selected to serve as comparison. None of the selected cases had periodontitis but seven of those had dental caries and were part of the dental caries group described above as well. We estimated power as 43% with the sample size we had. The prevalence of osteoporosis was set at 0.55, according to the May 2018 Interdisciplinary Symposium on Osteoporosis (nof.org).

Temporomandibular Joint Symptoms—We selected 1,202 women in child bearing age, 521 with a record of, at least, one symptom in the temporomandibular joint (clicks, sounds or pain) and 681 without any symptoms that were used for comparison. Their mean age was 35.3 years, ranging from 15 to 55 years. Power was estimated as 100% to detect a possible association with the studied sample size. The disease prevalence in the general population was set at 0.5.

Combined Phenotypes—We followed the European Federation of Periodontology (EFP) and European Organization for Caries Research (ORCA) joint recommendation and analyzed dental caries combined with periodontitis within the same individuals [Chapple et al., 2017]. In addition, we analyzed dental caries combined with periapical pathology and caries combined with periodontitis and periapical pathology. Osteoporosis and temporomandibular joint symptoms were excluded from this analysis since only 22 cases with confirmed osteoporosis/osteopenia were available and for temporomandibular joint symptoms no formal significant associations with the selected genes were found.

We excluded 24 participants that had missing information about the presence of periodontitis or periapical pathology since those conditions were taken into consideration for the combined analyses. We found that 794 individuals had periodontitis and recorded caries experience (high caries experience, 174 females and 149 males; low caries experience, 229 females, 242 males), 433 had periapical pathology and recorded caries experience (high caries experience, 57 females and 63 males; low caries experience, 158 females, 155 males), and 234 have periodontitis, periapical pathology and high caries experience (high caries experience, 31 females and 48 males; low caries experience, 90 females, 107 males). Combining the disease phenotypes aims to generate a more homogeneous group.

Selection of Genes and Single Nucleotide Polymorphisms

Since *RHEB* is a molecule located at the center of the mTOR pathway and can be repressed by *TSC1 and 2*, we choose genes immediately up and downstream of *RHEB*. *RHEB*'s role also impacts ER stress [Fan et al., 2017]. Twenty-seven single nucleotide polymorphisms (SNPs) in eight genes were selected (*ERN1* -rs196929, rs196950, rs11655020, rs16947425 and rs1874087, *XBP1* -rs2097461 and rs2239815, *RPTOR* -rs2289764, rs1012117, rs11651724, rs4255830 and rs4396582, *TSC1* -rs1050700, *TSC2* -rs1051771, rs7187438 and rs2073636, *RHEB* -rs3753151, rs2299967, rs2374261 and rs1109089, *RICTOR* -

rs1239265, rs13166875, rs1423688 and rs2043112 and *mTOR* -rs11580061, rs1010447 and rs11121718). We prioritized genes to be studied considering: [Fan et al.] previous reports of expression in diseased tissues, and [Fan et al.] previous reports of association with bone diseases. In the case of temporomandibular joint symptoms, four polymorphisms were tested in *ERN1* (rs11655020, rs1874087, rs196950 and rs196929) and two in *XBP1* (rs2097461 and rs2239815).

SNPs were selected based on published reports and/or their locations in the genes, based on their likelihood to have functional consequences (i.e., located in promoter regions, exons or near exon/intron boundaries), or if considered tag SNPs as surrogates for the linkage disequilibrium blocks surrounding the gene of interest. We used information from the NCBI dbSNP (http://www.ncbi.nlm.nih.gov/snp) and the HapMap Project (http://www.hapmap.org) databases for selecting SNPs to be studied.

Genotyping

Genomic DNA was extracted from whole saliva using established protocols. Genotypes were generated blindly to clinical diagnosis status. Reactions were carried out using Taqman chemistry [Ranade et al., 2001] in volumes of 3.0 μl in an ABI PRISM Sequence Detection System 7900 (Applied Biosystems, Foster City, CA, USA). Applied Biosystems supplied assays and reagents. The results were analyzed using SDS software version 1.7 (Applied Biosystems). PCR reactions were repeated twice when necessary.

