UNESP - Universidade Estadual Paulista
"Júlio de Mesquita Filho"
Faculdade de Odontologia de Araraquara

Thamiris Cirelli

Investigação da suscetibilidade genética à periodontite crônica associada ao diabetes mellitus tipo 2

Araraquara

2020

UNESP - Universidade Estadual Paulista

"Júlio de Mesquita Filho"

Faculdade de Odontologia de Araraquara

Thamiris Cirelli

Investigação da suscetibilidade genética à periodontite crônica associada ao diabetes mellitus tipo 2

Tese apresentada à Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araraquara para obtenção do título de Doutor em Odontologia, na Área de Periodontia

Orientadora Profa. Dr. Raquel Mantuanelli Scarel Caminaga

Araraquara

2020

Cirelli, Thamiris
Investigação da suscetibilidade genética à periodontite crônica associada ao diabetes mellitus tipo 2 / Thamiris Cirelli.- Araraquara: [s.n.], 2020
118 f.; 30 cm.

Tese (Doutorado em Odontologia) – Universidade Estadual Paulista, Faculdade de Odontologia
Orientadora: Profa. Dra. Raquel Mantuaneli Scarel Caminaga

1. Polimorfismo genético 2. Periodontite 3. Diabetes mellitus I. Título

Ficha catalográfica elaborada pela Bibliotecária Marley C. Chiusoli Montagnoli, CRB/5646
Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araraquara
Diretoria Técnica de Biblioteca e Documentação

Thamiris Cirelli

Investigação da suscetibilidade genética à periodontite crônica associada ao diabetes mellitus tipo 2

Comissão julgadora

Tese para obtenção do grau de doutor em Periodontia

Presidente e orientador Profa. Dra. Raquel Mantuaneli Scarel Caminaga

2º Examinador Profa. Dra. Letícia Helena Theodoro

3º Examinador Prof. Dr. Renato Corrêa Viana Casarin

4º Examinador Profa. Dra. Andreia Bufalino

5º Examinador Profa. Dra. Silvana Regina Peres Orrico

Araraquara, 24 de setembro de 2020.

DADOS CURRICULARES

Thamiris Cirelli

NASCIMENTO: 09/03/1993 – Descalvado – São Paulo

FILIAÇÃO: Jeronimo Cirelli Junior

Paula Adriana Pires Maringolo Cirelli

2011 - 2015 Curso de Graduação em Odontologia – Faculdade de Odontologia de Araraquara – FOAr Universidade Estadual Paulista – UNESP

2017 - 2019 Curso de Especialização em Periodontia – Faculdade de Odontologia de Araraquara – FOAr Universidade Estadual Paulista – UNESP.

2016 - 2020 Curso de Pós-Graduação em Odontologia, Área de concentração em Periodontia – Nível Doutorado - Faculdade de Odontologia de Araraquara – FOAr Universidade Estadual Paulista – UNESP.

Dedico este trabalho...

Aos ***meus pais***, Jeronimo Cirelli Junior e Paula Adriana Pires Maringolo Cirelli pois sem eles, NADA seria possível. Agradeço por todo amor, carinho e dedicação pela nossa família desde SEMPRE. Pela educação e ensinamento que levarei para toda minha VIDA. São exemplos de AMOR e HUMILDADE

Aos ***meus irmãos,*** Lucas Antônio Cirelli e Lívia Cirelli, por toda paciência e companheirismo, estaremos juntos para sempre.

Agradeço especialmente...

À ***Deus***, por estar presente em todos os momentos da minha vida, obrigada por iluminar e guiar meu caminho e minhas escolhas.

À ***Santa Josefina Bakhita*** que intercede junto ao senhor para que cresçamos no Seu amor. É exemplo de fé e humildade.

Aos meus pais, ***Jeronimo Cirelli Junior*** e ***Paula Adriana Pires Maringolo Cirelli*** que sempre me apoiaram e lutaram junto comigo pelas minhas escolhas, muito obrigada por ensinar o que é amor, humildade, respeito e compaixão. Hoje sou o reflexo de vocês, e sou muito grata por isso, são meus exemplos de vida, onde encontro meu porto seguro. Aos meus irmãos ***Lucas A. Cirelli*** e ***Livia Cirelli***, que são meus companheiros desde sempre, me apoiando e me ajudando. Agradeço a Deus por ter vocês.

Meu namorado, ***Arthur Galleti Lima,*** por estar do meu lado em todos os momentos, escutando e apoiando todas as minhas escolhas, pelo amor e carinho, por ser essa pessoa maravilhosa que faz parte da minha vida.

A minha *Grande Familía*, meus pais, irmãos e ***Guilherme Boneli*** e ***Cíntia Belli Vischi***. Por estarem sempre comigo, apoiando minhas escolhas. Muito obrigada pelos momentos que compartilhamos juntos. Um agradecimento especial à minha sobrinha ***Cecília Vischi Cirelli*** que nos trouxe muito amor e alegria.

À minha orientadora ***Profa. Dra. Raquel Scarel Caminaga*** pela oportunidade de conhecer a pesquisa, por ter me acolhido. Obrigada por toda ajuda, atenção, paciência e principalmente por estar disposta a fazer o melhor. Agradeço e admiro sua dedicação.

Ao ***Prof. Dr. Joni Augusto Cirelli***, que sempre esteve de braços abertos me acolhendo e ajudando para que eu conseguisse alcançar meus objetivos de melhor forma possível.

À minha amiga-irmã, ***Joaquina Santos Diniz***, simplesmente por TUDO! Por estar do meu lado desde o começo, escutando e apoiando todas as minhas escolhas (mesmo de longe). Obrigada pela amizade e por ser essa pessoa maravilhosa que fez e sempre fará parte da minha vida.

À minha amiga, ***Marina Costa***, por estar do meu lado em todos os momentos. Sua amizade foi o melhor presente que a especialização me deu. Agradeço pelos momentos de alegria que compartilhamos juntas e por me escutar SEMPRE, mesmo estando longe. Tenho certeza que nossa amizade será para a vida toda.

À minha amiga, ***Suzane Cristina Pigossi***, pela parceria. Por estar sempre disposta a me ajudar compartilhando seu conhecimento. Agradeço pelos momentos divertidos que passamos juntos. Que nossa amizade permaneça por muitos anos.

Ao meu amigo, ***Diego Bussaneli***, por todos os momentos que passamos juntos. Admiro muito você como pessoa e profissional. Também agradeço pela sua ajuda durante todo o doutorado.

À minha amiga, ***Ingra Gagno Nicchio*** pela amizade e pelos momentos felizes juntas. Agradeço pelo seu companheirismo diário. Admiro a profissional que está se tornando.

À minha amiga, ***Andressa Nogueira***, entrou na minha vida esse ano e se tornou uma pessoa muito especial. Agradeço pelo seu companheirismo diário e me ensinar tanto em Mainz. À minha amiga, ***Camila Marcantonio***, que se tornou uma pessoa muito especial para mim. Agradeço pelos momentos que passamos juntas e por você ser essa pessoa tão carinhosa e especial.

Ao meu amigo, ***Rafael Nepomuceno***, pelos momentos divertidos que passamos juntos e pela ajuda na execução deste trabalho.

A todos meus meus companheiros de laboratório de ***Genética Humana,*** Sâmia Tfaile, Profa. Ticiana Capote, Karen Poquechoque, Marco Hidalgo, Bruna Rodrigues, por fazerem os dias de trabalho serem mais leves e por me ajudarem sempre que preciso. Ana Cláudia Rios, Jéssica Govea, Bárbara Roque, Karina Ferreira alunas de iniciação científica que me deram a oportunidade de ensinar e aprender muito. Tenho sorte de fazer parte desse grupo de pesquisa.

Agradeço...

À ***Faculdade de Odontologia de Araraquara***, na pessoa de seu Diretor, Prof. Dr. Edson Alves de Campos e da Vice-Diretora, Profa. Dra. Patrícia P. Nordi Sasso Garcia , responsável pela minha formação e pela estrutura oferecida para a realização dessa pesquisa.

Ao ***Coordenador do Curso de Pós-Graduação em Odontologia***, Área de Periodontia, Prof. Dr. Joni Augusto Cirelli, e a todos os ***docentes do Curso de Pós-Graduação do Programa de Periodontia***, sempre dispostos a ensinar, pela disponibilidade e dedicação ao programa e aos seus alunos.

Aos ***docentes do curso de Especialização em Periodontia***, Profa. Dra. Rosemary Adriana Chiérici Marcantonio, Prof. Dr. Carlos Rossa Júnior, Prof. Dr. Joni Augusto Cirelli e Prof. Dr. José Eduardo Cézar Sampaio que me tornaram especialista e pela dedicação ao curso.

À **CAPES:**O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de financiamento 001.

À **FAPESP** – Fundação de Amparo à Pesquisa do Estado de São Paulo (Processo nº2014/13295-1, 2016/03753-8, 2016/08070-6, 2016/18313-3, 2018/26367-1) pelo apoio financeiro para realização dessa pesquisa.

Aos ***Pacientes***, que colaboraram com a pesquisa, contribuindo com a realização dos exames clínicos e coletas e que com amabilidade compreenderam meu desafio e dividiram esta responsabilidade comigo! Muito obrigada!

Aos funcionários da Disciplina de Periodontia, ***Isabela, Claudinha e Suleima***, sempre dispostos a ajudar, pelo carinho e apoio aos alunos.

Aos ***funcionários do Departamento de Morfologia*** pelo apoio na execução desse trabalho.

Aos funcionários da ***Seção de Pós-Graduação***, pela paciência e dedicação aos alunos.

Aos ***funcionários da Biblioteca*** pela disposição de sempre.

A **todos** que, direta ou indiretamente, colaboraram e tornaram possível a realização deste trabalho.

"Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar. Mas o mar seria menor se lhe faltasse uma gota".

(Madre Teresa de Calcutá)

Cirelli T. Investigação da suscetibilidade genética à periodontite crônica associada ao diabetes mellitus tipo 2 [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2020.

RESUMO

Periodontite (P) e Diabetes Mellitus Tipo 2 (DM2) são doenças com grande prevalência na população, e o número de pacientes afetados por ambas as doenças, como comorbidades, tem se tornando cada vez maior. Além disso, a periodontite e a DM2 são doenças complexas que possuem mecanismos patogênicos comuns, mas o potencial componente genético que pode ser compartilhado entre essas doenças permanece pouco investigado. Marcadores genéticos de risco para cada uma dessas doenças foram identificados, mas devem ser avaliados em diferentes populações, para serem validados. Além disso é importante verificar se tais marcadores genéticos isolados seriam úteis na identificação da ocorrência conjunta dessas doenças como comorbidades. O objetivo deste estudo em uma população brasileira foi dividido em 2 capítulos: (i) Avaliar se os polimorfismos de nucleotídeo único (SNPs) nos genes *IL10, IL1A, IL1B, IL4, TNFA, IL6, OPG, RANK e RANKL*, "classicamente" relacionados à periodontite, podem estar associados à suscetibilidade ao DM2 e também com ambas as doenças concomitantemente; (ii) avaliar 12 SNPs selecionados a partir de estudos de associação ampla do genoma (GWAS) e bioinformática previamente associados a DM2 ou à periodontite isoladamente, como marcadores genéticos para DM2, ou periodontite, ou ambas doenças como comorbidades. Considerando o cálculo amostral, 956 pacientes foram submetidos a exame periodontal completo, além da análise bioquímica do seu perfil glicêmico e lipídico. Foram investigados pacientes com DM2 e P (Grupo DM2+P, n=239 pacientes); e para comparação dos resultados foram considerados pacientes sem DM2 com P severa ou moderada (Grupo Periodontite, n=358), e também pacientes sem DM2 e sem P, sendo considerados saudáveis (Grupo Controle, n=356). Assim, o grupo Periodontite foi considerado controle para o DM2 e o Grupo Controle foi considerado controle para a DM2 e a P. Após exame periodontal completo e realização dos exames bioquímicos, foi obtido o bochecho de cada paciente para extração do DNA pelo método Salting-out. Ao todo, 21 polimorfismos de nucleotídeo único (SNP) foram investigados por meio do sistema de genotipagem OpenArray. As associações entre SNPs e patologias foram testadas por regressões logísticas múltiplas, ajustando-se à idade, sexo e hábito de fumar. Também investigamos se houve efeito influenciado pelo sexo ou tabagismo em cada SNP na ocorrência desses fenótipos. Referente ao capítulo 1 verificou-se que o SNP rs1143634-GA *(IL1B)* apresentou probabilidade significativamente menor de desenvolver DM2+P em toda a população, mas principalmente em mulheres (OR ajustado = 0,37, IC 95% = 0,16 - 0,88, p = 0,02), enquanto mulheres portadoras do genótipo CT rs224320 *(IL4)* foram mais suscetíveis a desenvolver DM2+P (OR ajustado = 1,81, IC 95% = 1,04-3,15, p = 0,03). Homens portadores do genótipo rs1800795-CC *(IL6)* apresentaram menor probabilidade de desenvolver DM2+P (OR ajustado = 0,12, IC 95% = 0,02 - 0,70, p = 0,01). No capítulo 2, considerando a correção de Bonferroni, identificou-se que o genótipo rs7957197-TA (gene *HNF1A*) foi associado à maior suscetibilidade ao DM2+P (Periodontite versus DM2+P, OR = 1,95; IC 95% = 1,26–3,02; p = 0,003) em toda população e também para os homens comparados às mulheres. Mulheres portadoras do genótipo rs77544840-GT (gene *CDKAL1*) também mostraram suscetibilidade ao DM2+P (P versus P+DM2, OR = 2,49; IC 95% = 1,41–4,42; p = 0,002). Os pacientes homozigotos para o alelo mais raro (GG) do rs7018475 (gene *CDKN2B*) mostraram suscetibilidade para desenvolverem DM2+P (todo o controle versus todos os DM2+P, OR = 3,91; IC95% = 1,76–8,70; p = 0,001), bem como para mulheres e nunca fumantes. Portanto, pode-se concluir que SNPs nos genes *IL1B, IL4* e *IL6* demonstraram associação influenciada

pelo sexo com periodontite concomitante ao DM2, aumentando as evidências de um componente genético comum entre essas doenças. As variantes genéticas rs7957197 (*HNF1A*) e rs77544840 (*CDKAL1*) foram validadas nos brasileiros como marcadores de risco para DM2+P. O rs7018475 (*CDKN2B*) foi associado com maior risco para o desenvolvimento de periodontite juntamente com DM2 (DM2+P). Como esses achados eram específicos ao sexo, mais estudos com critérios de seleção semelhantes são necessários para confirmar os resultados observados e investigar o papel funcional desses SNPs no contexto do fenótipo de sexo e doença.

Palavras chave: Polimorfismo genético. Periodontite. Diabetes Mellitus.

Cirelli T. Investigation of genetic susceptibility to chronic periodontitis associated with type 2 diabetes mellitus [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2020.

ABSTRACT

Periodontitis (P) and Diabetes Mellitus Type 2 (T2DM) are diseases with a high prevalence in the population, and the number of patients affected by both diseases, such as comorbidities, has been increasing. In addition, periodontitis and T2DM are complex diseases that have common pathogenic mechanisms, but the potential genetic component that can be shared between these diseases remains poorly investigated. Genetic risk markers for each of these diseases have been identified, but they must be evaluated in different populations to be validated. In addition, it is important to verify whether such isolated genetic markers would be useful in identifying the joint occurrence of these diseases as comorbidities. The objective of this study in a Brazilian population was divided into 2 publications: (i) To assess whether single nucleotide polymorphisms (SNPs) in the *IL10*, *IL1A*, *IL1B*, *IL4*, *TNFA*, *IL6*, *OPG*, *RANK* and *RANKL* genes, "classically" related to periodontitis, may be associated with susceptibility to T2DM and also with both diseases concomitantly; (ii) evaluate 12 SNPs selected from studies of genome wide association (GWAS) and bioinformatics previously associated with T2DM or periodontitis alone, as genetic markers for T2DM, or periodontitis, or both diseases as comorbidities. Considering the sample size, 956 patients underwent a complete periodontal examination, in addition to biochemical analysis of their glycemic and lipid profile. Patients with T2DM and P (Group T2DM+P, n=239 patients) were investigated; and for comparison of results, patients without T2DM with severe or moderate P (Periodontitis Group, n = 358) were considered, and also patients without T2DM and without P, being considered healthy (Control Group, n = 356). Thus, the Periodontitis group was considered control for T2DM and the Control Group was considered control for T2DM and P. After a complete periodontal examination and biochemical tests, each patient's mouthwash was obtained for DNA extraction using the Salting- out. The 21 single nucleotide polymorphisms (SNP) were investigated using the OpenArray genotyping system. The associations between SNPs and pathologies were tested by multiple logistic regressions, adjusting for age, sex and smoking. We also investigated whether there was an effect influenced by sex or smoking in each SNP on the occurrence of these phenotypes. Regarding piblication 1, it was found that the SNP rs1143634-GA (*IL1B*) was significantly less likely to develop P+T2DM in the entire population, but mainly in women (adjusted OR = 0.37, 95% CI = 0.16 - 0.88, p = 0.02), while women with the CT rs224320 (*IL4*) genotype were more susceptible to developing P+T2DM (adjusted OR = 1.81, 95% CI = 1.04-3.15, p = 0.03). Men with the rs1800795-CC (*IL6*) genotype were less likely to develop T2DM (adjusted OR = 0.12, 95% CI = 0.02 - 0.70, p = 0.01). In publication 2, considering the Bonferroni's correction, it was found that the rs7957197-TA genotype (*HNF1A* gene) was associated with greater susceptibility to T2DM (Periodontitis versus P+T2DM, OR=1.95; 95% CI=1.26 – 3.02; p=0.003) in the entire population and also for men compared to women. Women with the rs77544840-GT genotype (*CDKAL1* gene) also showed susceptibility to T2DM (P versus P+T2DM, OR = 2.49; 95% CI = 1.41–4.42; p = 0.002). The patients homozygous for the rarer (GG) allele of rs7018475 (*CDKN2B* gene) showed susceptibility to develop P+T2DM (all control versus all P + T2DM, OR=3.91; 95% CI=1.76–8, 70; p=0.001), as well as for women and never smokers. Therefore, it can be concluded that SNPs in the *IL1B*, *IL4* and *IL6* genes demonstrated an association influenced by sex with periodontitis concomitant with T2DM, increasing the evidence of a common genetic component between these diseases. The genetic

variants rs7957197 (*HNF1A*) and rs77544840 (*CDKAL1*) have been validated in Brazil as risk markers for T2DM. Rs7018475 (*CDKN2B*) was associated with an increased risk of developing periodontitis along with T2DM (P + T2DM). As these findings were sex specific, further studies with similar selection criteria are needed to confirm the observed results and to investigate the functional role of these SNPs in the context of the sex and disease phenotype.

Keywords: Genetic polymorphism. Periodontitis. Diabetes Mellitus.

SUMÁRIO

1 INTRODUÇÃO 15

2 PROPOSIÇÃO.....20
2.1 Proposição Geral.....20
2.2 Proposições Específicas 20

3 PUBLICAÇÕES 21
3.1 Publicação 1.....21
3.2 Publicação 2.....52

4 DISCUSSÃO 83

5 CONCLUSÃO 91

REFERÊNCIAS 92

APÊNDICE 104

ANEXO 118

1 INTRODUÇÃO

A Doença Periodontal (DP) é a maior causa de perdas dentárias em adultos[1]. A DP é caracterizada como uma doença imunoinflamatória multifatorial mediada pelo hospedeiro, que ocorre em resposta à disbiose microbiana, afetando as estruturas de suporte moles e duras que envolvem os dentes e causando destruição do tecido gengival conjuntivo, perda óssea alveolar, mobilidade e perda dentária[2]. Assim, o fator etiológico primário da DP são micro-organismos periodontopatogênicos como *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia* e *Aggregatibacter actinomycetemcomitans*[3,4].

Como já mencionado, de acordo com Page e Kornman[5], a DP é iniciada e perpetuada por um grupo bacteriano específico. Apesar de essenciais, as bactérias sozinhas são insuficientes para que a doença ocorra. Atualmente é bem estabelecido que o dano tecidual gerado na DP é atribuído principalmente pela resposta do hospedeiro, e não pela ação bacteriana direta e de seus produtos[4-6]. A função primordial da cascata inflamatória é proteger o organismo contra agentes estranhos; porém, quando a resposta imune não é suficiente para eliminar os micro-organismos invasores, a persistência de mediadores inflamatórios pode levar a injúrias ao tecido, com consequente formação de bolsa periodontal, perda de tecido conjuntivo, destruição do ligamento periodontal e reabsorção de osso alveolar, culminando com a perda do elemento dentário[7]. Diversos fatores relacionados ao hospedeiro, como fatores ambientais (tabagismo), doenças sistêmicas e herança genética passaram a ser estudados para compreender como participam para o início e progressão da DP[5,8]. A influência genética pode explicar, em parte, porque a DP pode se manifestar de diferentes formas clínicas na população[9]. Em um clássico estudo com gêmeos monozigóticos e dizigóticos foi constatado que o fator genético tem forte contribuição para o acometimento da DP crônica, e estima-se que esta doença possui aproximadamente 50% de hereditariedade, mesmo após ajustes para variáveis comportamentais[10].

Atualmente a DP, assim como o Diabetes Mellitus (DM), tem sido considerada uma doença complexa ou multifatorial, em que fatores genéticos e ambientais exercem um papel essencial na patogênese[10]. Assim como a periodontite, a DM tem alto grau de herdabilidade, estimado em 72% por meio de metanálise de estudos em gêmeos[11]. Uma característica importante das doenças complexas, como a DM e a DP, é o fato de serem poligênicas, ou seja, inúmeros genes estão envolvidos na suscetibilidade e na severidade da doença, cada qual garantindo uma pequena contribuição.

O Diabetes Mellitus é uma doença metabólica que envolve primariamente os carboidratos, seguido dos lipídeos e proteínas, sendo caracterizado pela hiperglicemia resultante de defeitos na secreção de insulina, em sua ação ou em ambos. Como resultado direto da hiperglicemia e do desequilíbrio osmótico, uma tríade clínica clássica de sintomas é desenvolvida, e inclui polifagia, polidipsia e poliúria. O aparecimento da doença está relacionado a fatores de risco genéticos, ambientais e comportamentais[12]. A prevalência do diabetes mellitus aumenta em todo o mundo, sendo estimado que mais de 693 milhões de indivíduos sejam afetados até 2045[13]. Atualmente, os tipos mais comuns de DM são tipo 1 (DM1), em que as células beta do pâncreas são destruídas por autoimunidade, levando à uma deficiência absoluta de insulina, e o tipo 2 (DM2), onde há graus variados de diminuição de secreção e também da resistência à insulina[14].

A periodontite pode ser reconhecida como a sexta maior complicação associada ao diabetes[15], sendo que foi detectada maior extensão e severidade da doença periodontal em indivíduos portadores de diabetes do que naqueles não portadores da doença[16-18]. A inter-relação bi-direcional entre DP e DM tem sido estudada há vários anos[19, 20]. A premissa para tal inter-relação entre DP e DM é a presença de citocinas pró-inflamatórias, bactérias e toxinas na DP que são liberadas localmente no tecido periodontal e adentram na circulação sistêmica, influenciando tecidos e órgãos à distância. Ao mesmo tempo, as citocinas pró-inflamatórias sistemicamente envolvidas no DM infiltram-se nos tecidos periodontais e agravam a condição periodontal, resultando numa relação bidirecional[19, 21]. Assim, acredita-se que a associação entre a DP e o DM resulta de dois mecanismos distintos: 1) uma relação causal direta, na qual as complicações do DM agem como modificadores da expressão da DP; 2) ou que a presença de citocinas inflamatórias da DP possam levar a alterações no metabolismo da glicose e de lipídios[22].

Considerando-se o primeiro mecanismo, há evidências de que a prevalência e a severidade da DP são maiores em indivíduos com diabetes em comparação a não diabéticos[19, 21-24]. Foi constatado que pacientes com bom controle glicêmico são menos propensos à destruição periodontal[25]. Estudos têm demonstrado que os produtos finais da glicação avançada (AGEs), formada como resultado da hiperglicemia e da hiperlipidemia, podem alterar o fenótipo de tipos celulares específicos por meio de um receptor de alta afinidade da superfície celular (RAGE). Isso pode alterar as funções biológicas do tecido conjuntivo periodontal por meio de interações célula-matriz[26], além de transformar macrófagos e polimorfonucleares em células hiper-responsivas a bactérias periodontopatogênicas, o que leva a maior secreção de citocinas pró-inflamatórias como IL-1, IL-6 e TNF-α[27]. Em

concordância, segundo Kinane et al.[28] 2017 e Bartold et al.[29] 2007 o diabetes pode ser considerado mais um fator modificador da periodontite já existente do que propriamente um agente causador da doença.

O segundo mecanismo para associação entre a DP e o DM, ou seja, que a DP pode influenciar a condição sistêmica, foi demonstrado que a bacteremia e a endotoxemia associadas à DP podem elevar os níveis séricos de citocinas, como TNF-α, PGE2, IL-1 e IL-6, fomentando a periodontite. Sugere-se que o tecido conjuntivo periodontal inflamado atue como reservatório para esses mediadores, o que poderia acarretar em alterações no metabolismo da glicose e dos lipídios, induzindo à resistência insulínica[30].

Lembrando que o DM2 e DP são doenças multifatoriais, a carga genética pode atuar cumulativamente ou interagir com outros fatores de risco influenciando a ocorrência e a severidade de ambas as doenças. O metabolismo glicêmico sofre influência de polimorfismos em alguns genes que codificam enzimas importantes. Polimorfismos genéticos são formas variantes (alelos) de um lócus específico do cromossomo, que coexistem naturalmente na população humana. São portanto, variações normais do genoma humano, no qual o alelo mais raro ocorre com uma frequência maior que 1% na população. Os polimorfismos surgem como resultado de mutações, como inserções, deleções ou substituições de bases nucleotídicas, podendo gerar uma proteína não funcional ou alterar a expressão da referida proteína. O tipo de polimorfismo mais comumente relatado na literatura é o Polimorfismo de Nucleotídeo Único (*Single Nucleotide Polimorphism - SNP*), que consiste em uma variação da identidade de um nucleotídeo singular num sítio particular do genoma[31].

