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Variantes da interleucina-1 e suscetibilidade genética à reabsorção radicular apical externa

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Summary

Resumo

Introdução: A reabsorção radicular apical externa (RRAE) é uma sequela iatrogénica resultante do tratamento ortodôntico. A sua etiologia é complexa e influenciada pela associação de múltiplos fatores de risco como variantes ambientais, biológicas e genéticas. Tem sido desenvolvido muito trabalho de investigação na tentativa de identificar marcadores polimórficos de ADN para a RRAE, que permitam definir o risco de suscetibilidade pré-tratamento. Do mesmo modo, têm vindo a ser descritas variantes genéticas para a periodontite crónica (PC) que poderiam melhorar a avaliação do prognóstico da doença e auxiliar na definição do plano do tratamento. Os genes associados às vias da interleucina-1 estão entre os mais estudados para ambas as doenças. Ambas as patologias, RRAE e PC, estão dependentes de mecanismos imunoinflamatórios e da resposta do metabolismo do osso alveolar. Curiosamente, na RRAE a remodelação óssea é protetora, enquanto na PC a perda de tecido ósseo é uma característica da doença. É, portanto, expectável que estas doenças multifatoriais partilhem alguns genes candidatos.

Objetivo: O nosso objetivo é verificar se os mesmos polimorfismos genéticos influenciam a suscetibilidade de um paciente para a RRAE e/ou Periodontite crónica em direções opostas.

Materiais e métodos / Metodologia de pesquisa: Foi realizada uma análise crítica dos resultados da literatura relativamente aos dois polimorfismos mais estudados em ambas as doenças: rs1800587 do gene da Interleucina-1 alfa (IL1A) e rs1143634 do gene da interleucina-1 beta (IL1B). A pesquisa eletrónica foi realizada nas bases de dados MEDLINE/PubMed e EBSCOhost. Os títulos e resumos dos artigos foram rastreados com base em critérios de inclusão e exclusão. Outra literatura relevante foi adicionada manualmente.

Resultados: A pesquisa eletrónica gerou um total de 382 artigos. Vinte e um artigos satisfaziam os critérios de inclusão e exclusão: 12 estudos caso-controlo, 2 estudos retrospectivos e 5 meta-análises. Foi elaborada uma tabela de resumo dos resultados dos estudos incluídos.

Discussão: Na última década vários estudos de associação genética foram conduzidos com o objectivo de investigar a relação entre os polimorfismos da IL1 e os fenótipos estudados. A maioria dos estudos não encontra uma associação estatisticamente significativa entre o polimorfismo da IL1A (rs1800587) e o aumento da suscetibilidade para a RRAE ou para a PC. Para o polimorfismo da IL1B (rs1143634),

os resultados foram mais consistentes, sendo que a presença do alelo C foi frequentemente associada ao aumento da suscetibilidade para a RRAE, enquanto a presença de alelo T foi preferencialmente associada a uma suscetibilidade aumentada para a PC. No entanto, os resultados e as conclusões de muitos estudos são discordantes.

Conclusão: Os resultados da literatura relativos ao polimorfismo da IL1B (rs1143634) apoiam a nossa hipótese inicial de um perfil genético oposto entre RRAE e a PC. Contudo os resultados para o polimorfismo da IL1A não confirmam esta teoria, impondo a avaliação de outros polimorfismos. Esta relação entre as duas patologias pode ser útil na seleção de genes candidatos de suscetibilidade. Essas variantes genéticas podem, no futuro, integrar modelos preditivos que permitam a otimização do tratamento ortodôntico. Além disso, a suscetibilidade para as duas doenças pode ser prevista ao mesmo tempo, através da caracterização dos mesmos *loci*.

Palavras-Chaves: "Ortodontia", "Reabsorção Radicular", "Polimorfismos Genéticos", "Interleucina-1", "Periodontite Crónica".

Abstract

Introduction: External apical root resorption (EARR) is an iatrogenic outcome of orthodontic treatment, with complex etiology, influenced by the association of multiple risk factors, including environmental, biological and genetic variants. Many investigations have searched for polymorphic DNA markers for EARR in order to define a pre-treatment risk. Similarly, gene variants have been reported in chronic periodontitis (CP) that are associated with disease prognosis and may assist in treatment planning. Genes associated with interleukin-1 pathways are among the most studied for EARR and CP. Both diseases, EARR and CP, are dependent on immunoinflammatory mechanisms and alveolar bone metabolism, but if EARR benefits from bone remodelling, CP is characterized by osteolytic damaged. Then, it is expectable that these two multifactorial diseases share some candidate genes.

Aim: Our aim was to verify if the same genetic polymorphisms influence patient's susceptibility to EARR and/or Chronic periodontitis in opposite directions.

Material and methods / Search methodology: A critical analysis of literature's results concerning two of the most studied polymorphisms in both diseases, rs1800587 from Interleukin-1 alpha (IL1A) gene and rs1143634 from interleukin-1 beta (IL1B) gene, was performed. The electronic search included MEDLINE/PubMed and EBSCOhost databases. The titles and abstracts of the articles were screened, based on inclusion and exclusion criteria. Other relevant literature was manually added.

Results: The electronic search yielded a total of 382 articles. Twenty one articles met the inclusion and exclusion criteria: 12 case-control, 2 retrospective studies and 5 meta-analyses. A summary table of the study's results was performed.

Discussion: In the last decade several genetic association studies have been conducted to investigate the relation between IL1 polymorphisms and the studied phenotypes. Most of the studies could not prove the association between *IL1A* rs1800587 and susceptibility for EARR or for CP. For the *IL1B* rs1143634 polymorphism, results were more consistent, showing that the presence of allele C was often linked to an increased susceptibility for EARR, whereas the presence of allele T was preferentially associated with an increased susceptibility for CP. However, the results and conclusions remain conflicting.

Conclusion: The literature results on *IL1B* rs1143634 polymorphism supports our initial hypothesis of an opposite genetic profile between EARR and CP. Results of IL1A

polymorphism were contradictory even for each pathology. Additional genetically based studies are still required in order to provide supplementary information and support this theory. The use of this knowledge will assist in the design of gene-candidate studies aiming to identify the genetic profile of susceptibility to EARR. Also, the susceptibility for the two diseases may be predicted at the same time, through characterization of the same *loci*. These genetic variants may, in the future, integrate predictive models allowing optimization of orthodontic treatment.