Data Analyses

Allele frequencies and Hardy–Weinberg equilibrium were calculated. Association analyses were performed comparing genotypes between affected individuals and their respective comparison group as implemented in PLINK [Purcell et al., 2007]. P-values below 0.002 (0.05/27; the denominator is the number of genetic markers tested) were considered statistically significant. Additional analysis was performed combining patients that have more than one oral disease phenotype (periodontitis, periapical pathology, and caries experience), as well as the three oral phenotypes combined.

Results

We found nominal associations between each of the five phenotypes studied and mTOR or ER stress genes (Table 2). Significant associations were found between periapical lesions due to deep caries lesions in dentin and *RHEB* (p=0.0002), osteoporosis/osteopenia and *mTOR* (p=6.0E-8), *RAPTOR* (p=0.002), *RICTOR* (p=0.001), *ERN1* (p=5.0E-5), and *TSC2* (p=4.0E-5), and dental caries and *RPTOR* (p=0.0008). When combining patients that had concomitant dental caries, periodontitis, and periapical pathology, several markers in *RHEB* showed association (Table 3).

Discussion

Although caries is most times a preventable disease, it is still very common all over the world. Many factors can play a role in the dental caries phenotype, such as the high level of consumption of sugar and carbs, the oral microbiota, exposure to fluorides from the drinking

water and/or dentifrices, and poor oral hygiene. Even though all these factors contribute for the disease development, evidence suggests that individual genetic variation plays a role in the disease process as well [Vieira et al., 2012].

The genetic factors related to the host, that are represented by genes involving enamel formation, saliva composition, dietary preferences, and immune response [Shuler, 2001], can be involved in caries susceptibility. Genes in the *mTOR* (mammalian target of rapamycin) pathway are involved in dental mineralization [Kim et al., 2011] and hence could contribute to caries susceptibility. In addition, fluoride induces ER (endoplasmic reticulum) stress and interferes with enamel proteinase secretion [Wei et al., 2013], which may result in alterations of the enamel that can modify individual caries susceptibility.

mTORC1 is a ternary complex containing *mTOR, RAPTOR* (Regulatory Associated Protein of *mTOR*) and G-BetaL. *MTORC1* regulates VEGF (Vascular Endothelial Growth Factor) by inducing HIF1α [Brugarolas et al., 2003]. The association between genetic variation in *RAPTOR* and cases with more severe caries experience may be related to a mechanism that involves individuals more susceptible to caries lesion progression in enamel and dentine.

When the caries phenotype is defined as having caries or not having caries, DMFT score 0 versus DMFT score 1 or higher, it may not be feasible to detect genes that may contribute to the severity of the disease. This is probably the general limitation of the work done by us and others, both including candidate gene approaches [Tannure et al., 2012a; Tannure et al., 2012b] and genome wide scan analyses [Shaffer et al., 2011; Wang et al., 2012]. If anybody with any recorded DMFT score is assigned to the disease group, this group will have individuals with very little caries experience (DMFT scores 1 or 2) along with individuals with much higher DMFT scores. We believe a DMFT score of 1 is not under the same influences of a DMFT score of 10, having one decayed tooth is much less of a severe disease than having 10 teeth affected for example. Although it may be surprising for many, Pittsburgh is the largest city of the Appalachian region, which is one of the poorest areas of the US, and consequently a region with very poor health indicators, including oral health indicators [Anjomshoaa et al., 2009]. The phenotyping scheme used in our study takes into account ages and distribution of caries experience in the study participants. Individuals with very severe caries experience (DMFT higher than 10 by 20 years of age) demonstrated an association with a genetic variant in *RAPTOR*. We have previously tested modifying the cut-off thresholds of those definitions and shown that there are no dramatic differences when the threshold is moved slightly (DMFT cut-off 9 in comparison to 10 or 11; [Deeley et al., 2008] [Shimizu et al., 2013], but the limitation continues to be that we lose statistical power when trying to make more distinct groups eliminating intermediate values (comparing caries free with individuals with DMFT 10 or higher).

