UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

JÉSSICA LIMA MELCHIADES

Impact of *Slc11a1* gene variants on the host response patterns and in the determination of periodontal diseases resistance and susceptibility phenotypes

Impacto das variantes do gene *Slc11a1* nos padrões de resposta do hospedeiro e na determinação de fenótipos de resistência e susceptibilidade às doenças periodontais

BAURU
2019

JÉSSICA LIMA MELCHIADES

Impact of *Slc11a1* gene variants on the host response patterns and in the determination of periodontal diseases resistance and susceptibility phenotypes

Impacto de variantes do gene *Slc11a1* nos padrões de resposta do hospedeiro e na determinação de fenótipos de resistência e susceptibilidade às doenças periodontais

Dissertação apresentada a Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Mestre em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Biologia Oral.

Orientador: Prof. Dr. Gustavo Pompermaier Garlet

BAURU

2019

Melchiades, Jéssica Lima

Impact of *Slc11a1* gene variants on the host response patterns and in the determination of periodontal diseases resistance and susceptibility phenotypes . – Bauru, 2019.
58p. : il. ; 31cm.

Dissertação (Mestrado) – Faculdade de Odontologia de Bauru. Universidade de São Paulo

Orientador: Prof. Dr. Gustavo Pompermaier Garlet

Comitê de Ética da UNG
CAAE:32465714.4.1001.5506
Data:12/08/14

FOLHA DE APROVAÇÃO

DEDICATÓRIA

Dedico este trabalho,

*Primeiramente, a **Deus**, pela Graça de estar ao meu lado em todos os momentos, bons e ruins, me dando capacidade física e mental para chegar até aqui, e realizar este sonho.*

*Aos meus pais **Neide** e **Ezequiel**, que nunca mediram esforços para me ajudar a chegar onde cheguei. Amo muito vocês. Agradeço por sempre estarem ao meu lado.*

*Aos todos os meus **familiares e amigos** que sempre me apoiaram e me impulsionaram nos momentos de dificuldade.*

AGRADECIMENTOS ESPECIAIS

Em especial agradeço,

O meu orientador ***Prof. Dr. Gustavo Pompermaier Garlet****, por me orientar na elaboração deste trabalho, pela paciência, dedicação, disponibilidade, tranquilidade e compreensão em todos os momentos de dificuldade. Obrigada pela confiança que depositou em mim. Saiba que você contribuiu de forma imensurável para meu amadurecimento cientifico e profissional. Para mim, você é um exemplo de profissional e também como pessoa. Muito obrigada.*

Ao ***Guilherme*** *que me ajudou durante todo o início de minha carreira acadêmica, me concedendo todo apoio necessário.*

À ***Angélica****, por ser essa amiga e confidente que esteve do meu lado me escutando e me mostrando sempre o bom que a vida tem para oferecer.*

À ***Michelle*** *por ser esse porto seguro dando toda ajuda necessária para a conclusão deste trabalho, e sendo uma excelente amiga.*

Ao ***Prof. Dr. Lucas Rasmussen*** *que foi o primeiro professor a me orientar e me inspirar como profissional e pessoa dentro da vida acadêmica.*

Aos meus amigos queridos pelos momentos de descontração ***João Cleber e Naiara.***

Aos ***professores do Departamento de Ciências Biológicas da FOB/USP****, sempre dedicados à arte de ensinar, pelo acolhimento e pelos ensinamentos recebidos.*

Aos alunos do departamento de Histologia: ***André, Ana Carolina, Carol, Dani, Ever, Luan, Nath, Nádia, Paulinha, Rafael, Rafaella, Ricardo, Rodrigo e Suelen****, pela convivência maravilhosa que temos no laboratório, pelos momentos de paciência, dedicação e compreensão. Agradeço a colaboração e amizade.*

Às técnicas do laboratório de Histologia, ***Tânia, Danielle e Patrícia****, pelo profundo senso de dedicação e responsabilidade e pela inestimável ajuda prestada neste trabalho.*

*À querida secretária **Teresa**, não só pela capacidade de realizar todas as suas tarefas com dedicação e eficiência, mas principalmente pelo bom-humor, alegria e vontade de realizá-las. Sua dedicação e boa-vontade sempre fizeram a diferença!*

*A todos os demais **professores e funcionários da FOB-USP**, por estarem sempre dispostos a ajudar.*

*À **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, a CAPES**, pela bolsa concedida no início do meu mestrado.*

*À **FAPESP** pela bolsa concedida (Número do Processo: 2017/11463-2).*

Meus sinceros Agradecimentos

Jéssica Lima Melchiades

AGRADECIMENTOS INSTITUCIONAIS

Ao Prof. Dr. Vahan Agopyan, *digníssimo reitor da Universidade de São Paulo;*

Ao Prof. Dr. Pedro Vitoriano de Oliveira, *digníssimo Secretário Geral da Universidade de São Paulo;*

Ao Prof. Dr. Carlos Ferreira dos Santos, *digníssimo Diretor da Faculdade de Odontologia de Bauru da Universidade de São Paulo;*

Ao Prof. Dr. Guilherme dos Reis Pereira Janson, *digníssimo Vice-diretor da Faculdade de Odontologia de Bauru da Universidade de São Paulo;*

Ao Prof. Dr. José Henrique Rubo, *digníssimo Prefeito do Campus da Faculdade de Odontologia de Bauru da Universidade de São Paulo;*

À Prof. Dra. Izabel Regina Fischer Rubira Bullen, *digníssima Presidente da Pós-Graduação da Faculdade de Odontologia de Bauru da Universidade de São Paulo;*

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, *pela bolsa concedida no início do meu mestrado;*

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), *órgão de fomento deste trabalho que me concedeu a bolsa até a conclusão deste trabalho (número de processo: 2017/11463-2).*

E a todos que, de alguma maneira, tornaram este sonho realidade...

...Meu muito obrigada!

"Só há duas maneiras de viver a vida: a primeira é vivê-la como se os milagres não existissem. A segunda é vivê-la como se tudo fosse milagre"

Albert Einstein

RESUMO

Estudos em humanos e em modelos experimentais tem demonstrado a influência de múltiplos *loci* genéticos na determinação de fenótipos de susceptibilidade/resistência à periodontite. Dentre estes genes, o *Slc11a1*, cujas funções pleiotrópicas incluem a regulação da atividade macrófagos e linfócitos, tem potencial papel na modulação da resistência/susceptibilidade às doenças periodontais. Nesse contexto, nosso grupo demonstrou que camundongos das linhagens AIRmax e AIRmin, caracterizados pela predominância de variantes distintas de alelos do *Slc11a1*, associadas a diferentes padrões de resposta imune e inflamatória, apresentam fenótipos distintos de resistência/susceptibilidade à periodontite experimental. Ainda, variantes genéticas (SNPs) no *Slc11a1* se mostram associadas a diferentes doenças infecciosas em humanos. Neste contexto, este estudo teve como objetivo correlacionar os polimorfismos genéticos rs17228995, rs17235409, rs2290708, rs2695343, rs3731865 do gene *Slc11a1* com perfis de resistência/susceptibilidade às doenças periodontais em humanos, assim avaliar o impacto de variantes hipo/hiperresponsivas na periodontite experimental em camundongos. Para tanto, foram analisados 444 pacientes com periodontite crônica (CP), 476 indivíduos saudáveis (H) e 207 indivíduos com gengivite crônica (CG) para as análises de associação, e subgrupos para análise de possíveis correlações entre expressão/genótipo (CP=127, H=63) e para ensaios in vitro (H=29). Na análise dos genótipos, apenas os ensaios para a caracterização dos SNPs rs2290708 e rs37371865 se mostraram efetivos na descriminação alélica, os demais ensaios foram considerados tecnicamente inefetivos. Os polimorfismos rs2290708 e rs37371865 se mostraram associados ao risco de periodontite, sendo os genótipos CT+TT e GC+CC e alelos T e C (respectivamente) mais frequentes no grupo CP. Além da associação na abordagem caso controle, observamos que a presença dos alelos polimórficos T (rs2290708) e C (rs37371865) se mostrou associada ao aumento de expressão de TNF-α, IL-1β, IL-6, RANKL e RANKL/OPG nas lesões periodontais. Não foram observadas diferenças no padrão de colonização microbiológica de sítios com periodontite crônica com relação aos SNPs rs2290708 e rs37371865. A análise *in vitro* reforça a natureza hiper-reativa dos alelos polimórficos T (rs2290708) e C (rs37371865), uma vez que a produção de citocinas inflamatórias por macrófagos portadores de tais alelos se mostra aumentada frente ao estímulo por LPS. Finalmente, observamos que variantes hipereativas do *Slc11a1*, caracterizadas nas linhagens murinas AIRmin e AIRmax, se mostram associadas ao aumento de perda óssea

alveolar, influxo de leucócitos e maior produção de citocinas pró-inflamatórias na periodontite experimental. Dessa forma, é possível concluir que polimorfismos funcionais no gene *Slc11a1* associados ao aumento da responsividade inflamatória, e influenciam o risco ao desenvolvimento de periodontite em humanos e em modelo experimental.

Palavras-chave: Doença Periodontal. Gene *Slc11a1*. SNPs.