Key Words: "Orthodontics", "Root Resorption", "Genetic Polymorphisms", "Interleukin-1"**,** "Chronic Periodontitis".

Abbreviations

2D – Two dimensions

3D – Three dimensions

5' - End of nucleotide sequence with the carbon phosphate 5 of free pentose

ATP - Adenosine triphosphate

C - Cytocine

CI – Confidence interval

CP – Chronic periodontitis

DNA - Deoxyribonucleic acid

EARR - External apical root resorption

IL1 – Interleukin-1

IL1A - Interleukin-1 alpha

IL1B - Interleukin-1 beta

IL1A - Interleukin-1 alpha gene

IL1B - Interleukin-1 beta gene

IL1RN – Antagonist receptor of IL1 gene

n – Number

NLRP3 - Nod-like receptor pyrin domain containing 3

OPG - Osteoprogerin

OR - Odd ratio: statistic term which translate the risk of a condition, > 1 high risk, < 1 low risk, = 1 identical risk

P2X7 – Purigenic receptor P2X7

PDL – Periodontal ligament

Phe – Phenylalanine residue

RANK - Receptor activator of nuclear factor-k B

RANKL - Receptor activator of nuclear factor-k B ligand

SNP – Single nucleotide polymorphism

T - Thymine

UTR – Untranslated region

The nomenclature used for the genes is according to *HUGO gene nomenclature committee*.

1. Introduction

In an orthodontic treatment, the desired positioning of teeth is achieved by the application of forces that induce teeth movement. This phenomenon depends directly on alveolar bone remodeling (coupled and balanced bone resorption and formation) around the tooth (Iglesias-Linares et al. 2016).

External apical root resorption (EARR) is an iatrogenic outcome of orthodontic treatment, and may result in irreversible root shortening (Al-Qawasmi et al. 2003). However, it can occur in the absence of orthodontic treatment (Gulden et al. 2009; Linhartova et al. 2013; Wu et al. 2013; Sharab et al. 2015) with an incidence of 7 % to 13 % (Hartsfield et al. 2004; Hartsfield 2009; Linhartova et al. 2013). The apical portion of the root is usually the most affected, due to three main reasons: the concentration of orthodontic forces upon root apex, the presence of periodontal fibers with different orientations and the existence of a more friable cementum (cellular cementum) in the apical region (Hartsfield et al. 2004).

EARR is usually diagnosed during routine X-ray imaging. Different techniques, such as periapical (Bastos Lages et al. 2009), occlusal (Sharab et al. 2015), panoramic (Gulden et al. 2009; Tomoyasu et al. 2009; Iglesias-Linares et al. 2012; Linhartova et al. 2013; Pereira et al. 2014; Sharab et al. 2015; Pereira et al. 2016), lateral cephalogram radiographs (Tomoyasu et al. 2009; Iglesias-Linares et al. 2012; Linhartova et al. 2013) and, recently, cone beam computed tomography, have been used to diagnose and classify the severity of EARR. The scale of Levander and Malmgren (Fig. 1) is the most referenced among the studies (Pereira 2014; Sharab et al. 2015).
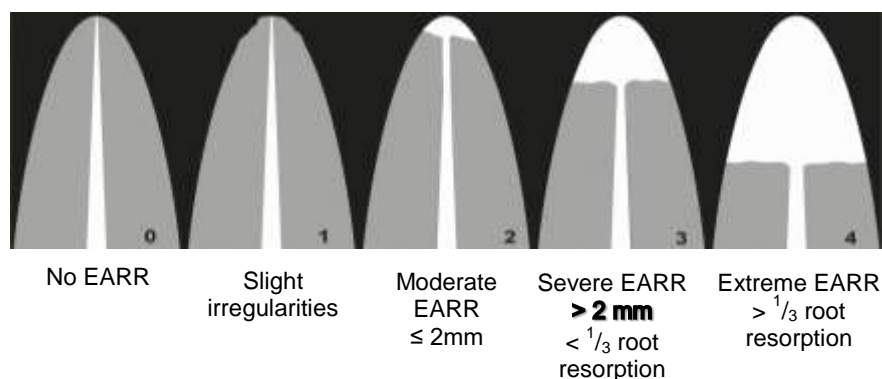

Figure 1 - Classification scale of Levander and Malmgren (Maues et al. 2015).

Approximately one-third of orthodontic patients suffer from moderate EARR, and 2-5 % from a severe form (Hartsfield et al. 2004). Although EARR may occur in any teeth, the most affected ones are the maxillary incisors, followed by the mandibular incisors (Al-Qawasmi et al. 2003; Hartsfield et al. 2004; Gulden et al. 2009; Linhartova et al. 2013; Pereira et al. 2016).

EARR is a complex phenotype influenced by the association of several environmental and host risk factors. Some are orthodontic treatment related factors, such as duration of treatment, maxillary premolar extraction (Mohandesan et al. 2007; Nanekrungsan et al. 2012; Pereira et al. 2014; Pereira et al. 2016), type of forces (Pandis et al. 2008) and sort of orthodontic appliance (Pandis et al. 2008; Pereira et al. 2014; Pereira 2014; Pereira et al. 2016). Multiple individual biological variations, such as age, systemic conditions, root shape, malocclusion type (Nanekrungsan et al. 2012), gender (Mohandesan et al. 2007; Pereira et al. 2014; Pereira et al. 2016) and genetic predisposition have also been implicated in EARR development. Newman (Newman 1975) was the first researcher to describe family clustering of EARR. Heritability estimates using sib-pair (Harris et al. 1997; Hartsfield et al. 2004) and twin studies (Ngan et al. 2004) have been fairly high, from 50 % to 84 %. Population and animal studies also supports that this is a genetically influenced trait involving multiple genes (Brezniak and Wasserstein 1993).