Os polimorfismos genéticos são muito úteis em estudos da área de Genética de Populações. As frequências dos genótipos e alelos podem variar entre grupos de pacientes doentes e de saudáveis. Estudos relatam que variações alélicas, como os polimorfismos em genes que codificam citocinas, podem afetar a suscetibilidade e a progressão da DP[7, 32-35]. O primeiro estudo demonstrando a associação entre DP e um polimorfismo genético, no caso no gene *IL1*, foi de Kornman et al.[36] em 1997. A partir de então, muitos estudos vêm sendo realizados com o objetivo de associar diversos polimorfismos em vários genes com a periodontite. Existem mais de 14 milhões de SNPs distribuídos no genoma que têm sido investigados em associação a doenças, mas apenas uma pequena parcela destes foi estudado em relação à DP[37]. Os primeiros estudos investigando a suscetibilidade genética à periodontite crônica na população Brasileira são de 2002, utilizando a técnica da Reação em Cadeia da Polimerase (PCR) aliada à de Polimorfismo de Comprimento de Fragmento de Restrição (RFLP), ou seja, a PCR-RFLP[38]. A partir de então, várias outras pesquisas têm sido realizadas

na população Brasileira a fim de identificar marcadores genéticos que possam estar associados com a suscetibilidade ou predisposição à DP[20, 39-41]. Nota-se até os dias atuais uma tendência mundial em identificar marcadores genéticos de suscetibilidade à DP, principalmente focando-se em genes relacionados ao sistema imunoinflamatório.

Em estudos de varredura ampla do genoma, ou seja (genome wide association study – GWAS) enfocando DM2 em mais de 4 mil pacientes europeus investigando simultaneamente milhares de SNPs, foi verificada associação dos polimorfismos E23K (rs5219) no gene *KCNJ11* e rs7903146 no gene *TCF7L2* com forte susceptibilidade ao DM2[42, 43]. Estudos enfocando genes do sistema imune foram realizados investigando a suscetibilidade genética à DP juntamente com DM2, em grupos amostrais pequenos, mas mesmo assim mostraram resultados interessantes. Em um estudo realizado em 100 pacientes com DM2, verificou-se que aqueles que apresentavam o genótipo C/T ou T/T para o polimorfismo +3954 no gene *IL1B* tinham um risco 3,8 vezes aumentado de desenvolver DP[23]. Outro polimorfismo em gene de citocina, no caso o gene *IL6*, foi associado ao DM, pois o genótipo GG do polimorfismo –174 foi um forte fator de suscetibilidade para DP entre os pacientes com DM1[44].

Como as frequências dos alelos polimórficos variam entre as diferentes etnias, resultados de associação de polimorfismos genéticos com suscetibilidade a doenças também costumam variar[45, 46]. Portanto, um determinado polimorfismo que tenha sido previamente associado à uma doença precisa ser validado na nossa população. No caso da possível relação com o DM, é necessário incluir a avaliação rigorosa do controle glicêmico (e também lipídico) à avaliação periodontal. Para que um polimorfismo possa ser efetivamente considerado um marcador genético, é necessário realizarem-se estudos com populações maiores e de diferentes etnias utilizando critérios clínicos padronizados. Somente isso poderá permitir comparações de resultados e efetivamente esclarecer se um determinado polimorfismo teria poder preditivo para uma doença em uma determinada população[20]. Subsequentemente, quando um determinado alelo está associado com a doença, estudos enfocando a genética funcional podem ser realizados para investigar a possível influência do referido polimorfismo na expressão do gene e seu papel na etiologia e patogênese da doença[47,48]. Afinal, um polimorfismo pode influenciar no nível de secreção de uma determinada proteína, provocando variações nas respostas imunológica e inflamatória individuais frente a uma agressão bacteriana[49].

Para otimizar a análise de vários SNPs, atualmente tem sido empregadas tecnologias de alto desempenho, como *microarrays*. A partir de 2009 surgiram as primeiras publicações

utilizando-se um protocolo baseando em uma tecnologia de alto desempenho e rendimento de genotipagem por PCR em tempo real, conhecido como Plataforma de Genotipagem TaqMan® OpenArray® da empresa Life Technologies[50]. Tal tecnologia atua por meio de um chip contendo sondas para detecção de alelos de diferentes SNPs, assim esse chip de genotipagem permite a análise simultânea de no mínimo 16 e máximo 256 SNPs. Uma das principais vantagens desse método é que o pesquisador escolhe os SNPs que deseja investigar, comunicando-se com a empresa que desenvolverá o chip de modo "customizado". O chip assemelha-se a uma lâmina que contém os ensaios em nano-cavidades, requerendo apenas a adição da amostra quantificada de DNA dos pacientes. A manipulação e distribuição das amostras no chip de OpenArray® é realizada por um pipetador automático, o que aumenta a precisão e diminui erros de pipetagem. Apesar de aparentemente mais caro, na verdade, em comparação a técnicas de genotipagem convencionais (onde cada SNP é investigado de cada vez), o uso da plataforma OpenArray® é mais econômica quando pretende-se analisar diversos polimorfismos[51]. Tal tecnologia está em processo de difusão, constando desde 2007, 40 trabalhos publicados empregando essa tecnologia. Em um estudo realizado em uma população brasileira, a plataforma de genotipagem OpenArray® provou ser altamente eficaz (taxa de precisão de 96,99%) para a genotipagem de SNPs suspeitos de estarem relacionados à perda auditiva[51]. O resultado de genotipagem com o OpenArray foi validado por meio de outras técnicas como sequenciamento direto, PCR multiplex e PCR-RFLP.

Apesar de existirem vários estudos enfocando a associação de polimorfismos genéticos com a Periodontite, revisões da literatura e mais recentemente, meta-análises, mais estudos ainda se fazem necessários, pois:(i) a maioria dos estudos investigaram SNPs em genes relacionados ao sistema imune, de modo que outros genes com funções biológicas importantes foram pouco ou nunca investigados; (ii) Polimorfismos em genes que codificam enzimas costumam influenciar o perfil metabólico do paciente, que pode relacionar-se à sua condição periodontal; e existem poucos estudos que investigaram a condição metabólica sistêmica do paciente em associação com a condição periodontal, principalmente relacionando-se à potencial influência da sua carga genética.

2 PROPOSIÇÃO

A proposição deste trabalho foi dividido em seções, apresentando a seguir a proposição geral e os objetivos específicos de acordo com cada uma das publicações.

2.1 Proposição Geral

O objetivo deste estudo foi investigar a suscetibilidade genética à periodontite na presença ou não de Diabetes Mellitus tipo 2 e prospectar a sua possível relação com o perfil glicêmico e lipídico do paciente, buscando-se assim, marcadores genéticos da periodontite potencialmente associados à comorbidades como o DM2 na população do sudeste do Brasil.

2.2 Proposições Específicas

Publicação 1- O objetivo deste estudo foi avaliar se os polimorfismos de nucleotídeo único (SNPs) nos genes *IL10*, *IL1A*, *IL1B*, *IL4*, *IL6*, *TNFA*, *OPG, RANK* e *RANKL*, previamente relacionados à periodontite, também podem estar associados ao DM2 e também à periodontite concomitante ao T2DM (P+T2DM).

Publicação 2- Avaliar 12 polimorfismos de nucleotídeo único (SNPs) (rs5219, rs5215, rs7957197, rs2943641, rs2062377, rs7754840, rs7018475, rs1531343, rs6712932, rs352140, rs187238, rs187238, rs387238, rs387238, rs387238 e rs3826782) previamente associados a DM2 ou à periodontite isoladamente a partir de estudos de ampla associação do genoma (GWAS) e de bioinformática como possíveis marcadores genéticos para a periodontite e/ou DM2.

3 PUBLICAÇÕES

A seção Publicações a seguir, foi dividia em duas subseções de acordo com o objetivo de cada uma das publicações.

3.1 Publicação 1*

Genetic polymorphisms in the *Interleukins IL1B, IL4* and *IL6* are associated with concomitant periodontitis and type 2 diabetes mellitus in Brazilian patients

Running title: *IL1B, IL4, IL6* polymorphisms in periodontitis and T2DM

Thamiris Cirelli [1,2], Rafael Nepomuceno[1,2], Ana Cláudia S Rios[2], Silvana R P Orrico[1,3], Joni A Cirelli[1], Letícia H Theodoro [4], Silvana P. Barros [5], Raquel M Scarel-Caminaga[2]

1. Department of Diagnosis and Surgery, São Paulo State University – UNESP, School of Dentistry at Araraquara, Araraquara, SP, Brazil
2. Department of Morphology, Genetics, Orthodontics and Pediatric Dentistry, São Paulo State University – UNESP, School of Dentistry at Araraquara, Araraquara, SP, Brazil
3. Advanced Research Center in Medicine, Union of the Colleges of the Great Lakes (UNILAGO), São José do Rio Preto, SP, 15030-070, Brazil
4. Department of Surgery and Integrated Clinic, São Paulo State University – UNESP, School of Dentistry at Araçatuba, Araçatuba, SP, Brazil
5. Department of Periodontology, University of North Carolina at Chapel Hill – UNC, School of Dentistry, Chapel Hill, NC, USA

Correspondence:

Raquel Mantuaneli Scarel-Caminaga, PhD

Department of Morphology, Genetics, Orthodontics and Pediatric Dentistry, School of Dentistry at Araraquara, UNESP – São Paulo State University, CP 331, CEP 14801-903, Araraquara, SP, Brazil, E-mail: raquel.caminaga@unesp.br

* Este artigo segue as normas do periódico em que está publicado “Journal of Periodontal Reasearch”.

This is the accepted version of the following article: Cirelli T, Nepomuceno R, Rios ACS, Orrico SRP, Cirelli JA, Theodoro LH, Barros SP, Scarel-Caminaga RM. Genetic polymorphisms in the Interleukins IL1B, IL4, and IL6 are associated with concomitant periodontitis and type 2 diabetes mellitus in Brazilian patients. J Periodontal Res. 2020 Jul 9. doi: 10.1111/jre.12784. Epub ahead of print. PMID: 32648256, which has been published in final form at [doi.org/10.1111/jre.12784].

Conflict of interest and source of funding

The authors have stated explicitly that there are no conflicts of interest in connection with this article. This study was supported by the Foundation for Research Support of State of São Paulo – FAPESP, Grants 2018/26367-1, 2016/08070-6, 2016/03753-8, 2016/18313-3 and 2014/13295-1 and National Council for Scientific and Technological Development (CNPq) Grants 445336/2014-5 and 304570/2017-6.

ABSTRACT

Objective: To assess whether single nucleotide polymorphisms (SNPs) in the *IL10*, *IL1A*, *IL1B*, *IL4*, *TNFA*, *IL6*, *OPG*, *RANK* and *RANKL* genes, "classically" related with periodontitis, could be associated with susceptibility to T2DM, and also with both diseases concomitantly.

Background: There are common pathogenic mechanisms in type 2 diabetes mellitus (T2DM) and periodontitis, but the knowledge of the genetic aspect of this is limited. In patients affected by concomitant T2DM and periodontitis, whose incidence is increasing, there is scarce information regarding the gene-phenotype association, including whether there are genes able to influence both diseases as comorbidities.

Methods: Periodontal clinical parameters and biochemical profile (Insulin, Fasting Glycemia, HbA1c, Triglycerides, Total Cholesterol, HDL-cholesterol, LDL-cholesterol) data were obtained from 894 individuals divided into three groups: Healthy (H; n=347), Periodontitis (P; n=348) and Periodontitis+T2DM (P+T2DM; n=199). DNA from oral epithelial cells was collected for genotyping. Associations between SNPs and pathologies were tested by multiple logistic regression models, adjusting for age, sex, and smoking habits. We also investigated whether there are sex or smoking effects of each SNP in these phenotypes.

Results: The rs1143634-GA (*IL1B)* SNP showed significantly less likely to develop P+T2DM for all population and mainly for women (adjusted OR=0.37, 95% CI=0.16 - 0.88), while women carrying the rs224320 CT (*IL4)* were more susceptible to develop P+T2DM (adjusted OR=1.81, 95% CI=1.04-3.15). Men carrying the rs1800795-CC (*IL6)* genotype were less likely to develop T2DM (adjusted OR=0.12, 95% CI=0.02 - 0.70, p=0.01).

Conclusions: Some SNPs in the *IL1B*, *IL4*, and *IL6* genes demonstrated sex-influenced association with concomitant periodontitis and T2DM, increasing the evidence of a common

genetic component between these diseases and contributing with the understanding of their common pathogenic mechanisms.

Keywords: Periodontal Diseases, Single Nucleotide Polymorphism, Interleukin-1beta, Interleukin-6, Interleukin-4

Introduction

Periodontitis is characterized as a host-mediated multifactorial immunoinflammatory disease that occurs in response to microbial dysbiosis, affecting the soft and the hard supporting structures encompassing the teeth and cause destruction of connective gingival tissue, alveolar bone loss, tooth mobility and tooth loss. [1] It is considered the main cause of tooth loss in adults, affecting approximately 10-15% of the world population. [2] The genetic makeup, environmental factors and the presence of systemic diseases play an essential role in the pathogenesis of periodontitis. [3] Diabetes mellitus is a metabolic disorders characterized by hyperglycemia caused by defects in insulin secretion, insulin action, or both. It constitutes a serious global public health burden with 371 million cases estimated worldwide in 2012 and is expected to rise to 642 million in 2040. [4] A more common form, type 2 diabetes mellitus (T2DM), is due to a combination of insulin resistance and impaired insulin secretion. In adults, about 90–95% of all diagnosed cases of diabetes correspond to T2DM, [5] and during the last two decades, periodontitis has been recognized as the sixth complication of T2DM. [6] As inflammation can promote insulin resistance and dysregulated glycemia [7,8] it was hypothesized that periodontitis might affect glycemic control in diabetic patients. [7] Diabetes and periodontitis are complex chronic diseases with an established bidirectional relationship. [8-10] Like periodontitis, T2DM is considered a multifactorial and complex disease; therefore, the host's genetic make up influence may explain, in part, why these diseases can manifest in different clinical forms in the population. [10]

An important characteristic of complex diseases, such as T2DM and periodontitis, is the fact that they are polygenic, that is, a large number of genes are involved in the susceptibility and severity of the disease, each guaranteeing a small contribution. The abundance of genetic variation in the human genome, mainly single nucleotide polymorphisms (SNP), has made them targets to explain differences in the risk of common diseases. [10,11] There has been extensive investigations regarding the potential genetic association with periodontitis. In the last decades, some polymorphisms mainly in genes related to the immune response and bone remodeling, have been preferentially chosen in many candidate gene studies. [12-14] Indeed, it is notable in the literature that those genes seemed to be like "classically periodontally

investigated polymorphisms", which can be evidenced by the development of many meta-analyses [15-18] in genes such as (i) (-819 C>T) in the *IL-10* gene, which encodes the important anti-inflammatory cytokine [19] ; (ii) (-889 G>A) in the *IL1A* gene, which encodes the IL1-1A cytokine facilitating and amplifying the inflammatory response by induction of adhesion molecules to cellular infiltrate and monocyte stimulation with bone resorption [15] ; (iii) (+3954 G>A) in the *IL1B* gene which plays an important role as a mediator of various inflammatory responses in periodontitis [20] ; (iv) (-590 C>T) in the *IL4* gene that encodes a cytokine involved in the process of inflammation [16] ; (v) (-308 G>A) in the *Tumor Necrosis Factor Alpha* (*TNFA)* gene which encodes a proinflammatory cytokine [21] ; (vi) (-174 C>G*)* in the *Interleukin-6* (*IL-6*) gene which encodes an important multifunctional cytokine. [22] Bone remodeling results from the balance between osteoblast and osteoclast activity. [23] In this context, the main molecular signaling pathway that has been studied regarding periodontitis involves the action of RANK (Receptor Activator of NF-Kappa-B), RANKL (Receptor Activator of NF-Kappa-B Ligand) and OPG (Osteoprotegerin) proteins, which are encoded by *TNFRSF11A*, *TNFSF11and TNFRSF11B* gene respectively.

The polygenic aspect of the T2DM as a multifactorial disease had been evidenced by studies showing the influence of many gene variants in the disease susceptibility. [11,24] Studies have suggested that there is a fraction of gene variants shared between T2DM and periodontitis. [7] However, this aspect requires a more broadly investigation, by amplifying the number of genes which pathways could influence both diseases. This additional knowledge will contribute significantly to better understand the common pathogenic mechanisms between T2DM and periodontitis.

Thus, considering some similarities in the etiopathogenesis of T2DM and periodontitis, including the fact that they are common diseases characterized by a potent inflammatory response to various stimuli [7] and the increasing incidence of patients concomitantly affected by T2DM and periodontitis, we hypothesize that polymorphisms "classically" investigated in periodontitis, and never or scarcely studied regarding T2DM, could be associated with susceptibility to T2DM, and also with both diseases as comorbidities.

Therefore, the objective of this study was to assess whether single nucleotide polymorphisms (SNPs) in the *IL10, IL1A, IL1B, IL4, IL6, TNFA, OPG, RANK,* and *RANKL* genes, previously investigated related with periodontitis could be also associated with susceptibility to T2DM, or concomitant periodontitis and T2DM (P+T2DM). That way we

could contribute with the gene-phenotype understanding of those diseases, and their common pathogenic mechanisms.

1. Material and Methods

1.1. Study subjects, sample collection, and DNA extraction

This study was performed in accordance with the Helsinki Declaration of 1975 (revised in 1983) and was approved by the Ethics in Human Research Committee of the Araraquara School of Dentistry (UNESP; Protocol number 57/04). All the patients signed written informed consent before any study-related procedures (APÊNDICE).

The individuals were recruited through random selection from the population of subjects treated from 2010 to 2018 in the Department of Periodontology, at School of Dentistry at Araraquara, São Paulo State University (UNESP), Brazil. The study participants were at least 30 years old and genetically unrelated. Participants were excluded from the study if they had chronic or systemic diseases (i.e. HIV infection or immunosuppressive chemotherapy) if they had received systemic antibiotics in the previous 3 months, if they received periodontal treatment in the previous 6 months, if they had less than 10 natural teeth (excluding third molars) or if they were pregnant or lactating. The sample size calculation was assessed by the G* Power Calculator, version 3.182, considering the parameters: logistic regression; two-tail, OR: 1.5; Pr (Y = 1 / X = 1) H0 = 0.2; alpha of 0.0056 (0.05/ 9 SNPs) and 80% power, R2=0. We found that each group should be composed of at least 150 patients to have sufficient power to attest to the association of the allele(s) with the diseased phenotype.

According to periodontal clinical examination and diagnosis of T2DM, the selected individuals were divided into three groups: (i) Healthy group (H) with 347 healthy or mild periodontitis individuals; (ii) Periodontitis group (P) with 348 patients diagnosed with moderate or severe periodontitis and the (iii) Periodontitis + T2DM group (P+T2DM) with 199 patients with moderate or severe periodontitis associated with the presence the T2DM.

History of smoking was defined according to Miyake et al., 2014 as follows: (i) never smoked or (ii) ever smoked (including patients those who are current smokers and former smokers). [25] Former smokers were patients that reported they had stopped smoking for at least one year. Regarding smoking status, patients were classified as smoker when they reported smoking on a daily basis for at least one year, and former smoker when they had stopped smoking for at least one year. [25]

The criteria for the definition of periodontitis were according to the CDC / AAP (Centers

for Disease Control and Prevention / American Academy of Periodontology). [26] All fully erupted teeth, except third molars and teeth with extensive destruction of the clinical crown were evaluated following clinical signs and parameters at four interproximal sites around each tooth: probing pocket depth (PPDi) and clinical attachment level (CALi), measured to the nearest millimeter by a UNC-15 periodontal probe. The presence of visible plaque, marginal bleeding, and bleeding on probing (BOP) were registered as a percentage of the total number of interproximal sites. According to the periodontal condition, the patients from the Periodontitis group were subsequently subdivided into Moderate periodontitis (P_M) and Severe periodontitis (P_S).

The study participants underwent a physical examination to assess: waist circumference (cm), waist/hip ratio, weight (kg) and height (m) to obtain the body mass index (BMI), in m/Kg^2 of each individual, and they were referred to the Clinical Analysis Laboratory to collect blood by venipuncture to perform the tests: Hemogram (ABX Micros 60), Insulin (chemiluminescence method), Fasting Glycemia (modified Bondar and Mead method) (Kit Labtest), HbA1c (turbidimetric inhibition immunoassay method) (Kit Roche), Triglycerides (enzymatic-Trinder method) (Kit Labtest), Total Cholesterol (enzymatic-Trinder method) (Kit Labtest) and HDL-cholesterol (High-density lipoprotein, enzymatic method-Labtest kit), the fractions of VLDL (Very Low-Density Lipoprotein) and LDL-cholesterol (Low-density lipoprotein) were calculated according to the Friedewald equation. [27]

The diagnosis of T2DM of each patient was obtained from their physician. The T2DM participants were divided according to their metabolic control well-controlled-T2DM whether the glycated hemoglobin (HbA1c) was until 7% (P+T2DM_well) and poorly controlled-T2DM whether the HbA1c ≥7.1% (P+T2DM_poor) [28,29] (American Diabetes Association).

The buccal epithelial samples were collected from a mouthwash of each patient with glucose 3% for DNA extraction. Genomic DNA was extracted from buccal mucosa epithelial cells from each patient, using the methods of salting-out. [30] The purity of genomic DNA was assessed on a microvolume UV spectrophotometer (NanoDrop® 2000, Thermo Scientific) and quantified on a fluorometer (Qubit® 2.0 fluorometer, Invitrogen). Samples were used whether they presented $A_{260/280}$ ratio between 1.7 and 2.0 and the concentration was adjusted to 50 ng/μL in T.E buffer (TrisHCl 10mM, EDTA 0,1 M, SDS 0,5% - pH 8.0).

1.2. Polymerase Chain Reaction (PCR) amplification and SNP Genotyping

The single-nucleotide polymorphisms (SNPs) in the *IL10*, *IL1A*, *IL1B*, *IL4*, *TNFA*, *IL6*,

OPG, *RANK* and *RANKL* genes (Table 1), selected for this study, were genotyped utilizing 2.5 μL of each DNA sample (total of 125 ng of genomic DNA) in each well of a 384-well plate in the presence of 2.5 μL of genotyping qPCR master mix (2X TaqMan®OpenArray® Genotyping Master Mix, Applied Biosystems), according to the manufacturer's recommendation. The reaction was performed in the presence of specific pairs of primers and TaqMan probe (Table 1) on a qPCR thermocycler (QuantStudio 12K Flex Real-Time PCR System, Applied Biosystems). The results were analyzed using online genotyping analysis software (Applied Biosystems™ Analysis Software, Genotyping Analysis Module, version 3.2), by an automated grouping of sequences deemed by the analysis software to have a similar High-Resolution Melt (HRM) curve shapes and temperature melting. The quality value of the genotype data point was determined by a threshold above 90%.

1.3. Statistical analysis

Demographic, periodontal clinical parameters and results of biochemical and physical examinations data were expressed as the mean (Standard Deviation) for numerical data and as frequency distributions for categorical data. Differences in the frequencies of demographic factors were tested using the ANOVA test with posttest Tukey's or chi-squared analysis using the software IBM® SPSS® Statistics (Version 20) and GraphPad Prism 5.01. The data from genotyping was analyzed on PLINK software [31] to detect differences in distributions for each SNP between the groups of patients, according to periodontal and T2DM status. The adjusted multiple logistic regression analysis was performed by STATA software, version 12.0 (Statistics/Data Analysis, Stata Corporation, College Station, Tex., USA).

We tested for Hardy–Weinberg equilibrium with the chi-squared goodness-of-fit for biallelic markers test. The association between genotypes and diseases was computed using logistic regression. A multivariate logistic regression analysis assuming the additive model adjusting for potential confounding factors, such as age, sex, and smoking status were used for testing associations between SNPs and the presence of the two diseases (periodontitis and T2DM): Control group *versus* Periodontitis group, Control group *versus* Periodontitis+T2DM group, Periodontitis group *versus* Periodontitis+T2DM group. The effect size was expressed as an odds ratio (OR) with a 95% confidence interval (CI). Experiment-wise significance (*p-value* = 0.005) was determined by Bonferroni correction based on the total number of markers genotyped of the study. Furthermore, the same analysis was stratified by sex to identify the sex-specific effects of all SNPs on periodontitis and T2DM, by the additive model using

multiple logistic regression adjusted by age and smoking. Similarly, to identify the effects of smoking habits with those SNPs, we stratified the population by smoking status and executed the multiple logistic regression adjusting by age and sex.

2. Results

Table 2 presents the demographic characteristics of the subjects. The statistical difference can be observed in the age distribution between the groups, with the Healthy group showing the lowest mean age (43.7). Also, there is a predominance of women and non-smoking subjects in all groups. Regarding the periodontal clinical parameters, it can be observed that the subjects in the control group have a larger number of teeth, less visible plaque index, as well as less probing bleeding rate. Regarding the periodontitis group, both groups presented clinical parameters compatible with the presence of the disease, and the group with T2DM presents clinical parameters of a more severe and advanced periodontal disease, with a higher percentage of sites with PPDi $\geq$ 5mm and lower interproximal clinical attachment level. Regarding the glycemic profile of subjects, we can state that subjects in the T2DM group have clinical parameters that prove the presence of the disease according to ADA (American Diabetes Association) criteria. [29] Besides, subjects in the periodontitis group have a worse condition compared to subjects in the control group. From the lipid profile, we can observe that the systemic condition of the subjects worsens according to the sum of the presence of the diseases, for example, subjects in the Control group have the lowest triglyceride level and the worst levels belongs to the T2DM + periodontitis group, which present both diseases (Table 2).

All SNPs presented a minor allele frequency (MAF) greater than 0.05, and the genotype distributions of the SNPs did not deviate from the Hardy – Weinberg equilibrium in the control subjects, as can be viewed in Table 3 for the *IL1B, IL4,* and *IL6* genes, and for the other investigated genes in the Supplementary Table 1.

In Table 4, the effect of each SNP in the periodontitis association was assessed by multiple logistic regressions, with adjustments for sex, age, and smoking habits as confounding factors. The rs1143634 SNP in the *IL1B* gene was compared between Healthy and P + T2DM and showed that subjects carrying the GA genotype were significantly less likely to develop T2DM together with periodontitis compared to GG subjects (adjusted OR 0.61, 95% CI: 0.38 - 0.98, p = 0.04). This fact was also confirmed by sex-specific analysis, stating that GA women were 0.37 less likely to develop both diseases (adjusted OR 0.37, 95%

CI: 0.16 - 0.88, p = 0.02). Continuing the sex-specific analyses, women with CT genotype for the rs224325 polymorphism in the *IL4* gene in the Healthy group, when compared to women with periodontitis and T2DM with CC genotype are more likely to develop both diseases (adjusted OR 1.81, 95% CI: 1.04-3.15,p= 0.03). For rs1800795 polymorphism in the *IL6* gene, men with CC genotype are less likely to develop T2DM and periodontitis when compared to GG men in the Healthy group (adjusted OR 0.12, 95% CI: 0.02 - 0.70, p = 0.01). This fact was also found among non-smoking subjects, but when comparing the heterozygous genotype (CG, adjusted OR 0.12, 95% CI: 0.02 - 0.70, p = 0.01) (Table 4). For the other investigated SNPs it was not found significant associations regarding the presence of the diseases (Supplementary Table 2).

Considering the interesting results obtained in Table 4, we sought to know whether the intensity of the diseased phenotype would reflect in stronger genetic findings. In Table 5 we demonstrated that not the degree of periodontitis (Moderate/ Severe) or the glycemic control of T2DM (well/ poorly controlled) could impact the genetic analyses.