This is the first study that has provided evidence for association between a gene in the *mTOR* pathway and caries. This information may turn out to be relevant for the tissue engineering effort focused in regenerating dentin and the creation of more biocompatible approaches to rehabilitate dental tissue destruction, since *RAPTOR* particularly regulates cell growth in response to nutrient and insulin levels [Foster et al., 2010].

We found that periodontal disease and osteoporosis were associated with single nucleotide polymorphisms (SNPs) in *ERN1*. This result is remarkable, particularly for osteoporosis, due to our study sample size, and should be taken cautiously. *ERN1* is involved in the development of secretory cells and organs [Reimold et al., 2000] as well as osteoclastogenesis [Tohmonda et al., 2015], suggesting that *ERN1* deficiency may induce osteopenia/osteoporosis with a slow bone turnover. Recent evidence indicates that the inhibitory effects of *TSC1/TSC2* is mediated through *TSC2* inactivation of *RHEB* and it has been postulated that *TSC1/TSC2* complex inhibits *mTOR* signaling, that pathway is a central regulator of proliferation and cellular growth. Without this functional signaling, cells at the inflammatory sites cannot regenerate, and consequently there will be periodontal tissue destruction or higher predisposition for pulp inflammation even under slow progressing deep caries lesions in dentin. This process will be aggravated by the swollen gingiva and accumulation of plaque.

In this present study, we have done a combined analysis of three phenotypes with the intention of finding a pleiotropic effect of the genes typed in the studied phenotypes. Pleiotropy is when one gene appears to affect more than one unrelated phenotypic trait. Previous studies looking at genetic variants were inconclusive in evidencing an association between caries and periodontitis; none of the gene variants that showed association with periodontitis had been associated with caries before [Nibali et al., 2017]. In our study, both rs2374261 and rs1109089 markers in *RHEB* showed associations under the dominant model with individuals that had caries and periodontitis. This approach might be efficient in finding pleiotropic genes associated with oral phenotypes and help identify individuals with poor oral health outcomes. We believe that sophisticating phenotype descriptions [Vieira, 2018] and looking for patterns of disease affection [Koruyucu et al., 2018; Weber et al., 2018] based on common underlying mechanisms (i.e. inflammation) are more promising approaches for identifying genes contributing to poor oral health outcomes than genome-wide association studies that use very crude phenotypical descriptions such as “caries-free” versus ‘caries-affected.”

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Table 1.

Definition of caries experience based on age and DMFT (decayed, missing due to caries and filled teeth) scores. The thresholds were defined based on the DMFT distribution in the studied group by age [Kuchler et al., 2014].

Caries Experience Level	DMFT/dmft	Sample (N=1,481)
Children and teenagers (from 6 to 19 years of age)		
Less Severe Caries Experience	0–3	73
More Severe Caries Experience	4 or higher	138
Young Adults (from 20 to 39 years of age)		
Less Severe Caries Experience	0–10	215
More Severe Caries Experience	11 or higher	241
Middle Age (from 40 to 59 years of age)		
Less Severe Caries Experience	0–15	152
More Severe Caries Experience	16 or higher	384
Elderly (60 years of age and older)		
Less Severe Caries Experience	0–20	95
More Severe Caries Experience	21 or higher	183

Table 2.

Summary results highlighting nominal (between 0.05 and 0.0025) and statistically significant (equal or lower than 0.002, marked in bold) p-values for allele and genotype association analyses between periapical lesions, periodontal disease, osteoporosis, dental caries experience, and temporomandibular joint symptoms and the evaluated markers. Odds Ratios [Koruyucu et al.] and 95% confidence intervals (C.I.) were calculated for the significant results (p<0.002) of allele frequency comparisons.