EARR process involves molecular pathways that promote activation of cells responsible for alveolar bone remodelling and root demineralization, through the formation of resorption pits (Iglesias-Linares et al. 2016). Orthodontic mechanical loading leads to compression of the periodontal ligament (PDL), with decrease or even interruption of the microcirculation, with consequent anoxia and sterile necrosis (corresponding to the hyaline tissue observed in histologic preparations) (Bastos Lages et al. 2009). The necrotic tissues will be eliminated by resident PDL and bone marrow-derived hematopoietic precursor cells, like macrophages (Hartsfield 2009; Iglesias-Linares and Hartsfield 2017). During the inflammatory process, pro-inflammatory cytokines, such as tumor necrosis factor (TNFα), interleukin-1 (IL1), other cytokines and ATP (adenosine triphosphate) are released. These mediators promote cellular activation of osteoblasts and osteoclast (responsible for bone remodelling), as well as induce the differentiation of odontoclasts and cementoclasts. The periodontal ligament seems to play a key role on the EARR process, because it is the origin of the cellular components for the root resorption mechanisms (Pereira 2014).

During orthodontic treatment, alveolar bone is submitted to direct compression in one surface, and tension in the opposite surface. The applied forces will induce local release of mediators and trigger mechanotransduction pathways. They predominantly

activate osteoclasts in the compression surface, and osteoblasts in the tension surface, assuring an adequate bone remodeling, necessary for tooth movement and stabilization. If there is disequilibrium between remodeling of alveolar bone and mechanical loading, resistance and repair capacity of periapical tissues will be exceeded and EARR may occur. Destruction of cementoblasts and activation of cementoclasts will initiate mineral resorption resulting in exposure of dentin (Prakash and Nagar 2013; Feller et al. 2016; Iglesias-Linares and Hartsfield 2017). The exposed dentin is more likely to be attacked by odontoclastic cells (Hartsfield 2009; Prakash and Nagar 2013), since the clastic cells have a greater affinity towards the root dentin than to the bone (Iglesias-Linares and Hartsfield 2017). At the end of orthodontic treatment, root resorption ceases and some cellular cementum is laid up (Feller et al. 2016).

The role of IL1 in alveolar bone remodelling, and indirectly in EARR, is, at least, partially mediated by two cellular pathways: RANK/RANKL/OPG and P2XR7.

Osteoclasts are multinucleated giant cells that are differentiated from hematopoietic cells of myeloid lineage. Receptor activator of nuclear factor-k B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) are essential molecules for differentiation of osteoclasts from their precursors. RANKL is a membrane protein belonging to the tumor necrosis factor superfamily, that is expressed in osteoblasts. RANKL binds to its receptor, RANK, on the surface of osteoclasts precursors, activating them. Osteoprotegerin (OPG) is also released by osteoblasts and protects the bone from excessive resorption, by competing with RANKL for the binding to RANK. IL1, TNFα and other cytokines, promote osteoclastogenesis indirectly, by increasing the expression of RANKL and M-CSF by stromal cells and T cells, and also by acting directly on osteoclasts precursors to synergize with RANKL in driving osteoclastogenesis (Teitelbaum 2006; Takayanagi 2007). IL1 cytokine is able to stimulate osteoclasts on its own, however, if the osteoclasts are previously activated by RANKL, these cells will become more responsive to IL1B (Pereira 2014).

The P2X purinergic receptor ligand-gated ion channel 7 (P2XR7) is an ionic channel ATP dependent, present in the surface of macrophages, osteoblasts, osteoclasts and osteocytes (Iglesias-Linares and Hartsfield 2017) and it was recently identified in PDL cells of orthodontic patients (Viecilli et al. 2009). In immunoinflammatory cells and in osteoclasts precursors, activation of P2XR7 by ATP leads to overexpression of cytokines, namely IL1B (Hartsfield 2009; Pereira et al. 2016; Iglesias-Linares and Hartsfield 2017). These mediators act as chemo attractants of phagocytic cells, in the resorption pit. Autocrine and paracrine (by immunoinflammatory cells) IL1 stimulation of osteoclasts cells and the activation of RANK/RANKL/OPG

pathway will enhance bone remodelling process (Hartsfield 2009; Iglesias-Linares and Hartsfield 2017).

Though having a different primary etiology, periodontitis shares molecular pathways with EARR. Periodontitis is a chronic, immunoinflammatory multifactorial disease, with microbial, environmental (e.g. tobacco) and individual etiologic factors. It is characterized by the destruction of the tooth-connective tissues and supporting bone (Kornman et al. 1997; Armitage 1999), being responsible for premature tooth loss (Isaza-Guzman et al. 2016). Although bacterial infection is the key factor for the initiation of periodontal diseases, individual factors are important, with approximately 50-60 % of the clinical variance, severity and progression, explained by genetic variations (Kornman et al. 1997; Karimbux et al. 2012; Wu et al. 2015)

Periodontitis can be classified in chronic periodontitis (CP) or aggressive periodontitis (AP) (Armitage 1999). The most frequent type of periodontitis is chronic periodontitis, which is present in 10-30 % (Nikolopoulos et al. 2008; Deng et al. 2013; Mao et al. 2013) of adults, whether the severe forms may be found in 7-13 % of the population (Nikolopoulos et al. 2008; Wu et al. 2015). Due to its lower frequency, aggressive periodontitis is less studied.

In CP, biological substances produced by bacterial agents induce an inflammatory response in periodontal tissues (Mendonça et al. 2015). High levels of IL1 have been found in gingival crevicular fluid (GCF) of patients with periodontitis, with the levels being proportional to the severity of the symptoms (Karimbux et al. 2012; Yin et al. 2016). Therefore, IL1 expression in gingival crevicular fluid is one of the most reliable predictors of periodontitis progression and severity (Wu et al. 2015). In periodontal diseases, this cytokine is the most active inducer of osteoclastic activity and, consequent alveolar bone resorption characteristic of the disease (Deng et al. 2013).

Chronic periodontitis can be influenced by the activation of the NLRP3 inflammasome in immunoinflammatory cells, mainly of myeloid lineage, in response to several bacterial, chemical and physical agents. After sensing danger signals, NLRP3 triggers a molecular cascade that leads to caspase-1 activation resulting in maturation and secretion of proinflammatory cytokines, IL1 and IL18 (Isaza-Guzman et al. 2016).