Discussion

This study, as a strong aspect, presented a detailed periodontal clinical profile as well as the metabolic profile of patients. Based on these data, we observed that patients with both periodontitis and T2DM showed the worst periodontal clinical and metabolic condition in comparison with the control group or the patients with only periodontitis (Table 2). This finding reinforces the evidence of the association between T2DM and periodontitis. [7] Epidemiological studies and clinical trials had shown that diabetes patients with difficulties in controlling serum glucose levels are more likely to suffer from periodontitis, compared with those having sufficient metabolic control of diabetes and people without diabetes, while periodontitis affects the management of diabetes control. [7,32] Conversely, some studies failed to demonstrate the association between the degree of glycemic control and periodontitis. [33,34] Therefore, aiming to contribute with the present knowledge about the association of both diseases, this study was the first to demonstrate that SNPs previously related with periodontitis are also associated with susceptibility to T2DM and with the combination of both diseases. Some polymorphisms, such as *ADIPOQ* (rs1501299 and rs17300539), *LEPR*

(rs1137101 and rs1045895), *IRS2* (rs1805092), *GRB14* (rs10195252 and rs3923113) and *PPARG* (rs1801282) were associated with uncontrolled diabetes and overweight condition. [35] However, we were the first to investigate the selected SNPs studied here, the genetic load associated with glycemic control and degree of periodontitis.

It is well known that the IL-1ß is involved in the tissue destruction associated with periodontal disease due to its proinflammatory and bone-resorptive properties. It is shown to be overexpressed in the periodontal tissue and gingival crevicular fluid of affected subjects, where it appears to play a central role in the regulation of inflammatory genes, including other interleukins, chemokines, and adhesion molecules. [36,37] Because of the etiologic complexity of periodontitis, the role of IL-1ß continues to be investigated, [38] and clinical studies have been conducted to assess the impact of polymorphisms in the *IL1* genes also in association with the subgingival microbiota. [39,40] Carriers of the rare genotypes were more frequently affected with certain species, primarily those of the red and orange complex. [40] This interdependence was mainly observed with increasing pocket depth. [39] Schulz et. al, 2011 showed that a genetically dependent change in IL-1β expression could increase gingival inflammation and gingival crevicular fluid flow, in association with subgingival dysbiosis. [40]

This is the first study to investigate the potential association of SNPs classically associated with periodontitis, with concomitant T2DM and periodontitis (as comorbidities). In the literature, polymorphisms in the *IL1* gene are the most prominently investigated in periodontitis studies. Kormman et al. 1997 were the first to report the susceptibility of the rs114363 *IL1B* (+3954*)* SNP, and an analysis of a composite genotype, with the presence of periodontitis. [41] Since then, numerous following studies have shown a high variety of results, which permitted the development of some meta-analyses. [12,15] A meta-analysis performed in 2012 that had eligible ten studies with larger samples showed the OR=1.54 for the association between the rs1143634 *IL1B* SNP and periodontitis. [15] Another meta-analysis, evaluating 54 case-control studies, with a total of 4924 patients with chronic periodontitis and 4452 healthy controls, found that the minor allele of the rs1143634 was associated with periodontitis (OR=1.35), while the major allele was associated with the healthy individuals (OR = 0.74). [12] Linhartova et al 2019, investigated this same SNP in the *IL1B* gene in Czech T2DM with/without periodontitis, but no significant difference between the diseased and the healthy groups was found. [13] Our present study has not demonstrated an association between the rs1143634 with the periodontitis alone, but different from Linhartova et al 2019, [13] we observed significantly less likely to develop periodontitis in conjunction with T2DM in Brazilian studied population carrying the GA heterozygote genotype, mainly after the

stratification of population by sex (Table 4). Interestingly, GA individuals (heterozygotes for the rs1143634) produced higher levels of IL-1β in the gingival crevicular fluid, as reported by Lavu et al. 2017, but they did not notice any difference in IL-1β levels between major and minor allele carriers. [42] In agreement, a genotype-dependent influence on IL-1β secretion was also demonstrated by Pociot et al (1992) [43] and Santtila et al. (1998), which found that allele 2 of the IL-1B polymorphism was associated with decreased IL-1β secretion. [44] Even though, the association between the *IL1B* gene polymorphisms influencing the IL-1β cytokine levels is not fully clarified. [38] Therefore, we suggest that further investigation of the IL-1β levels regarding the rs1143634-GA, should be performed enrolling a larger population and also considering evaluation of sex influence. It can be noted that, even after decades of studies regarding this SNP and periodontitis in ethnic different populations, the literature still shows a high variability of results, probably associated with the experimental design, differences between of sample number, non-homogeneous or uncorrected samples for classical variables such as age, sex, smoking and race and geographical location dependence. There is also interest in the evaluation of the genetic risk to the periodontitis degree. A genome-wide association study did not show a significant association between the rs1143634 polymorphism and occurrence of moderate or severe chronic periodontitis, [45] similar to our findings showed in Table 5.

The interleukin-4 inhibits the persistence of macrophages in periodontitis lesions and down-regulates CD14, one of the major receptors for the periodontopathogen *Porphyromonas gingivalis* LPS and thus leads to decreased tissue destruction. [46] Michel et al. (2001) found that the *IL4* (-590 C>T) rs2243250 SNP was significantly associated with early-onset periodontitis in German patients, [47] and Gonzales et al. 2007 demonstrated an association between the *IL4* gene -590 T/T genotype and German patients with aggressive periodontitis. [48] However, no relationship was observed in that *IL4* gene polymorphism with chronic periodontitis in the Turkish, [49] Swedish Caucasian [50] and Iranian [51] populations. Anovazzi et al, 2010 showed that individuals carrying at least one T allele (for-590 SNP; adjusted OR=3.04, 95% CI=1.66-5.54) were more susceptible to periodontitis, even after adjusting for age, gender, smoking status, and polymorphism covariates for the Brazilian population. [14] A meta-analysis study with 37 studies involving 4,385 patients with periodontitis and 5,168 controls individuals showed no association between the isolated rs2243250 SNP with periodontitis. [16] Also, meta-analyses have limitations, the most relevant is the high heterogeneity among the studies included, mainly regarded to variability in the selected clinical parameters and the ethnically different populations. When considering that SNP of

part of a haplotype, Anovazzi et al, 2010 identified a haplotype in the *IL4* gene associated with lower risk to periodontitis. In a subsequent study, that named *IL4* protection haplotype was associated with high-production of IL-4 mRNA and protein levels and higher levels of anti-inflammatory cytokines, while the susceptible haplotype produced higher levels of pro-inflammatory cytokines [52]. Interestingly, subsequent studies demonstrated that the IL-4-susceptible haplotype was associated with the high levels of periodontopathogens that could, in a synergistic way, contribute to the periodontium infection and development of periodontitis in subjects carrying this haplotype. [53,54] Other studies have investigated the role of *IL4* gene polymorphisms in the development of periodontitis and indicated that cytokine gene expression is influenced by genetic polymorphisms and these variations appear to be linked with the progression of disease. [55] In particular, polymorphisms in the *IL4* gene may have a significant impact on the host response. In human beings, the *IL4* gene maps to the long arm of chromosome 5 (q31–33) within a cluster of other cytokine genes, including *IL5*, *IL12*, and *IL13*. [56] IL-4 has been shown to stimulate B-cell proliferation, to regulate immunoglobulin class switching (IgG1 and IgE), and to promote T-cell development. [57] Thus, genetic polymorphisms in the *IL4* gene may increase periodontal disease severity by altering IL-4 levels. Indeed, polymorphisms in the *IL4* gene have been implicated in the regulation of IL-4 production, and studies suggest that the 590 C/T*T allele, which is in tight linkage disequilibrium with the 33 C/T*T allele, is associated with increased IL-4 expression. [58] IL-4 is a Th2-derived, pleomorphic cytokine with anti-inflammatory properties [59] recognized as a modulator of macrophage function that downregulates CD14 levels [60] and inhibits the secretion of PGE2, IL-1, IL-6, and tumor necrosis factor (TNF). [61] Indeed, human studies have linked reduced gingival and serum IL-4 levels with periodontal disease [62] and resorption of alveolar bone [47] and IL4 mRNA levels were also reported to be significantly repressed in diseased compared with healthy peri-implant tissues. [63]

The present study did not show an association of this SNP with the occurrence of isolated periodontitis, but with the development of periodontitis together with T2DM (P+T2DM) in female patients carrying the rs2243250-CT. To our knowledge, this is the first study to report a sexually dimorphic potential role of an SNP in the *IL4* gene with susceptibility to concomitant periodontitis and T2DM. Interestingly, the relationship between the *IL4* (-590) rs2243250 SNP and the susceptibility to T2DM had been showing in different populations, such as Egyptians that showed positive associations with T2DM in the dominant, codominant, and overdominant models of inheritance. [64] In contrast, T2DM caucasian patients showed after logistic regression analysis adjusted for gender, age, diabetes duration, and glycated

hemoglobin, no association between the rs2243250 and the risk for Diabetic nephropathy (OR= 1.06; 95% CI= 0.37-3.05; p = 0.9). [65]

The SNP rs1800795 (-174 C>G) in the *IL6* gene is associated with susceptibility to chronic periodontitis in the German [66] and Brazilian [67] populations. Increased expression of IL-6 has been associated with periodontal disease severity in Brazilian individuals. [67] According to Costa et al. 2010, elderly Brazilian women carrying the GG genotype were most severely affected by chronic periodontitis. [68] This polymorphism was also associated with increased odds of detecting *A. actinomycetemcomitans*, *P. gingivalis and T. forsythensis* in patients with aggressive periodontitis, after adjustment for age, ethnicity, smoking and periodontitis extent, supporting the hypothesis that complex interactions between microbiota and host genome may be at the basis of susceptibility to that disease. [69] Regarding the *IL6* (-174 C>G) SNP, the G allele is associated with elevated IL-6 protein expression by increasing its production. Likewise, the *IL6* (−597 G>A) polymorphism also elevated the IL-6 production and increased the inflammatory response. [67] Nibali et al., found an association of *IL6* polymorphisms with periodontitis progression in the Caucasian population. [69] However, studies enrolling the Asiatic population there are conflicting results. [70] The IL-6 is an important modulator of the inflammatory response in periodontal tissues that could also activate osteoclasts and induce differentiation of B-cells acting synergistically with other factors like IL-1 and TNF-α. [71] In comparison to the control group, a higher level of IL-6 was detected in the crevicular fluid of the periodontal disease group; specifically, IL-6 was significantly higher in aggressive and chronic periodontitis patients. [61] Increased expression was found in gingivitis, but the difference was not statistically significant. [61] Despite it was demonstrated in a British population that the C allele of rs1800795 SNP was associated with lower levels of IL-6, [72] Moreira et al., 2006 showed in Brazilians a higher expression of IL-6 correlated with clinical attachment loss and the rs1800795 CC genotype, which was more frequent in the control group. [67] Conversely, in a German population, the CC genotype was associated with an elevated risk of developing periodontitis. [66] The rs1800795 SNP tends to be quite polymorphic in Caucasians, while Asian and African populations are almost monomorphic for the G allele. Interestingly, the severity of gingivitis resulted associated with polymorphisms of the *IL6* gene in a Russian male population. [73] These data appear to indicate that the rs1800795 in the *IL6* promoter could modulate the IL-6 expression in periodontal tissues and carriers of the variant allele could be protected from periodontal diseases, including both gingivitis and chronic periodontitis. In a meta-analysis including 18 case-control studies comprising 1,616 periodontitis cases and 1,511 controls suggested that the

rs1800795 SNP in the *IL6* gene may be associated a protection against to periodontitis, since the CC vs. GG inheritance model reduced the risk of general and overall periodontitis by 60% (95% CI = 0.25-0.65, p < 0.01) and 31% (95% CI = 0.38-0.97, p = 0.04) respectively. [17] In another meta-analysis, for Brazilian and for Caucasian populations, the GG genotype played as a risk factor to periodontitis. [18] In addition, the rs1800795 SNP was already associated with T2DM, [74,75] besides obesity [76] and lots of types of cancer. [77,78] Here, our analysis of the rs1800795 SNP (*IL6* gene) showed no significant results for the susceptibility to periodontitis, but when focusing the association of both T2DM and periodontitis, we found that the men with CC genotype were 88% less prone to develop P + T2DM, with a true effect on the population between 98% and 30% (adjusted OR= 0.12, 95% CI= 0.02 - 0.70, p = 0.01).

In this study we firstly demonstrated a sex-influenced association of the rs1143634 *(IL1B)*, rs2243250 (*IL4)* and rs1800795 (*IL6)* SNPs, previously associated with periodontitis, with concomitant periodontitis and T2DM. Case-control studies [79], as well *in vitro* [80] and *in vivo* [81] studies, indicate the influence of steroid hormones on inflammation, immune response and bone metabolism, demonstrating the existence of an association of periodontitis with sex hormone levels.

Different studies have shown that for every inflammatory disease, a large number of different common susceptibility alleles have been identified. [24,45] This observation, together with highly variable disease phenotypes between close relatives, despite high genetic heritability, shaped the current hypothesis proposing that the specific disease phenotype can be attributed to the effects of combinations of many genetic risk alleles and their interactions with internal and external factors. [10] Periodontitis and T2DM are recognized complex diseases, whose cause cannot be attributed to a specific genetic variability, but rather to a set of genetic variants or epigenetic influence, which in association with habits and environment, can influence the onset and progression of the disease. [10,11]

This study has some limitations. Despite the literature-based SNPs being chosen, it is known that the genes investigated here have a variety of other SNPs, and it is interesting to investigate whether other polymorphisms and their possible haplotypes are relevant in individuals with periodontitis and T2DM. Moreover, because there is a high prevalence of periodontitis in patients with T2DM and we were interested in evaluating these diseases associations, we have not investigated individuals solely affected by T2DM. In addition, we decided not to apply the Bonferroni correction (BC) to our results since it would reduce the α-value to 0.0056 (0.05/9 SNP), and could prevent the identification of discrete biological

effects. The use of BC would increase the type II error, [82] which would lead us to reject a hypothesis of a true biological association. True associations may be lost once a correction for multiple testing is implemented. [83] We also acknowledge that additional studies are necessary to investigate the functional role of these SNPs in the periodontal disease and the T2DM context and to verify the possible influence of sex in the transcriptional or translational levels of the encoded proteins.

In spite of these limitations, our study presents relevant aspects, such as all patients and controls were selected from the same geographical area (São Paulo State, Southeast of Brazil) and were of a similar socioeconomic status, besides the mentioned complete periodontal exams and biochemical data of the selected patients. Importantly, we concluded that there was the sex-influenced association of the three SNPs rs1143634 (*IL1B* gene), rs2243250 (*IL4* gene) and rs1800795 (*IL6* gene) with the presence of periodontitis together with T2DM, as comorbidities, in a Brazilian population.

References

1. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Clin Periodontol.* 2018;45 Suppl 20:S149-S161.
2. Frencken JE, Sharma P, Stenhouse L, Green D, Laverty D, Dietrich T. Global epidemiology of dental caries and severe periodontitis - a comprehensive review. *J Clin Periodontol.* 2017;44 Suppl 18:S94-S105.
3. Michalowicz BS, Diehl SR, Gunsolley JC, et al. Evidence of a substantial genetic basis for risk of adult periodontitis. *Journal of periodontology*. 2000;71(11):1699-1707.
4. Ogurtsova K, da Rocha Fernandes JD, Huang Y, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes research and clinical practice.* 2017;128:40-50.
5. Control CfD, Prevention. National diabetes statistics report, 2017. *Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services.* 2017.
6. Loe H. Periodontal disease. The sixth complication of diabetes mellitus. *Diabetes care.* 1993;16(1):329-334.
7. Polak D, Shapira L. An update on the evidence for pathogenic mechanisms that may link periodontitis and diabetes. *J Clin Periodontol.* 2018;45(2):150-166.
8. Baeza M, Morales A, Cisterna C, et al. Effect of periodontal treatment in patients with periodontitis and diabetes: systematic review and meta-analysis. *Journal of applied oral science : revista FOB.* 2020;28:e20190248.
9. Nelson RG, Shlossman M, Budding LM, et al. Periodontal disease and NIDDM in Pima Indians. *Diabetes care.* 1990;13(8):836-840.
10. Schaefer AS. Genetics of periodontitis: Discovery, biology, and clinical impact. *Periodontology 2000.* 2018;78(1):162-173.
11. Kaul N, Ali S. Genes, Genetics, and Environment in Type 2 Diabetes: Implication in Personalized Medicine. *DNA and cell biology.* 2016;35(1):1-12.

12. da Silva FRP, Vasconcelos A, de Carvalho Franca LF, Di Lenardo D, Nascimento HMS, Vasconcelos DFP. Association between the rs1143634 polymorphism in interleukin-1B and chronic periodontitis: Results from a meta-analysis composed by 54 case/control studies. *Gene.* 2018;668:97-106.
13. Borilova Linhartova P, Poskerova H, Tomandlova M, et al. Interleukin-1 Gene Variability and Plasma Levels in Czech Patients with Chronic Periodontitis and Diabetes Mellitus. *International journal of dentistry.* 2019;2019:6802349.
14. Anovazzi G, Kim YJ, Viana AC, et al. Polymorphisms and haplotypes in the interleukin-4 gene are associated with chronic periodontitis in a Brazilian population. *Journal of periodontology.* 2010;81(3):392-402.
15. Karimbux NY, Saraiya VM, Elangovan S, et al. Interleukin-1 gene polymorphisms and chronic periodontitis in adult whites: a systematic review and meta-analysis. *Journal of periodontology.* 2012;83(11):1407-1419.
16. Jia XW, Yuan YD, Yao ZX, et al. Association between IL-4 and IL-4R Polymorphisms and Periodontitis: A Meta-Analysis. *Disease markers.* 2017;2017:8021279.
17. Zhao B, Li R. The association between periodontitis and interleukin-6 genetic polymorphism -174 G/C: A meta-analysis. *Arch Oral Biol.* 2018;96:13-20.
18. Zhu J, Guo B, Fu M, et al. Interleukin-6-174G/C Polymorphism Contributes to Periodontitis Susceptibility: An Updated Meta-Analysis of 21 Case-Control Studies. *Disease markers.* 2016;2016:9612421.
19. de Waal MR, Yssel H, Roncarolo M, Spits H, De Vries J. Interleukin-10. *Current opinion in Immunology.* 1992;4(3):314.
20. Hong SJ, Kang SW, Kim SK, Kim YS, Ban JY. Lack of Association between Interleukin-1beta Gene Polymorphism (rs16944) and Chronic Periodontitis: From a Case-Control Studies to an Updated Meta-Analysis. *Disease markers.* 2018;2018:8287026.
21. Javed F, Al-Hezaimi K, Salameh Z, Almas K, Romanos GE. Proinflammatory cytokines in the crevicular fluid of patients with peri-implantitis. *Cytokine.* 2011;53(1):8-12.
22. Fonseca JE, Santos MJ, Canhao H, Choy E. Interleukin-6 as a key player in systemic inflammation and joint destruction. *Autoimmunity reviews.* 2009;8(7):538-542.
23. Silva I, Branco JC. Rank/Rankl/opg: literature review. *Acta reumatologica portuguesa.* 2011;36(3):209-218.
24. Diabetes Genetics Initiative of Broad Institute of H, Mit LU, Novartis Institutes of BioMedical R, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science.* 2007;316(5829):1331-1336.
25. Miyake Y, Tanaka K, Arakawa M. IL3 rs40401 polymorphism and interaction with smoking in risk of asthma in Japanese women: the Kyushu Okinawa Maternal and Child Health study. *Scandinavian journal of immunology.* 2014;79(6):410-414.
26. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. *Journal of periodontology.* 2007;78(7 Suppl):1387-1399.
27. Nordestgaard BG, Langsted A, Mora S, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points—a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. *European heart journal.* 2016;37(25):1944-1958.
28. American Diabetes A. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. *Diabetes care.* 2019;42(Suppl 1):S13-S28.

29. American Diabetes A. 6. Glycemic Targets: Standards of Medical Care in Diabetes-2019. *Diabetes care.* 2019;42(Suppl 1):S61-S70.
30. Aidar M, Line SR. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. *Brazilian dental journal.* 2007;18(2):148-152.
31. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81(3):559-575.
32. Preshaw PM, Alba AL, Herrera D, et al. Periodontitis and diabetes: a two-way relationship. *Diabetologia.* 2012;55(1):21-31.
33. Zielinski MB, Fedele D, Forman LJ, Pomerantz SC. Oral health in the elderly with non-insulin-dependent diabetes mellitus. *Special care in dentistry : official publication of the American Association of Hospital Dentists, the Academy of Dentistry for the Handicapped, and the American Society for Geriatric Dentistry.* 2002;22(3):94-98.
34. Sastrowijoto S, Van der Velden U, Van Steenbergen T, et al. Improved metabolic control, clinical periodontal status and subgingival microbiology in insulin-dependent diabetes mellitus: A prospective study. *Journal of Clinical Periodontology.* 1990;17(4):233-242.
35. Kasim NB, Huri HZ, Vethakkan SR, Ibrahim L, Abdullah BM. Genetic polymorphisms associated with overweight and obesity in uncontrolled Type 2 diabetes mellitus. *Biomarkers in medicine.* 2016;10(4):403-415.
36. Mastrangelo F, Dedola A, Cattoni F, et al. Etiological periodontal treatment with and without low-level laser therapy on IL-1beta level in gingival crevicular fluid: an in vivo multicentric pilot study. *Journal of biological regulators and homeostatic agents.* 2018;32(2):425-431.
37. Ozel N, Aksoy A, Kirzioglu FY, Doguc DK, Aksoy TA. Evaluation of interleukin-1beta level and oxidative status in gingival crevicular fluid during rapid maxillary expansion. *Arch Oral Biol.* 2018;90:74-79.
38. Boukortt KN, Saidi-Ouahrani N, Boukerzaza B, et al. Association analysis of the IL-1 gene cluster polymorphisms with aggressive and chronic periodontitis in the Algerian population. *Arch Oral Biol.* 2015;60(10):1463-1470.
39. Socransky SS, Haffajee AD, Smith C, Duff GW. Microbiological parameters associated with IL-1 gene polymorphisms in periodontitis patients. *J Clin Periodontol.* 2000;27(11):810-818.
40. Schulz S, Stein JM, Altermann W, et al. Single nucleotide polymorphisms in interleukin-1gene cluster and subgingival colonization with Aggregatibacter actinomycetemcomitans in patients with aggressive periodontitis. *Human immunology.* 2011;72(10):940-946.
41. Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol.* 1997;24(1):72-77.
42. Lavu V, Venkatesan V, Venugopal P, et al. Clinical Relevance of Cytokines Gene Polymorphisms and Protein Levels in Gingival Cervical Fluid from Chronic Periodontitis Patients. *Iranian journal of immunology : IJI.* 2017;14(1):51-58.
43. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. *European journal of clinical investigation.* 1992;22(6):396-402.
44. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. *Scandinavian journal of immunology.* 1998;47(3):195-198.

45. Divaris K, Monda KL, North KE, et al. Exploring the genetic basis of chronic periodontitis: a genome-wide association study. *Hum Mol Genet.* 2013;22(11):2312-2324.
46. Lauener RP, Goyert SM, Geha RS, Vercelli D. Interleukin 4 down-regulates the expression of CD14 in normal human monocytes. *European journal of immunology.* 1990;20(11):2375-2381.
47. Michel J, Gonzales JR, Wunderlich D, Diete A, Herrmann JM, Meyle J. Interleukin-4 polymorphisms in early onset periodontitis. *J Clin Periodontol.* 2001;28(5):483-488.
48. Gonzales JR, Mann M, Stelzig J, Bodeker RH, Meyle J. Single-nucleotide polymorphisms in the IL-4 and IL-13 promoter region in aggressive periodontitis. *J Clin Periodontol.* 2007;34(6):473-479.
49. Kara N, Keles GC, Sumer P, et al. Association of the polymorphisms in promoter and intron regions of the interleukin-4 gene with chronic periodontitis in a Turkish population. *Acta odontologica Scandinavica.* 2007;65(5):292-297.
50. Donati M, Berglundh T, Hytonen AM, Hahn-Zoric M, Hanson LA, Padyukov L. Association of the -159 CD14 gene polymorphism and lack of association of the -308 TNFA and Q551R IL-4RA polymorphisms with severe chronic periodontitis in Swedish Caucasians. *J Clin Periodontol.* 2005;32(5):474-479.
51. Hooshmand B, Hajilooi M, Rafiei A, Mani-Kashani KH, Ghasemi R. Interleukin-4 (C-590T) and interferon-gamma (G5644A) gene polymorphisms in patients with periodontitis. *J Periodontal Res.* 2008;43(1):111-115.
52. Anovazzi G, Medeiros MC, Pigossi SC, et al. Functional Haplotypes in Interleukin 4 Gene Associated with Periodontitis. *PloS one.* 2017;12(1):e0169870.
53. Finoti LS, Anovazzi G, Pigossi SC, et al. Periodontopathogens levels and clinical response to periodontal therapy in individuals with the interleukin-4 haplotype associated with susceptibility to chronic periodontitis. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology.* 2013;32(12):1501-1509.
54. Cirelli T, Finoti LS, Corbi SCT, et al. Absolute quantification of Aggregatibacter actinomycetemcomitans in patients carrying haplotypes associated with susceptibility to chronic periodontitis: multifaceted evaluation with periodontitis covariants. *Pathogens and disease.* 2017;75(7).
55. Nares S. The genetic relationship to periodontal disease. *Periodontology 2000.* 2003;32:36-49.
56. Marsh DG, Neely JD, Breazeale DR, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science.* 1994;264(5162):1152-1156.
57. Salgame P, Abrams JS, Clayberger C, et al. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. *Science.* 1991;254(5029):279-282.
58. Rosenwasser LJ, Borish L. Genetics of atopy and asthma: the rationale behind promoter-based candidate gene studies (IL-4 and IL-10). *American journal of respiratory and critical care medicine.* 1997;156(4 Pt 2):S152-155.
59. Shapira L, van Dyke TE, Hart TC. A localized absence of interleukin-4 triggers periodontal disease activity: a novel hypothesis. *Medical hypotheses.* 1992;39(4):319-322.
60. Lauener RP, Goyert SM, Geha RS, Vercelli D. Interleukin 4 down-regulates the expression of CD14 in normal human monocytes. *Eur J Immunol.* 1990;20(11):2375-2381.