ABSTRACT

Impact of *Slc11a1* gene variants on the host response patterns and in the determination of periodontal diseases resistance and susceptibility phenotypes

Studies in humans and experimental models have demonstrated the influence of multiple genetic loci on the determination of susceptibility/resistance phenotypes to periodontitis. Among these genes, *Slc11a1*, whose pleiotropic functions include the regulation of macrophages and lymphocyte activity, has a potential role in the modulation of resistance/susceptibility to periodontal diseases. In this context, our group demonstrated that AIRmax and AIRmin mice, characterized by the predominance of distinct variants of *Slc11a1* alleles, associated to different patterns of immune and inflammatory response, present distinct phenotypes of resistance/susceptibility to experimental periodontitis. Furthermore, genetic variants (SNPs) in *Slc11a1* are shown to be associated with different infectious diseases in humans. In this context, this study aimed to correlate the genetic polymorphisms rs17228995, rs17235409, rs2290708, rs2695343, rs3731865 of the gene *Slc11a1* with profiles of resistance/susceptibility to periodontal diseases in humans, thus to evaluate the impact of hypo/hyperresponsive variants on experimental periodontitis in mice. Forty-five patients with chronic periodontitis (CP), 476 healthy individuals (H) and 207 individuals with chronic gingivitis (CG) were analyzed for association analysis, and subgroups were analyzed for analysis of possible correlations between expression / genotype (CP = 127, H = 63) and for in vitro tests (H = 29). In the analysis of the genotypes, only the assays for the characterization of the SNPs rs2290708 and rs3731865 were effective in the allelic discrimination, the other tests were considered technically ineffective. The polymorphisms rs2290708 and rs3731865 were shown to be associated with the risk of periodontitis, with a higher frequency of the genotypes CT+TT and GC+CC and the alleles T and C (respectively) in the CP group. In addition to the association in the control case approach, the presence of the polymorphic T (rs2290708) and C (rs37371865) alleles was shown to be associated with increased TNF-α, IL-1β, IL-6, RANKL and RANKL/OPG periodontal lesions. No differences were observed in the pattern of microbiological colonization of sites with chronic periodontitis with respect to the SNPs rs2290708 and rs37371865. In vitro analysis reinforces the hyper-reactive nature of the polymorphic alleles T (rs2290708) and C (rs37371865), since the production of inflammatory cytokines by macrophages bearing such alleles is shown to be increased in response to LPS

stimulation. Finally, we observed that hypereactive variants of *Slc11a1*, characterized in the AIRmin and AIRmax murine are associated with increased alveolar bone loss, leukocyte influx, and increased production of proinflammatory cytokines in experimental periodontitis. Thus, it is possible to conclude that functional polymorphisms in the *Slc11a1* gene, associated with increased inflammatory responsiveness, influence the risk to the development of periodontitis in humans and in mice experimental model.

Keywords: Periodontal diseases. *Slc11a1* Gene. SNPs

LIST OF ABREVIATIONS

AIRmax	Maximal Inflammatory Reactions
AIRmin	Minimal Inflammatory Reactions
BOP	Bleeding on Probing
CAL	Clinical Attachment Loss
CG	Chronic Gingivitis
CP	Chronic Periodontitis
DNA	Desoxyribonucleic acid
H	Healthy
IL	Interleukin
LPS	Lypopolysaccharide
M1	Macrophages exhibit high levels of pro-inflammatory
M2	Macrophages exhibit high levels of anti-inflammatory
mRNA	messenger RNA
OPG	Osteoprotegerin
PD	Probing Depth
PDs	Periodontal Diseases
R	Resistance
RANKL	Receptor activator of nuclear factor kappa-B ligand
S	Susceptibility
SNP	Single Nucleotide Polymorphism
TNF	Tumor Necrosis Factor

SUMMARY

1 INTRODUCTION

1 INTRODUCTION

Periodontal diseases (PDs) are chronic and multifactorial in nature, involving in its pathogenesis microbial, inflammatory and immunological factors, that together can be modulated by local, environmental, genetic and epigenetic factors, which ultimately result in alterations of the teeth protection and sustentation tissues (MARTINEZ and HOLT, 1999; KINANE and LAPPIN, 2001; LALLA *et al.*, 2003). Even in clinical health conditions, periodontal tissues are in close proximity with potentially pathogenic microorganisms, and to some extent coexist successfully with such microbes without deleterious consequences. However, qualitative and quantitative changes occur with the development of the bacterial biofilm, generating and intense antigenic load on the gingival sulcus, usually associated to significant changes in the pattern of host response, with in turn are associated with the appearance of clinical signs of the disease (KINANE and LAPPIN, 2001; LALLA *et al.*, 2003).

In this context, the understanding of the interrelationship between the host inflammatory immune response and the bacterial biofilm is fundamental to explain the pathogenesis of PDs. The complexity of the subgingival biofilm has been noticed from the primary Van Leeuwenhoek's microscopic observation of and evolved with modern microbial culture and identification techniques. Using the DNA-DNA hybridization checkerboard technique, five fundamental bacterial complexes were identified. The red complex, composed of *Porfiromonas gingivalis*, *Treponema denticola* and *Tenerella forsytia*; the orange complex, composed of *Fusobacterium nucleatum*, *Prevotella intermedia*, *Prevotella nigricens*, *Micromonas micros*, *Campilobacter rectus* e *Campilobacter showae*, are strongly associated with clinical parameters of periodontitis. Unlike the red and orange complex, the green complex (*Capnocytophaga spp*, *Aggregatibacter actinomycetemcomitans* serotype a and *Eikenella corrodens*), yellow (species of streptococci) and purple (*Actinomyces odontolyticus* and *Veillonella parvula*) are usually associated with periodontal health. The recognized periodontal pathogen *Aggragatibacter actinomycetemcomitans* serotype b is not routinely isolated in association with any other pathogen and is not classified in any of the clusters (SOCRANSKY *et al.*, 1998).

It is important to note that although the presence of pathogenic microorganisms is associated with PD occurrence and severity, their presence is not mandatorily associated with the occurrence of periodontitis (such microorganisms can be isolated from healthy periodontal

sites), as it is not obligatory for the development of periodontitis. In fact, it is believed that periodontitis is associated with ecological modifications in oral microflora, where the lack of beneficial or health-associated microorganisms may be as or more important than the presence of pathological microorganisms. This qualitative change is the relative proportions within the microbial biofilm, known as dysbiosis, has been associated with multiple pathologies in addition to periodontitis (SOCRANSKY and HAFFAJEE, 2005) such as gastroesophageal reflux (YANG *et al.*, 2009), otitis media (USVIATSOV *et al.*, 2000) and ulcerative colitis (FRANK *et al.*, 2007), among others (MARSH, 1994). In a general context, such changes in of proportions of microorganisms in the subgingival biofilm (dysbiosis) result in the induction of a chronic inflammatory and immunological response by the host, such response being an important factor in the establishment of pathological changes associated with periodontitis (PEYYALA and EBERSOLE, 2013; PEYYALA *et al.*, 2013).

Indeed, although the association of different microbial complex with the occurrence and severity of PDs, it has been shown that the amplification and progression of these processes are highly dependent of the host's immune and inflammatory response in response to bacteria or their products (GENCO *et al.*, 1998; MATRICARDI e RONCHETTI, 2001; GIBSON *et al.*, 2006). Healthy tissues maintain a mild and permanent inflammatory state known as subclinical inflammation, characterized by selective (and slight) expression of chemokines and cytokines, which guide the constant infiltration of neutrophils through the junctional epithelium, supposed to control the growth and tissue invasion of the biofilm. On the other hand, sites with periodontitis are characterized by increased expression of multiple inflammatory mediators, infiltration of different leukocyte subpopulations, and disruption of the normal architecture (BEREZOW e DARVEAU, 2011). In this way, the presence of the microbial stimulus associated with host responsiveness, which involves the intensity and nature of the developed response and the self-regulation capacity of the system, are determinants of the development of periodontitis (KORNMAN *et al.*, 1997).

In other words, while the presence of specific microorganisms is required to the establishment and progression of periodontitis, the pattern of host response triggered by the microbial stimuli seems to be the major determinant of the development of the disease. Generally, host inflammatory mediators have been associated with tissue destruction, while anti-inflammatory mediators counteract and attenuate disease progression. With the discovery of several T-cell subsets bearing distinct immunoregulatory properties, this pro- vs. anti-inflammatory scenario became more complex, and a series of studies has hypothesized

protective or destructive roles for Th1, Th2, Th17, and Treg subpopulations of polarized lymphocytes. Interestingly, the "protective vs. destructive" archetype is usually considered in a framework related to tissue destruction and disease progression. However, it is important to remember that periodontal diseases are infectious inflammatory conditions, and recent studies have demonstrated that cytokines (TNF-α and IFN-γ) considered harmful in the context of tissue destruction play important roles in the control of periodontal infection. On the other hand, immunoregulatory elements such as Th2 and Treg subsets seems to limit the tissue damage without critically impairing the protection against the infection. Therefore, a delicate balance between anti-microbial defenses and immunoregulatory circuits seems to operate towards the protection of the host with a minimal collateral damage to the periodontal tissues (GARLET, 2010).

In this context, extrinsic (i.e. environmental) and intrinsic factors that can modulate the nature and intensity of inflammatory immune response can contribute to the modulation of disease severity. Basically, intrinsic factors correspond to genetic variants, the most common being the ones that affect single nucleotides, called single nucleotide polymorphism (SNP). The SNPs may occur in a coding region of the DNA and entail the substitution, addition or deletion of an amino acid, altering the protein structure with profound biological effects or engaging in a promoter region, resulting in the inhibition or stimulation of gene expression, as well as also may not generate any changes when affected in non-coding regions (SCHORK *et al.*, 2000).