As mentioned before, the IL1 cytokine has important roles as molecular mediator of immunity and inflammatory reactions, by regulating cellular differentiation, proliferation, migration and apoptosis (Kornman et al. 1997; Wu et al. 2015). There are two forms of IL1: interleukin-1 alpha (IL1A) and interleukin-1 beta (IL1B). IL1A is a mediator of local inflammation and a regulator of intracellular events (Nikolopoulos et al. 2008; Yin et al. 2016). IL1B is a powerful extracellular bone-resorptive cytokine

released by activated fibroblasts and macrophages (Al-Qawasmi et al. 2003; Nikolopoulos et al. 2008; Sharab et al. 2015; Yin et al. 2016). Although both of these cytokines have a similar tertiary structure, IL1B is fifteen times more potent in inducing bone resorption (Pereira 2014). In tissues, they act as primary activators of early chemoattractant cytokines and facilitate migration of leucocytes, by inducing the expression of adhesion molecules (Deng et al. 2013).

IL1-dependent cellular pathways seem to have a pivotal role in both EARR and periodontitis. In fact, increased levels of IL1B have also been found in gingival crevicular fluid and gingival tissues of orthodontic patients (Al-Qawasmi et al. 2003; Gulden et al. 2009; Linhartova et al. 2013). In periodontitis, IL1B is responsible for the initiation and progression of the pathology through direct bone resorption or stimulation of secondary pathways (Grigoriadou et al. 2010). Yet, in orthodontic treatment, IL1B action favours bone remodelling, therefore, if there is defective IL1B production, root may be exposed to excessive mechanical loading and EARR occurs. Genetic variants that influence IL1B expression levels may have opposing effects on the susceptibility to the related phenotypes, EARR and periodontitis.

Great effort has been directed to the identification of functional gene variants that contribute to inter-individual susceptibility, to multifactorial diseases. Many investigations have searched for polymorphic DNA markers for EARR in order to define a pre-treatment risk. Similarly, gene variants in chronic periodontitis have been reported that could define prognosis and assist in treatment planning.

The IL1 gene cluster is present on the human chromosome 2q14, and includes *IL1A* gene (IL1-alpha)*, IL1B* gene (IL1-beta) and th*e IL1RN* gene (IL1 receptor antagonist), a competitive inhibitor for the proinflammatory cytokines (Al-Qawasmi et al. 2003). Single nucleotide polymorphisms (SNPs) of *IL1A* and *IL1B* genes are the genetic variants most frequently studied in association with CP and EARR.

Taking into account the pathophysiology of EARR and periodontitis, it is expectable that these two multifactorial diseases share some candidate genes, and that for each locus, the allelic variant that increases the susceptibility to one phenotype, decreases the susceptibility to the other one.

2. Aim

The goal of our work is to clarify and understand the hypothesis of a genetic relationship between two diseases with similar physiopathology, the EARR and the CP.

3. Materials and methods / Search methodology

The methodology applied is a bibliographic search of the currently available literature concerning two of the most studied genetic variants in *IL1A* and *IL1B* genes, with critical evaluation of the included studies.

This review of literature was performed according to the PICO methodology (Patient, intervention, comparison, outcome), establishing the following question:

- (P) Population: orthodontic patients with EARR and patients with chronic periodontal disease;
- (I) Intervention: *IL1A* (rs1800587 C>T) or *IL1B* (rs1143634 C>T) genetic polymorphisms;
- (C) Comparison: genetic profile associated with the severity of the disease;
- (O) Outcome: verify if when a DNA *loci* is described as a risk factor for one of these diseases, EARR or CP, it should it be considered as a candidate *loci* for the other one.

Review question following PICO methodology:

Can *IL1A* (rs1800587 C>T) or *IL1B* (rs1143634 C>T) genetic polymorphisms influence patient's susceptibility to EARR and/or Chronic Periodontitis in opposite directions?

3.1. Search strategy

An electronic search was performed on Medline/PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) and EBSCOhost (http://search.ebscohost.com/) primary databases at January 2017.

Different "MeSH Terms" were combined with the Boolean connector "AND" or "OR": "orthodontics", "periodontitis", "chronic periodontitis", "root resorption", "genetic polymorphisms", "interleukin-1". The keywords "external apical root resorption" were also used.

In MEDLINE/PubMed database three separate searches were performed:

1) (((orthodontics[MeSH Terms]) OR periodontitis[MeSH Terms]) AND interleukin-1[MeSH Terms]) AND genetic polymorphism[MeSH Terms];

2) (periodontitis[MeSH Terms] OR chronic periodontitis[MeSH Terms]) AND orthodontics[MeSH Terms];

3) "external apical root resorption".

In EBSCOhost database two research equations were performed:

1) "orthodontics" AND "root resorption" AND "genetic polymorphism" AND "interleukin-1";

2) "periodontitis" AND "genetic polymorphisms" AND "interleukin-1".

The "Abstract", "Full text", "10 years", "English" and "Portuguese" filters were activated. No restriction was made on type of article.

3.2. Inclusion and exclusion criteria for the literature review

Inclusion criteria were:

1. Studies that assessed EARR in orthodontically treated patients;
2. Studies reporting association of orthodontics and periodontitis;
3. Studies that connected the *IL1A* (rs1800587 C>T) and/or *IL1B* (rs1143634 C>T) gene polymorphisms with external apical root resorption;
4. Studies that connected the *IL1A* (rs1800587 C>T) and/or *IL1B* (rs1143634 C>T) gene polymorphisms with non-syndromic chronic periodontal disease.

Exclusion criteria were:

1. Articles that did not meet the inclusion criteria;
2. Animal or cell culture laboratory studies;
3. Association of interleukin-1 genetic variations and post-orthodontic external root resorption in endodontically-treated teeth;
4. Studies of interleukin-1 polymorphisms in patients with periodontitis and a concomitant systemic disease (for which IL1 gene variants contribute to the genetic susceptibility of the disease) and syndromic pathology;
5. Dataset including only aggressive form of periodontal disease;
6. Systematic and narrative reviews of literature;
7. Records analysed in included meta-analysis;
8. Duplicated records;
9. Insufficient data.

3.3. Allelic variants reference

Genetic variants references are frequently changing, explaining the heterogeneity in nomenclature found in literature. For easier understanding, in this paper, we used the same nomenclature (according to SNP database) for each locus, independently of how it was referred in the original paper. The variants searched for *IL1A* and *Il1B* genes are described in table I.

Table I - Allelic variants of *IL1A* and *IL1B* genes more frequently associated with external apical root resorption and chronic periodontitis.