61. Corcoran ML, Stetler-Stevenson WG, Brown PD, Wahl LM. Interleukin 4 inhibition of prostaglandin E2 synthesis blocks interstitial collagenase and 92-kDa type IV collagenase/gelatinase production by human monocytes. *J Biol Chem.* 1992;267(1):515-519.
62. Buhlin K, Hultin M, Norderyd O, et al. Risk factors for atherosclerosis in cases with severe periodontitis. *J Clin Periodontol.* 2009;36(7):541-549.
63. Duarte PM, de Mendonca AC, Maximo MB, Santos VR, Bastos MF, Nociti Junior FH. Differential cytokine expressions affect the severity of peri-implant disease. *Clinical oral implants research.* 2009;20(5):514-520.
64. Alsaid A, El-Missiry M, Hatata el S, Tarabay M, Settin A. Association of IL-4-590 C>T and IL-13-1112 C>T gene polymorphisms with the susceptibility to type 2 diabetes mellitus. *Disease markers.* 2013;35(4):243-247.
65. Zavrsnik M, Letonja J, Makuc J, Seruga M, Cilensek I, Petrovic D. Interleukin-4 (IL4) -590C/T (rs2243250) gene polymorphism is not associated with diabetic nephropathy (DN) in Caucasians with type 2 diabetes mellitus (T2DM). *Bosnian journal of basic medical sciences.* 2018;18(4):347-351.
66. Babel N, Cherepnev G, Babel D, et al. Analysis of tumor necrosis factor-alpha, transforming growth factor-beta, interleukin-10, IL-6, and interferon-gamma gene polymorphisms in patients with chronic periodontitis. *Journal of periodontology.* 2006;77(12):1978-1983.
67. Moreira PR, Lima PM, Sathler KO, et al. Interleukin-6 expression and gene polymorphism are associated with severity of periodontal disease in a sample of Brazilian individuals. *Clinical and experimental immunology.* 2007;148(1):119-126.
68. Costa AM, Guimaraes MC, de Souza ER, Nobrega OT, Bezerra AC. Interleukin-6 (G-174C) and tumour necrosis factor-alpha (G-308A) gene polymorphisms in geriatric patients with chronic periodontitis. *Gerodontology.* 2010;27(1):70-75.
69. Nibali L, D'Aiuto F, Donos N, et al. Association between periodontitis and common variants in the promoter of the interleukin-6 gene. *Cytokine.* 2009;45(1):50-54.
70. Komatsu Y, Tai H, Galicia JC, et al. Interleukin-6 (IL-6)--373 A9T11 allele is associated with reduced susceptibility to chronic periodontitis in Japanese subjects and decreased serum IL-6 level. *Tissue antigens.* 2005;65(1):110-114.
71. Irwin CR, Myrillas TT. The role of IL-6 in the pathogenesis of periodontal disease. *Oral diseases.* 1998;4(1):43-47.
72. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest.* 1998;102(7):1369-1376.
73. Foster CB, Lehrnbecher T, Samuels S, et al. An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. *Blood.* 2000;96(7):2562-2567.
74. Saxena M, Agrawal CG, Srivastava N, Banerjee M. Interleukin-6 (IL-6)-597 A/G (rs1800797) & -174 G/C (rs1800795) gene polymorphisms in type 2 diabetes. *The Indian journal of medical research.* 2014;140(1):60-68.
75. Plataki MN, Zervou MI, Samonis G, Daraki V, Goulielmos GN, Kofteridis DP. Association of the Interleukin-6 rs1800795 Polymorphism with Type 2 Diabetes Mellitus in the Population of the Island of Crete, Greece. *Genet Test Mol Biomarkers.* 2018;22(7):448-452.
76. Gholami M, Sharifi F, Shahriari S, Khoshnevisan K, Larijani B, Amoli MM. Association of interleukin-6 polymorphisms with obesity: A systematic review and meta-analysis. *Cytokine.* 2019;123:154769.

77. Marangon AV, Colli CM, Cardozo DM, et al. Impact of SNPs/Haplotypes of IL10 and IFNG on the Development of Diffuse Large B-Cell Lymphoma. *Journal of immunology research.* 2019;2019:2137538.
78. Banescu C, Tripon F, Trifa AP, et al. Cytokine rs361525, rs1800750, rs1800629, rs1800896, rs1800872, rs1800795, rs1800470, and rs2430561 SNPs in relation with prognostic factors in acute myeloid leukemia. *Cancer medicine.* 2019;8(12):5492-5506.
79. Meisel P, Krause T, Cascorbi I, et al. Gender and smoking-related risk reduction of periodontal disease with variant myeloperoxidase alleles. *Genes and immunity.* 2002;3(2):102-106.
80. Lee DJ, Wu L, Shimono M, et al. Differential Mechanism of Periodontitis Progression in Postmenopause. *Frontiers in physiology.* 2018;9:1098.
81. Steffens JP, Wang X, Starr JR, Spolidorio LC, Van Dyke TE, Kantarci A. Associations Between Sex Hormone Levels and Periodontitis in Men: Results From NHANES III. *Journal of periodontology.* 2015;86(10):1116-1125.
82. Vieira AR, Bayram M, Seymen F, Sencak RC, Lippert F, Modesto A. In Vitro Acid-Mediated Initial Dental Enamel Loss Is Associated with Genetic Variants Previously Linked to Caries Experience. *Frontiers in physiology.* 2017;8:104.
83. Vieira AR, McHenry TG, Daack-Hirsch S, Murray JC, Marazita ML. Candidate gene/loci studies in cleft lip/palate and dental anomalies finds novel susceptibility genes for clefts. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2008;10(9):668-674.

Table 1. Information regarding the nine genotyped polymorphisms

SNP	Assay ID	Position†, Alleles and type of SNP	Gene (SNP)	MAF‡		Call rate (%)§
rs1800871	C___1747362_10	chr1:g. 206946634, A>G *Intragenic, Transition Substitution*	***IL10* (-819)**	**A|A**	0.34	96.78
rs1800587	C___9546481_20	chr2:g. 113542960, G>A *UTR 5, Intragenic, Transition Substitution*	***IL1A (-889)***	**A|A**	0.28	94.97
rs1143634	C___9546517_10	chr2:g. 113590390, G>A *Intragenic, Transition Substitution, Silent Mutation*	***IL1B* (+3954)**	**A|A**	0.19	96.78
rs2243250	C__16176216_10	chr5:g.132009154, C>T, *Intragenic, Transition Substitution*	***IL4 (-590*)**	**T|T**	0.29	97.02
rs1800629	C___7514879_10	chr6:g.31543031, G>A *Intragenic, Transition Substitution*	***TNFA (-308*)**	**A|A**	0.11	96.36
rs1800795	C___1839697_20	chr7:g.22766645, C>G *Intragenic, Transversion Substitution*	***IL6 (-174)***	**C|C**	0.25	97.20
rs2062377	C___1970989_10	chr8:g.120007420, T>A *Intron, Intragenic, Transversion Substitution*	***TNFRSF11B (alias OPG)***	**T|T**	0.37	92.40
rs9533090	C___1444411_10	chr13:g. 42951449, C>T *Intergenic, Transition Substitution*	***upstream TNFSF11*(alias *RANKL)***	**T|T**	0.38	89.23
rs884205	C__30370382_10	chr18:g. 60054857, A>C *UTR 3, Intragenic, Transversion Substitution*	***TNFRSF11A* (alias *RANK*)**	**A|A**	0.17	89.47

† Genome Reference Consortium Human Build 37 (GRCh37.p13) or hg19; ‡ MAF: Minor Allele Frequencie this study;
§ Represents the amount of genotyping performed with success (all subjects), in percentage, for OpenArray™ plate and assays

Table 2. Comparison of demographic characteristics, periodontal parameters, biochemical and physical data among the groups

Demographic Characteristics	Healthy n=347	Periodontitis n=348	Periodontitis+T2DM n=199
Age - Mean (±SD)	43.7 (±11.0)[a]	49.1 (±9.3)[b]	56.3 (±9.4)[c]
Sex (%), Mean (±SD)			
Males	114 (32.8) [a]	142 (40.8) [a]	82 (41.2) [a]
Females	233 (67.2) [a]	206 (59.2) [a]	117 (58.8) [a]
Smoking habits (%), Mean (±SD)			
Never	270 (77.8) [a]	223 (64.4) [a]	111 (55.8) [a]
Ever	77 (22.2) [a]	124 (35.6) [a]	78 (39.2) [a]
Periodontal Parameters - Mean (±SD**)**			
Number of teeth	25.5 (3.4)[a]	22.8 (4.5)[b]	20.4 (5.5)[c]
Visible plaque (% of sites)	24.7 (20.2)[a]	45.9 (28.0)[b]	56.5 (26.9)[c]
Marginal bleeding (% of sites)	8.9 (11.9) [a]	20.1 (23.3) [b]	40.4 (24.1)[c]
BOP (% of sites)	3.4 (5.2)[a]	40.2 (23.2)[b]	50.4 (27.3)[c]
PPDi ≤ 4mm (% of sites)	99.9 (0.3)[a]	86.7 (14.1)[b]	79.7 (22.4)[c]
PPDi ≥ 5mm (% of sites)	0.08 (0.35)[a]	13.2 (14.1)[b]	20.3 (22.4)[c]
CALi ≤ 3mm (% of sites)	99.4 (0.92)[a]	68.2 (21.4)[b]	56.8 (27.4)[c]
CALi = 4-5mm (% of sites)	0.05 (0.87)[a]	20.2 (11.9)[b]	24.9 (15.4)[c]
CALi ≥ 6mm (% of sites)	0.04 (0.23)[a]	11.6 (13.7)[b]	18.3 (20.7)[c]
Biochemical and Physical data Median (Minimum-Maximum)			
Fasting Blood Glucose	91.5 (80.0-99.0) [a]	92.0 (60.0-99.0) [a]	153.1 (83.0-467.9) [b]
HbA_{1c}	5.4 (4.8-5.6) [a]	5.6 (4.4-5.6) [a]	7.9 (4.5-14.3) [b]
Insulin	7.4 (3.6-37.8) [a]	9.9 (4.1-32.4) [a]	13.8 (3.2-161.0) [b]
Total cholesterol	173.0 (123.0-321.0) [a]	176.0 (137.0-273.0) [ab]	193.9 (39.0-306.0) [b]
HDL cholesterol	54.9 (31.0-90.0) [a]	53.0 (30.0-88.0) [a]	44.0 (24.0-350.6) [b]
LDL cholesterol	98.0 (50.6-232.6) [a]	96.2 (17.0-732.0) [a]	109.2 (7.5-523.0) [a]
Triglycerides	105.0 (41.0-286.0) [a]	134.0 (48.0-710.0) [b]	155.0 (31.0-765.0) [b]
BMI (m/kg2)	27.3 (18.0-41.6) [a]	26.6 (19.3-38.2) [a]	29.4 (18.5-47.2) [b]
Waist/ Hip Ratio (cm)	0.86 (0.08-1.1) [a]	0.89 (0.65-1.1) [a]	0.95 (0.08-1.2) [b]

a,b,c = different letter means statistical significant difference among groups ($p < 0.05$). All the quantitative intergroup comparisons were made by with One-way ANOVA, followed by Tukey´ test for Age and Periodontal Parameters. The categorical sex and smoking habits intergroup comparisons were made using the Chi squared test. Biochemical and Physical data were analyzed by Kruskal-Wallis, followed by Dunn test. Abbreviations: BOPi= Interproximal Bleeding on Probing; P= Periodontitis; PPDi= Interproximal Probing Pocket Depth; CALi= Level of Interproximal Clinical Attachment; SD, standard deviation.

Table 3. Alleles and genotypes frequencies of the most relevant SNPs investigated in the studied groups.

	Healthy n=347	**Periodontitis n= 348**	**Periodontitis+T2DM n= 199**
rs1143634-*IL1B*	n (%)	n (%)	n (%)
Alleles G/A	554 (79.8)/ 136 (19.6)	549 (79.3)/ 135 (19.5)	318 (79.9)/ 70 (17.6)
MAF	0.20	0.19	0.18
Genotypes			
GG	217 (62.5)	222 (64.1)	130 (65.3)
GA	120 (34.6)	105 (30.3)	58 (29.1)
AA	8 (2.3)	15 (4.3)	6 (3.0)
HWE	0.09	0.61	1.00
rs2243250-*IL4*	n (%)	n (%)	0.05
Alleles C/T	494 (71.2)/ 192 (27.6)	486 (70.2)/ 198 (28.6)	263 (66.1)/ 109 (27.4)
MAF	0.28	0.29	0.29
Genotypes			
CC	181 (52.2)	173 (50.0)	92 (46.2)
CT	132 (38.0)	140 (40.5)	79 (39.7)
TT	30 (8.6)	29 (8.4)	15 (7.5)
HWE	0.42	0.89	0.85
rs1800795-*IL6*	n (%)	n (%)	
Alleles G/C	491 (70.7)/ 203 (29.2)	518 (74.9)/ 178 (25.7)	303 (76.1)/ 95 (23.9)
MAF	0.29	0.26	0.24
Genotypes			
GG	176 (50.7)	193 (55.6)	117 (58.8)
GC	139 (40.1)	132 (37.9)	69 (34.7)
CC	32 (9.2)	23 (6.6)	13 (6.5)
HWE	0.60	1.00	0.56

HWE: Hardy Weinberg Equilibrium; MAF: Minor Allele Frequencies

Table 4. Multiple Logistic Regression adjusted for age, sex and smoking statuses and stratified by sex and smoking, of the SNPs investigated that showed the most relevant results

	Healthy *versus* Periodontitis		**Healthy *versus* P+T2DM**		**Periodontitis *versus* P+T2DM**	
	Adj OR (95% CI)	***p*-value**	**Adj OR (95% CI)**	***p*-value**	**Adj OR (95% CI)**	***p*-value**
rs1143634-*IL1B*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
GG	Ref		Ref			
GA	0.87 (0.61 – 1.22)	0.43	**0.61 (0.38 – 0.98)**	**0.04**	0.86 (0.56 – 1.32)	0.49
AA	1.64 (0.65- 4.10)	0.29	0.53 (0.12 – 2.22)	0.38	0.37 (0.12 – 1.17)	0.09
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
GG	Ref		Ref		Ref	
GA	0.75 (0.43 – 1.30)	0.30	**0.38 (0.16 – 0.88)**	**0.02**	0.68 (0.34 – 1.35)	0.27
AA	4.51 (0.50 – 40.5)	0.18	0.56 (0.04 – 7.48)	0.17	0.48 (0.64 – 2.12)	0.33
Female ‡						
GG	Ref		Ref		Ref	
GA	0.95 (0.61-1.45)	0.80	**0.37 (0.16 – 0.88)**	**0.02**	0.68 (0.34 – 1.35)	0.26
AA	1.17 (0.40-3.45)	0.77	0.56 (0.04 – 7.48)	0.66	0.48 (0.10 – 2.12)	0.33
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
GG	Ref		Ref		Ref	
GA	0.79 (0.53 – 1.18)	0.25	0.79 (0.53 – 1.18)	0.25	0.85 (0.48 – 1.47)	0.55
AA	1.12 (0.38 – 3.32)	0.84	1.11 (0.37 – 3.32)	0.84	0.26 (0.04 – 1.45)	0.12
Ever Smoking ¶						
GG	Ref		Ref		Ref	
GA	1.12 (0.58 – 2.14)	0.72	0.75 (0.32 – 1.77)	0.51	0.86 (0.43 – 1.72)	0.67
AA	4.80 (0.56 – 40.89)	0.15	1.48 (0.11 – 19.43)	0.76	0.60 (0.12 – 3.04)	0.54
rs2243250-*IL4*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref			
CT	1.06 (0.76 – 1.48)	0.71	1.45 (0.92 – 2.29)	0.10	1.10 (0.73 – 1.67)	0.49
TT	0.98 (0.55- 1.76)	0.96	0.86 (0.36 – 2.05)	0.74	0.87 (0.41 – 1.87)	0.73
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
CT	0.78 (0.44 – 1.36)	0.38	0.87 (0.38 – 1.97)	0.73	1.01 (0.52 – 1.99)	0.96
TT	0.41 (0.15 – 1.10)	0.07	0.35 (0.07– 1.62)	0.18	0.50 (0.51 – 6.71)	0.36
Female ‡						
CC	Ref		Ref		Ref	
CT	1.25 (0.83-1.90)	0.28	**1.81 (1.04 – 3.15)**	**0.03**	1.12 (0.66 – 1.91)	0.44
TT	1.61 (0.78-3.36)	0.19	1.34 (0.46 – 3.91)	0.55	0.65 (0.25 – 1.67)	0.37
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
CT	1.07 (0.72 – 1.59)	0.37	1.64 (0.95 – 2.84)	0.07	1.17 (0.69 – 1.99)	0.53

TT	1.25 (0.63 – 2.48)	0.51	1.20 (0.41 – 3.51)	0.74	0.71 (0.26 – 1.92)	0.50
Ever Smoking ¶						
CC	Ref		Ref		Ref	
CT	1.01 (0.55 – 1.89)	0.95	1.18 (0.52 – 2.72)	0.68	1.12 (0.56 – 2.27)	0.33
TT	0.55 (0.19 – 1.58)	0.27	0.47 (0.12 – 2.02)	0.31	1.40 (0.39 – 4.93)	0.59
rs1800795-*IL6*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
GG	Ref		Ref			
GC	0.89 (0.63 – 1.23)	0.48	0.68 (0.43 – 1.07)	0.09	0.85 (0.57 – 1.29)	0.46
CC	0.60 (0.33- 1.10)	0.10	0.60 (0.26 – 1.36)	0.22	0.95 (0.44 – 2.04)	0.95
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
GG	Ref		Ref		Ref	
GC	0.68 (0.39 – 1.19)	0.18	0.52 (0.23 – 1.16)	0.11	0.96 (0.50 – 1.84)	0.90
CC	0.37 (0.13-1.07)	0.07	**0.12 (0.02-0.70)**	**0.01**	0.43 (0.09 – 1.99)	0.28
Female ‡						
GG	Ref		Ref		Ref	
GC	1.02 (0.67 – 1.54)	0.90	0.77 (0.44 – 1.34)	0.36	0.78 (0.46 – 1.32)	0.36
CC	0.76 (0.36 – 1.61)	0.48	1.00 (0.39 – 2.52)	0.99	1.24 (0.50 – 3.07)	0.25
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
GG	Ref		Ref		Ref	
GC	**0.56 (0.33 – 0.98)**	**0.04**	**0.57 (0.32 – 0.98)**	**0.04**	0.67 (0.39 – 1.12)	0.13
CC	0.91 (0.34 – 2.40)	0.85	0.91 (0.34 – 2.40)	0.85	1.12 (0.44 – 2.87)	0.25
Ever Smoking ¶						
GG	Ref		Ref		Ref	
GC	0.85 (0.44 – 1.63)	0.68	0.96 (0.41 – 2.21)	0.92	1.08 (0.54 – 2.16)	0.81
CC	0.42 (0.15-1.17)	0.09	0.25 (0.05-1.13)	0.07	0.59 (0.15 – 2.32)	0.45

Bold font indicates $p < 0.05$;

† OR with 95% CI were estimated by multiple logistic regression models after controlling for age, sex and smoking.

‡ OR with 95% CI were estimated by multiple logistic regression models after controlling for age and smoking.

¶ OR with 95% CI were estimated by multiple logistic regression models after controlling for age and sex.

Abbreviations: OR, odds ratio; CI, confidence interval

Table 5. Multiple Logistic Regression of the subgroups according to periodontal and glycemic condition adjusted for age, sex and smoking status, considering the SNPs investigated that showed the most relevant results.

	Control *versus* P_M		**Control *versus* P_S**		**Control *versus* P+T2DM_well**		**Control *versus* P+T2DM_poor**	
	Adj OR (95% CI)	***p*-value**	**Adj OR (95% CI)**	***p*-value**	**Adj OR (95% CI)**	***p*-value**	**Adj OR (95% CI)**	***p*-value**
rs1143634-*IL1B*								
All patients	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
GG	Ref		Ref		Ref		Ref	
GA	0.89 (0.54 – 1.46)	0.65	0.92 (0.63 – 1.34)	0.67	0.56 (0.27 – 1.15)	0.12	0.62 (0.35 – 1.09)	0.10
AA	0.57 (0.21 – 1.56)	0.28	0.65 (0.34 – 1.28)	0.21	0.65 (0.20 – 2.09)	0.46	0.68 (0.26 – 1.78)	0.43
rs2243250-*IL4*								
All patients								
CC	Ref		Ref		Ref		Ref	
CT	0.97 (0.58 – 1.61)	0.92	0.56 (0.56 – 1.22)	0.35	0.86 (0.42 – 1.75)	0.67	0.31 (0.32 – 1.04)	0.07
TT	1.18 (0.23 – 2.89)	0.84	2.04 (0.73 – 5.74)	0.17	2.07 (0.42 – 10.19)	0.37	0.34 (0.03 – 3.32)	0.35
rs1800795-*IL6*								
All patients	Adjusted OR†							
GG	Ref		Ref		Ref		Ref	
GC	1.19 (0.72-1.98)	0.56	1.02 (0.70 – 1.49)	0.88	1.63 (0.82 – 3.25)	0.16	1.52 (0.86 – 2.68)	0.14
CC	1.12 (0.47-2.63)	0.78	0.93 (0.48 – 1.81)	0.84	0.66 (0.17 – 2.62)	0.56	0.70 (0.23 – 2.11)	0.53

Abbreviations: OR, odds ratio; CI, confidence interval; P_M: patients with moderate periodontitis (from the periodontitis group), P_S: patients with severe periodontitis (from the periodontitis group), P+T2DM_well: patients with glycated hemoglobin (HbA1c) < 7%; P+T2DM_poor: patients with poorly controlled-T2DM whether the HbA1c ≥7.1%.

Supplementary Table 1. Alleles and genotypes frequencies of the SNPs not associated with the phenotypes.

	Healthy n=347	**Periodontitis n= 348**	**Periodontitis+T2DM n= 199**
rs1800871-*IL10*	n (%)	n (%)	n (%)
Alleles G/A	460 (66.3)/ 228 (32.8)	445 (64.3)/ 239 (34.53)	253 (63.6)/ 129 (32.4)
MAF	0.33	0.35	0.34
Genotypes			
GG	154 (44.4)	144 (41.6)	83 (41.7)
GA	152 (43.8)	157 (45.4)	87 (43.7)
AA	38 (10.9)	41 (11.8)	21 (10.5)
HWE	1.00	0.91	0.87
rs1800587-*IL1A*	n (%)	n (%)	n (%)
Alleles G/A	492 (70.9)/ 190 (27.4)	489 (70.7)/ 187 (27.0)	251 (63.1)/ 103 (25.9)
MAF	0.28	0.28	0.29
Genotypes			
GG	174 (50.1)	184 (53.2)	97 (48.7)
GA	144 (41.5)	121 (34.9)	57 (28.6)
AA	23 (6.6)	33 (9.5)	23 (11.5)
HWE	0.42	0.06	**0.005**
rs1800629-*TNFA*	n (%)	n (%)	
Alleles G/A	607 (87.4)/ 77 (11.1)	604 (87.3)/ 76 (11.0)	339 (85.2)/ 45 (11.3)
MAF	0.11	0.11	0.12
Genotypes			
GG	270 (77.8)	269 (77.7)	152 (76.4)
GA	67 (19.3)	66 (19.1)	35 (17.6)
AA	5 (1.44)	5 (1.44)	5 (2.5)
HWE	0.78	0.59	0.14
rs2062377- *OPG*	n (%)	n (%)	
Alleles A/T	383 (55.2)/ 235 (33.9)	371 (53.6)/ 235 (33.9)	246 (61.8)/124 (31.2)
MAF	0.38	0.39	0.33
Genotypes			
AA	119 (34.3)	99 (28.5)	85 (42.7)
AT	128 (36.9)	146 (41.9)	76 (38.2)
TT	41 (11.8)	32 (9.2)	24 (12.1)
HWE	0.47	0.55	0.32
rs9533090-*RANK*	n (%)	n (%)	
Alleles C/T	366 (52.7)/ 210 (30.2)	344 (49.7)/ 210 (30.3)	222 (55.8)/ 140 (35.2)
MAF	0.36	0.38	0.39
Genotypes			
CC	119 (34.3)	99 (28.4)	67 (33.7)
CT	128 (36.9)	146 (41.9)	88 (44.2)
TT	41 (11.8)	32 (9.2)	26 (13.1)
HWE	0.52	0.06	0.87
rs884205-*RANKL*	n (%)	n (%)	

Alleles C/A	485 (69.9)/ 103 (14.8)	490 (70.4)/ 104 (14.9)	315 (79.1)/ 57 (14.3)
MAF	0.17	0.18	0.15
Genotypes			
CC	201 (57.9)	202 (58.0)	133 (67.2)
CA	83 (23.9)	86 (24.7)	49 (24.7)
AA	10 (2.9)	9 (2.6)	4 (2.0)
HWE	0.68	1.00	1.00

HWE: Hardy Weinberg Equilibrium; MAF: Minor Allele Frequencies

Supplementary Table 2. Multiple Logistic Regression adjusted for age, sex and smoking statuses and stratified by sex and smoking, considering the SNPs which showed non significant results

	Healthy *versus* Periodontitis		**Healthy *versus* P+T2DM**		**Periodontitis *versus* P+T2DM**	
	Adj OR (95% CI)	***p*-value**	**Adj OR (95% CI)**	***p*-value**	**Adj OR (95% CI)**	***p*-value**
rs1800871-*IL10*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
GG	Ref		Ref			
GA	1.09 (0.78 – 1.52)	0.60	1.19 (0.76 – 1.87)	0.45	1.03 (0.0.67 – 1.55)	0.90
AA	1.05 (0.62- 1.77)	0.85	0.97 (0.48 – 1.97)	0.93	0.87 (0.46 – 1.68)	0.69
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
GG	Ref		Ref		Ref	
GA	1.07 (0.61 – 1.89)	0.80	0.65 (0.29 – 1.46)	0.29	0.75 (0.38 – 1.49)	0.41
AA	1.46 (0.59 – 3.61)	0.41	1.94 (0.56 – 6.81)	0.30	0.96 (0.36 – 2.51)	0.94
Female ‡						
GG	Ref		Ref		Ref	
GA	1.11 (0.73 – 1.68)	0.63	1.59 (0.90 – 2.78)	0.10	1.25 (0.74 – 2.11)	0.41
AA	0.89 (0.46 – 1.69)	0.71	0.63 (0.24 – 1.62)	0.34	0.74 (0.29 – 1.87)	0.53
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
GG	Ref		Ref		Ref	
GA	0.94 (0.63 – 1.39)	0.75	1.31 (0.75 – 2.28)	0.34	1.25 (0.74 – 2.10)	0.40
AA	0.79 (0.43 – 1.47)	0.46	0.90 (0.38 – 2.10)	0.80	1.11 (0.49 – 2.50)	0.81
Ever Smoking ¶						
GG	Ref		Ref		Ref	
GA	1.65 (0.87 – 3.13)	0.12	0.87 (0.38 – 1.97)	0.62	0.63 (0.31 – 1.29)	0.21
AA	2.25 (0.80 – 6.28)	0.12	1.19 (0.30 – 4.74)	0.80	0.59 (0.19 – 1.79)	0.35
rs1800587-*IL1A*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
GG	Ref		Ref			
GA	0.82 (0.59 – 1.14)	0.24	0.78 (0.48 – 1.24)	0.28	0.92 (0.59 – 1.42)	0.71
AA	1.33 (0.73- 2.42)	0.35	1.52 (0.71 – 3.26)	0.27	1.27 (0.66 – 2.44)	0.48
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
GG	Ref		Ref		Ref	
AG	0.95 (0.54 – 1.66)	0.85	0.60 (0.26 – 1.41)	0.29	0.79 (0.38 – 1.62)	0.52