Gene	SNP	Periapical Lesions		Periodontitis		Osteoporosis		Caries		TMD	
		p-value		p-value		p-value		p-value		p-value	
		Allele	Genotype	Allele	Genotype	Allele	Genotype	Allele	Genotype	Allele	Genotype
ERN1	196929	0.04	NS	0.008	0.02	NS	NS	NS	NS	NS	NS
	196950	0.03	NS	0.04	NS	NS	NS	NS	NS	NS	NS
	11655020	NS	NS	NS	NS	NS	0.05	0.01	0.02	0.04	0.05
	16947425	NS	NS	NS	NS	0.003	**5.0E-5**	NS	NS	NS	NS
XBP1	2097461	NS	0.04	NS	NS	0.004	0.004	NS	NS	NS	NS
	2239815	NS	0.05	NS	NS	0.03	NS	NS	NS	NS	NS
RAPTOR	2289764	NS	NS	0.01	NS	NS	NS	0.05	NS	NS	NS
	11651724 (OR, 95% C.I. for allele)	NS	NS	NS	NS	NS	**0.002**	**0.0008** (1.50; 1.18–1.91)	0.004	NS	NS
	1012117	NS	NS	NS	NS	NS	0.02	NS	NS	NS	NS
TSC1	1050700	NS	NS	0.04	NS	NS	NS	NS	NS	NS	NS
TSC2	1051771	NS	NS	NS	NS	0.01	**4.0E-5**	NS	NS	NS	NS
RHEB	3753151	NS	**0.0002**	NS	NS	S	NS	NS	NS	NS	NS
RICTOR	1239265	NS	NS	0.07	NS	0.007	**0.001**	NS	NS	NS	NS
MTOR	11580061 (OR, 95% C.I. for allele)	NS	NS	NS	NS	**6.0E-8** (8.51; 3.4-21.26)	**0.0004**	NS	NS	NS	NS

NS= Non-statistically significant

Table 3.

Summary of all nominal (p-values between 0.05 and 0.002) and significant results from the combined analysis of patients with caries, periodontitis and periapical lesions (bold indicates statistically significant p-values under the threshold 0.002).

	Gene	SNP	p-value	Genotypic Model
Patients with Periodontal Disease and Caries	*RAPTOR*	rs1012117	0.05	Recessive
	RHEB	rs1109089	0.02	Dominant
	RICTOR	rs1423688	0.03	Recessive
	RAPTOR	rs2374261	0.02	Genotype
	RHEB	rs2374261	0.004	Dominant
	RAPTOR	rs4396582	0.03	Genotype
	RPTOR	rs4396582	0.03	Dominant
Patients with Periapical Lesions and Caries	*RHEB*	rs1109089	**0.002**	Genotype
	RHEB	rs1109089	**0.0006**	Allelic
	RHEB	rs1109089	**0.0007**	Dominant
	RHEB	rs2374261	0.003	Genotype
	RHEB	rs2374261	**0.0007**	Allelic
	RHEB	rs2374261	**0.001**	Dominant
	ERN1	rs16947425	0.04	Recessive
	ERN1	rs196950	0.04	Allelic
	XBP1	rs2239815	0.04	Dominant
Patients with Periapical Lesions, Periodontitis, and Caries	*RHEB*	rs1109089	0.005	Genotype
	RHEB	rs1109089	0.003	Allelic
	RHEB	rs1109089	**0.001**	Dominant
	RHEB	rs2374261	**0.0009**	Genotype
	RHEB	rs2374261	**0.0003**	Allelic
	RHEB	rs2374261	**0.0002**	Dominant
	ERN1	rs16947425	**0.04**	Allelic
	RAPTOR	rs4396582	**0.001**	Allelic