Locus	Reference SNPs	Description	Alias	Function	Other information
IL1A	rs1800587	NM_000575.4:c.-949C>T	- 889C>T	5'UTR	Allele 1 = C Allele 2 = T
IL1B	rs1143634	NM_000576.2:c.315C>T	+3953C>T +3954C>T	Phe105Phe	Allele 1 = C Allele 2 = T

SNP database: https://www.ncbi.nlm.nih.gov/snp

4. Results

The electronic search yielded a total of 382 publications. The duplicated records were removed. Through title and abstract screening based on the inclusion and exclusion criteria 21 publications were selected and accessed for the review.

Additional relevant data, for the introduction and discussion, was added from the review excluded group and from manual search.

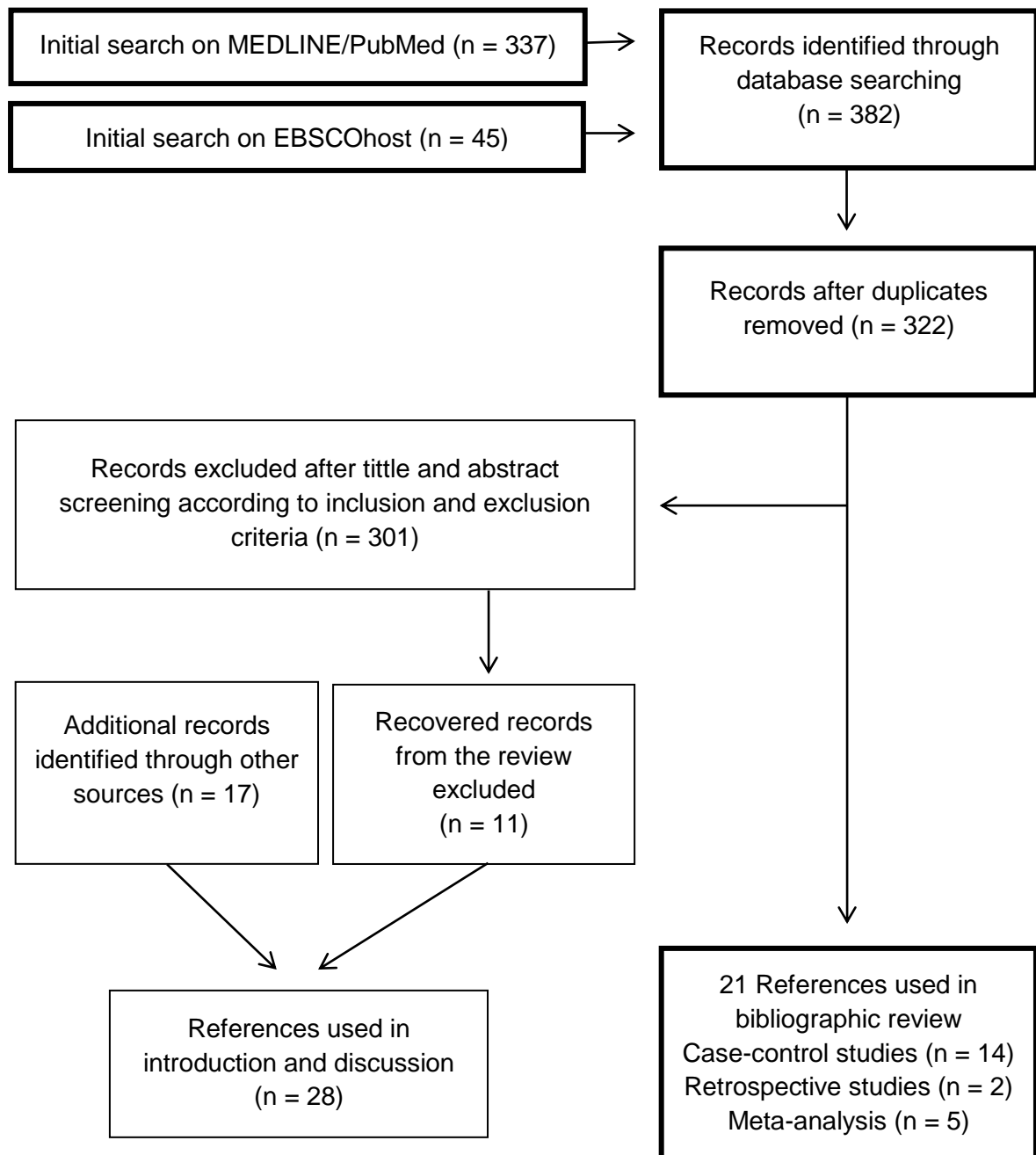A total of 49 bibliographic references were included in this work.

Figure 2 – Flow chart of study selection process.

Table II – Results of studies meeting inclusion criteria: association genetic studies and linkage genetic studies between specific genetic variants and external apical root resorption / chronic periodontitis.

SNP	Disease	Reference	Type of study	Sample characteriscs		Results
				Country (Ethnicity)	Sample size (case/control)	
IL1A rs1800587 (C>T)	External apical root resorption (EARR)	Gulden et al. (2009)	Case-control	Germany (Caucasian)	45/44	Association of genotype TT with EARR (OR = 9.3, %95 CI 1.1-77.8, p < 0.032).
		Iglesias-Linares et al. (2012)	Case-control	Spain (Caucasian)	25/29	No statistical association
		Linhartova et al. (2013)	Case-control	Czech Republic (Caucasian)	32/74	No statistical association
		Sharab et al. (2015)	Case-control	USA (Caucasian)	67/67	No statistical association
	Chronic Periodontitis (CP)	Nikolopoulos et al. (2008)	Meta-analysis	Caucasian, Asians, "other"	1002/878 11 Studies	No statistical association
		Karimbux et al. (2012)	Systematic review and Meta-analysis	Caucasian	25 Studies (8 MA)	No statistical association for allele T, though (OR = 1.48, 95% CI 1.17-1.86, p = 0.083, I^2:44.3%).
		Mao et al. (2013)	Meta-analysis	Caucasian, Asians, "mixed-ethnicity in Brazilians"	2122/1794 23 Studies	For Caucasians and Asians, association of T allele with increased risk of CP (OR = 1.29, 95% CI = 1.15-1.44, p < 0.001). Significant association was also found in the other genetic models TT vs. CC (OR = 1.59); CT vs. CC (OR = 1.30); (CT+TT) vs. CC (OR = 1.40) and TT vs. (CT+CC) (OR = 1.47).
		Lavu et al. (2015)	Case-control	India (Asian)	200/200	No statistical association