AA	2.26 (0.85 – 6.00)	0.10	2.35 (0.68 – 8.15)	0.17	1.58 (0.64 – 3.87)	0.32
Female ‡						
GG	Ref		Ref		Ref	
AG	0.76 (0.49-1.14)	0.19	0.85 (0.48 – 1.51)	0.24	0.79 (0.38 – 1.62)	0.52
AA	0.93 (0.43-2.04)	0.86	1.02 (0.37 – 2.89)	0.17	1.58 (0.64 – 13.87)	0.32
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
GG	Ref		Ref		Ref	
AG	0.82 (0.56 – 1.22)	0.33	0.96 (0.55 – 1.68)	0.89	1.10 (0.64 – 1.89)	0.71
AA	1.06 (0.51 – 2.22)	0.86	1.18 (0.44 – 3.18)	0.73	1.17 (0.46 – 2.98)	0.74
Ever Smoking ¶						
GG	Ref		Ref		Ref	
AG	0.81 (0.43 – 1.52)	0.52	0.45 (0.18 – 1.09)	0.08	0.70 (0.32 – 1.52)	0.37
AA	2.10 (0.70 – 6.35)	0.18	2.21 (0.59 – 8.30)	0.24	1.39 (0.52 – 3.69)	0.50
rs1800629-*TNFA*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
GG	Ref		Ref			
GA	1.00 (0.68 – 1.49)	0.98	0.92 (0.54 – 1.58)	0.78	0.94 (0.56 – 1.54)	0.56
AA	0.93 (0.26- 3.44)	0.91	1.83 (0.36 – 9.49)	0.35	1.77 (0.42 – 7.35)	0.43
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
GG	Ref		Ref		Ref	
GA	0.84 (0.43 – 1.61)	0.60	1.36 (0.56 – 3.35)	0.49	1.29 (0.59 – 2.81)	0.64
AA	Empty		empty		1.10 (0.15 – 8.13)	0.92
Female ‡						
GG	Ref		Ref		Ref	
GA	1.12 (0.68 – 1.84)	0.46	0.78 (0.38 – 1.55)	0.47	0.75 (0.38 – 1.45)	0.40
AA	0.42 (0.07 – 2.33)	0.32	0.99 (0.15 – 6.52)	0.99	0.42 (0.42 – 27.03)	0.25
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
GG	Ref		Ref		Ref	
GA	1.08 (0.67 – 1.74)	0.75	1.00 (0.52 – 1.93)	0.99	0.99 (0.53 – 1.86)	0.98
AA	0.54 (0.12 – 2.46)	0.43	1.02 (0.15 – 6.89)	0.97	1.51 (0.23 – 9.62)	0.66
Ever Smoking ¶						
GG	Ref		Ref		Ref	
GA	0.86 (0.68 – 1.74)	0.68	0.83 (0.32 – 2.16)	0.70	0.82 (0.35 – 1.93)	0.65
AA	Empty		empty		4.16 (0.34 – 50.9)	0.26
rs2062377- *OPG*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
AA	Ref		Ref			
AT	1.01 (0.71 – 1.45)	0.93	0.79 (0.49 – 1.27)	0.33	0.72 (0.47 – 1.11)	0.14
TT	1.04 (0.63- 1.72)	0.85	0.83 (0.43 – 1.62)	0.60	0.69 (0.38 – 1.27)	0.23
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
AA	Ref		Ref		Ref	
AT	1.24 (0.67 – 2.31)	0.69	0.93 (0.40 – 2.17)	0.88	0.54 (0.26 – 1.11)	0.09
TT	2.02 (0.82-4.98)	0.12	1.89 (0.58-6.16)	0.29	0.74 (0.29 – 1.84)	0.52

Female ‡						
AA	Ref		Ref		Ref	
AT	0.92 (0.59 – 1.43)	0.70	0.74 (0.41 – 1.32)	0.31	0.85 (0.49 – 1.48)	0.57
TT	0.75 (0.41 – 1.38)	0.36	0.52 (0.22 – 1.24)	0.14	0.60 (0.25 – 1.42)	0.25
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
AA	Ref		Ref		Ref	
AT	0.91 (0.59 – 1.40)	0.04	0.62 (0.35 – 1.10)	0.10	0.66 (0.39 – 1.14)	0.13
TT	0.97 (0.54 – 1.74)	0.94	0.58 (0.24 – 1.38)	0.22	0.43 (0.44 – 0.98)	0.04
Ever Smoking ¶						
AA	Ref		Ref		Ref	
AT	1.28 (0.66 – 2.48)	0.74	1.36 (0.57 – 3.24)	0.47	0.84 (0.40 – 1.78)	0.67
TT	1.23 (0.47-3.23)	0.67	1.51 (0.49 - 4.56)	0.46	1.52 (0.56 – 4.16)	0.41
rs9533090-*RANK*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref			
CT	1.06 (0.73 – 1.54)	0.73	0.80 (0.49 – 1.31)	0.38	0.90 (0.57 – 1.42)	0.66
TT	0.89 (0.34- 2.30)	0.80	0.89 (0.25 – 3.13)	0.85	0.73 (0.21 – 2.55)	0.62
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
CT	1.69 (0.88 – 3.24)	0.11	1.63 (0.64 – 4.10)	0.30	1.07 (0.52 – 2.21)	0.85
TT	2.55 (0.26-24.91)	0.42	1.83 (0.15-21.56)	0.48	0.81 (0.12 – 5.26)	0.82
Female ‡						
CC	Ref		Ref		Ref	
CT	0.83 (0.52 – 1.31)	0.43	0.61 (0.33 – 1.11)	0.10	0.83 (0.46 – 1.49)	0.54
TT	0.58 (0.18 – 1.83)	0.35	0.58 (0.12 – 2.91)	0.51	0.68 (0.12 – 3.84)	0.68
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
CT	1.19 (0.77 – 1.84)	0.42	0.88 (0.48 – 1.59)	0.10	0.96 (0.55 – 1.69)	0.90
TT	0.71 (0.22 – 2.32)	0.57	0.66 (0.17 – 3.50)	0.63	0.72 (0.13 – 4.03)	0.04
Ever Smoking ¶						
CC	Ref		Ref		Ref	
CT	0.77 (0.38 – 1.56)	0.47	0.69 (0.28 – 1.70)	0.42	0.79 (0.37 – 1.73)	0.57
TT	1.29 (0.22-7.76)	0.77	1.59 (0.19 – 13.13)	0.67	0.84 (0.12-5.95)	0.87
rs884205-*RANKL*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref			
CA	1.28 (0.89 – 1.86)	0.18	1.28 (0.80 – 2.07)	0.30	0.98 (0.62 – 1.53)	0.94
AA	0.99 (0.57- 1.72)	0.98	1.21 (0.60 – 2.46)	0.59	1.32 (0.67 – 2.59)	0.41
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
CA	1.30 (0.68 – 2.46)	0.42	0.79 (0.34 – 1.85)	0.59	0.78 (0.38 – 1.61)	0.50
AA	1.14 (0.47-2.77)	0.76	0.78 (0.23-2.66)	0.69	0.99 (0.34 – 2.78)	0.97
Female ‡						

CC	Ref		Ref		Ref	
CA	1.29 (0.82 – 2.02)	0.27	1.63 (0.90 – 2.95)	0.10	1.14 (0.65 – 2.03)	0.54
AA	0.91 (0.45 – 1.87)	0.81	1.49 (0.62 – 3.61)	0.37	1.61 (0.65 – 3.95)	0.29
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
CA	1.31 (0.84 – 2.03)	0.22	1.68 (0.93 – 3.00)	0.08	1.15 (0.66 – 2.01)	0.61
AA	0.86 (0.45 – 1.67)	0.67	1.01 (0.40 – 2.53)	0.98	0.91 (0.36 – 2.30)	0.85
Ever Smoking ¶						
CC	Ref		Ref		Ref	
CA	1.25 (0.62 – 2.46)	0.54	0.69 (0.28 – 1.64)	0.39	0.78 (0.36 – 1.68)	0.52
AA	1.43 (0.48 -4.21)	0.51	1.44 (0.41 – 5.08)	0.57	2.53 (0.81-7.90)	0.10

Bold font indicates $p < 0.05$; *indicates statistical significance after Bonferroni correction ($p < 0.01$).

† OR with 95% CI were estimated by multiple logistic regression models after controlling for age, sex and smoking.

‡ OR with 95% CI were estimated by multiple logistic regression models after controlling for age and smoking.

¶ OR with 95% CI were estimated by multiple logistic regression models after controlling for age and sex.

Abbreviations: OR, odds ratio; CI, confidence interval

3.2 Publicação 2*

Gene variants previously identified by Genome-Wide studies to risk for Periodontitis or Type 2 Diabetes Mellitus: validation in a Brazilian population

Thamiris Cirelli [1,2], Rafael Nepomuceno[1,2], Jéssica Marina Goveia [2], Silvana R P Orrico[1,3], Joni A Cirelli[1], Letícia Helena Theodoro [4], Silvana P. Barros [5], Raquel M Scarel-Caminaga[2]

6. Department of Diagnosis and Surgery, São Paulo State University – UNESP, School of Dentistry at Araraquara, Araraquara, SP, Brazil
7. Department of Morphology and Pediatric Clinics, São Paulo State University – UNESP, School of Dentistry at Araraquara, Araraquara, SP, Brazil
8. Advanced Research Center in Medicine, Union of the Colleges of the Great Lakes (UNILAGO), São José do Rio Preto, SP, 15030-070, Brazil
9. Department of Surgery and Integrated Clinic, São Paulo State University – UNESP, School of Dentistry at Araçatuba, Araçatuba, SP, Brazil
10. Department of Periodontology, University of North Carolina at Chapel Hill – UNC, School of Dentistry, Chapel Hill, NC, USA

Correspondence:

Raquel Mantuaneli Scarel-Caminaga, PhD

Department of Morphology, School of Dentistry at Araraquara, UNESP – São Paulo State University, CP 331, CEP 14801-903, Araraquara, SP, Brazil, E-mail: raquel.caminaga@unesp.br

* Este artigo segue as normas do periódico “International Journal of Molecular Sciences” para o qual submetemos para publicação.

ABSTRACT

Periodontitis and Type 2 Diabetes Mellitus (T2DM) are complex diseases with common pathogenic mechanisms and increased prevalence affecting simultaneously the same patients. However, what genetic component might be shared between these diseases remains unclear. (SNPs) rs5219, rs5215, rs7957197, rs2943641, rs2062377, rs7754840, rs7018475, rs1531343, rs6712932, rs352140, rs187238, rs3826782 selected from genome wide association studies previously related with T2DM or periodontitis, were sought to be validated as gene markers for T2DM and/or periodontitis in a Brazilians. Periodontal, glycemic and lipid data was obtained from 953 individuals divided into three groups: Control (n=356), Periodontitis (P; n=358) and Periodontitis together with T2DM (P+T2DM; n=239). DNA from oral epithelial cells was used for genotyping by OpenArray. Associations between SNPs and pathologies were tested by multiple logistic regressions, adjusting for age, sex, and smoking habits. The rs7957197-TA genotype was associated with increased susceptibility to T2DM for all population, and for men. Women carrying the rs77544840-GT genotype (*CDKAL1* gene) also showed susceptibility to T2DM. The rs7018475-GG (*CDKN2B* gene) showed susceptibility to P+T2DM population, for female, and never smokers. The rs7957197 (*HNF1A*) and rs77544840 (*CDKAL1)* were validated in Brazilians as risk markers for T2DM. The rs7018475 (*CDKN2B*) was associated with higher risk for the development of P+T2DM.

Keywords: Periodontal Disease, Diabetes mellitus type 2, Single Nucleotide Polymorphism, Hepatocyte nuclear factor-1 alpha, Cyclin dependent kinase inhibitor 2B, CDK5 regulatory subunit associated protein 1 like 1

Introduction

Complex diseases are caused by a combination of genetic, environmental, and lifestyle habits (smoking, stress, diet, etc.). In such cases, environment and lifestyle factors only act in the short term and they are often shared with individuals who do not develop the disease [1]. Periodontitis is a complex oral disease, entailing a dysbiotic oral microbial shift and a deregulated host inflammatory response that contribute to progressive periodontal tissue destruction [2]. Approximately 47% of adults aged 30 years and older had Periodontitis, with 30% presenting moderate and 8.5% the severe form of the disease [3]. The existence of a genetic component to periodontitis has been confirmed in twin studies, which estimated that 38–82% of populational variability in the clinical parameters of periodontal disease was attributable to genetic factors [4-6]. The heritability of Periodontitis was estimated to be around 50%, which was unaltered following covariate adjustments for smoking, dental hygiene, age, and gender [6].

Type 2 Diabetes Mellitus (T2DM), another complex disease, is considered a metabolic disorder characterized by hyperglycemia caused by a combination of insulin resistance and impaired secretion [7]. The relationship between periodontitis and T2DM have been studied for years demonstrating that the presence of one condition tends to increase the risk and severity of the other. Therefore, nowadays it is widely accepted that T2DM is a risk factor for increased prevalence and severity of periodontitis [8, 9]. Conversely, periodontitis is an important factor for increased risk of diabetic complications in patients with diabetes [10], being periodontitis considered the sixth complication of T2DM [11].

Genetic research may explain why the host response often differs markedly between individuals with similar environmental backgrounds and comparable lifestyle habits [1]. By studying gene variants, mainly single nucleotide polymorphisms (SNPs), it is possible the identification of genetic risk factors associated with complex diseases, such as Periodontitis and T2DM, to permit in the future the prevention and treatment strategies improvement for these diseases [12].

Genome-wide (GWAS) are hypothesis-free studies which simultaneously test millions polymorphisms spread across the genome of a single patient, providing an unbiased approach for identifying the genetic factors of susceptibility to a disease. A number of GWAS have reported novel findings for complex diseases, and interestingly, most of these genes have not been thought of as likely candidates before [13]. Based on GWAS results, some genetic variants are currently considered gene markers for T2DM in the *KCNJ11, HNF1A, IRS1,*

TCF7L2, CDKAL1, CDKN2B, RPSAP52, GPR45 genes [14-20], while others were found by GWAS like *EMR1* [13] or bioinformatics approaches for periodontitis association, such as the *TLR9, IL18* genes [21].

The genetic risk factors found in the GWAS need to be validated in independent populations. A GWAS result is 'replicated' when it is reproduced in a population similar to that of the original study; such was done by [22] and [23]. However, when the GWAS result is reproduced in a different population (i.e. ethnically distinct), the result is considered 'validated' [24]. Considering that the mentioned gene markers for T2DM/ Periodontitis prospected from GWAS have not yet been validated in the Brazilian population, and the scarce knowledge on the genetic component potentially shared between them, the purpose of the present study was to investigate whether 12 SNPs (rs5219, rs5215, rs7957197, rs2943641, rs2062377, rs7754840, rs7018475, rs1531343, rs6712932, rs352140, rs187238, rs3826782) would be validated in a Brazilian population, as markers for T2DM, or Periodontitis, or those diseases together, as comorbidities.

Material and Methods

This study was performed in accordance with the Helsinki Declaration of 1975, as revised in 1983, and was approved by the Ethics in Human Research Committee of the Araraquara School of Dentistry (UNESP; Protocol number 50/06). The sample size calculation was assessed by the G*Power Calculator, version 3.182, considering the parameters: logistic regression; two tail, OR: 1.5; Pr (Y = 1 / X = 1) H0 = 0.2; alpha of 0.0042 (0,05/ 12 SNPs) and 80% power, R2=0. We found that each group should be composed by at least 476 patients (159 each group) to have sufficient power to attest the association of the allele(s) with the diseased phenotype. Eight hundred and ninety four (894) individuals were randomly selected from the population of patients treated from 2010 to 2018 at the Department of Periodontology, School of Dentistry at Araraquara, São Paulo State University (UNESP), Brazil. The participants were at least 30 years old, with at least 10 teeth (excluding third molars), without any chronic or systematic disease (i.e. HIV infection, immunosuppressive chemotherapy) or treatment with systemic antiobiotic in the previous 3 months or received periodontal treatment in the previous 6 months, no pregnant or lactating.

A questionnaire about smoking status was applied to each patient, and the history of smoking was defined as having smoked at least once per day for at least 1 year [25]. Therefore, patients were classified as never smokers, or ever smokers (current smokers +

former smokers). By utilizing a University of North Carolina-15 periodontal probe at four sites (interproximal sites) around each tooth of the participants, the clinical periodontal parameters were assessed: interproximal probing pocket depth (PPDi), interproximal clinical attachment level (CALi), bleeding on probing (BOP), visible plaque and marginal bleeding. All fully erupted teeth, except third molars and retained roots were examined. From peripheral blood of patients the glycemic profile was assessed: Fasting Glycemia (modified Bondar and Mead method, Labtest Kit), HbA1c (turbidimetric inhibition immunoassay method, Roche)and Insulin (chemiluminescence method); as well the lipid profile: Triglycerides (enzymatic-Trinder method, Labtest Kit), Total Cholesterol (enzymatic-Trinder method, Labtest) and HDL-cholesterol (enzymatic method, Labtest kit), and the fractions of VLDL and LDL-cholesterol were calculated according to the Friedewald equation [26].

According to their periodontal status and glycemic profile, patients were divided into three groups: (i) Control = 356 individuals with healthy periodontium or mild periodontitis and without T2DM; (ii) Periodontitis = 358 patients with moderate or severe periodontitis and without T2DM; (iii) Periodontitis+T2DM = 239 patients affected by T2DM with moderate or severe periodontitis. The criteria for definition of periodontitis were according the CDC / AAP (Centers for Disease Control and Prevention / American Academy of Periodontology) [27]. The diagnosis of T2DM was medical responsibility. The T2DM patients were divided according to their metabolic control in well-controlled-T2DM whether the glycated hemoglobin (HbA1c) was until 7% (P+T2DM_well) and poorly controlled-T2DM whether the HbA1c ≥7.1% (P+T2DM_poor) [28, 29]. According to the periodontal condition, the patients from Periodontitis group were divided into Moderate periodontitis (P_M) and Severe periodontitis (P_S).

2.2. Patient's SNPs Genotyping

Genomic DNA was obtained from buccal epithelial cells after a mouthwash, being extracted using standard protocols involving proteinase K digestion, 8M ammonium acetate and subsequent ethanol precipitation [30]. The genomic DNA was quantified by NanoDrop® 2000 spectrophotometer (Thermo Scientific) and a Qubit® 2.0 fluorometer (Invitrogen). The samples showing $A_{260/280}$ ratio between 1.7 and 2.0 and a concentration of 50 ng/μL were selected (APÊNDICE).

The SNPs selected from previous GWAS to this study here, were genotyped utilizing 2.5 μL of each individual DNA sample (total of 125 ng of genomic DNA) in each well of a

384-well plate in the presence of 2.5 μL of genotyping qPCR master mix (2X TaqMan®OpenArray® Genotyping Master Mix, Thermo Fisher Scientific), according to the manufacturer's recommendation. The reaction was performed in the presence of specific pairs of primers and TaqMan probe (Table 1) on a qPCR thermocycler (QuantStudio 12K Flex Real-Time PCR System, Thermo Fisher Scientific). The results were analyzed using an online genotyping analysis software (Applied Biosystems™ Analysis Software, Genotyping Analysis Module, version 3.2). The quality value of the genotype data point was determined by a threshold above 90%.

2.3. Statistical analyses

Demographic, periodontal clinical parameters and results of biochemical and physical examinations were expressed as the mean (± standard deviation) for numerical data, and as frequency distributions for categorical data. Differences in the frequencies of data were tested using the ANOVA test with Tukey's posttest (age, periodontal and biochemical) or the chi-squared test (sex and smoking) using the software IBM® SPSS® Statistics (version 20) and GraphPad Prism 5.01. To detect differences in the allele and genotype distributions of each SNP between the patients' groups, and to test the Hardy–Weinberg equilibrium, we utilized the PLINK software [31]. All other analyses were performed by STATA software, version 12.0 (Statistics/Data Analysis, Stata Corporation, College Station, Tex., USA).

The association between genotypes and diseases was computed using multivariate logistic regression analyses assuming the additive model and adjusting for age, sex and smoking status (as confounding factors), when we considered all population. The effect size was expressed as an odds ratio (OR) with a 95% confidence interval (CI). Experiment-wise significance (*p-value* = 0.0042) was determined by Bonferroni's correction based on the total number of markers genotyped of the study. Furthermore, the same analysis was stratified: (i) by sex, to identify the sex-specific effects of each SNPs on the pathological phenotype, but multiple logistic regressions were adjusted by age and smoking; (ii) by smoking status, adjusting the multiple logistic regressions by age and sex.

Results

We found statistical difference among the groups for the mean age, belonging the younger patients (43.6 years mean) to the control group. There were predominance of females, and never smokers in the studied population (Table 2). Regarding periodontal parameters, as

expected, we observed that the Control patients did not have loss of periodontal support, with most of interproximal clinical attachment level (iCAL ≤ 3mm) of and interproximal probing pocket depth (iPPD ≤ 4mm). Comparing the groups with periodontitis, the patients with T2DM had the worst condition, with higher number of visible plaque (56.5%), marginal bleeding (50.8%) and bleeding on probing (40.2%). Consequently, T2DM had a high loss of periodontal support (PPDi ≥ 5mm = 20.5%, CALi ≥ 6m =: 18.5%).

Demonstrating to follow the clinical criteria, T2DM patients showed significantly increased levels of fasting glucose, HbA1c, and insulin levels compared with nondiabetic patients in the other groups. Since the lipid parameters were not considered to divide the patients into the groups, it is possible to note that in each group there are some patients with altered levels of lipids, being the highest levels found in the P+T2DM. Interestingly, the higher parameters of obesity, the BMI ($p<0.05$) and waist/hip proportion was observed in the P+T2DM group (Table 2).

The genetic analysis in the Table 3 showed that every SNPs were in Hardy Weinberg Equilibrium (HWE) in the control group, and the rs2521634 was the SNP presenting the lowest minor allele frequency (MAF=0.04) (Supplementary Table 1). To more accurately evaluate the strength of any association and to eliminate the distortion caused by confounding effects (sex, age and smoking), the additive model in multiple logistic regressions were performed (Table 3). Considering the comparison of Periodontitis versus P+T2DM, after the Bonferroni's correction, the heterozygotes TA for the rs7957197 SNP in the *HNF1A* gene were significantly associated with increased susceptibility to T2DM, since both groups were affected by periodontitis. For the rs2062377 (*TCF7L2* gene) and the rs77754840 (*CDKAL1* gene), two of the most important gene markers of T2DM, demonstrated for the all studied Brazilians, association with T2DM (P versus P+T2DM) for the TT genotype in the *TCF7L2* (OR= 2.22; 95% CI= 1.24–4.01; p = 0.007), and CG genotype in the *CDKAL1* gene (OR=1.73; 95% CI= 1.13 – 2.66; p= 0.01), but those p values did not reach the statistical threshold of $p<0.0042$ by the Bonferroni's correction for multiple comparisons. Similarly, we found a weak association of the rs2943641-*IRS1* with a lower chance to develop Periodontitis and T2DM (P+T2DM). For the SNP rs3826782 in the *EMR1* no association could be found in this study. Evaluating the susceptibility of all Brazilians to Periodontitis (Control versus P group), the rs1531343-*RPSAP52* gene (OR= 1.52; 95% CI= 1.06 – 2.19; p = 0.02) showed to increase this susceptibility, while the rs77754840-*CDKAL1* and the rs7957197-*HNF1A* genes demonstrated lower chance to develop Periodontitis.

It was tested whether sex or smoking status had specific effects in the selected SNPs by stratifying the population by sex, and after by smoking status (ever- or never-smokers) using the additive model in multiple logistic regressions. Considering the Bonferroni's correction, female carrying the rs7754840-CG (*CDKAL1* gene) had significant increased chance to develop T2DM (OR=2.49; 95% CI= 1.41 – 4.42; $p = 0.002$), while a weaker effect was observed for female carrying the rs2062377-TT (*TCF7L2* gene) and the rs187238- GG (*IL18* gene). In the analysis of Periodontitis+T2DM, we found that women carrying the rs7018475-GG (*CDKN2B* gene) demonstrated increased susceptibility to both as comorbidities (Control versus P+T2DM group, OR= 3.91; 95% CI= 1.76–8.70; $p = 0.001$).

The more relevant genetic association found for men was regarding the rs7957197-TA (*HNF1A* gene) that were more susceptible to T2DM (P versus P+T2DM group, OR= 3.06; 95% CI= 1.51–6.17; $p = 0.002$), even after the Bonferroni's correction (Table 3).

Regarding to smoking status, the statistically significant result after the Bonferroni's correction was found for patients carrying the rs7018475-GG (*CDKN2B* gene) who never smoked had higher chance to develop both diseases (Control versus P+T2DM group, OR= 4.28; 95% CI= 1.89–9.63; $p = 0.000$) (Table 3).

In the Table 5, we subdivided the Periodontitis group by the severity of periodontitis into the P_M (moderate), and P_S (severe), as well we subdivided the P+T2DM into the well-controlled (HbA1c $\leq$ 7.0% and $\geq$5.8%) patients (P+T2DM_well) and those poorly glycemic controlled (HbA1c $\geq$ 7.1%) patients (P+T2DM_poor). For the Control *versus* P_S, individuals carrying the rs7957197-TA (*HNF1A* gene) demonstrated significant association with the lower chance to develop severe periodontitis (OR= 0.53; 95% CI= 0.35–0.80; $p = 0.002$), even after the Bonferroni's correction. Patients with TT genotype for the rs2062377-*TCF7L2* were more susceptible to develop P+T2DM_well controlled, while and the GG genotype for rs7018475-*CDKN2B* were associated with the same diseases, but at a poorly controlled T2DM. Regarding the chance to develop severe periodontitis, heterozygotes for the rs7754840-*CDKAL1* were lower prone to develop it (OR= 0.48; 95% CI= 0.28–0.83; $p = 0.009$).

Discussion

This is the first study to sought for whether the genetic markers to each T2DM or Periodontitis, identified by GWAS, could be also markers for both Periodontitis and T2DM

(as comorbidities). We were able to validate the rs7957197 (*HNF1A* gene) and rs7018475 (*CDKN2B* gene) as genetic markers of susceptibility to both complex diseases (P+T2DM), while the rs7754840 (*CDKAL1* gene) demonstrated to be a lower risk marker to develop them. Those results found in a Brazilian population were statistically significant even after the Bonferroni's correction (p value lower than 0.0042). The present study contributes with the evidence that there are genetic component shared between those complex diseases [26][32].