Despite controversies in the literature (considered in the sequence) (KORNMAN *et al.*, 1997; KORNMAN, 2008; BRUNNER *et al.*, 2010; LAINE and CRIELAARD, 2012; LAINE *et al.*, 2013) periodontitis has been associated with SNPs in multiple genes, each of which allegedly contributes in a small proportion to the increased relative risk of the disease, by which can be considered modifying genes (HART *et al.*, 2000; BRUNNER *et al.*, 2010; LAINE e CRIELAARD, 2012; LAINE *et al.*, 2013). It is believed that genetic risk factors can influence the natural history of periodontitis, increasing the probability of suffering the disease, being part of the causal chain and relating and increasing the effect of other risk factors (LOOS *et al.*, 2005). Studies in twins has shown that from 32 to 82% of individual variation in clinical parameters of periodontitis (CAL, PD, BOP, GI and PI) could be attributed to genetic factors, and even considering statistical adjustments for known environmental risk factors, genetic variations would be explained up to 50% of the variability of chronic periodontitis in adults (MICHALOWICZ *et al.*, 1991; MICHALOWICZ *et al.*, 2000).

To date, the possible genetic influence on the development of PDs has been generally studied through case-control approaches focusing on candidate genes, in which a "case" group (patients with periodontitis) is compared to a "control" group (patients presenting periodontal health) to determine the frequency of the polymorphic alleles in the different groups. Such approaches have identified a multiplicity of associations between the presence of polymorphisms in genes related to initiation, effector response and regulation of immune response to infection, and an increased risk of presenting periodontitis, although most of the associations describe are inconsistent and/or controversial (KORNMAN *et al.*, 2002; LAINE *et al.*, 2002). In this context, the case-control definitions (or lack of proper definitions) used in this approach can be considered the major factor responsible for the literature controversies previously mentioned. Indeed, limitations in the experimental design generally used in such studies may make it difficult to identify the possible risk factors in their real magnitude (GARLET *et al.*, 2012). Using an alternative case-control definitions approach, which primarily considers the exposure to etiological/microbial factors, our group demonstrated that the use of a control group of patients presenting periodontal health with adequate microbial control, and therefore not exposed to bacterial challenge (such as the control group "classic" traditionally used in these studies), limits both the correct identification of the *odds ratio* of a given SNP and the sampling power by up to 60% (GARLET *et al.*, 2012). Alternatively, the use of a control group exposed to the risk factor in an equivalent manner, but which does not present a destructive inflammatory manifestation of the periodontal tissues unlike to the "case" group, and consequently can be considered as are "resistant", can fulfill the requirements of a proper case-control design to study the genetic basis of an infectious disease. In this approach, in periodontitis context 'resistant' subjects are represented by chronic gingivitis, which are exposed to a microbial challenge but in longitudinal evaluations does not experience progression to periodontitis, and theoretically demonstrates the existence of a "resistant" genotype more appropriate to the case-control analysis (GARLET *et al.*, 2012).

Additionally, in order to overcome the limitations of the classic case-control studies usually performed in humans, supplementary approaches have been successfully used in studies in the field. Indeed, in addition to simply test the possible association between a given genotype and susceptibility clinical phenotype, it is possible to perform functional host response evaluations to understand the genetic influence on specific elements of host inflammatory and immune elements, and its subsequent impact on expression of the disease phenotype (GEMMELL *et al.*, 2000; TROMBONE, CARDOSO*, et al.*, 2009). In this context, previous

studies from our research group identified that certain gene variants, regardless of the modest genetic association in the classic case-control approach, were associated with different expression profiles of inflammatory and immunological mediators in the periodontal tissues. As examples, we can mention IL10-592 SNP whose AA/CA polymorphic genotype is shown to be associated with lower levels of IL-10 anti-inflammatory cytokine expression in periodontal tissues (CLAUDINO *et al.*, 2008); and IL1B-954 and TNFA-308 SNPs, which are respectively related to higher levels of expression of IL-1β and TNF-γ and with clinical parameters of periodontitis (FERREIRA *et al.*, 2008; TROMBONE, CARDOSO*, et al.*, 2009). It is important to highlight that such functional analysis also showed that even when considering gene variants specifically related to inflammatory cytokines, the presence of certain microorganisms (i.e. red complex) had a more significant and evident association with increased transcript levels (mRNA) for cytokine inflammatory conditions than the SNPs investigated (FERREIRA *et al.*, 2008; TROMBONE, CARDOSO*, et al.*, 2009). Indeed, our research group has previously shown that although MMP1-1607 SNP was proven to be functional, being related increased MMP-1 expression in periodontal tissues, the presence of classic periodontopathogens has a preponderant effect on the levels of expression of MMP-1 mRNA *(*REPEKE *et al., 2009)*. Such study also demonstrated that the strong and persistent microbial and inflammatory stimuli overcome the genetic predisposition to MMP-1 expression (REPEKE *et al., 2009)*. In another study, Tbet-related SNP TBX21-1993 T/C (rs4794067), which is essential for lymphocyte polarization to the Th1 pattern, was found to have an immediate relation to Tbet expression levels in gingival tissue, but not with the prototypical cytokine IFNγ (CAVALLA *et al.*, 2015), adding some complexity to the genetic influence over host response patterns in periodontal environment. Despite the lack of a direct correlation with Th1-type response, TBX21-1993 T/C (rs4794067) was found to be associated with increased risk for the development of periodontitis, independently of the pattern of periodontal infection (CAVALLA *et al.*, 2015).

It is also important to consider that certain genetic variants may account for the risk of periodontitis by modulating the patterns of microbial colonization of periodontal tissues. In a recent study, our group demonstrated that the polymorphism rs2521634 proved significantly associated with *Tannerella. forsythia, Actinomyces gerencseriae, Fusobacterium periodonticum, and Prevotella nigrescens*; rs10010758 and rs6667202 were associated with increased counts of *Porphyromonas gingivalis*; and rs10043775 proved significantly associated with decreased counts of *Prevotella intermedia*; comprising therefore a strong connection

between the host's genetic profile and the occurrence of chronic periodontitis-associated bacteria (CAVALLA *et al.*, 2018).

Thus, independent studies suggest that both microbial and genetic factors may play a significant role in determining the host response associated with periodontitis, and consequently, may influence the outcome of the disease. Still, it is imperative to highlight the complexity of the disease model. From the genetic viewpoint, we must consider the potential participation of multiple genes in the determination of resistance and susceptibility phenotypes to periodontal diseases. Additionally, it is mandatory to consider the additional complexity underlying periodontitis pathogenesis, which involves of microorganism-host interaction, possible systemic and environmental cofactors (or modifying factors) that increase the complexity of human studies.

In this context, experimental models using mouse strains with known genetic backgrounds and well characterized host response phenotypes may comprise interesting tools for studying the genetic basis of resistance and susceptibility to periodontal diseases, pointing to candidate genes that regulate such phenotype, and whose homologous genes in humans could have similar role. In fact, studies comparing different classical strains of isogenic mice (BAKER, 2005; SHUSTERMAN, DURRANT*, et al.*, 2013; SHUSTERMAN, SALYMA*, et al.*, 2013; HIYARI *et al.*, 2015) demonstrate in a cause and effect manner the influence of the genetic background on host response and susceptibility to experimental periodontitis. Indeed, experimental studies performed in mice strains with different genetic backgrounds, support the genetic influence in the determination of resistance/susceptibility phenotypes to periodontitis. Early reports, still in the 1960´s, described opposite profiles for isogenic strains STR/N and DBA/2JN regarding the susceptibility to experimental periodontitis (BAKER *et al.,* 1961). Subsequent studies involving other strains, such as BALB/c, C57Bl/6, AKR/J, A/J,129/J and SJL/J, also reported the existence of resistance and susceptibility genotypes (BAKER *et al.*, 2000; BAKER and ROOPENIAN, 2002; HART *et al.*, 2004; BAKER, 2005). Furthermore, studies demonstrated the heritability of susceptibility to alveolar bone loss by generating recombinant lines derived from 5 standard isogenic lines, reinforcing the genetic aspect of susceptibility to experimental periodontitis (HIYARI *et al.*, 2015). However, despite the clear influence of the genetic background in the resistance/susceptibility to experimental periodontitis, the number and identity of such genetic determinants remains unclear.

In this context, our group demonstrated that mice genetically selected for maximum (AIRmax) our minimum (AIRmin) inflammatory reaction also had different phenotypes when submitted to the induction of periodontitis (TROMBONE, CARDOSO*, et al.*, 2009). Importantly, such variation was associated with different patterns of immune and inflammatory response presented by such strains. These mice strains were established by the Laboratory of Immunogenetics of the Butantan Institute by selective bi-directional reproduction in in order to understand the genetic basis of the immunological and inflammatory response, and in order to study the effect of different genotypes and phenotypes in relation to the immune and inflammatory response, (IBANEZ *et al.*, 1992; ARAUJO *et al.*, 1998; VIGAR *et al.*, 2000; CARNEIRO *et al.*, 2002; PETERS *et al.*, 2007). Such strains derive from a genetically heterogeneous founding population (F0) produced through the cross-linking of eight lines of isogenic mice of independent origin (A/J, DBA/2J, P/J, SWR/J, SJL/J, CBA/J, BALB/cJ e C57BL/6J) (STIFFEL *et al.*, 1990). The cross-linking of these strains was performed based on the intensity of the inflammatory reaction generated by the injection of the phylogenetic agent Biogel into the subcutaneous tissue of the animal, and mating between animals with higher or lower inflammatory response relative to the normal distribution of the resulting mouse population in each generation. From the 20th generation of selective mating it was mating accepted that the strains reached the maximum of phenotypic separation (called the selection limit), in which the allele(s) conferring the maximum and minimum inflammatory response are fixed in homozygosis AIRmax and AIRmin lines (IBANEZ *et al.*, 1992). In fact, the AIRmax and AIRmin lines present significant differences in the ability of the inflammatory response to various inflammatory agents (VASQUEZ-BRAVO,1996) (CARNEIRO *et al.*, 2002), constituting in a suitable model to study the mechanisms of the inflammatory/immunological response in different infectious models (ARAUJO *et al.*, 1998; BIOZZI *et al.*, 1998; VIGAR *et al.*, 2000; MARIA *et al.*, 2003; PETERS *et al.*, 2007). The AIRmax and AIRmin strains were successfully used in previous studied by our research group (TROMBONE, FERREIRA*, et al.*, 2009; TROMBONE *et al.*, 2010), in a model of experimental periodontitis and arthritis/periodontitis comorbidity models, in which the dichotomous inflammatory phenotypes were confirmed, and the variations of the AIRmax and AIRmin animals are due to a distinct inflammatory profile involving the modulation of the expression of several inflammatory mediators simultaneously, such as TNF-α, IL-1b and IL-6 (VIGAR *et al.*, 2000; DI PACE *et al.*, 2006).