Table II (continued)

IL1B rs1143634 (C>T)	External apical root resorption (EARR)	Bastos Lages et al. (2009)	Case-control	Brazil (Mixed)	23/38	Association of C allele with EARR (OR = 4.0). Significant association was also found in the other genetic models TT vs. CT+CC (OR = 4.00, 95% CI = 1.23-12.9; p = 0.0349), TT vs. CC (OR = 7.33; 95% CI = 1.81-29.6; p = 0.0095).
		Gulden et al. (2009)	Case-control	Germany (Caucasian)	45/49	No statistical association
		Tomoyasu et al. (2009)	Case-control	Japan (Asian)	27/24	No statistical association
		Iglesias-Linares et al. (2012)	Case-control	Spain (Caucasian)	25/29	Association of genotype CC with EARR (OR = 3.47, 95% CI = 1.12-10.72, p = 0.027).
		Pereira et al. (2014; 2016)	Retrospective: dependent variable % EARRmax	Portugal (Caucasian)	195 Orthodontic patients	No statistical association
		Linhartova et al. (2013)	Case-control	Czech Republic (Caucasian)	32/74	No statistical association
		Sharab et al. (2015)	Case-control	USA (Caucasian)	67/67	No statistical association
	Chronic Periodontitis (CP)	Nikolopoulos et al. (2008)	Meta-analysis	Caucasian, Asians, other	1470/2328 20 Studies	Association of T allele carriers with CP (OR = 1,447, 95% CI = 1.129-1.854, I^2=52.4%).
		Karimbux et al. (2012)	Systematic review and Meta-analysis	Whites	27 Studies (10 MA)	Association of *IL1B* marker and CP (OR = 1.54, 95% CI 1.03-2.30, p < 0.001, I^2:77.3%).
		Deng et al. (2013)	Meta-analysis	Caucasian, Asians, other	3095/2839 36 Studies	Association of T allele carriers with CP (OR = 1.33). Significant association was also found in the T allele vs C allele genetic model (OR = 1.30, 95% CI = 1.05-1.60, p < 0.001).

Table II (continued)

		Atanasovxka-Stojanovska et al. (2013)	Case-control	Macedonia (caucasian)	114/301	No statistical association
		Amirisetty et al. (2015)	Case-control	North India (Asian)	58/62	Significant association was also found in the T allele vs C allele genetic model (OR= 2.7, 95% CI = 1.13-6.1, p = 0.02, x^2 :5.2).
		Boukortt et al. (2015)	Case-control	Algerian population	151/128	No statistical association
		Mendonça et al. (2015)	Case-control	Brazil	134/213	No statistical association
		Lavu et al. (2015)	Case-control	India (Asian)	200/200	Association of T allele carriers with CP (OR = 1.51, 95% CI = 1.07-2.11, p = 0.017).
IL1B rs1143634 (C>T)	Chronic Periodontitis (CP)	Isaza-Guzmán et al. (2016)	Case-control	Colombia	124/81	No statistical association
		Ribeiro et al. (2016)	Case-control	Brazil	52/50	Association of T allele with CP (OR = 2.84, 95% CI = 1.44-5.62).
		Yin et al. (2016)	Meta-analysis	Mixed population	236/286	Association of T allele (OR=1.51, 95% CI = 1.14/2.00, p= 0.004) and T carriers with periodontitis risk (OR = 1.53, 95% CI = 1.06-2.21, p = 0.02).

EARR - External apical root resorption; CP - chronic periodontitis; *IL1A* - interleukin-1 alfa gene; *IL1B* - interleukin-1 beta gene; OR - odds ratio; p - P value; MA - meta-analysis; I^2 - heterogeneity statistic: 30-60 % may represent moderate heterogeneity, 50-90 % may represent substantial heterogeneity; C - cytocine; T - thymine; 95 % CI - 95 % confidence interval.

5. Discussion

For a successful prevention and treatment planning of diseases, identification of the risk factors is a crucial step. In complex phenotypes, like EARR and CP, this is a particularly difficult task. In the last two decades, efforts have been focusing on the role and influence of the genetic component (Nieto-Nieto et al. 2017).

The localization and characterization of genomic variations of human populations allowed the identification of genetic susceptibility for multiple common diseases. These DNA variants have a high frequency in the population (superior to 1%, so they may be described as polymorphisms), a low penetrance and may be divided in functional (interfering in genic expression) or non-functional (considered only as DNA markers) (Grigoriadou et al. 2010). Two strategies, based on association studies, have been successfully used to identify these susceptibility *loci*: gene-candidate and genome wide approaches, with the last one being mainly reserved for multicentre studies (Hartsfield 2008).

Given to the common presence of IL1 cytokines in physiopathology of the studied phenotypes, the purpose of this paper is to clarify and understand the hypothesis of a genetic relationship between EARR and CP by the analysis of *IL1A* rs1800587 or *IL1B* rs1143634 genetic polymorphisms.

The SNP rs1800587 of *IL1A* is present in a regulator region (5'UTR) and it is in allelic disequilibrium with *IL1A* rs17561 (+4845G>T) in exon 5. Due to this fact, many reports combine the two polymorphisms and show the results together, or choose one of them, considering the simplicity and availability of genetic tests. Despite these two variants are highly concordant, the perfect match cannot be stated as 100 % (Grigoriadou et al. 2010). Thus, in this paper, only studies directly analysing variant *IL1A* rs1800587 and meta-analysis with joint results were selected for analysis.

The SNP rs1143634 of *IL1B*, in exon 5, is a synonym variant (Phe105Phe), but with a putative role in the genetic expression (Pereira et al. 2014).

In periodontal disease, Kornman et al. (1997) primarily reported the association of allele T of both *IL1A* rs1800587 and *IL1B* rs1143634 polymorphisms with the severity of the phenotype in Caucasians non-smokers. They also reported that the presence of the composite *IL1* genotype resulted in an increase of IL1A and IL1B production in crevicular fluid.