Other interesting results were observed here, in spite of not following the Bonferroni's correction such as the increased risk to Periodontitis+T2DM associated with the rs2062377 (*TCF7L2* gene), rs1531343 (*RPSAP52* gene) and rs187238 *(IL18* gene); while a lower risk to Periodontitis+T2DM was identified with the rs5219 and rs5215 (*KCNJ11* gene), rs2943641 (*IRS1* gene), rs6712932 (*GPR45* gene) and rs352140 (*TLR9* gene). We consider those results biologically relevant, as explained following, and because it is important to take into mind that the application of Bonferroni's correction (BC) is controverse. Despite the BC is widespread used in population genetics, it has been constantly discussed since the BC may not guarantee a prudent result due to an irrelevant null hypothesis, reluctance to reject the null hypothesis, different interpretations of identical *p*-values, and inflated type II error [33].

The rs352140 (*TLR9* gene) and rs187238 *(IL18* gene) SNPs were firstly associated with periodontitis by integrative gene ranking method, a bioinformatics tool to prioritization of candidate genes identification [21]. TLR9 is a member of the TLR family. Rojo-Botello et al.[34] reported that the expression of TLR9 was higher in patients with periodontitis when compared with healthy individuals. Kajita et al.[35] compared the expression levels of TLR9 between patients with gingivitis and those with periodontitis and found that he expression of TLR9 was significantly higher in periodontitis lesions than gingivitis lesions. Holla **et al**.[36] have concluded that *TLR9* haplotypes may be associated with the patient's susceptibility to chronic periodontitis, while another studies with Indians [37] and Caucasians [38] did not find association with periodontitis. The SNP rs352140 (*TLR9* gene) was investigated regarding T2DM but no association was found [39, 40]. In our study, we have a higher number of patients and more detailed periodontal and glycemic profile then the cited studies, and here we could associate the rs352140 with lower risk to the development of Periodontitis and T2DM. The IL-18 cytokine has been implicated in inflammation and modulates the activity of macrophages and neutrophils [41, 42]. Our findings were in accordance with some studies [43, 44], including a meta-analysis with a total of 576 periodontitis patients and 458 healthy controls, that showed the association with increased risk to periodontitis [45].

The protein encoded by the *KCNJ11* (potassium inwardly rectifying channel subfamily J member 11) gene is an integral membrane protein and inward-rectifier type potassium channel. Potassium channels are present in most mammalian cells, where they participate in a wide range of physiologic responses [46]. The rs5219 [47, 48] and rs5215 [49, 50] SNPs in the *KCNJ11* gene were associated with T2DM by GWAS. Referring to the rs5215 polymorphism, 3 studies showed strong associations between this variant and T2DM [51-53], whereas other studies showed the opposite, i.e. no association with T2DM [54-57]. In the present study we associated the rs5215 SNP with lower risk to T2DM in our population. For the rs5219 SNP, we did not validate here the previous findings of association to T2DM [58-60]. Also, we did not validate here in the studied Brazilian population the genetic variant rs3826782 in the *EMR1* gene as a gene marker of periodontitis, as previously identified by Divaris et al, 2013 [13].

As a member of the insulin receptor substrate (IRS) protein family, the protein coded by the *IRS1* gene play a key pathogenic role in the insulin-stimulated signal pathway and then in the development of T2DM [61]. The genetic variant, rs2943641, located adjacent to the *IRS1* gene was associated with T2DM, insulin resistance, hyperinsulinemia, and adiposity by GWAS [62, 63]. There are no consistent results about the association between the rs2943641 SNP and T2DM, as demonstrated by a recent meta-analysis which showed that the codominant model of rs2943641 was associated with T2DM risk in Caucasian, not in overall population [64]. It may be caused by the different genetic backgrounds and environmental factors that can affect their sensitivity to particular genomic variants.

For the rs6712932 (G*PR45* gene) SNP, although GWAS studies including four different populations showed association with T2DM (The DiaGen Consortium)[17], here we did not find the association with T2DM, but we are the first to show the association of this SNP with Periodontitis. The rs1531343 (*RPSAP52* gene) SNP were already associated with T2DM in Europeans by GWAS [14, 18, 19, 49], but in the Brazilian population we did not validate that result, since we just had found a weak association of this SNP with the P+T2DM.

One of the most reproducible risk genes for diabetes identified across different ethnic populations is Cyclin dependent kinase 5 (CDK), which regulates protein subunit 1 homolog 1 (*CDKAL1*) [65]. In 2007, using the results obtained from the GWAS studies of the Diabetes Genetic Initiative (DGI) [14], Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION) [18], Wellcome Trust Case Control Consortium (WTCCC), and the United Kingdom Type 2 Diabetes Genetics Consortium (UKT2D) [14] the rs7754840 G/C polymorphism in the *CDKAL1* gene was reported to be strongly associated

with T2DM. Here we observed that the heterozygote genotype of the rs7754840 SNP in the *CDKAL1* was associated with increased risk to the development of T2DM in all patients, but mainly in female. This result validate in the Brazilian studied population those previous findings, altogether a meta-analysis with 70141 subjects that showed similar results in the recessive model for the homozygote genotype (OR= 1.58, 95% CI= 1.20-2.08, $p < 0.001$)[66]. Moreover, there are a lot of studies showing the association of this SNP with gestational diabetes [67-69]. To our knowledge is the first time that the rs7754840 SNP in the *CDKAL1* was associated with lower risk to develop periodontitis, indeed we could validated its association with T2DM (OR= 2.49; 95% CI= 1.41–4.42; $p = 0.002$).

The rs70184759 (*CDKN2B* gene) was associated with T2DM by Huang et al, 2012 [20]. Later, Sanghera et al 2012 [15], addressed the importance of such polymorphism for the pathophysiology of the complex T2DM phenotype. In the present study we validated in our Brazilian population the susceptibility to T2DM (Table 3), and we originally showed the association of rs70184759-GG (*CDKN2B)* with the higher risk to T2DM + periodontitis in female and never smokers (Table 3), as well with the poor controlled T2DM + periodontitis affected patients (Table 4). This SNP is different from 51 tagging SNPs previously investigated by Schaefer et al. 2011 [70] comprising the *CDKN2BAS* and adjacent *CDKN2A* and *CDKN2B* genes which identified association with aggressive periodontitis in different European populations. Since the *CDKN2B* gene was associated with other inflammatory diseases, such as coronary heart disease [71], and lymphoblastic leukemia [72], more studies should be made to better understand the inter-relationship of periodontitis and systemic complex diseases. The *CDKN2B* gene encodes a cyclin-dependent kinase inhibitor and the expression of this gene was found to be dramatically induced by TGF beta, which suggested its role in the TGF beta induced growth inhibition (ref).

Regarding the Hepatocyte Nuclear Factor 1α (*HNF1A*) gene, which result in reduced concentrations of ATP in β cells, leading to lower insulin secretion [73, 74], is associated with many cases of Maturity-onset diabetes of the young (MODY), a heritable monogenic form of diabetes [75]. Some GWAS identified the rs7957197 SNP at the *HNF1A* as a genetic marker of T2DM [16, 50, 65]. The rs7957197 SNP in the *HNF1A* gene affects *in vivo* and *in vitro* glycaemic traits in the context of T2DM [76]. In our study we verified that the heterozygote genotype of the rs7957197 (*HNF1A)* was significantly associated with increased risk to develop periodontitis together with T2DM (P+T2DM) (Table 4). Interestingly, when considering the periodontitis alone, the same genotype indicated lower risk to general periodontitis (Table 4), by mainly to severe periodontitis (Table 5).

This study has some limitations. According to Parra et al., 2003, is not recommended grouping Brazilians into ethnic groups based on their skin color and other physical characteristics associated with racial divisions [77]. All genetic studies enrolling a Brazilian population should consider the nature of its admixed ethnic origin, since Brazilians form one of the most heterogeneous populations in the world, resulting of the 5 centuries of inter-ethnic crosses between Europeans, Africans, Asians and autochthonous Amerindians [78]. Because of this fact, we did not explore this characteristic in any of our genetic analysis. Altogether, we also not deeper explore here the reasons why the sex or smoking habits might influence the gene-phenotype associations. Further studies with a specific design to assess these aspects are necessary to investigate the functional role of these SNPs in the context of periodontal disease and T2DM. In spite of each studied group here have sufficient power (at least 159 patients) to detect genetic significant associations, as increasing the number of patients to be enrolled, as higher the results confidence, as well it will increase the chance to find patients affected by only T2DM. A different study design in the recruitment and selection criteria might overcome the limitation of this study about the absence of a T2DM group without Periodontitis as comorbidity. On the other hand, as positive points of our study we highlight that all patients had the accurate diagnosis to be part of a group (such as the complete periodontal exams) and they were of a similar socioeconomic status and selected from the same geographical area (Araraquara region, São Paulo State, Southeast of Brazil).

In conclusion, the genetic variants rs7957197 (*HNF1A*), rs77544840 (*CDKAL1*) and rs7018475 (*CDKN2B*) were validated in Brazilians as risk markers for T2DM, while the rs7018475 (*CDKN2B*) was associated with higher risk for the development of Periodontitis together with T2DM. Moreover, there were a weak association between the rs5219 and rs5215-*KCNJ11,* rs6712932-*GPR45,* rs1531343-*RPSAP52,* rs352140-*TLR9* with only Periodontitis, and the SNPs rs5215- *KCNJ11,* rs7957197-*HNF1A*, rs7903146-*TCF7L2*, rs1531343-*RPSAP52,* rs352140-*TLR9,* rs7903146-*TCF7L2* with Periodontitis+T2DM (as comorbidity) in Brazilians by performing this case-control analysis. These findings may be useful in public health genomics and future advanced clinical practice.

Conflict of interest and source of funding

The authors have stated explicitly that there are no conflicts of interest in connection with this article. This study was supported by the Foundation for Research Support of State of São Paulo – FAPESP, Grants 2014/13295-1, 2016/03753-8, 2016/18313-3, 2016/08070-6 and

2018/26367-1; and National Council for Scientific and Technological Development (CNPq) Grants 445336/2014-5 and 304570/2017-6.

Authors contribution

All authors have made substantial contributions to conception and design of the study. TC, RN, JMG, RMSC, SRPO, JAC and LHT been involved in data collection and data analysis. TC, RN, JMG, SPB and RMSC have been involved in data interpretation. TC, RN, JMG, SRPO, JAC, LHT, SPB and RMSC drafted the manuscript, revised it critically, and have given final approval of the version to be published.

References

1. Schaefer, A. S., Genetics of periodontitis: Discovery, biology, and clinical impact. *Periodontology 2000* **2018,** 78, (1), 162-173.
2. Berezow, A. B.; Darveau, R. P., Microbial shift and periodontitis. *Periodontology 2000* **2011,** 55, (1), 36-47.
3. Thornton-Evans, G.; Eke, P.; Wei, L.; Palmer, A.; Moeti, R.; Hutchins, S.; Borrell, L. N.; Centers for Disease, C.; Prevention, Periodontitis among adults aged >/=30 years - United States, 2009-2010. *MMWR supplements* **2013,** 62, (3), 129-35.
4. Michalowicz, B. S.; Aeppli, D. P.; Kuba, R. K.; Bereuter, J. E.; Conry, J. P.; Segal, N. L.; Bouchard, T. J., Jr.; Pihlstrom, B. L., A twin study of genetic variation in proportional radiographic alveolar bone height. *J Dent Res* **1991,** 70, (11), 1431-5.
5. Michalowicz, B. S.; Aeppli, D.; Virag, J. G.; Klump, D. G.; Hinrichs, J. E.; Segal, N. L.; Bouchard, T. J., Jr.; Pihlstrom, B. L., Periodontal findings in adult twins. *Journal of periodontology* **1991,** 62, (5), 293-9.
6. Michalowicz, B. S.; Diehl, S. R.; Gunsolley, J. C.; Sparks, B. S.; Brooks, C. N.; Koertge, T. E.; Califano, J. V.; Burmeister, J. A.; Schenkein, H. A., Evidence of a substantial genetic basis for risk of adult periodontitis. *Journal of periodontology* **2000,** 71, (11), 1699-707.
7. King, G. L.; Shiba, T.; Oliver, J.; Inoguchi, T.; Bursell, S. E., Cellular and molecular abnormalities in the vascular endothelium of diabetes mellitus. *Annual review of medicine* **1994,** 45, 179-88.
8. Tan, W. C.; Tay, F. B.; Lim, L. P., Diabetes as a risk factor for periodontal disease: current status and future considerations. *Annals of the Academy of Medicine, Singapore* **2006,** 35, (8), 571-81.
9. Southerland, J. H.; Taylor, G. W.; Moss, K.; Beck, J. D.; Offenbacher, S., Commonality in chronic inflammatory diseases: periodontitis, diabetes, and coronary artery disease. *Periodontology 2000* **2006,** 40, 130-43.
10. Zhou, X.; Zhang, W.; Liu, X.; Zhang, W.; Li, Y., Interrelationship between diabetes and periodontitis: role of hyperlipidemia. *Arch Oral Biol* **2015,** 60, (4), 667-74.
11. Loe, H., Periodontal disease. The sixth complication of diabetes mellitus. *Diabetes care* **1993,** 16, (1), 329-34.
12. Song, L.; Yao, J.; He, Z.; Xu, B., Genes related to inflammation and bone loss process in periodontitis suggested by bioinformatics methods. *BMC oral health* **2015,** 15, 105.
13. Divaris, K.; Monda, K. L.; North, K. E.; Olshan, A. F.; Reynolds, L. M.; Hsueh, W. C.; Lange, E. M.; Moss, K.; Barros, S. P.; Weyant, R. J.; Liu, Y.; Newman, A. B.;

Beck, J. D.; Offenbacher, S., Exploring the genetic basis of chronic periodontitis: a genome-wide association study. *Hum Mol Genet* **2013,** 22, (11), 2312-24.

14. Diabetes Genetics Initiative of Broad Institute of, H.; Mit, L. U.; Novartis Institutes of BioMedical, R.; Saxena, R.; Voight, B. F.; Lyssenko, V.; Burtt, N. P.; de Bakker, P. I.; Chen, H.; Roix, J. J.; Kathiresan, S.; Hirschhorn, J. N.; Daly, M. J.; Hughes, T. E.; Groop, L.; Altshuler, D.; Almgren, P.; Florez, J. C.; Meyer, J.; Ardlie, K.; Bengtsson Bostrom, K.; Isomaa, B.; Lettre, G.; Lindblad, U.; Lyon, H. N.; Melander, O.; Newton-Cheh, C.; Nilsson, P.; Orho-Melander, M.; Rastam, L.; Speliotes, E. K.; Taskinen, M. R.; Tuomi, T.; Guiducci, C.; Berglund, A.; Carlson, J.; Gianniny, L.; Hackett, R.; Hall, L.; Holmkvist, J.; Laurila, E.; Sjogren, M.; Sterner, M.; Surti, A.; Svensson, M.; Svensson, M.; Tewhey, R.; Blumenstiel, B.; Parkin, M.; Defelice, M.; Barry, R.; Brodeur, W.; Camarata, J.; Chia, N.; Fava, M.; Gibbons, J.; Handsaker, B.; Healy, C.; Nguyen, K.; Gates, C.; Sougnez, C.; Gage, D.; Nizzari, M.; Gabriel, S. B.; Chirn, G. W.; Ma, Q.; Parikh, H.; Richardson, D.; Ricke, D.; Purcell, S., Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* **2007,** 316, (5829), 1331-6.
15. Sanghera, D. K.; Blackett, P. R., Type 2 Diabetes Genetics: Beyond GWAS. *Journal of diabetes & metabolism* **2012,** 3, (198).
16. Saxena, R.; Elbers, C. C.; Guo, Y.; Peter, I.; Gaunt, T. R.; Mega, J. L.; Lanktree, M. B.; Tare, A.; Castillo, B. A.; Li, Y. R.; Johnson, T.; Bruinenberg, M.; Gilbert-Diamond, D.; Rajagopalan, R.; Voight, B. F.; Balasubramanyam, A.; Barnard, J.; Bauer, F.; Baumert, J.; Bhangale, T.; Bohm, B. O.; Braund, P. S.; Burton, P. R.; Chandrupatla, H. R.; Clarke, R.; Cooper-DeHoff, R. M.; Crook, E. D.; Davey-Smith, G.; Day, I. N.; de Boer, A.; de Groot, M. C.; Drenos, F.; Ferguson, J.; Fox, C. S.; Furlong, C. E.; Gibson, Q.; Gieger, C.; Gilhuijs-Pederson, L. A.; Glessner, J. T.; Goel, A.; Gong, Y.; Grant, S. F.; Grobbee, D. E.; Hastie, C.; Humphries, S. E.; Kim, C. E.; Kivimaki, M.; Kleber, M.; Meisinger, C.; Kumari, M.; Langaee, T. Y.; Lawlor, D. A.; Li, M.; Lobmeyer, M. T.; Maitland-van der Zee, A. H.; Meijs, M. F.; Molony, C. M.; Morrow, D. A.; Murugesan, G.; Musani, S. K.; Nelson, C. P.; Newhouse, S. J.; O'Connell, J. R.; Padmanabhan, S.; Palmen, J.; Patel, S. R.; Pepine, C. J.; Pettinger, M.; Price, T. S.; Rafelt, S.; Ranchalis, J.; Rasheed, A.; Rosenthal, E.; Ruczinski, I.; Shah, S.; Shen, H.; Silbernagel, G.; Smith, E. N.; Spijkerman, A. W.; Stanton, A.; Steffes, M. W.; Thorand, B.; Trip, M.; van der Harst, P.; van der, A. D.; van Iperen, E. P.; van Setten, J.; van Vliet-Ostaptchouk, J. V.; Verweij, N.; Wolffenbuttel, B. H.; Young, T.; Zafarmand, M. H.; Zmuda, J. M.; Look, A. R. G.; consortium, D.; Boehnke, M.; Altshuler, D.; McCarthy, M.; Kao, W. H.; Pankow, J. S.; Cappola, T. P.; Sever, P.; Poulter, N.; Caulfield, M.; Dominiczak, A.; Shields, D. C.; Bhatt, D. L.; Zhang, L.; Curtis, S. P.; Danesh, J.; Casas, J. P.; van der Schouw, Y. T.; Onland-Moret, N. C.; Doevendans, P. A.; Dorn, G. W., 2nd; Farrall, M.; FitzGerald, G. A.; Hamsten, A.; Hegele, R.; Hingorani, A. D.; Hofker, M. H.; Huggins, G. S.; Illig, T.; Jarvik, G. P.; Johnson, J. A.; Klungel, O. H.; Knowler, W. C.; Koenig, W.; Marz, W.; Meigs, J. B.; Melander, O.; Munroe, P. B.; Mitchell, B. D.; Bielinski, S. J.; Rader, D. J.; Reilly, M. P.; Rich, S. S.; Rotter, J. I.; Saleheen, D.; Samani, N. J.; Schadt, E. E.; Shuldiner, A. R.; Silverstein, R.; Kottke-Marchant, K.; Talmud, P. J.; Watkins, H.; Asselbergs, F. W.; de Bakker, P. I.; McCaffery, J.; Wijmenga, C.; Sabatine, M. S.; Wilson, J. G.; Reiner, A.; Bowden, D. W.; Hakonarson, H.; Siscovick, D. S.; Keating, B. J., Large-scale gene-centric meta-analysis across 39 studies identifies type 2 diabetes loci. *Am J Hum Genet* **2012,** 90, (3), 410-25.
17. Salonen, J. T.; Uimari, P.; Aalto, J. M.; Pirskanen, M.; Kaikkonen, J.; Todorova, B.; Hypponen, J.; Korhonen, V. P.; Asikainen, J.; Devine, C.; Tuomainen, T. P.;

Luedemann, J.; Nauck, M.; Kerner, W.; Stephens, R. H.; New, J. P.; Ollier, W. E.; Gibson, J. M.; Payton, A.; Horan, M. A.; Pendleton, N.; Mahoney, W.; Meyre, D.; Delplanque, J.; Froguel, P.; Luzzatto, O.; Yakir, B.; Darvasi, A., Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. *Am J Hum Genet* **2007,** 81, (2), 338-45.

18. Scott, L. J.; Mohlke, K. L.; Bonnycastle, L. L.; Willer, C. J.; Li, Y.; Duren, W. L.; Erdos, M. R.; Stringham, H. M.; Chines, P. S.; Jackson, A. U.; Prokunina-Olsson, L.; Ding, C. J.; Swift, A. J.; Narisu, N.; Hu, T.; Pruim, R.; Xiao, R.; Li, X. Y.; Conneely, K. N.; Riebow, N. L.; Sprau, A. G.; Tong, M.; White, P. P.; Hetrick, K. N.; Barnhart, M. W.; Bark, C. W.; Goldstein, J. L.; Watkins, L.; Xiang, F.; Saramies, J.; Buchanan, T. A.; Watanabe, R. M.; Valle, T. T.; Kinnunen, L.; Abecasis, G. R.; Pugh, E. W.; Doheny, K. F.; Bergman, R. N.; Tuomilehto, J.; Collins, F. S.; Boehnke, M., A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* **2007,** 316, (5829), 1341-5.
19. Sladek, R.; Rocheleau, G.; Rung, J.; Dina, C.; Shen, L.; Serre, D.; Boutin, P.; Vincent, D.; Belisle, A.; Hadjadj, S.; Balkau, B.; Heude, B.; Charpentier, G.; Hudson, T. J.; Montpetit, A.; Pshezhetsky, A. V.; Prentki, M.; Posner, B. I.; Balding, D. J.; Meyre, D.; Polychronakos, C.; Froguel, P., A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* **2007,** 445, (7130), 881-5.
20. Huang, J.; Ellinghaus, D.; Franke, A.; Howie, B.; Li, Y., 1000 Genomes-based imputation identifies novel and refined associations for the Wellcome Trust Case Control Consortium phase 1 Data. *European journal of human genetics : EJHG* **2012,** 20, (7), 801-5.
21. Zhan, Y.; Zhang, R.; Lv, H.; Song, X.; Xu, X.; Chai, L.; Lv, W.; Shang, Z.; Jiang, Y.; Zhang, R., Prioritization of candidate genes for periodontitis using multiple computational tools. *Journal of periodontology* **2014,** 85, (8), 1059-69.
22. Munz, M.; Richter, G. M.; Loos, B. G.; Jepsen, S.; Divaris, K.; Offenbacher, S.; Teumer, A.; Holtfreter, B.; Kocher, T.; Bruckmann, C.; Jockel-Schneider, Y.; Graetz, C.; Munoz, L.; Bhandari, A.; Tennstedt, S.; Staufenbiel, I.; van der Velde, N.; Uitterlinden, A. G.; de Groot, L.; Wellmann, J.; Berger, K.; Krone, B.; Hoffmann, P.; Laudes, M.; Lieb, W.; Franke, A.; Dommisch, H.; Erdmann, J.; Schaefer, A. S., Genome-wide association meta-analysis of coronary artery disease and periodontitis reveals a novel shared risk locus. *Scientific reports* **2018,** 8, (1), 13678.
23. Munz, M.; Willenborg, C.; Richter, G. M.; Jockel-Schneider, Y.; Graetz, C.; Staufenbiel, I.; Wellmann, J.; Berger, K.; Krone, B.; Hoffmann, P.; van der Velde, N.; Uitterlinden, A. G.; de Groot, L.; Sawalha, A. H.; Direskeneli, H.; Saruhan-Direskeneli, G.; Guzeldemir-Akcakanat, E.; Keceli, H. G.; Laudes, M.; Noack, B.; Teumer, A.; Holtfreter, B.; Kocher, T.; Eickholz, P.; Meyle, J.; Doerfer, C.; Bruckmann, C.; Lieb, W.; Franke, A.; Schreiber, S.; Nohutcu, R. M.; Erdmann, J.; Loos, B. G.; Jepsen, S.; Dommisch, H.; Schaefer, A. S., A genome-wide association study identifies nucleotide variants at SIGLEC5 and DEFA1A3 as risk loci for periodontitis. *Hum Mol Genet* **2018,** 27, (5), 941-942.
24. Igl, B. W.; Konig, I. R.; Ziegler, A., What do we mean by 'replication' and 'validation' in genome-wide association studies? *Human heredity* **2009,** 67, (1), 66-8.
25. Miyake, Y.; Tanaka, K.; Arakawa, M., IL3 rs40401 polymorphism and interaction with smoking in risk of asthma in Japanese women: the Kyushu Okinawa Maternal and Child Health study. *Scandinavian journal of immunology* **2014,** 79, (6), 410-4.
26. Nordestgaard, B. G.; Langsted, A.; Mora, S.; Kolovou, G.; Baum, H.; Bruckert, E.; Watts, G. F.; Sypniewska, G.; Wiklund, O.; Borén, J., Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including

flagging at desirable concentration cut-points—a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. *European heart journal* **2016,** 37, (25), 1944-1958.