Aiming to explore the mechanisms by which the AIRmax and AIRmin lines have their distinct phenotypes determined, genetic studies were conducted, and identified *Slc11a1* (Solute carrier Family 11a member 1) as one of the genes responsible for the differential response. Located on the chromosome 2q35, the *Slc11a1* gene contains 15 exons and plays a determinant role in the immune and inflammatory response, impacting the host's susceptibility to pathogens and autoimmune diseases. Described for the first time in mice for their functions in regulation of resistance and susceptibility to infectious agents, this gene was previously called Nramp1 (Natural resistance-associated macrophage protein -1) (FORBES and GROS, 2001). *Slc11a1* encode a highly hydrophilic integral membrane protein with 12 transmembrane domains and with a glycosylated extracellular cycle (EJGHAL *et al.*, 2014), and presents pleiotropic functions, such as the transport of essential ions, like Fe^{+2}, protons and other divalent cations (Zn^{+2} and Mn^{+2}) (FRITSCHE *et al.*, 2007). Regarding the modulation of the immune and inflammatory response, *Slc11a1* regulates the activity of macrophages reflecting in its activation and consequently in the production of nitric oxide, TNF-α, IL-1 (KITA *et al.*, 1992; RAMARATHINAM *et al.*, 1993), and also influence the activation of Th1 and Th2 lymphocytes (ARCHER *et al.*, 2015). Therefore, despite the limited information regarding the mechanisms by which *Slc11a1* modulates immune responses, it has been considered a candidate gene to influence the susceptibility to autoimmune and infectious diseases (ARCHER *et al.*, 2015).

In relation to the AIRmin and AIRmax strains, the allelic variants of *Slc11a1* are called R or S alleles since they confer resistance (R) or susceptibility (S) certain infections/diseases (ARAUJO *et al.*, 1998). In fact, subsequent studies demonstrated significant differences in the frequency of these alleles in the AIRmax and AIRmin lineage, with the R allele being predominant in AIRmax animals, while the presence of allele S is characteristic of the AIRmin strain (ARAUJO *et al.*, 1998). In humans, although there are no variations identical to the R and S alleles of *Slc11a1* described in mice, polymorphisms with similar potential immunoregulatory impact have been identified and associated with susceptibility to different infectious diseases (FATTAHI-DOLATABADI *et al.*, 2016). These polymorphisms vary in the location of coding regions, still involving missense mutations or silent substitutions (ABEL *et al.*, 1998; BELLAMY *et al.*, 1998; ALCAIS *et al.*, 2000; GREENWOOD *et al.*, 2000; FATTAHI-DOLATABADI *et al.*, 2016).

Among the SNPs described in the *Slc11a1* gene, rs17228995, rs17235409, rs2290708 rs2695343 and rs3731865 can be considered as SNP tags since are described in the literature as

important markers of susceptibility or resistance to various infectious diseases (SAPKOTA *et al.*, 2012; BIBERT *et al.*, 2017). *Slc11a1*SNP rs17235409 was used for the study of susceptibility to cutaneous leishmaniasis, along with eight other SNPs from this same gene. This study demonstrated a strong linkage disequilibrium between the SNPs rs17235409 in exon 15 and the insertion/deletion of the polymorphism rs17235416. However, the polymorphisms of the *Slc11a1* gene did not influence the susceptibility to the development of cutaneous leishmaniasis (SOPHIE *et al.*, 2017). The SNP rs2290708, has little information in the literature. The only study that brings information about it is also unique to a single population, the Pakistani and the susceptibility to cutaneous leishmaniasis, and a mentioned above there was no significant difference between the distribution of alleles and genotypes (SOPHIE *et al.*, 2017). Three previously described polymorphisms point to a statistically significant difference for the susceptibility to tuberculosis in African American and Caucasian individuals for the *Slc11a1* gene, being rs3731865, rs3731863 and rs17221959. And those African American individuals who had the rs3731865 polymorphism with the CG and GG genotypes had a higher risk of developing tuberculosis with CT and TT genotypes presented a decreased risk for the disease when the analysis was performed in *multilocus (*VELEZ *et al., 2009).* In addition to infectious diseases, type 1 diabetes was also associated with SNP rs3731865 (YANG *et al.*, 2011). The polymorphic allele of the SNPs rs3731865 has been associated with an increased risk of suffering from otitis media in a pediatric population in Australia, but relative to the SNPs rs2276631 and rs2695343 this significant statistical difference does not hold (RYE *et al.*, 2013). Together, the evidence indicates that the polymorphic variants in the *Slc11a1* gene may be associated with a variety of risk phenotypes in infectious diseases, making it an interesting candidate for an association study in chronic periodontitis.

With regard to periodontal diseases, a single recent study investigated the possible association of variants in *Slc11a1* with such conditions. In a genetic association study including 75 patients with chronic periodontitis and 50 healthy controls, the association of the rs17235409 and rs2276631 polymorphisms in the *Slc11a1* gene with the periodontal disease phenotype was described, with the polymorphic allele having a protective effect for chronic periodontitis (KADKHODAZADEH *et al.*, 2016). It is noteworthy that even with relatively small experimental N identified a possible risk variant in the *Slc11a1* gene. However, no other type of correlation (with host response patterns or microbial colonization patterns) were investigated, as was a single population with a distinct genetic background of the Brazilian population, was tested.

Therefore, this study goal is to correlate the genetic polymorphisms rs17228995, rs17235409, rs2290708, rs2695343, rs3731865 of the gene *Slc11a1* with profiles of resistance/susceptibility to periodontal diseases in humans, thus to evaluate the impact of hypo/hyperresponsive variants on experimental periodontitis in mice.

2 ARTICLE

2 ARTICLE

Journal of Leucocyte Biology

Hiper reactive variants of *Slc11a1* gene are associated with increased inflammatory responsiveness and comprise a risk factor for periodontal diseases: evidences from human and experimental periodontitis

Jéssica Lima Melchiades[1], Franco Cavalla[1,2], Michelle de Campos Soriani Azevedo[1], Priscila Maria Colavite [1], Marcelo Faveri [3], Magda Feres [3], Andrea Borrego [4], Marcelo de Franco [5], Ariadne Letra[6,7], Renato Menezes da Silva[6], Gustavo Pompermaier Garlet[1]

1. School of Dentistry of Bauru, University of Sao Paulo (FOB/USP) – Department of Biological Science, Bauru, Brazil;
2. University of Chile (UChile), Faculty of Dentistry, Department of Conservative Dentistry, Santiago, Chile;
3. Dental Research Division, Department of Periodontology, Guarulhos University, São Paulo, Brazil.
4. Laboratory of Immunogenetics, Butantan Institute, Secretary of Health, Government of the State of São Paulo, SP, Brazil.
5. Diagnostic Section, Pasteur Institute, Secretary of Health, Government of the State of São Paulo, SP, Brazil.
6. University of Texas Health Science Center at Houston, Department of Endodontics, School of Dentistry, Houston, Texas, USA;
7. University of Texas Health Science Center at Houston, Department of Diagnostic and Biomedical Science, and Center for Craniofacial Research, Houston, Texas, USA;

***Corresponding author**
Gustavo Pompermaier Garlet
Bauru School of Dentistry (FOB/USP) - Department of Biological Sciences
Al. Octávio Pinheiro Brisola, 9-75 - CEP 17012-901 – Bauru - SP - Brazil
Phone +55 (14) 3235-8274 - Fax +55 (14) 3223-4679
Email: garletgp@usp.br

CONFLICT OF INTEREST DISCLOSURE: The authors declare no conflict of interest.

LIST OF ABREVIATIONS

AIRmax	Maximal Inflammatory Reactions
AIRmin	Minimal Inflammatory Reactions
BOP	Bleeding on Probing
CAL	Clinical Attachment Loss
CG	Chronic Gingivitis
CP	Chronic Periodontitis
DNA	Desoxyribonucleic acid
H	Healthy
IL	Interleukin
LPS	Lypopolysaccharide
M1	Macrophages exhibit high levels of pro-inflammatory
M2	Macrophages exhibit high levels of anti-inflammatory
mRNA	messenger RNA
OPG	Osteoprotegerin
PD	Probing Depth
PDs	Periodontal Diseases
R	Resistance
RANKL	Receptor activator of nuclear factor kappa-B ligand
S	Susceptibility
SNP	Single Nucleotide Polymorphism
TNF	Tumor Necrosis Factor