Al-Qawasmi et al. (2003) were the leading investigators to describe and establish an association between *IL1B* rs1143634 SNP and susceptibility to EARR. Al-Qawasmi first suggested that IL1B is responsible for clastic cells stimulation during tooth movement, and that a reduced production of IL1B would lead to a lower

resorption of the cortical bone at the PDL interface, resulting in a continuous stress, concentrated in the root, causing necrotic areas in the PDL and triggering root resorption. The *IL1B* SNP has been shown to influence IL1B production in monocytes *in vitro* (Pociot et al. 1992), and *in vivo*, to enhance IL1 production in the crevicular fluid of orthodontic patients (Iwasaki et al. 2006). Specifically, allele C was associated to low production of IL1B, meaning it may increase susceptibility to EARR.

Since Kornman et al. (1997) and Al-Qawasmi et al. (2003), several studies have been conducted to evaluate the role of these polymorphisms in susceptibility to EARR and periodontitis in different ethnic populations, with controversial and inconclusive results (table II).

After the screening of the published literature and the application of the inclusion and exclusion criteria, eight studies provided data on the association between *IL1A* SNP with EARR or CP. Of the four studies that examined the *IL1A* variant and EARR, only one (Gulden et al. 2009) obtained statistically relevant results. The German sample provided by Gulden, associates the presence of TT genotype with root resorption (p<0.032, OR = 9.3, 95 % CI 1.1-77.8, p = 0.040).

In CP, three meta-analysis and one case-control study were assessed. Although allele T is generally described as more frequent among patients, only Mao et al. (2013) reported an overall significant association for allele T and genotypes including this allele: TT vs. CC (OR = 1.59); CT vs. CC (OR = 1.30); (CT+TT) vs. CC (OR = 1.40) and TT vs. (CT+CC) (OR = 1.47). The fact that the risk increases with the number of T alleles, supports the role of this genetic variant in the susceptibility to CP. Yet, Mao et al. (2013) only found a significant association for Caucasian and Asians subgroups, but not for the mixed Brazilians population they included in their study, demonstrating the heterogeneity between different ethnicities. A patient-based study in Asian population did not support IL1A polymorphism association with CP (Lavu et al. 2015).

Over the last decade, many case-control, retrospective studies and meta-analysis have been conducted to investigate the relation between the *IL1B* rs1143634 (+3954C>T) SNP and risk of EARR in orthodontic patients. However, results have been conflicting. Fulfilling the selection criteria, six retrospective case-control studies (Bastos Lages et al. 2009; Gulden et al. 2009; Tomoyasu et al. 2009; Iglesias-Linares et al. 2012; Linhartova et al. 2013; Sharab et al. 2015) and two retrospective studies (Pereira et al. 2014; Pereira et al. 2016) were included.

A significant association was reported by two studies, one from Brazil (Bastos Lages et al. 2009) and another from Spain (Iglesias-Linares et al. 2012). Bastos Lages et al. (2009) described that orthodontic patients carrying the C allele had 4 times more

chances of developing EARR. Also, CC subjects had 7.3 more chances of developing EARR, in comparison to TT individuals. The Spanish sample provided by Iglesias et al. (2012) revealed that patients homozygous for allele C had a 3.47-fold higher risk of developing EARR (OR = 3.47, 95% CI = 1.12-10.72, p = 0.027). These two reports agree with the first description of *IL1B* polymorphism as a genetic marker for EARR made by Al-Qawasmi et al. (2003). Thus, these three studies support the hypothesis that enhanced levels of IL1B (associated to T allele carriers, especially homozygous) might be a protective factor against orthodontic-induced EARR. An increased remodeling of alveolar bone, associated with higher expression of IL1B would reduce root resorption and accelerate tooth movement (Al-Qawasmi et al. 2003; Bastos Lages et al. 2009; Iglesias-Linares et al. 2012).

However, some patient-based studies could not prove this association (Gulden et al. 2009; Tomoyasu et al. 2009; Linhartova et al. 2013; Pereira et al. 2014; Sharab et al. 2015; Pereira et al. 2016).

One meta-analysis has also been performed by Wu et al. (2013). Nevertheless, their evaluation for the association between IL1B polymorphism and the risk of EARR was not considered for our review, because a study of endodontically treated teeth was included in the quantitative analysis. Since pulp vitality can influence the root resorption process and the entire sample is composed by root-filled teeth, the study may act as a confounding factor for the meta-analysis results and compromise its validity.

The association between *IL1B* SNP and CP has been the target of numerous investigations, among different ethnic groups. However, a consensus has not yet been reached. In our review, four meta-analysis and seven case-control studies were analysed. Two Asian (Amirisetty et al. 2015; Lavu et al. 2015) and one Brazilian (Ribeiro et al. 2016) case-control studies associated the T allele with CP. Lavu et al. (2015) included a large sample (200 cases and 200 controls) and excluded any form of tobacco from the confounding factors, resulting in an increasing reliability in the results. Even though Amirisetty et al. (2014) and Ribeiro et al. (2016) studied different populations, their results were similar (OR = 2.7 and OR = 2.84, respectively). In contrast, Atanasovxka-Stojanovska et al. (2013), Boukortt et al. (2015), Mendonça et al. (2015) and Isaza-Guzman et al. (2016) reported a lack of association between the SNP and CP.

Many meta-analyses have emerged to clarify the relationship between IL1 polymorphisms and periodontitis risk. According to Nikolopoulos et al. (2008) and Deng et al. (2013) meta-analysis on Caucasian and Asian populations, carriers of T allele have a higher risk to develop CP (OR = 1,447 and OR = 1.30, respectively). Similarly, Karimbux et al. (2012) found that there is an association of IL1B marker and CP, but

only in Caucasian population. More recently, Yin et al. (2016), in a mixed population, confirmed these conclusions, showing an association of T allele (OR = 1.51, 95% CI = 1.14/2.00, p = 0.004) and T carriers (OR = 1.53, 95% CI = 1.06-2.21, p = 0.02) with periodontitis risk. Therefore, considering the results of the previous data, there is a consistent observation of association of allele T with chronic periodontitis.

From the reviewed data, we can conclude that the evidence of a relationship between the SNP rs1800587 of *IL1A* and EARR is reduced (only one study found a significant association) (Gulden et al. 2009). This could be, in part, explained by the fact that IL1A is fifteen times less powerful than IL1B (Pereira 2014). Susceptibility for both EARR and periodontitis was associated with the presence of allele T, not supporting the theory of opposite genetic profiles.