27. Page, R. C.; Eke, P. I., Case definitions for use in population-based surveillance of periodontitis. *Journal of periodontology* **2007,** 78, (7 Suppl), 1387-99.
28. American Diabetes, A., 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. *Diabetes care* **2019,** 42, (Suppl 1), S13-S28.
29. American Diabetes, A., 6. Glycemic Targets: Standards of Medical Care in Diabetes-2019. *Diabetes care* **2019,** 42, (Suppl 1), S61-S70.
30. Aidar, M.; Line, S. R., A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. *Brazilian dental journal* **2007,** 18, (2), 148-52.
31. Purcell, S.; Neale, B.; Todd-Brown, K.; Thomas, L.; Ferreira, M. A.; Bender, D.; Maller, J.; Sklar, P.; de Bakker, P. I.; Daly, M. J.; Sham, P. C., PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **2007,** 81, (3), 559-75.
32. Polak, D.; Shapira, L., An update on the evidence for pathogenic mechanisms that may link periodontitis and diabetes. *J Clin Periodontol* **2018,** 45, (2), 150-166.
33. Ye, Z.; Wang, Z.; Hou, Y., Does Bonferroni correction "rescue" the deviation from Hardy-Weinberg equilibrium? *Forensic science international. Genetics* **2020,** 46, 102254.
34. Rojo-Botello, N. R.; Garcia-Hernandez, A. L.; Moreno-Fierros, L., Expression of toll-like receptors 2, 4 and 9 is increased in gingival tissue from patients with type 2 diabetes and chronic periodontitis. *J Periodontal Res* **2012,** 47, (1), 62-73.
35. Kajita, K.; Honda, T.; Amanuma, R.; Domon, H.; Okui, T.; Ito, H.; Yoshie, H.; Tabeta, K.; Nakajima, T.; Yamazaki, K., Quantitative messenger RNA expression of Toll-like receptors and interferon-alpha1 in gingivitis and periodontitis. *Oral microbiology and immunology* **2007,** 22, (6), 398-402.
36. Holla, L. I.; Vokurka, J.; Hrdlickova, B.; Augustin, P.; Fassmann, A., Association of Toll-like receptor 9 haplotypes with chronic periodontitis in Czech population. *J Clin Periodontol* **2010,** 37, (2), 152-9.
37. Ashok, N.; Warad, S.; Kalburgi, N. B.; Bilichodmath, S.; Prabhakaran, P. S.; Tarakji, B., Toll-like receptor 9 gene polymorphism in chronic and aggressive periodontitis patients. *Journal of Indian Society of Periodontology* **2014,** 18, (6), 723-7.
38. Sahingur, S. E.; Xia, X. J.; Gunsolley, J.; Schenkein, H. A.; Genco, R. J.; De Nardin, E., Single nucleotide polymorphisms of pattern recognition receptors and chronic periodontitis. *J Periodontal Res* **2011,** 46, (2), 184-92.
39. Liu, F.; Lu, W.; Qian, Q.; Qi, W.; Hu, J.; Feng, B., Frequency of TLR 2, 4, and 9 gene polymorphisms in Chinese population and their susceptibility to type 2 diabetes and coronary artery disease. *Journal of biomedicine & biotechnology* **2012,** 2012, 373945.
40. Wifi, M. A.; Assem, M.; Elsherif, R. H.; El-Azab, H. A.; Saif, A., Toll-like receptors-2 and -9 (TLR2 and TLR9) gene polymorphism in patients with type 2 diabetes and diabetic foot. *Medicine* **2017,** 96, (17), e6760.
41. Neighbors, M.; Xu, X.; Barrat, F. J.; Ruuls, S. R.; Churakova, T.; Debets, R.; Bazan, J. F.; Kastelein, R. A.; Abrams, J. S.; O'Garra, A., A critical role for interleukin 18 in primary and memory effector responses to Listeria monocytogenes that extends beyond its effects on Interferon gamma production. *The Journal of experimental medicine* **2001,** 194, (3), 343-54.
42. Leung, B. P.; Culshaw, S.; Gracie, J. A.; Hunter, D.; Canetti, C. A.; Campbell, C.; Cunha, F.; Liew, F. Y.; McInnes, I. B., A role for IL-18 in neutrophil activation. *J Immunol* **2001,** 167, (5), 2879-86.

43. Tanaka, K.; Miyake, Y.; Hanioka, T.; Furukawa, S.; Miyatake, N.; Arakawa, M., The IL18 Promoter Polymorphism, rs1946518, Is Associated with the Risk of Periodontitis in Japanese Women: The Kyushu Okinawa Maternal and Child Health Study. *The Tohoku journal of experimental medicine* **2017,** 243, (3), 159-164.
44. Martelli, F. S.; Mengoni, A.; Martelli, M.; Rosati, C.; Fanti, E., IL-18 gene promoter polymorphisms are only moderately associated with periodontal disease in Italian population. *Clinical cases in mineral and bone metabolism : the official journal of the Italian Society of Osteoporosis, Mineral Metabolism, and Skeletal Diseases* **2012,** 9, (3), 153-6.
45. Li, Z. G.; Li, J. J.; Sun, C. A.; Jin, Y.; Wu, W. W., Interleukin-18 promoter polymorphisms and plasma levels are associated with increased risk of periodontitis: a meta-analysis. *Inflammation research : official journal of the European Histamine Research Society ... [et al.]* **2014,** 63, (1), 45-52.
46. Haghvirdizadeh, P.; Mohamed, Z.; Abdullah, N. A.; Haghvirdizadeh, P.; Haerian, M. S.; Haerian, B. S., KCNJ11: Genetic Polymorphisms and Risk of Diabetes Mellitus. *Journal of diabetes research* **2015,** 2015, 908152.
47. Timpson, N. J.; Lindgren, C. M.; Weedon, M. N.; Randall, J.; Ouwehand, W. H.; Strachan, D. P.; Rayner, N. W.; Walker, M.; Hitman, G. A.; Doney, A. S.; Palmer, C. N.; Morris, A. D.; Hattersley, A. T.; Zeggini, E.; Frayling, T. M.; McCarthy, M. I., Adiposity-related heterogeneity in patterns of type 2 diabetes susceptibility observed in genome-wide association data. *Diabetes* **2009,** 58, (2), 505-10.
48. Suzuki, K.; Akiyama, M.; Ishigaki, K.; Kanai, M.; Hosoe, J.; Shojima, N.; Hozawa, A.; Kadota, A.; Kuriki, K.; Naito, M.; Tanno, K.; Ishigaki, Y.; Hirata, M.; Matsuda, K.; Iwata, N.; Ikeda, M.; Sawada, N.; Yamaji, T.; Iwasaki, M.; Ikegawa, S.; Maeda, S.; Murakami, Y.; Wakai, K.; Tsugane, S.; Sasaki, M.; Yamamoto, M.; Okada, Y.; Kubo, M.; Kamatani, Y.; Horikoshi, M.; Yamauchi, T.; Kadowaki, T., Identification of 28 new susceptibility loci for type 2 diabetes in the Japanese population. *Nature genetics* **2019,** 51, (3), 379-386.
49. Zeggini, E.; Weedon, M. N.; Lindgren, C. M.; Frayling, T. M.; Elliott, K. S.; Lango, H.; Timpson, N. J.; Perry, J. R.; Rayner, N. W.; Freathy, R. M.; Barrett, J. C.; Shields, B.; Morris, A. P.; Ellard, S.; Groves, C. J.; Harries, L. W.; Marchini, J. L.; Owen, K. R.; Knight, B.; Cardon, L. R.; Walker, M.; Hitman, G. A.; Morris, A. D.; Doney, A. S.; Wellcome Trust Case Control, C.; McCarthy, M. I.; Hattersley, A. T., Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* **2007,** 316, (5829), 1336-41.
50. Zeggini, E.; Scott, L. J.; Saxena, R.; Voight, B. F.; Marchini, J. L.; Hu, T.; de Bakker, P. I.; Abecasis, G. R.; Almgren, P.; Andersen, G.; Ardlie, K.; Bostrom, K. B.; Bergman, R. N.; Bonnycastle, L. L.; Borch-Johnsen, K.; Burtt, N. P.; Chen, H.; Chines, P. S.; Daly, M. J.; Deodhar, P.; Ding, C. J.; Doney, A. S.; Duren, W. L.; Elliott, K. S.; Erdos, M. R.; Frayling, T. M.; Freathy, R. M.; Gianniny, L.; Grallert, H.; Grarup, N.; Groves, C. J.; Guiducci, C.; Hansen, T.; Herder, C.; Hitman, G. A.; Hughes, T. E.; Isomaa, B.; Jackson, A. U.; Jorgensen, T.; Kong, A.; Kubalanza, K.; Kuruvilla, F. G.; Kuusisto, J.; Langenberg, C.; Lango, H.; Lauritzen, T.; Li, Y.; Lindgren, C. M.; Lyssenko, V.; Marvelle, A. F.; Meisinger, C.; Midthjell, K.; Mohlke, K. L.; Morken, M. A.; Morris, A. D.; Narisu, N.; Nilsson, P.; Owen, K. R.; Palmer, C. N.; Payne, F.; Perry, J. R.; Pettersen, E.; Platou, C.; Prokopenko, I.; Qi, L.; Qin, L.; Rayner, N. W.; Rees, M.; Roix, J. J.; Sandbaek, A.; Shields, B.; Sjogren, M.; Steinthorsdottir, V.; Stringham, H. M.; Swift, A. J.; Thorleifsson, G.; Thorsteinsdottir, U.; Timpson, N. J.; Tuomi, T.; Tuomilehto, J.; Walker, M.; Watanabe, R. M.; Weedon, M. N.; Willer, C. J.; Wellcome Trust Case Control, C.; Illig, T.; Hveem, K.;

Hu, F. B.; Laakso, M.; Stefansson, K.; Pedersen, O.; Wareham, N. J.; Barroso, I.; Hattersley, A. T.; Collins, F. S.; Groop, L.; McCarthy, M. I.; Boehnke, M.; Altshuler, D., Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nature genetics* **2008,** 40, (5), 638-45.

51. Chavali, S.; Mahajan, A.; Tabassum, R.; Dwivedi, O. P.; Chauhan, G.; Ghosh, S.; Tandon, N.; Bharadwaj, D., Association of variants in genes involved in pancreatic beta-cell development and function with type 2 diabetes in North Indians. *Journal of human genetics* **2011,** 56, (10), 695-700.
52. Liu, Z.; Zhang, Y. W.; Feng, Q. P.; Li, Y. F.; Wu, G. D.; Zuo, J.; Xiao, X. H.; Fang, F. D., [Association analysis of 30 type 2 diabetes candidate genes in Chinese Han population]. *Zhongguo yi xue ke xue yuan xue bao. Acta Academiae Medicinae Sinicae* **2006,** 28, (2), 124-8.
53. Sakamoto, Y.; Inoue, H.; Keshavarz, P.; Miyawaki, K.; Yamaguchi, Y.; Moritani, M.; Kunika, K.; Nakamura, N.; Yoshikawa, T.; Yasui, N.; Shiota, H.; Tanahashi, T.; Itakura, M., SNPs in the KCNJ11-ABCC8 gene locus are associated with type 2 diabetes and blood pressure levels in the Japanese population. *Journal of human genetics* **2007,** 52, (10), 781-793.
54. Koo, B. K.; Cho, Y. M.; Park, B. L.; Cheong, H. S.; Shin, H. D.; Jang, H. C.; Kim, S. Y.; Lee, H. K.; Park, K. S., Polymorphisms of KCNJ11 (Kir6.2 gene) are associated with Type 2 diabetes and hypertension in the Korean population. *Diabetic medicine : a journal of the British Diabetic Association* **2007,** 24, (2), 178-86.
55. Hotta, K.; Kitamoto, A.; Kitamoto, T.; Mizusawa, S.; Teranishi, H.; So, R.; Matsuo, T.; Nakata, Y.; Hyogo, H.; Ochi, H.; Nakamura, T.; Kamohara, S.; Miyatake, N.; Kotani, K.; Komatsu, R.; Itoh, N.; Mineo, I.; Wada, J.; Yoneda, M.; Nakajima, A.; Funahashi, T.; Miyazaki, S.; Tokunaga, K.; Masuzaki, H.; Ueno, T.; Chayama, K.; Hamaguchi, K.; Yamada, K.; Hanafusa, T.; Oikawa, S.; Yoshimatsu, H.; Sakata, T.; Tanaka, K.; Matsuzawa, Y.; Nakao, K.; Sekine, A., Association between type 2 diabetes genetic susceptibility loci and visceral and subcutaneous fat area as determined by computed tomography. *Journal of human genetics* **2012,** 57, (5), 305-10.
56. Odgerel, Z.; Lee, H. S.; Erdenebileg, N.; Gandbold, S.; Luvsanjamba, M.; Sambuughin, N.; Sonomtseren, S.; Sharavdorj, P.; Jodov, E.; Altaisaikhan, K.; Goldfarb, L. G., Genetic variants in potassium channels are associated with type 2 diabetes in a Mongolian population. *Journal of diabetes* **2012,** 4, (3), 238-42.
57. Heni, M.; Ketterer, C.; Thamer, C.; Herzberg-Schafer, S. A.; Guthoff, M.; Stefan, N.; Machicao, F.; Staiger, H.; Fritsche, A.; Haring, H. U., Glycemia determines the effect of type 2 diabetes risk genes on insulin secretion. *Diabetes* **2010,** 59, (12), 3247-52.
58. Abdelhamid, I.; Lasram, K.; Meiloud, G.; Ben Halim, N.; Kefi, R.; Samb, A.; Abdelhak, S.; Houmeida, A., E23K variant in KCNJ11 gene is associated with susceptibility to type 2 diabetes in the Mauritanian population. *Primary care diabetes* **2014,** 8, (2), 171-5.
59. Hu, C.; Zhang, R.; Wang, C.; Wang, J.; Ma, X.; Lu, J.; Qin, W.; Hou, X.; Wang, C.; Bao, Y.; Xiang, K.; Jia, W., PPARG, KCNJ11, CDKAL1, CDKN2A-CDKN2B, IDE-KIF11-HHEX, IGF2BP2 and SLC30A8 are associated with type 2 diabetes in a Chinese population. *PloS one* **2009,** 4, (10), e7643.
60. Zhou, D.; Zhang, D.; Liu, Y.; Zhao, T.; Chen, Z.; Liu, Z.; Yu, L.; Zhang, Z.; Xu, H.; He, L., The E23K variation in the KCNJ11 gene is associated with type 2 diabetes in Chinese and East Asian population. *Journal of human genetics* **2009,** 54, (7), 433-5.

61. Sesti, G.; Federici, M.; Hribal, M. L.; Lauro, D.; Sbraccia, P.; Lauro, R., Defects of the insulin receptor substrate (IRS) system in human metabolic disorders. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2001,** 15, (12), 2099-111.
62. Rung, J.; Cauchi, S.; Albrechtsen, A.; Shen, L.; Rocheleau, G.; Cavalcanti-Proenca, C.; Bacot, F.; Balkau, B.; Belisle, A.; Borch-Johnsen, K.; Charpentier, G.; Dina, C.; Durand, E.; Elliott, P.; Hadjadj, S.; Jarvelin, M. R.; Laitinen, J.; Lauritzen, T.; Marre, M.; Mazur, A.; Meyre, D.; Montpetit, A.; Pisinger, C.; Posner, B.; Poulsen, P.; Pouta, A.; Prentki, M.; Ribel-Madsen, R.; Ruokonen, A.; Sandbaek, A.; Serre, D.; Tichet, J.; Vaxillaire, M.; Wojtaszewski, J. F.; Vaag, A.; Hansen, T.; Polychronakos, C.; Pedersen, O.; Froguel, P.; Sladek, R., Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. *Nature genetics* **2009,** 41, (10), 1110-5.
63. Haghani, K.; Bakhtiyari, S., The study on the relationship between IRS-1 Gly972Arg and IRS-2 Gly1057Asp polymorphisms and type 2 diabetes in the Kurdish ethnic group in West Iran. *Genet Test Mol Biomarkers* **2012,** 16, (11), 1270-6.
64. Li, Q.; Qiao, Y.; Wang, C.; Zhang, G.; Zhang, X.; Xu, L., Associations between two single-nucleotide polymorphisms (rs1801278 and rs2943641) of insulin receptor substrate 1 gene and type 2 diabetes susceptibility: a meta-analysis. *Endocrine* **2016,** 51, (1), 52-62.
65. Voight, B. F.; Scott, L. J.; Steinthorsdottir, V.; Morris, A. P.; Dina, C.; Welch, R. P.; Zeggini, E.; Huth, C.; Aulchenko, Y. S.; Thorleifsson, G.; McCulloch, L. J.; Ferreira, T.; Grallert, H.; Amin, N.; Wu, G.; Willer, C. J.; Raychaudhuri, S.; McCarroll, S. A.; Langenberg, C.; Hofmann, O. M.; Dupuis, J.; Qi, L.; Segre, A. V.; van Hoek, M.; Navarro, P.; Ardlie, K.; Balkau, B.; Benediktsson, R.; Bennett, A. J.; Blagieva, R.; Boerwinkle, E.; Bonnycastle, L. L.; Bengtsson Bostrom, K.; Bravenboer, B.; Bumpstead, S.; Burtt, N. P.; Charpentier, G.; Chines, P. S.; Cornelis, M.; Couper, D. J.; Crawford, G.; Doney, A. S.; Elliott, K. S.; Elliott, A. L.; Erdos, M. R.; Fox, C. S.; Franklin, C. S.; Ganser, M.; Gieger, C.; Grarup, N.; Green, T.; Griffin, S.; Groves, C. J.; Guiducci, C.; Hadjadj, S.; Hassanali, N.; Herder, C.; Isomaa, B.; Jackson, A. U.; Johnson, P. R.; Jorgensen, T.; Kao, W. H.; Klopp, N.; Kong, A.; Kraft, P.; Kuusisto, J.; Lauritzen, T.; Li, M.; Lieverse, A.; Lindgren, C. M.; Lyssenko, V.; Marre, M.; Meitinger, T.; Midthjell, K.; Morken, M. A.; Narisu, N.; Nilsson, P.; Owen, K. R.; Payne, F.; Perry, J. R.; Petersen, A. K.; Platou, C.; Proenca, C.; Prokopenko, I.; Rathmann, W.; Rayner, N. W.; Robertson, N. R.; Rocheleau, G.; Roden, M.; Sampson, M. J.; Saxena, R.; Shields, B. M.; Shrader, P.; Sigurdsson, G.; Sparso, T.; Strassburger, K.; Stringham, H. M.; Sun, Q.; Swift, A. J.; Thorand, B.; Tichet, J.; Tuomi, T.; van Dam, R. M.; van Haeften, T. W.; van Herpt, T.; van Vliet-Ostaptchouk, J. V.; Walters, G. B.; Weedon, M. N.; Wijmenga, C.; Witteman, J.; Bergman, R. N.; Cauchi, S.; Collins, F. S.; Gloyn, A. L.; Gyllensten, U.; Hansen, T.; Hide, W. A.; Hitman, G. A.; Hofman, A.; Hunter, D. J.; Hveem, K.; Laakso, M.; Mohlke, K. L.; Morris, A. D.; Palmer, C. N.; Pramstaller, P. P.; Rudan, I.; Sijbrands, E.; Stein, L. D.; Tuomilehto, J.; Uitterlinden, A.; Walker, M.; Wareham, N. J.; Watanabe, R. M.; Abecasis, G. R.; Boehm, B. O.; Campbell, H.; Daly, M. J.; Hattersley, A. T.; Hu, F. B.; Meigs, J. B.; Pankow, J. S.; Pedersen, O.; Wichmann, H. E.; Barroso, I.; Florez, J. C.; Frayling, T. M.; Groop, L.; Sladek, R.; Thorsteinsdottir, U.; Wilson, J. F.; Illig, T.; Froguel, P.; van Duijn, C. M.; Stefansson, K.; Altshuler, D.; Boehnke, M.; McCarthy, M. I.; investigators, M.; Consortium, G., Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nature genetics* **2010,** 42, (7), 579-89.

66. Tuerxunyiming, M.; Mohemaiti, P.; Wufuer, H.; Tuheti, A., Association of rs7754840 G/C polymorphisms in CDKAL1 with type 2 diabetes: a meta-analysis of 70141 subjects. *International journal of clinical and experimental medicine* **2015,** 8, (10), 17392-405.
67. Noury, A. E.; Azmy, O.; Alsharnoubi, J.; Salama, S.; Okasha, A.; Gouda, W., Variants of CDKAL1 rs7754840 (G/C) and CDKN2A/2B rs10811661 (C/T) with gestational diabetes: insignificant association. *BMC research notes* **2018,** 11, (1), 181.
68. Guo, F.; Long, W.; Zhou, W.; Zhang, B.; Liu, J.; Yu, B., FTO, GCKR, CDKAL1 and CDKN2A/B gene polymorphisms and the risk of gestational diabetes mellitus: a meta-analysis. *Archives of gynecology and obstetrics* **2018,** 298, (4), 705-715.
69. Liang, Z.; Wang, L.; Liu, H.; Chen, Y.; Zhou, T.; Heianza, Y.; Leng, J.; Li, W.; Yang, X.; Shen, Y.; Gao, R.; Hu, G.; Qi, L., Genetic susceptibility, lifestyle intervention and glycemic changes among women with prior gestational diabetes. *Clinical nutrition* **2019**.
70. Schaefer, A. S.; Richter, G. M.; Dommisch, H.; Reinartz, M.; Nothnagel, M.; Noack, B.; Laine, M. L.; Folwaczny, M.; Groessner-Schreiber, B.; Loos, B. G.; Jepsen, S.; Schreiber, S., CDKN2BAS is associated with periodontitis in different European populations and is activated by bacterial infection. *Journal of medical genetics* **2011,** 48, (1), 38-47.
71. Huang, K.; Zhong, J.; Li, Q.; Zhang, W.; Chen, Z.; Zhou, Y.; Wu, M.; Zhong, Z.; Lu, S.; Zhang, S., Effects of CDKN2B-AS1 polymorphisms on the susceptibility to coronary heart disease. *Molecular genetics & genomic medicine* **2019,** 7, (11), e955.
72. Jang, W.; Park, J.; Kwon, A.; Choi, H.; Kim, J.; Lee, G. D.; Han, E.; Jekarl, D. W.; Chae, H.; Han, K.; Yoon, J. H.; Lee, S.; Chung, N. G.; Cho, B.; Kim, M.; Kim, Y., CDKN2B downregulation and other genetic characteristics in T-acute lymphoblastic leukemia. *Experimental & molecular medicine* **2019,** 51, (1), 4.
73. Triggs-Raine, B. L.; Kirkpatrick, R. D.; Kelly, S. L.; Norquay, L. D.; Cattini, P. A.; Yamagata, K.; Hanley, A. J.; Zinman, B.; Harris, S. B.; Barrett, P. H.; Hegele, R. A., HNF-1alpha G319S, a transactivation-deficient mutant, is associated with altered dynamics of diabetes onset in an Oji-Cree community. *Proc Natl Acad Sci U S A* **2002,** 99, (7), 4614-9.
74. Ostoft, S. H.; Bagger, J. I.; Hansen, T.; Pedersen, O.; Faber, J.; Holst, J. J.; Knop, F. K.; Vilsboll, T., Glucose-lowering effects and low risk of hypoglycemia in patients with maturity-onset diabetes of the young when treated with a GLP-1 receptor agonist: a double-blind, randomized, crossover trial. *Diabetes care* **2014,** 37, (7), 1797-805.
75. Andersen, G.; Hansen, T.; Pedersen, O., Genetics of common forms of glycaemia with pathological impact on vascular biology: are we on the right track? *Current molecular medicine* **2005,** 5, (3), 261-74.
76. Jonsson, A.; Ladenvall, C.; Ahluwalia, T. S.; Kravic, J.; Krus, U.; Taneera, J.; Isomaa, B.; Tuomi, T.; Renstrom, E.; Groop, L.; Lyssenko, V., Effects of common genetic variants associated with type 2 diabetes and glycemic traits on alpha- and beta-cell function and insulin action in humans. *Diabetes* **2013,** 62, (8), 2978-83.
77. Parra, F. C.; Amado, R. C.; Lambertucci, J. R.; Rocha, J.; Antunes, C. M.; Pena, S. D., Color and genomic ancestry in Brazilians. *Proc Natl Acad Sci U S A* **2003,** 100, (1), 177-82.
78. Alves-Silva, J.; da Silva Santos, M.; Guimaraes, P. E.; Ferreira, A. C.; Bandelt, H. J.; Pena, S. D.; Prado, V. F., The ancestry of Brazilian mtDNA lineages. *Am J Hum Genet* **2000,** 67, (2), 444-61.

Table 1. Information regarding the 12 genotyped Single Nucleotide Polymorphisms (SNPs)

SNP	Assay ID	Position†, Alleles and type of SNP	Gene (SNP)	MAF‡		Call rate (%)§
rs5219	C__11654065_10	chr11:g.17409572T>A, Stop Gained	***KCNJ11***	**T|T**	0.31	96.51
rs5215	C___2991148_10	Chr11:g.17408630C>T, Missense Variant	***KCNJ11***	**A|A**	0.36	97.07
rs7957197	C__29094181_10	Chr12:g.121460686T>A, Intron Variant	***HNF1A***	**A|A**	0.35	96.65
rs2943641	C___1533178_10	Chr2:g.227093745T>C, Intergenic Variant	***IRS1***	**T|T**	0.17	96.09
rs7903146	C__29347861_10	Chr10:g.112998590 C>T, Intron Variant	***TCF7L2***	**T|T**	0.26	96.36
rs7754840	C__29246232_10	Chr6:g.20661019G>C , Intron Variant	***CDKAL1***	**G|G**	0.33	96.64
rs7018475	C__11259127_20	Chr9:g. 22137686T>G, Regulatory region variant	***CDKN2B***	**G|G**	0.32	96.92
rs1531343	C___1692218_10	chr12:g. 65781114G>C, Intron Variant	***RPSAP52***	**C|C**	0.33	96.93
rs6712932	C___2099176_10	Chr2:g. 105221141T>C, Substitution	***GPR45***	**T|T**	0.48	93.85
rs352140	C___2301954_20	chr18:g.52222681C>T, Synonymous Variant	***TLR9***	**T|T**	0.17	96.51
rs187238	C___2408543_10	chr11: g.112164265C>G, Upstream Variant	***IL18***	**G|G**	0.31	97.34
rs3826782	C__27506722_10	chr19:g.6887725G>A, Intron Variant	***EMR1***	**A|A**	0.46	95.67

† Genome Reference Consortium Human Build 37 (GRCh37.p13) or hg19

‡MAF: Minor allele frequence found for the total polulation investigated here.

§ Represents the amount of genotyping performed with success (all subjects), in percentage, for OpenArray™ plate and assays

Table 2. Comparison of demographic characteristics and periodontal parameters (comparisons performed by chi-square test or ANOVA with posttest Tukey's)

	Control n=356	Periodontitis n=358	Periodontitis+T2DM n=239
Demographic Characteristics			
Age, Mean (±SD)	43.6 (±11.1)[a]	49.1 (±9.5)[b]	56.2 (±9.5)[c]
Sex, n (%)			
Males	112 (32.5)	143 (41.1)	83 (41.3)
Females	233 (67.5)	205 (58.9)	118 (58.7)
Smoking habits, n (%)			
Never	268 (77.7)	224 (64.9)	115 (57.2)
Ever	77 (22.3)	124 (35.6)	86 (42.8)
Periodontal Parameters, Mean (±SD)			
Number of teeth	25.5 (±3.4)[a]	22.8 (±4.6)[b]	20.5 (±5.4)[c]
Visible plaque (% of sites)	20.4 (±20.0)[a]	45.7 (±28.2)[b]	56.4 (±26.9)[c]
Marginal bleeding (% of sites)	3.3 (±4.9)[a]	40.4 (±23.3)[b]	50.8 (±26.9)[b]
BOP (% of sites)	8.7 (±11.7)[a]	20.1 (±23.5)[b]	40.2 (±23.4)[c]
PPDi ≤ 4mm (% of sites)	99.9 (±0.30)[a]	86.6 (±14.3)[b]	79.9 (±22.5)[c]
PPDi ≥ 5mm (% of sites)	0.07 (±0.3)[a]	13.4 (±14.3)[b]	20.5 (±22.5)[c]
CALi ≤ 3mm (% of sites)	99.5 (±0.8)[a]	68.1 (±21.6)[b]	56.5 (±27.2)[c]
CALi = 4-5mm (% of sites)	0.5 (±0.7)[a]	20.1 (±11.9)[b]	24.9 (±15.3)[c]
CALi ≥ 6mm (% of sites)	0.04 (±0.2)[a]	11.8 (±14.2)[b]	18.5 (±20.6)[c]
Biochemical and Physical data, Mean (±SD)			
Fasting Blood Glucose	90.8 (±6.1)[a]	92.4 (±7.5)[a]	173.2 (±71.6)[b]
HbA_{1c}	5.3 (±0.26)[a]	5.4 (±0.27)[a]	9.2 (±10.32)[b]
Insulin	10.2 (±7.3)[a]	13.1 (±8.5)[a]	21.8 (±23.2)[b]
Total cholesterol	177.6 (±34.8)[a]	186.8 (±32.4)[ab]	196.3 (±46.5)[b]
HDL cholesterol	53.5 (±14.9)[a]	53.7 (±14.5)[a]	49.6 (±31.0)[a]
LDL cholesterol	101.5 (±31.7)[a]	99.2 (±30.9)[a]	112.5 (±57.5)[a]
Triglycerides	121.8 (±67.7)[a]	168.0 (±107.9)[b]	185.0 (±108.0)[b]
BMI (m/Kg^2)	27.2 (±5.1)[a]	27.1 (±4.2)[a]	30.2 (±5.3)[b]
Waist / Hip Ratio (cm)	0.85 (±0.18)[a]	0.88 (±0.09)[a]	1.03 (±0.78)[a]

Different letter: $p < 0.05$; Abbreviations: P, periodontitis; SD, standard deviation; BOP, bleeding on probing; PPDi, interproximal probing pocket depth; CALi, interproximal clinical attachment level.