ABSTRACT

Studies in humans and experimental models have demonstrated the influence of multiple genetic loci on the determination of susceptibility/resistance phenotypes to periodontitis. Among these genes, *Slc11a1*, whose pleiotropic functions include the regulation of macrophages and lymphocyte activity, has a potential role in the modulation of resistance/susceptibility to periodontal diseases. In this context, our group demonstrated that AIRmax and AIRmin mice, characterized by the predominance of distinct variants of *Slc11a1* alleles, associated to different patterns of immune and inflammatory response, present distinct phenotypes of resistance/susceptibility to experimental periodontitis. Furthermore, genetic variants (SNPs) in *Slc11a1* are shown to be associated with different infectious diseases in humans. In this context, this study aimed to correlate the genetic polymorphisms rs17228995, rs17235409, rs2290708, rs2695343, rs3731865 of the gene *Slc11a1* with profiles of resistance/susceptibility to periodontal diseases in humans, thus to evaluate the impact of hypo/hyperresponsive variants on experimental periodontitis in mice. Forty-five patients with chronic periodontitis (CP), 476 healthy individuals (H) and 207 individuals with chronic gingivitis (CG) were analyzed for association analysis, and subgroups were analyzed for analysis of possible correlations between expression / genotype (CP = 127, H = 63) and for in vitro tests (H = 29). In the analysis of the genotypes, only the assays for the characterization of the SNPs rs2290708 and rs3731865 were effective in the allelic discrimination, the other tests were considered technically ineffective. The polymorphisms rs2290708 and rs3731865 were shown to be associated with the risk of periodontitis, with a higher frequency of the genotypes CT+TT and GC+CC and the alleles T and C (respectively) in the CP group. In addition to the association in the control case approach, the presence of the polymorphic T (rs2290708) and C (rs37371865) alleles was shown to be associated with increased TNF-α, IL-1β, IL-6, RANKL and RANKL/OPG periodontal lesions. No differences were observed in the pattern of microbiological colonization of sites with chronic periodontitis with respect to the SNPs rs2290708 and rs37371865. In vitro analysis reinforces the hyper-reactive nature of the polymorphic alleles T (rs2290708) and C (rs37371865), since the production of inflammatory cytokines by macrophages bearing such alleles is shown to be increased in response to LPS stimulation. Finally, we observed that hypereactive variants of *Slc11a1*, characterized in the AIRmin and AIRmax murine are associated with increased alveolar bone loss, leukocyte influx, and increased production of proinflammatory cytokines in experimental periodontitis. Thus, it

is possible to conclude that functional polymorphisms in the *Slc11a1* gene, associated with increased inflammatory responsiveness, influence the risk to the development of periodontitis in humans and in mice experimental model.

Keywords: Periodontal diseases, *Slc11a1* Gene and SNPs

3 DISCUSSION

3 DISCUSSION

Periodontal diseases are chronic and multifactorial involving in this pathogenesis microbial, inflammatory and immunological factors, that together can be modulated by local, environmental, genetic and epigenetic factors (MARTINEZ and HOLT, 1999; LALLA *et al.*, 2003). Periodontal disease initiation and propagation are triggered by the dysbiosis of the commensal oral microbiota, which then interacts with the immune defenses of the host, leading to inflammation and subsequently to the development of disease symptoms (KINANE *et al.*, 2017). In this context, understanding host mechanisms in the face of the microbial challenge are essential for the understanding and treatment of periodontal disease.

One of the factors that interfere in the response of the host that has been much studied are the genetic polymorphisms. Among the genes with potential to modulate periodontitis outcome, the predominance of *Slc11a1* hipo/hiper-responsive variants has been demonstrated to influence the severity of experimental periodontitis in mice. In humans, a recent study suggests that one SNP in *Slc11a1* could account for the risk to periodontitis development. Our results, showed that 639+22C/T (rs2290708) is related the development of chronic periodontal disease, since the CT and TT genotypes presented a statistically significant difference. The same relation was found for the same CT and TT genotypes when comparing the groups with chronic gingivitis and chronic periodontitis. This study comprises the first description of the potential association of such variant with periodontal disease. A previous study described the lack of association between rs2290708 with leishmania in Pakistani individuals (SOPHIE *et al.*, 2017). However, we must consider that the pathogenesis of leishmanial significantly differ for periodontitis pathogenesis, and also that Brazilian and Pakistani individuals present distinct genetic backgrounds.

Our results also demonstrate that rs3731865 was also associated with periodontitis. Indeed, allele C and the CT and TT genotypes are related to the presence of periodontal diseases, since its frequency is higher when comparing healthy groups and chronic gingivitis with that of periodontal diseases. Importantly, the frequency of rs3731865 was similar to that previously reported in the Brazilian population (BROCHADO *et al.*, 2016).

Also, polymorphism rs17235409 quoted initially to be studied is associated with a protective factor against periodontal diseases in the Iranian population. And as mentioned

above, due to technical problems this polymorphism was not analyzed (KADKHOZADEH *et al.*,2016). Additionally, rs3731865 was associated with the risk to other infectious conditions, such as susceptibility in tuberculosis in Turkish patients (ATES *et al.*, 2009), and in Brazilian populations was associated with the risk of leprosy (BROCHADO *et al.*, 2016).

While the case-control data from suggests the involvement of *Slc11a1* SNPs in periodontitis susceptibility/resistance, additional experimental approaches were conducted to support such potential association from the functional and mechanistic viewpoints. When analyzing the expression of cytokines taking into account the genotypes of the chronic periodontitis group of both polymorphisms, we noticed an increase in the expression of the cytokines of the innate immune response, TNF-α, IL-1β and IL-6 (Fig. 1), associated the presence of the T allele (rs2290708) and the allele C (rs3731865). To date, no previous studies have been investigated the possible association between *Slc11a1* and host response parameters *in vivo*, being the immunomodulatory effects of *Slc11a1* studied basically in experimental models (BAULER *et al.*, 2017; CORREA *et al.*, 2017). In human cells, the expression of *Slcl11a1* have been associated with enhancement of pro-inflammatory responses, promotes efficient resolution of infection, but is associated with autoimmunity and inflammation, such as type 1 diabetes (O'BRIEN *et al.*, 2008).

In periodontitis context, and it is already known that direct effect on the pathogenesis of periodontal diseases, TNF-α up-regulates the production of other classic pro-inflammatory innate cytokines, such IL-1β and IL-6 (OKADA *et al.*, 1997; GRAVES *et al.*, 2008; GARLET, 2010). And both IL-1β and IL-6 also have been characteristically associated with inflammatory cell migration and osteoclast genesis processes (GRAVES *et al.*, 2008; FONSECA *et al.*, 2009).This was also confirmed by our results, since the levels of RANKL expression and the RANKL/OPG ratio (Fig. 2), since in both polymorphisms these markers are with increased expression associated with polymorphic alleles. An earlier study, showed the blockage of RANKL by OPG leads to a reduction in alveolar bone loss throughout experimental periodontal disease in mice(JIN *et al.*, 2007), and analysis of experimental data supports results from human studies, since RANKL/OPG balance was associated with alveolar bone loss rate and experimental disease progression (GARLET *et al.*, 2006).Certainly, the absence of an exaggerated inflammatory response decreases bone loss in individuals with periodontal disease.

While a significant association between *Slc11a1* SNPs and host response parameters was observed in periodontal lesions, we must consider the complexity of periodontitis pathogenesis, where multiple factors can modulate host response and periodontitis outcome.

In this scenario, we observed that *Slc11a1* SNPs were not associated with variations in the patterns of periodontal infection. No difference was observed in the pattern of microbiological colonization of sites with chronic periodontitis with respect to SNPs rs2290708 and 3731865 (Fig.3). However, the presence of these microbial agents induces the microbial challenge, because they present lipopolysaccharide (LPS), bacterial DNA, diacyl lipopeptides and peptidoglycan, where there is poor oral hygiene favors the development of periodontal disease(MAHANONDA and PICHYANGKUL, 2007).But, strong evidence supporting a direct connection between the host's genetic profile, specifically rs2521634, rs10010758, rs666702 and rs10043775 polymorphisms and the occurrence of chronic periodontitis associated bacteria(CAVALLA *et al.*, 2018). It shows that there is an association between genetic polymorphisms and the presence of certain microorganisms.

In order to gain further insight into the potential modulation of host response ant periodontal environment, we next performed an *in vitro* analysis of macrophages derived from donors with distinct *Slc11a1* genotypes. Macrophages are considered key cells in periodontitis pathogenesis, in the view of its properties, which can range from pro-inflammatory M1 cells to pro-reparative M2 phenotype. Furthermore, the expression of *Slc11a1* have been associated with macrophage response *in vitro* increase after infection with osteopathogens (RYE *et al.*, 2013). *In vitro* analysis confirms the hyper-reactive response of the polymorphic alleles T (rs2290708) and C (rs3731865), because in both the presence of these alleles induced a greater production of TNF-α, IL-1β, IL-6 and IL-10 (Fig. 4). Although IL-6, has been produced by macrophage with increased LPS stimulation in the C (rs3731865) allele. This can be explained because high IL-6 levels are frequently observed in patients with chronic diseases, in addition to there is evidences that IL-6 is capable of mediating both proinflammatory effects. Thus, the increase in the LPS stimulus causes increases of IL-6 to occur as the balance between the proinflammatory and anti-inflammatory effects of IL-6 may influence the development of chronic inflammation and diseases (CRONSTEIN, 2007).

Finally, in order to test the impact of *Slc11a1* hipo/hiper-responsive variants in periodontitis outcome in a cause-and-effect manner, experimental periodontitis was induced in AIRmin (characterized by the predominance of 'S' hipo-responsive *Slc11a1* allele), AIRmax

(characterized by the predominance of 'R' hiper-responsive *Slc11a1* allele), AIRmaxRR and AIRmaxSS (strains presenting the AIRmax background but homozygous for R and S *Slc11a1* alleles). Ultimately, we observed that hyperactivity variants of *Slc11a1*, characterized in the AIRmin and AIRmax murine lines, are associated with increased alveolar bone leukocyte influx and increased production of TNF-α, IL-1β, IL-6 and IL-17 (Fig.5). Accordingly, a previous study demonstrated thatgenetic bases that result the differential phenotypes between the AIRmax and AIRmin strain, demonstrated that the gene *Slc11a1* ("solute carrier family 11a member 1") it is one of the genes responsible for the deferential response between the strains. *Slc11a1* alleles are named alleles R or S once they demonstrate resistance (R) or susceptibility (S) of determined infections/diseases (ARAUJO *et al.*, 1998; RIBEIRO *et al.*, 2003). Also, our data demonstrate that the presence of homozygous R and S *Slc11a1* genotypes in AIRmax background was also associated with a significant modulation of experimental periodontitis severity in mice.