Regarding IL1B, the involvement between this protein and the process of bone remodelling, which influences directly the process of root resorption, is well established. For *IL1B* SNP rs1143634, susceptibility to EARR and CP showed an opposite genetic profile, since the presence of C allele was associated with an increased susceptibility for EARR, whereas the presence of T allele was associated with an increased susceptibility for CP. Therefore, presumably, the carrier of the C allele has a predisposition for EARR and a diminished likelihood for developing CP, and vice-versa. This evidence, and the biological and molecular mechanisms involved in the two phenotypes raise an interesting question - When a DNA *loci* is described as a risk factor for one of these diseases, should it be considered as a candidate *loci* for the other one?

Although several researchers have explored the involvement of IL1 genetic variants in the risk of developing EARR and periodontal disease, results remain controversial.

Several possible factors can contribute to this inconsistency of results:

1. Complexity in the evaluation of the phenotype:

EARR definition and classification are still controversial. Most of the studies identify EARR through the Levander and Malmgren scale that define EARR as loss of tooth over 2 mm. This method may jeopardize the results accuracy, since variability of the tooth shape and the initial size of tooth are not considered. In order to overcome this limitation and maximize the accuracy, some studies (Gulden et al. 2009; Pereira et al. 2014; Pereira et al. 2016) calculate the crown/root (C/R) ratio. However, this criteria is only statistical and with no clinical correlation proven (Pereira 2014).

Another source of discrepancies is the type of imaging system used for EARR measurements. The different radiographs systems, as orthopantomograms, lateral cephalometric, periapical radiographs, 3D imaging, have different precision for

measuring root resorption. For instances, the magnification factor and distortion are critical limitations of panoramic measurements, despite being less critical for maxillary teeth (Pereira et al. 2014; Pereira et al. 2016). Also, the direct metric analysis of the panoramic films is not indicated due to their lack of reproducibility. The analysis of a 3D phenomenon in a two-dimensional (2D) image obviously limits accuracy, but the higher radiation doses and the costs of 3D systems still limit their use.

Independently of the image system used for diagnosis, as EARR is an active phenomenon, the radiological images cannot provide a truly state of evolution. The apical root resorption is only detected after 60-70% of root demineralization (5–6 months after orthodontic load) (Levander et al. 1998).

2. Multiplicity of low penetrance factors involved:

Being a multifactorial trait, tens to hundreds of genetic and non-genetic variables may be implicated, and penetrance is expected to be low, making it particularly difficult to prove their role, even studying large samples. For example, cellular response to IL1 involves multiple intricate molecular pathways. Even more, gene-gene and gene-environment (like applied forces) interactions may influence the occurrence of EARR (Atanasovska-Stojanovska et al. 2013; Mao et al. 2013; Isaza-Guzman et al. 2016). Epigenetic variants, either constitutional or acquired, may also interfere and have not been studied.

3. Population samples analysed:

Populations studied have different genetic backgrounds and polymorphisms frequencies are known to vary between populations (based on ethnic background) (Linhartova et al. 2013; Pereira et al. 2014; Pereira et al. 2016). Non-genetic confounding factors, such as root morphology, smoking status, concomitant systemic diseases, and treatment related factors, like the characteristics of orthodontic forces, influence EARR occurrence and are not always referred.

Furthermore, studies characteristics/design may contribute to the inconsistency of results. One of the most critical is the small sample size, as it limits the statistical power to identify variants of a low or moderate influence and promotes false associations (Linhartova et al. 2013; Wu et al. 2013; Pereira et al. 2014). In our review only 7 studies (Atanasovska-Stojanovska et al. 2013; Pereira et al. 2014; Boukortt et al. 2015; Lavu et al. 2015; Mendonça et al. 2015; Isaza-Guzman et al. 2016; Pereira et al. 2016) included more than 100 participants. Sample stratification is rarely analysed and may also interfere. Differences in patient selection criteria and even the diversity of genotyping techniques, some more prone to errors than others, are common between studies.

All these factors will increase the variation of results and the inconsistency of the conclusions.

The difficulty to perform a reliable comparison of the results is reflected in some limitations of this study: first, the small number of included eligible studies; second, some relevant researches could not be included in our analysis due to incomplete data or search restrictions; third, analysis of confounding variables (such as gender, smoking, or duration of treatment) was not taking into account; finally, differences between studies-design may lead to interpretation bias.

In extreme situations of EARR, the longevity of the tooth can be affected, compromising the results of a successful orthodontic treatment (Pereira et al. 2014). Thus, after completing the treatment, the severe cases of root resorption should be controlled by performing periapical radiographs every 6 months, particularly to the incisor teeth, always taking into account the ALARA principle ("as low as reasonably achievable") (Pereira 2014). Special cases of enhanced risk a 3 month follow-up is recommended (Levander et al. 1998).

While waiting for a clinical useful genetic profile, in order to reduce the risk of resorption, we must minimize the controllable variables, proposing less frequency of orthodontic appointments, avoiding premolar extraction or large distances of tooth movement, avoiding large duration of treatments, reducing the level of forces, among other actions.

A more in-depth data regarding both the individual genetic profile and clinical variables influencing phenotypes may allow a more informed decision by the clinician. In the daily practice, the support of a reliable predictive model for EARR could allow a personalized approach before, during and after the orthodontic treatment, improving results and diminishing the occurrence of this complication. Unfortunately, we are still waiting for diagnostic and prognostic markers that support therapeutic options and new molecular targets for conducted therapies.

To the best of our knowledge, this is the first study to propose a possible opposite genetic relationship between these two phenotypes. Our hypothesis should be treated as exploratory and requires further research.

6. Conclusion

The literature's results on *IL1B* rs1143634 polymorphism support our initial hypothesis of an opposite genetic profile between EARR and CP. Results of IL1A polymorphism were contradictory, even for each pathology.

Additional genetically based studies are still required in order to provide supplementary information and support this theory. The use of this knowledge will assist in the design of gene-candidate studies, aiming to identify the genetic profile of susceptibility to EARR. Also, the susceptibility for the two diseases may be predicted at the same time, through characterization of the same *loci*. These genetic variants may, in the future, integrate predictive models allowing optimization of orthodontic treatment.

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