4 CONCLUSION

4 CONCLUSION

Thus, it is possible to conclude that polymorphisms in the *Slc11a1* gene show to be functional, being associated to the increase of inflammatory responsiveness, and that consequently influence the risk to the development of periodontitis.

REFERENCES

REFERENCES

ABEL, L. et al. Susceptibility to leprosy is linked to the human NRAMP1 gene. **J Infect Dis,** v. 177, n. 1, p. 133-45, Jan 1998. ISSN 0022-1899 (Print)0022-1899 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9419180 >.

ALCAIS, A. et al. Granulomatous reaction to intradermal injection of lepromin (Mitsuda reaction) is linked to the human NRAMP1 gene in Vietnamese leprosy sibships. **J Infect Dis,** v. 181, n. 1, p. 302-8, Jan 2000. ISSN 0022-1899 (Print)0022-1899 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10608779 >.

ARAUJO, L. M. et al. Innate resistance to infection by intracellular bacterial pathogens differs in mice selected for maximal or minimal acute inflammatory response. **Eur J Immunol,** v. 28, n. 9, p. 2913-20, Sep 1998. ISSN 0014-2980 (Print)0014-2980 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9754578 >.

ARCHER, N. S.; NASSIF, N. T.; O'BRIEN, B. A. Genetic variants of SLC11A1 are associated with both autoimmune and infectious diseases: systematic review and meta-analysis. **Genes Immun,** v. 16, n. 4, p. 275-83, Jun 2015. ISSN 1476-5470 (Electronic)1466-4879 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25856512 >.

ATES, O. et al. NRAMP1 (SLC11A1) gene polymorphisms that correlate with autoimmune versus infectious disease susceptibility in tuberculosis and rheumatoid arthritis. **International Journal of Immunogenetics,** v. 36, n. 1, p. 15-19, Feb 2009. ISSN 1744-3121. Disponível em: < <Go to ISI>://WOS:000262667000003 >.

BAKER, P. J. Genetic control of the immune response in pathogenesis. **J Periodontol,** v. 76, n. 11 Suppl, p. 2042-6, Nov 2005. ISSN 0022-3492 (Print)0022-3492 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16277574 >.

BAKER, P. J.; DIXON, M.; ROOPENIAN, D. C. Genetic control of susceptibility to Porphyromonas gingivalis-induced alveolar bone loss in mice. **Infect Immun,** v. 68, n. 10, p. 5864-8, Oct 2000. ISSN 0019-9567 (Print)0019-9567 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10992496 >.

BAKER, P. J.; ROOPENIAN, D. C. Genetic susceptibility to chronic periodontal disease. **Microbes Infect,** v. 4, n. 11, p. 1157-67, Sep 2002. ISSN 1286-4579 (Print)1286-4579 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12361916 >.

BAULER, T. J. et al. Salmonella Meningitis Associated with Monocyte Infiltration in Mice. **American Journal of Pathology,** v. 187, n. 1, p. 187-199, Jan 2017. ISSN 0002-9440. Disponível em: < <Go to ISI>://WOS:000390829300019 >.

BELLAMY, R. et al. Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. **N Engl J Med,** v. 338, n. 10, p. 640-4, Mar 5 1998. ISSN 0028-4793 (Print)0028-4793 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9486992 >.

BEREZOW, A. B.; DARVEAU, R. P. Microbial shift and periodontitis. **Periodontol 2000,** v. 55, n. 1, p. 36-47, Feb 2011. ISSN 1600-0757 (Electronic)0906-6713 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21134227 >.

BIBERT, S. et al. Susceptibility to Mycobacterium ulcerans Disease (Buruli ulcer) Is Associated with IFNG and iNOS Gene Polymorphisms. **Front Microbiol,** v. 8, p. 1903, 2017. ISSN 1664-302X (Print)1664-302X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29046669 >.

BIOZZI, G. et al. Effect of genetic modification of acute inflammatory responsiveness on tumorigenesis in the mouse. **Carcinogenesis,** v. 19, n. 2, p. 337-46, Feb 1998. ISSN 0143-3334 (Print)0143-3334 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9498286 >.

BROCHADO, M. J. F. et al. Association of the solute carrier family 11 member 1 gene polymorphisms with susceptibility to leprosy in a Brazilian sample. **Memorias Do Instituto Oswaldo Cruz,** v. 111, n. 2, p. 101-105, Feb 2016. ISSN 0074-0276. Disponível em: < <Go to ISI>://WOS:000370522900003 >.

BRUNNER, J. et al. The capsule of Porphyromonas gingivalis reduces the immune response of human gingival fibroblasts. **BMC Microbiol,** v. 10, p. 5, Jan 11 2010. ISSN 1471-2180 (Electronic)1471-2180 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20064245 >.

CARNEIRO, A. S. et al. Local inflammatory reaction induced by Bothrops jararaca venom differs in mice selected for acute inflammatory response. **Toxicon,** v. 40, n. 11, p. 1571-9, Nov 2002. ISSN 0041-0101 (Print)0041-0101 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12419508 >.

CAVALLA, F. et al. Genetic Association with Subgingival Bacterial Colonization in Chronic Periodontitis. **Genes (Basel),** v. 9, n. 6, May 23 2018. ISSN 2073-4425 (Print)2073-4425 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29882907 >.

CAVALLA, F. et al. TBX21-1993T/C (rs4794067) polymorphism is associated with increased risk of chronic periodontitis and increased T-bet expression in periodontal lesions, but does not significantly impact the IFN-g transcriptional level or the pattern of periodontophatic bacterial infection. **Virulence,** v. 6, n. 3, p. 293-304, 2015. ISSN 2150-5608 (Electronic)2150-5594 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25832120 >.

CLAUDINO, M. et al. The broad effects of the functional IL-10 promoter-592 polymorphism: modulation of IL-10, TIMP-3, and OPG expression and their association with periodontal disease outcome. **J Leukoc Biol,** v. 84, n. 6, p. 1565-73, Dec 2008. ISSN 0741-5400 (Print)0741-5400 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18725394 >.

CORREA, M. A. et al. Slc11a1 (Nramp-1) gene modulates immune-inflammation genes in macrophages during pristane-induced arthritis in mice. **Inflammation Research,** v. 66, n. 11, p. 969-980, Nov 2017. ISSN 1023-3830. Disponível em: < <Go to ISI>://WOS:000412656700004 >.

CRONSTEIN, B. N. Interleukin-6--a key mediator of systemic and local symptoms in rheumatoid arthritis. **Bull NYU Hosp Jt Dis,** v. 65 Suppl 1, p. S11-5, 2007. ISSN 1936-9719 (Print)1936-9719 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17708739 >.

DI PACE, R. F. et al. Inverse genetic predisposition to colon versus lung carcinogenesis in mouse lines selected based on acute inflammatory responsiveness. **Carcinogenesis,** v. 27, n. 8, p. 1517-25, Aug 2006. ISSN 0143-3334 (Print)0143-3334 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16774945 >.

EJGHAL, R. et al. SLC11A1 polymorphisms and susceptibility to visceral leishmaniasis in Moroccan patients. **Acta Trop,** v. 140, p. 130-6, Dec 2014. ISSN 1873-6254 (Electronic)0001-706X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25151047 >.

FATTAHI-DOLATABADI, M. et al. NRAMP1 gene polymorphisms and cutaneous leishmaniasis: An evaluation on host susceptibility and treatment outcome. **Journal of Vector Borne Diseases,** v. 53, n. 3, p. 257-263, Sep 2016. ISSN 0972-9062. Disponível em: < <Go to ISI>://WOS:000387200400009 >.

FERREIRA, S. B., JR. et al. An interleukin-1beta (IL-1beta) single-nucleotide polymorphism at position 3954 and red complex periodontopathogens independently and additively modulate the levels of IL-1beta in diseased periodontal tissues. **Infect Immun,** v. 76, n. 8, p. 3725-34, Aug 2008. ISSN 1098-5522 (Electronic)0019-9567 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18541658 >.

FONSECA, J. E. et al. Interleukin-6 as a key player in systemic inflammation and joint destruction. **Autoimmunity Reviews,** v. 8, n. 7, p. 538-542, Jun 2009. ISSN 1568-9972. Disponível em: < <Go to ISI>://WOS:000267567300002 >.

FORBES, J. R.; GROS, P. Divalent-metal transport by NRAMP proteins at the interface of host-pathogen interactions. **Trends in Microbiology,** v. 9, n. 8, p. 397-403, Aug 2001. ISSN 0966-842X. Disponível em: < <Go to ISI>://WOS:000170462300011 >.

FRANK, D. N. et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. **Proceedings of the National Academy of Sciences of the United States of America,** v. 104, n. 34, p. 13780-13785, Aug 21 2007. ISSN 0027-8424. Disponível em: < <Go to ISI>://WOS:000249064700047 >.

FRITSCHE, G. et al. Modulation of macrophage iron transport by Nramp1 (Slc11a1). **Immunobiology,** v. 212, n. 9-10, p. 751-757, 2007. ISSN 0171-2985. Disponível em: < <Go to ISI>://WOS:000252620000008 >.

GARLET, G. P. et al. Cytokine pattern determines the progression of experimental periodontal disease induced by Actinobacillus actinomycetemcomitans through the modulation of MMPs, RANKL, and their physiological inhibitors. **Oral Microbiology and Immunology,** v. 21, n. 1, p. 12-20, Feb 2006. ISSN 0902-0055. Disponível em: < <Go to ISI>://WOS:000234405700003 >.

GARLET, G. P. et al. The use of chronic gingivitis as reference status increases the power and odds of periodontitis genetic studies - a proposal based in the exposure concept and clearer resistance and susceptibility phenotypes definition. **Journal of Clinical Periodontology,** v. 39, n. 4, p. 323-332, Apr 2012. ISSN 0303-6979. Disponível em: < <Go to ISI>://WOS:000301425600002 >.

GARLET, G. P. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. **J Dent Res,** v. 89, n. 12, p. 1349-63, Dec 2010. ISSN 1544-0591 (Electronic)0022-0345 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20739705 >.

GEMMELL, E. et al. The influence of genetic variation on the splenic T cell cytokine and specific serum antibody responses to Porphyromonas gingivalis in mice. **J Periodontol,** v. 71, n. 7, p. 1130-8, Jul 2000. ISSN 0022-3492 (Print)0022-3492 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10960020 >.

GENCO, C. A.; VAN DYKE, T.; AMAR, S. Animal models for Porphyromonas gingivalis-mediated periodontal disease. **Trends Microbiol,** v. 6, n. 11, p. 444-9, Nov 1998. ISSN 0966-842X (Print)0966-842X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9846362 >.

GIBSON, F. C., 3RD et al. Innate immune signaling and Porphyromonas gingivalis-accelerated atherosclerosis. **J Dent Res,** v. 85, n. 2, p. 106-21, Feb 2006. ISSN 0022-0345 (Print)0022-0345 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16434728 >.

GRAVES, D. T. et al. The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. **J Clin Periodontol,** v. 35, n. 2, p. 89-105, Feb 2008. ISSN 1600-051X (Electronic)0303-6979 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18199146 >.

GREENWOOD, C. M. et al. Linkage of tuberculosis to chromosome 2q35 loci, including NRAMP1, in a large aboriginal Canadian family. **Am J Hum Genet,** v. 67, n. 2, p. 405-16, Aug 2000. ISSN 0002-9297 (Print)0002-9297 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10882571 >.

HART, G. T. et al. Quantitative gene expression profiling implicates genes for susceptibility and resistance to alveolar bone loss. **Infect Immun,** v. 72, n. 8, p. 4471-9, Aug 2004. ISSN 0019-9567 (Print)0019-9567 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15271905 >.

HART, T. C.; MARAZITA, M. L.; WRIGHT, J. T. The impact of molecular genetics on oral health paradigms. **Crit Rev Oral Biol Med,** v. 11, n. 1, p. 26-56, 2000. ISSN 1045-4411 (Print)1045-4411 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10682900 >.

HIYARI, S. et al. Heritability of periodontal bone loss in mice. **J Periodontal Res,** v. 50, n. 6, p. 730-6, Dec 2015. ISSN 1600-0765 (Electronic)0022-3484 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25581386 >.

IBANEZ, O. M. et al. Genetics of nonspecific immunity: I. Bidirectional selective breeding of lines of mice endowed with maximal or minimal inflammatory responsiveness. **Eur J Immunol,** v. 22, n. 10, p. 2555-63, Oct 1992. ISSN 0014-2980 (Print)0014-2980 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/1396963 >.

JIN, Q. M. et al. RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis. **Journal of Periodontology,** v. 78, n. 7, p. 1300-1308, Jul 2007. ISSN 0022-3492. Disponível em: < <Go to ISI>://WOS:000248097100020 >.

KADKHODAZADEH, M. et al. Natural Resistance Associated Macrophage Protein 1 Gene Polymorphism is Associated with Chronic Periodontitis Not Peri-Implantitis in an Iranian Population: A Cross Sectional Study. **Acta Med Iran,** v. 54, n. 5, p. 323-9, May 2016. ISSN 1735-9694 (Electronic)0044-6025 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27309481 >.

KINANE, D. F.; LAPPIN, D. F. Clinical, pathological and immunological aspects of periodontal disease. **Acta Odontol Scand,** v. 59, n. 3, p. 154-60, Jun 2001. ISSN 0001-6357 (Print)0001-6357 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11501884 >.

KINANE, D. F.; STATHOPOULOU, P. G.; PAPAPANOU, P. N. Periodontal diseases. **Nat Rev Dis Primers,** v. 3, p. 17038, Jun 22 2017. ISSN 2056-676X (Electronic)2056-676X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28805207 >.

KITA, E. et al. Contribution of interferon gamma and membrane-associated interleukin 1 to the resistance to murine typhoid of Ityr mice. **J Leukoc Biol,** v. 51, n. 3, p. 244-50, Mar 1992. ISSN 0741-5400 (Print)0741-5400 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/1541907 >.

KORNMAN, K. S. Mapping the Pathogenesis of Periodontitis: A New Look. **Journal of Periodontology,** v. 79, n. 8, p. 1560-1568, Aug 2008. ISSN 0022-3492. Disponível em: < <Go to ISI>://WOS:000262441000010 >.

KORNMAN, K. S.; PAGE, R. C.; TONETTI, M. S. The host response to the microbial challenge in periodontitis: assembling the players. **Periodontol 2000,** v. 14, p. 33-53, Jun 1997. ISSN 0906-6713 (Print)0906-6713 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9567965 >.

KORNMAN, K.; DUFF, G.; REILLY, P. Re: A critical assessment of interleukin-1 (IL-1) genotyping when used in a genetic susceptibility test for severe chronic periodontitis. Greenstein G, Hart TC (2002;73 : 231-247). **Journal of Periodontology,** v. 73, n. 12, p. 1553-1556, Dec 2002. ISSN 0022-3492. Disponível em: < <Go to ISI>://WOS:000180411800020 >.

LAINE, M. L. et al. [Risk factors in adult periodontitis: polymorphism in the interleukin-1 gene family]. **Ned Tijdschr Tandheelkd,** v. 109, n. 8, p. 303-6, Aug 2002. ISSN 0028-2200 (Print)0028-2200 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12212456 >.

LAINE, M. L. et al. Modeling susceptibility to periodontitis. **J Dent Res,** v. 92, n. 1, p. 45-50, Jan 2013. ISSN 1544-0591 (Electronic)0022-0345 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23100272 >.

LAINE, M. L.; CRIELAARD, W. Functional foods/ingredients and periodontal diseases. **Eur J Nutr,** v. 51 Suppl 2, p. S27-30, Jul 2012. ISSN 1436-6215 (Electronic)1436-6207 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22535144 >.

LALLA, E. et al. Oral infection with a periodontal pathogen accelerates early atherosclerosis in apolipoprotein E-null mice. **Arterioscler Thromb Vasc Biol,** v. 23, n. 8, p. 1405-11, Aug 1 2003. ISSN 1524-4636 (Electronic)1079-5642 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12816879 >.

LOOS, B. G.; JOHN, R. P.; LAINE, M. L. Identification of genetic risk factors for periodontitis and possible mechanisms of action. **J Clin Periodontol,** v. 32 Suppl 6, p. 159-79, 2005. ISSN 0303-6979 (Print)0303-6979 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16128836 >.

MAHANONDA, R.; PICHYANGKUL, S. Toll-like receptors and their role in periodontal health and disease. **Periodontol 2000,** v. 43, p. 41-55, 2007. ISSN 0906-6713 (Print)0906-6713 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17214834 >.

MARIA, D. A. et al. Pulmonary adenoma susceptibility 1 (Pas1) locus affects inflammatory response. **Oncogene,** v. 22, n. 3, p. 426-32, Jan 23 2003. ISSN 0950-9232 (Print)0950-9232 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12545163 >.

MARSH, P. D. Microbial ecology of dental plaque and its significance in health and disease. **Adv Dent Res,** v. 8, n. 2, p. 263-71, Jul 1994. ISSN 0895-9374 (Print)0895-9374 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7865085 >.

MARTINEZ, F. D.; HOLT, P. G. Role of microbial burden in aetiology of allergy and asthma. **Lancet,** v. 354 Suppl 2, p. SII12-5, Sep 1999. ISSN 0140-6736 (Print)0140-6736 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10507253 >.

MATRICARDI, P. M.; RONCHETTI, R. Are infections protecting from atopy? **Curr Opin Allergy Clin Immunol,** v. 1, n. 5, p. 413-9, Oct 2001. ISSN 1528-4050 (Print)1473-6322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11964721 >.

MICHALOWICZ, B. S. et al. Evidence of a substantial genetic basis for risk of adult periodontitis. **J Periodontol,** v. 71, n. 11, p. 1699-707, Nov 2000. ISSN 0022-3492 (Print)0022-3492 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11128917 >.

MICHALOWICZ, B. S. et al. Periodontal findings in adult twins. **J Periodontol,** v. 62, n. 5, p. 293-9, May 1991. ISSN 0022-3492 (Print)0022-3492 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/2072240 >.

O'BRIEN, B. A. et al. Association of SLC11A1 promoter polymorphisms with the incidence of autoimmune and inflammatory diseases: A meta-analysis. **Journal of Autoimmunity,** v. 31, n. 1, p. 42-51, Aug 2008. ISSN 0896-8411. Disponível em: < <Go to ISI>://WOS:000257243300006 >.

OKADA, N. et al. Interleukin-6 production in human fibroblasts derived from periodontal tissues is differentially regulated by cytokines and a glucocorticoid. **J Periodontal Res,** v. 32, n. 7, p. 559-69, Oct 1997. ISSN 0022-3484 (Print)0022-3484 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9401927 >.

PETERS, L. C. et al. Slc11a1 (formerly NRAMP1) gene modulates both acute inflammatory reactions and pristane-induced arthritis in mice. **Genes Immun,** v. 8, n. 1, p. 51-6, Jan 2007. ISSN 1466-4879 (Print)1466-4879 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17122779 >.

PEYYALA, R. et al. Oral epithelial cell responses to multispecies microbial biofilms. **J Dent Res,** v. 92, n. 3, p. 235-40, Mar 2013. ISSN 1544-0591 (Electronic)0022-0345 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23300185 >.

PEYYALA, R.; EBERSOLE, J. L. Multispecies biofilms and host responses: "discriminating the trees from the forest". **Cytokine,** v. 61, n. 1, p. 15-25, Jan 2013. ISSN 1096-0023 (Electronic)1043-4666 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23141757 >.

RAMARATHINAM, L.; NIESEL, D. W.; KLIMPEL, G. R. Ity influences the production of IFN-gamma by murine splenocytes stimulated in vitro with Salmonella typhimurium. **J Immunol,** v. 150, n. 9, p. 3965-72, May 1 1993. ISSN 0022-1767 (Print)0022-1767 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8473743 >.

REPEKE, C. E. et al. Strong and persistent microbial and inflammatory stimuli overcome the genetic predisposition to higher matrix metalloproteinase-1 (MMP-1) expression: a mechanistic explanation for the lack of association of MMP1-1607 single-nucleotide polymorphism genotypes with MMP-1 expression in chronic periodontitis lesions. **J Clin Periodontol,** v. 36, n. 9, p. 726-38, Sep 2009. ISSN 1600-051X (Electronic)0303-6979 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19659894 >.

RIBEIRO, O. G. et al. Convergent alteration of granulopoiesis, chemotactic activity, and neutrophil apoptosis during mouse selection for high acute inflammatory response. **J Leukoc Biol,** v. 74, n. 4, p. 497-506, Oct 2003. ISSN 0741-5400 (Print)0741-5400 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12960266 >.

RYE, M. S. et al. Genetic and functional evidence for a role for SLC11A1 in susceptibility to otitis media in early childhood in a Western Australian population. **Infect Genet Evol,** v. 16, p. 411-8, Jun 2013. ISSN 1567-7257 (Electronic)1567-1348 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23538334 >.

RYE, M. S. et al. Genetic and functional evidence for a role for SLC11A1 in susceptibility to otitis media in early childhood in a Western Australian population. **Infect Genet Evol,** v. 16, p. 411-8, Jun 2013. ISSN 1567-7257 (Electronic)1567-1348 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23538334 >.

SAPKOTA, B. R. et al. Association of SLC11A1 (NRAMP1) polymorphisms with pulmonary Mycobacterium avium complex infection. **Human Immunology,** v. 73, n. 5, p. 529-536, May 2012. ISSN 0198-8859. Disponível em: < <Go to ISI>://WOS:000304024500016 >.

SCHORK, N. J.; FALLIN, D.; LANCHBURY, J. S. Single nucleotide polymorphisms and the future of genetic epidemiology. **Clinical Genetics,** v. 58, n. 4, p. 250-264, Oct 2000. ISSN 0009-9163. Disponível em: < <Go to ISI>://WOS:000089812900002 >.

SHUSTERMAN, A. et al. Genotype is an important determinant factor of host susceptibility to periodontitis in the Collaborative Cross and inbred mouse populations. **Bmc Genetics,** v. 14, Aug 9 2013. ISSN 1471-2156. Disponível em: < <Go to ISI>://WOS:000323121000001 >.

SHUSTERMAN, A. et al. Host Susceptibility to Periodontitis: Mapping Murine Genomic Regions. **Journal of Dental Research,** v. 92, n. 5, p. 438-443, May 2013. ISSN 0022-0345. Disponível em: < <Go to ISI>://WOS:000317715300008 >.

SOCRANSKY, S. S. et al. Microbial complexes in subgingival plaque. **J Clin Periodontol,** v. 25, n. 2, p. 134-44, Feb 1998. ISSN 0303-6979 (Print)0303-6979 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9495612 >.

SOCRANSKY, S. S.; HAFFAJEE, A. D. Periodontal microbial ecology. **Periodontol 2000,** v. 38, p. 135-87, 2005. ISSN 0906-6713 (Print)0906-6713 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15853940 >.

SOPHIE, M. et al. SLC11A1 polymorphisms and host susceptibility to cutaneous leishmaniasis in Pakistan. **Parasit Vectors,** v. 10, n. 1, p. 12, Jan 7 2017. ISSN 1756-3305 (Electronic)1756-3305 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28061874 >.

STIFFEL, C. et al. Genetics of acute inflammation: inflammatory reactions in inbred lines of mice and in their interline crosses. **Exp Clin Immunogenet,** v. 7, n. 4, p. 221-33, 1990. ISSN 0254-9670 (Print)0254-9670 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/2083094 >.

TROMBONE, A. P. et al. Experimental periodontitis in mice selected for maximal or minimal inflammatory reactions: increased inflammatory immune responsiveness drives increased alveolar bone loss without enhancing the control of periodontal infection. **J Periodontal Res,** v. 44, n. 4, p. 443-51, Aug 2009. ISSN 1600-0765 (Electronic)0022-3484 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18973535 >.

TROMBONE, A. P. et al. Periodontitis and arthritis interaction in mice involves a shared hyper-inflammatory genotype and functional immunological interferences. **Genes Immun,** v. 11, n. 6, p. 479-89, Sep 2010. ISSN 1476-5470 (Electronic)1466-4879 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20428191 >.

TROMBONE, A. P. et al. Tumor necrosis factor-alpha -308G/A single nucleotide polymorphism and red-complex periodontopathogens are independently associated with increased levels of tumor necrosis factor-alpha in diseased periodontal tissues. **J Periodontal Res,** v. 44, n. 5, p. 598-608, Oct 2009. ISSN 1600-0765 (Electronic)0022-3484 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19076989 >.

USVIATSOV, B.; PARSHUTA, L. I.; DOLGOV, V. A. [Microbial biocenosis in the mucous membranes of the nose and the middle ear in patients with purulent otitis]. **Zh Mikrobiol**

Epidemiol Immunobiol, n. 4 Suppl, p. 85-8, Jul-Aug 2000. ISSN 0372-9311 (Print)0372-9311 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12712523 >.

VELEZ, D. R. et al. Association of SLC11A1 with tuberculosis and interactions with NOS2A and TLR2 in African-Americans and Caucasians. **Int J Tuberc Lung Dis,** v. 13, n. 9, p. 1068-76, Sep 2009. ISSN 1815-7920 (Electronic)1027-3719 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19723394 >.

VIGAR, N. D. et al. Pristane-induced arthritis in mice selected for maximal or minimal acute inflammatory reaction. **Eur J Immunol,** v. 30, n. 2, p. 431-7, Feb 2000. ISSN 0014-2980 (Print)0014-2980 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10671198 >.

YANG, J. H. et al. Evidence of association with type 1 diabetes in the SLC11A1 gene region. **BMC Med Genet,** v. 12, p. 59, Apr 27 2011. ISSN 1471-2350 (Electronic)1471-2350 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21524304 >.

YANG, L. et al. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. **Gastroenterology,** v. 137, n. 2, p. 588-97, Aug 2009. ISSN 1528-0012 (Electronic)0016-5085 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19394334 >.

ANNEX

UNIVERSIDADE GUARULHOS - UNG

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: INFLUÊNCIA DO MOMENTO DA ADMINISTRAÇÃO DE METRONIDAZOL E AMOXICILINA NO TRATAMENTO DE INDIVÍDUOS COM PERIODONTITE CRÔNICA E DEFINIÇÃO DE POSSÍVEIS PERFIS - CLÍNICOS, MICROBIOLÓGICOS, IMUNOLÓGICOS E GENÉTICOS - COM DIFERENTES RESPOSTAS AO TRATAMENTO.

Pesquisador: Magda Feres Figueiredo

Área Temática:

Versão: 2

CAAE: 32465714.4.1001.5506

Instituição Proponente: Universidade Guarulhos - UNG

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 746.355

Data da Relatoria: 12/08/2014

Apresentação do Projeto:

Projeto está bem redigido, claro e bem fundamentado.

Objetivo da Pesquisa:

O objetivo está claro e pretende avaliar o melhor momento de administração de MTZ+AMX sistêmicos adjuntos à RAR no tratamento da periodontite crônica generalizada: a)na fase ativa da terapia periodontal,ou b) após a fase de cicatrização e reparo da terapia mecânica

Avaliação dos Riscos e Benefícios:

Estão descritos adequadamente.

Comentários e Considerações sobre a Pesquisa:

A casuística e o método adotados estão adequados para responder aos objetivos.

Considerações sobre os Termos de apresentação obrigatória:

O pesquisador apresentou todos os termos obrigatórios.

Recomendações:

Nada a declarar.

Endereço: Praça Tereza Cristina, 229
Bairro: Centro **CEP:** 07.023-070
UF: SP **Município:** GUARULHOS
Telefone: (11)2464-1779 **Fax:** (11)2464-1187 **E-mail:** comite.etica@ung.br

UNIVERSIDADE GUARULHOS - UNG

Continuação do Parecer: 746.355

Conclusões ou Pendências e Lista de Inadequações:

Foram adequados o TCLE e também os riscos.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Esta aprovação é válida pelo período previsto no cronograma postado. Enviar relatório final até 30/11/2020, via Plataforma Brasil.

GUARULHOS, 11 de Agosto de 2014

Assinado por:
Regina de Oliveira Moraes Arruda
(Coordenador)

Endereço: Praça Tereza Cristina, 229
Bairro: Centro **CEP:** 07.023-070
UF: SP **Município:** GUARULHOS
Telefone: (11)2464-1779 **Fax:** (11)2464-1187 **E-mail:** comite.etica@ung.br