Table 3. Multiple logistic regressions adjusted for age, sex and smoking statuses and stratified by sex and smoking

	Control *versus* Periodontitis		**Control *versus* P+T2DM**		**Periodontitis *versus* P+T2DM**	
	Adj OR (95% CI)	***p*-value**	**Adj OR (95% CI)**	***p*-value**	**Adj OR (95% CI)**	***p*-value**
rs5219-*KCNJ11*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref	
CT	0.78 (0.55 – 1.08)	0.13	0.84 (0.53 – 1.32)	0.45	0.89 (0.59 – 1.33)	0.57
TT	0.65 (0.37- 1.13)	0.13	0.67 (0.32 – 1.41)	0.29	0.77 (0.38 – 1.56)	0.47
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
CT	0.65 (0.37 – 1.15)	0.14	1.18 (0.52 – 2.67)	0.69	1.13 (0.59 – 2.19)	0.70
TT	1.21 (0.48 – 3.05)	0.69	1.44 (0.39 – 5.26)	0.58	0.65 (0.24 – 1.81)	0.42
Female ‡						
CC	Ref		Ref		Ref	
CT	0.86 (0.57 – 1.29)	0.47	0.72 (0.41 – 1.27)	0.26	0.74 (0.43 – 1.26)	0.27
TT	**0.41 (0.19 – 0.88)**	**0.02**	0.48 (0.19 – 1.24)	0.13	0.97 (0.36 – 2.63)	0.96
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
CT	**0.65 (0.44 – 0.96)**	**0.03**	0.94 (0.54 – 1.63)	0.83	1.31 (0.78 – 2.21)	0.31
TT	0.57 (0.29 – 1.16)	0.12	0.66 (0.26 – 1.68)	0.38	1.17 (0.47 – 2.95)	0.73
Ever Smoking ¶						
CC	Ref		Ref		Ref	
CT	1.19 (0.63 – 2.26)	0.58	0.61 (0.26 – 1.43)	0.26	**0.41 (0.19 – 0.83)**	**0.01**
TT	0.86 (0.33 – 2.21)	0.75	0.76 (0.21 – 2.76)	0.67	0.38 (0.12 – 1.18)	0.09
rs5215- *KCNJ11*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref			
AC	0.76 (0.55 – 1.06)	0.11	0.83 (0.53 – 1.30)	0.42	**0.58 (0.38 – 0.90)**	**0.02**
AA	0.67 (0.39- 1.17)	0.16	0.57 (0.27 – 1.22)	0.15	1.45 (0.81 – 2.60)	0.21
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
TC	0.65 (0.37 – 1.14)	0.13	0.97 (0.45 – 2.10)	0.94	1.07 (0.57 – 2.03)	0.83
TT	1.23 (0.50-2.99)	0.66	0.97 (0.26 – 3.58)	0.97	0.47 (0.16 – 1.34)	0.16
Female ‡						
CC	Ref		Ref		Ref	
TC	0.85 (0.56 – 1.27)	0.42	0.78 (0.45 – 1.34)	0.37	0.82 (0.49 – 1.37)	0.45
TT	**0.43 (0.21 – 0.91)**	**0.03**	0.46 (0.18 – 1.17)	0.10	0.91 (0.34 – 2.41)	0.84
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
TC	0.67 (0.45 – 0.99)	0.04	0.87 (0.51 – 1.49)	0.61	0.71 (0.36 – 1.40)	0.32
TT	0.61 (0.31 – 1.20)	0.15	0.80 (0.32 – 2.38)	0.65	0.74 (0.31 – 1.78)	0.51
Ever Smoking ¶						
CC	Ref		Ref		Ref	
TC	1.08 (0.58 – 2.02)	0.81	0.75 (0.34 – 1.66)	0.47	0.54 (0.27 – 1.06)	0.07
TT	0.85 (0.34-2.18)	0.74	0.63 (0.17 – 2.37)	0.49	0.31 (0.09 – 1.01)	0.05
rs7957197-*HNF1A*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
TT	Ref		Ref			
TA	**0.61 (0.43 – 0.88)**	**0.01**	0.98 (0.62 – 1.55)	0.94	**1.95 (1.26 – 3.02)**	**0.003***

AA	0.88 (0.39- 1.98)	0.76	1.10 (0.35 – 3.45)	0.87	1.18 (0.40 – 3.43)	0.77
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
TT	Ref		Ref		Ref	
TA	0.55 (0.30 – 1.02)	0.06	1.55 (0.70 – 3.43)	0.28	**3.06 (1.51 – 6.17)**	**0.002***
AA	0.39 (0.84-1.82)	0.23	1.78 (0.20-15.89)	0.61	1.33 (0.18 – 9.75)	0.78
Female ‡						
TT	Ref		Ref		Ref	
TA	0.65 (0.42 – 1.00)	0.05	0.77 (0.43 – 1.36)	0.37	1.43 (0.82 – 2.52)	0.21
AA	1.26 (0.48 – 3.27)	0.64	1.03 (0.26 – 3.99)	0.97	1.02 (0.29 – 3.64)	0.98
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
TT	Ref		Ref		Ref	
TA	**0.65 (0.42 – 0.98)**	**0.04**	0.91 (0.52 – 1.60)	0.75	1.59 (0.92 – 2.75)	0.10
AA	0.98 (0.37 – 2.62)	0.96	1.57 (0.45 – 5.40)	0.48	1.69 (0.50 – 5.75)	0.40
Ever Smoking ¶						
TT	Ref		Ref		Ref	
TA	0.53 (0.27 – 1.05)	0.07	1.08 (0.47 – 2.45)	0.86	**2.88 (1.34 – 6.19)**	**0.01**
AA	0.73 (0.18 - 2.95)	0.66	0.22 (0.01 – 4.05)	0.31	0.30 (0.02-3.87)	0.36
rs2943641- *IRS1*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref			
CT	1.20 (0.86 – 1.67)	0.29	0.71 (0.44 – 1.14)	0.16	**0.58 (0.38 – 0.90)**	**0.02**
TT	1.08 (0.62- 1.87)	0.79	1.75 (0.92 – 3.32)	0.09	1.45 (0.81 – 2.60)	0.21
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
CT	1.25 (0.70 – 2.21)	0.45	1.00 (0.42 – 2.39)	0.99	**0.46 (0.23 – 0.92)**	**0.03**
TT	1.13 (0.47-2.72)	0.78	1.50 (0.49-4.62)	0.48	0.88 (0.34 – 2.25)	0.78
Female ‡						
CC	Ref		Ref		Ref	
CT	1.13 (0.75 – 1.72)	0.55	0.63 (0.35 – 1.13)	0.12	0.68 (0.39 – 1.19)	0.17
TT	1.00 (0.49 – 2.03)	0.99	1.94 (0.89 – 4.26)	0.10	2.06 (0.97 – 4.38)	0.06
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
CT	1.16 (0.79 – 1.72)	0.45	0.58 (0.33– 1.04)	0.07	**0.55 (0.32 – 0.95)**	**0.03**
TT	1.27 (0.65 – 2.50)	0.48	1.76 (0.79 – 3.90)	0.16	1.20 (0.57 – 2.52)	0.64
Ever Smoking ¶						
CC	Ref		Ref		Ref	
CT	1.29 (0.68 – 2.44)	0.44	1.39 (0.59 – 3.28)	0.45	0.82 (0.38 – 1.74)	0.60
TT	0.79 (0.31- 2.02)	0.67	1.10 (0.34-3.58)	0.87	2.46 (0.91 – 6.63)	0.08
rs7903146-*TCF7L2*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref	
CT	0.93 (0.65 – 1.32)	0.69	1.26 (0.79 – 2.00)	0.32	1.05 (0.69 – 1.60)	0.79
TT	0.99 (0.56- 1.77)	0.99	**2.37 (1.25 – 4.51)**	**0.008**	**2.22 (1.24 – 4.01)**	**0.007**
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
CT	1.35 (0.75 – 2.45)	0.32	1.68 (0.71 – 3.93)	0.23	0.63 (0.32 – 1.22)	0.17
TT	1.06 (0.42 – 2.76)	0.89	2.99 (0.98– 9.06)	0.05	2.17 (0.85 – 5.56)	0.11
Female ‡						
CC	Ref		Ref		Ref	
CT	0.75 (0.48 - 1.16)	0.20	1.16 (0.67-2.04)	0.59	1.49 (0.87-2.56)	0.14
TT	1.01 (0.49-2.09)	0.97	**2.25 (1.02-5.02)**	**0.05**	**2.32 (1.09-4.94)**	**0.02**
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
CT	0.93 (0.61 – 1.41)	0.75	1.46 (0.83 – 2.60)	0.19	1.30 (0.77 –2.22)	0.33

TT	1.30 (0.67 – 2.51)	0.43	**2.93 (1.35 – 6.33)**	**0.006**	**2.19 (1.09 – 4.42)**	**0.03**
Ever Smoking ¶						
CC	Ref		Ref		Ref	
CT	0.89 (0.46 – 1.74)	0.74	0.92 (0.41 – 2.07)	0.85	0.76 (0.39 – 1.48)	0.42
TT	0.45 (0.14 – 1.44)	0.18	1.49 (0.47 – 4.71)	0.49	2.29 (0.77 – 6.89)	0.14
rs7754840-*CDKAL1*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref	
CG	**0.66 (0.47 – 0.92)**	**0.01**	1.19 (0.75 – 1.92)	0.45	**1.73 (1.13 – 2.66)**	**0.01**
GG	1.04 (0.63- 1.70)	0.88	1.58 (0.80 – 3.11)	0.19	1.50 (0.82 – 2.75)	0.18
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
CG	0.68 (0.39 – 1.20)	0.19	0.55 (0.23 – 1.33)	0.19	0.98 (0.50 – 1.94)	0.97
GG	0.77 (0.31 – 1.92)	0.58	0.74 (0.23 – 2.39)	0.62	1.67 (0.63 – 4.42)	0.29
Female ‡						
CC	Ref		Ref		Ref	
CG	**0.63 (0.41 – 0.97)**	**0.03**	1.61 (0.89 – 2.91)	0.11	**2.49 (1.41 – 4.42)**	**0.002***
GG	1.43 (0.74 – 2.77)	0.28	2.09 (0.89 – 4.92)	0.08	1.36 (0.61 – 3.02)	0.76
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
CG	**0.63 (0.42 – 0.95)**	**0.02**	1.08 (0.61 – 1.91)	0.78	1.57 (0.91 – 2.71)	0.10
GG	1.17 (0.64 – 2.13)	0.59	2.23 (1.01 – 4.91)	0.04	1.73 (0.83 – 3.58)	0.14
Ever Smoking ¶						
CC	Ref		Ref		Ref	
CG	0.71 (0.37 – 1.37)	0.31	1.47 (0.62 – 3.48)	0.37	1.93 (0.94 – 3.94)	0.07
GG	0.79 (0.33 – 1.91)	0.61	0.64 (0.17 – 2.44)	0.52	1.19 (0.38 – 3.67)	0.61
rs7018475-*CDKN2B*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
TT	Ref		Ref		Ref	
TG	1.21 (0.86 – 1.69)	0.26	1.21 (0.75 – 1.96)	0.43	0.90 (0.58 – 1.39)	0.63
GG	1.49 (0.86- 2.60)	0.16	**3.26 (1.63 – 6.56)**	**0.001***	1.81 (0.98 – 3.32)	0.05
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
TT	Ref		Ref		Ref	
TG	1.50 (0.86 – 2.62)	0.15	1.19 (0.52 – 2.70)	0.69	0.82 (0.41 – 1.64)	0.57
GG	1.61 (0.56 – 4.65)	0.37	1.39 (0.33 – 5.88)	0.65	1.40 (0.46 – 4.34)	0.55
Female ‡						
TT	Ref		Ref		Ref	
TG	1.07 (0.71 – 1.63)	0.73	1.26 (0.69 – 2.29)	0.44	0.96 (0.55 – 1.70)	0.91
GG	1.43 (0.74 – 2.77)	0.28	**3.91 (1.76 – 8.70)**	**0.001***	1.98 (0.96 – 4.12)	0.06
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
TT	Ref		Ref		Ref	
TG	0.98 (0.66 – 1.46)	0.95	1.19 (0.66 – 2.12)	0.56	1.15 (0.66 – 1.99)	0.62
GG	1.62 (0.83 – 3.15)	0.15	**4.28 (1.89 – 9.63)**	**0.000***	**2.12 (1.03 – 4.37)**	**0.04**
Ever Smoking ¶						
TT	Ref		Ref		Ref	
TG	**1.99 (1.05 – 3.77)**	**0.03**	1.32 (0.56 – 3.15)	0.53	0.62 (0.29 – 1.31)	0.22
GG	1.22 (0.44 – 3.36)	0.70	1.56 (0.41 – 5.96)	0.51	1.34 (0.45 – 4.26)	0.61
rs1531343-*RPSAP52*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
GG	Ref		Ref		Ref	
GC	**1.52 (1.06 – 2.19)**	**0.02**	**1.77 (1.11 – 2.81)**	**0.02**	1.12 (0.74 – 1.68)	0.59
CC	1.15 (0.49- 2.68)	0.34	0.34 (0.06 – 1.82)	0.21	0.36 (0.08 – 1.67)	0.19
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
GG	Ref		Ref		Ref	
GC	1.64 (0.87 – 3.09)	0.12	1.78 (0.73 – 4.35)	0.20	1.29 (0.65 – 2.54)	0.46

CC	1.12 (0.15 – 8.46)	0.91	2.95 (0.21 – 41.18)	0.42	1.70 (0.14 – 19.63)	0.67
Female ‡						
GG	Ref		Ref		Ref	
GC	1.49 (0.96 – 2.33)	0.07	**1.79 (1.03 – 3.10)**	**0.04**	1.05 (0.63 – 1.76)	0.84
CC	1.15 (0.46 – 2.91)	0.76	0.18 (0.02 – 1.58)	0.12	0.19 (0.02 – 1.59)	0.13
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
GG	Ref		Ref		Ref	
GC	1.51 (0.98 – 2.30)	0.06	1.59 (0.92 – 2.77)	0.10	1.05 (0.64 – 1.76)	0.83
CC	1.16 (0.43 – 3.13)	0.77	empty		empty	
Ever Smoking ¶						
GG	Ref		Ref		Ref	
GC	1.59 (0.79 – 3.19)	0.19	2.33 (0.97 – 5.62)	0.06	1.28 (0.64 – 2.58)	0.45
CC	1.12 (0.23 – 5.49)	0.88	1.82 (0.21 – 15.87)	0.59	1.13 (0.19 – 6.95)	0.89
rs6712932-*GPR45*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref	
CT	0.83 (0.59 – 1.15)	0.26	0.71 (0.44 –1.13)	0.15	0.88 (0.58 – 1.34)	0.57
TT	0.80 (0.48- 1.35)	0.41	0.69 (0.34 – 1.37)	0.28	0.77 (0.38 – 1.56)	0.19
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
CT	0.59 (0.33 – 1.08)	0.08	0.67 (0.29 – 1.53)	0.34	1.01 (0.52 – 1.90)	0.99
TT	**0.38 (0.15 – 0.95)**	**0.04**	0.82 (0.24 – 2.81)	0.75	1.46 (0.47 – 4.57)	0.51
Female ‡						
CC	Ref		Ref		Ref	
CT	0.96 (0.62 – 1.46)	0.83	0.79 (0.41 – 1.31)	0.30	0.69 (0.40 – 1.20)	0.20
TT	1.21 (0.64 – 2.28)	0.56	0.67 (0.28 – 1.62)	0.38	0.74 (0.32 – 1.66)	0.46
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
CT	1.03 (0.68 – 1.54)	0.88	0.81 (0.46 – 1.42)	0.46	0.69 (0.40 – 1.16)	0.16
TT	1.21 (0.66 – 2.19)	0.52	0.86 (0.38 – 1.95)	0.72	0.83 (0.39 - 1.76)	0.63
Ever Smoking ¶						
CC	Ref		Ref		Ref	
CT	**0.46 (0.24 – 0.89)**	**0.02**	0.52 (0.22 – 1.24)	0.14	1.04 (0.52 – 2.090)	0.89
TT	**0.23 (0.07 – 0.68)**	**0.008**	0.45 (0.11 – 1.79)	0.25	1.24 (0.30 – 5.17)	0.76
rs352140-*TLR9*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref	
CT	0.73 (0.50 – 1.07)	0.10	0.74 (0.45 – 1.22)	0.24	1.25 (0.81 – 1.93)	0.32
TT	1.66 (0.42- 1.04)	0.08	**0.47 (0.25 – 0.86)**	**0.02**	0.93 (0.54 – 1.58)	0.78
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
CT	1.03 (0.55 – 1.93)	0.92	1.02 (0.42 – 2.43)	0.97	1.21 (0.61 – 2.41)	0.58
TT	**0.39 (0.18 – 0.84)**	**0.02**	0.44 (0.15 – 1.24)	0.12	1.37 (0.58 – 3.23)	0.48
Female ‡						
CC	Ref		Ref		Ref	
CT	**0.60 (0.37 – 0.98)**	**0.04**	0.64 (0.34 – 1.18)	0.15	1.28 (0.72 – 2.26)	0.40
TT	0.84 (0.48 – 1.48)	0.55	0.48 (0.22 – 1.02)	0.06	0.73 (0.37 – 1.44)	0.36
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
CT	0.64 (0.41 – 1.01)	0.06	0.64 (0.35 – 1.17)	0.15	1.38 (0.79 – 2.40)	0.26
TT	0.65 (0.38 – 1.12)	0.12	**0.39 (0.18 – 0.84)**	**0.02**	0.75 (0.38 – 1.50)	0.42
Ever Smoking ¶						
CC	Ref		Ref		Ref	
CT	0.98 (0.49 – 1.95)	0.95	1.05 (0.51 – 2.16)	0.90	1.05 (0.51 – 2.16)	0.90
TT	0.65 (0.28 – 1.52)	0.32	1.19 (0.49 – 2.86)	0.70	1.19 (0.49 – 2.86)	0.70
rs187238-*IL18*						

All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref			
CG	0.91 (0.66 – 1.26)	0.57	1.02 (0.65 – 1.58)	0.95	1.03 (0.69 – 1.53)	0.90
GG	0.55 (0.27- 1.11)	0.10	1.13 (0.52 – 2.47)	0.75	2.24 (0.99 – 5.07)	0.05
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
CC	Ref		Ref		Ref	
CG	1.21 (0.69 – 2.11)	0.51	1.08 (0.49 – 2.39)	0.85	0.65 (0.34 – 1.23)	0.18
GG	0.69 (0.23 – 2.05)	0.50	0.81 (0.18 – 3.54)	0.78	1.07 (0.28 – 4.06)	0.92
Female ‡						
CC	Ref		Ref		Ref	
CG	0.79 (0.52-1.18)	0.25	1.04 (0.61 – 1.77)	0.90	1.36 (0.82 – 2.28)	0.24
GG	0.47 (0.19-1.20)	0.11	1.35 (0.54 – 3.38)	0.52	**3.62 (1.25 – 10.51)**	**0.02**
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
CC	Ref		Ref		Ref	
CG	0.86 (0.58 – 1.27)	0.44	0.97 (0.56 – 1.66)	0.90	1.12 (0.67 – 1.87)	0.66
GG	0.61 (0.27 – 1.36)	0.23	1.19 (0.48 – 2.92)	0.71	2.53 (0.97 – 6.64)	0.06
Ever Smoking ¶						
CC	Ref		Ref		Ref	
CG	1.06 (0.58 – 1.94)	0.85	1.15 (0.53 – 2.51)	0.72	0.82 (0.42 – 1.57)	0.54
GG	0.43 (0.11– 1.69)	0.23	1.04 (0.23 – 4.74)	0.96	1.48 (0.33 – 6.69)	0.61
rs3826782-*EMR1*						
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†	
GG	Ref		Ref			
GA	0.79 (0.50 – 1.12)	0.16	0.72 (0.34 – 1.43)	0.37	0.71 (0.38 – 1.33)	0.29
AA	1.33 (0.35- 5.02)	0.67	0.40 (0.03 – 4.66)	0.46	0.43 (0.04 – 3.71)	0.44
Male ‡	Adjusted OR‡		Adjusted OR‡		Adjusted OR‡	
GG	Ref		Ref		Ref	
GA	0.65 (0.33 – 1.31)	0.23	0.55 (0.16 – 1.91)	0.34	0.36 (0.13 – 1.03)	0.06
AA	0.46 (0.06-3.67)	0.47	0.53 (0.03-8.63)	0.66	0.81 (0.06 – 9.90)	0.87
Female ‡						
GG	Ref		Ref		Ref	
GA	0.79 (0.49 – 1.31)	0.37	0.88 (0.38 – 2.01)	0.76	1.05 (0.48 – 2.29)	0.90
AA	2.55 (0.45 – 14.56)	0.29	empty		empty	
Never Smoking ¶	Adjusted OR¶		Adjusted OR¶		Adjusted OR¶	
GG	Ref		Ref		Ref	
GA	0.87 (0.53 – 1.39)	0.56	0.96 (0.41 – 2.23)	0.91	0.79 (0.35 – 1.75)	0.56
AA	2.90 (0.51 – 16.53)	0.23	empty		empty	
Ever Smoking ¶						
GG	Ref		Ref		Ref	
GA	0.53 (0.25 – 1.12)	0.09	0.47 (0.15 – 1.49)	0.20	0.54 (0.37 – 1.49)	0.24
AA	0.38 (0.05-2.86)	0.35	0.40 (0.03 – 5.46)	0.49	0.86 (0.07-10.56)	0.90

Bold font indicates $p < 0.05$; *indicates statistical significance after Bonferroni correction ($p < 0.0042$).

† OR with 95% CI were estimated by multiple logistic regression models after controlling for age, sex and smoking.

‡ OR with 95% CI were estimated by multiple logistic regression models after controlling for age and smoking.

Never smokers = patients who never smoked; Ever smokers = current smokers + former smokers.

¶ OR with 95% CI were estimated by multiple logistic regression models after controlling for age and sex.

Abbreviations: OR, odds ratio; CI, confidence interval

Table 5. Multiple Logistic Regression adjusted for age, sex and smoking status stratified by different degrees of periodontitis (P_M, moderate periodontitis; P_S, severe periodontitis), as well by the T2DM glycemic control (P+T2DM_well, HbA1c ≤ 7.0% and ≥5.8%; P+T2DM_poor, HbA1c ≥ 7.1%). Median (minimum -maximum).

	Control *versus* P_M		**Control *versus* P_S**		**Control *versus* P+T2DM_well**		**Control *versus* P+T2DM_poor**	
	Adj OR (95% CI)	***p*-value**	**Adj OR (95% CI)**	***p*-value**	**Adj OR (95% CI)**	***p*-value**	**Adj OR (95% CI)**	***p*-value**
rs5219-*KCNJ11*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref		Ref	
CT	0.76 (0.47-1.24)	0.28	0.78 (0.54-1.13)	0.19	0.58 (0.28-1.24)	0.16	0.87 (0.49-1.54)	0.63
TT	**0.09 (0.02- 0.07)**	**0.02**	0.88 (0.49- 1.59)	0.68	0.41 (0.11-1.61)	0.20	0.83 (0.34-2.03)	0.69
rs5215-*KCNJ11*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref		Ref	
AC	0.79 (0.49 – 1.29)	0.35	0.75 (0.52 – 1.09)	0.09	0.61 (0.30 – 1.23)	0.17	0.90 (0.52 – 1.55)	0.70
AA	**0.18 (0.04 - 0.80)**	**0.02**	0.89 (0.50 – 1.58)	0.69	0.23 (0.05 – 1.15)	0.07	0.79 (0.33 – 1.90)	0.60
rs7957197-*HNF1A*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
TT	Ref		Ref		Ref		Ref	
TA	0.93 (0.55 – 1.57)	0.79	**0.53 (0.35 – 0.80)**	**0.002***	0.88 (0.42 – 1.85)	0.74	1.13 (0.64 – 2.00)	0.67
AA	1.15 (0.36- 3.65)	0.82	0.73 (0.29 – 1.82)	0.50	1.41 (0.24 – 8.27)	0.71	1.66 (0.47 – 5.88)	0.44
rs2943641- *IRS1*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref		Ref	
CT	1.38 (0.83 – 2.28)	0.22	1.15 (0.79 – 1.67)	0.46	1.01 (0.47 – 2.13)	0.99	0.72 (0.39 – 1.32)	0.29
TT	1.12 (0.47- 2.66)	0.80	1.09 (0.59 – 1.98)	0.79	1.62 (0.56 – 4.67)	0.37	2.12 (0.99 – 4.54)	0.06
rs2062377-*TCF7L2*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref		Ref	
CT	0.91 (0.52 – 1.58)	0.74	0.92 (0.62 – 1.36)	0.69	1.07 (0.50 – 2.28)	0.86	1.13 (0.64 – 2.01)	0.66
TT	1.31 (0.58- 2.95)	0.51	0.86 (0.44 – 1.66)	0.65	**3.42 (1.36 – 8.61)**	**0.009**	1.67 (0.72 – 3.82)	0.23
rs7754840-*CDKAL1*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref		Ref	
CG	**0.48 (0.28 – 0.83)**	**0.009**	0.72 (0.49 – 1.05)	0.09	1.23 (0.58 – 2.58)	0.58	1.22 (0.68 – 2.21)	0.49
GG	1.45 (0.75- 2.80)	0.26	0.79 (0.45 – 1.41)	0.44	1.28 (0.39 – 4.25)	0.68	1.98 (0.89 – 4.42)	0.09
rs7018475-*CDKN2B*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
TT	Ref		Ref		Ref		Ref	
TG	1.07 (0.64 – 1.76)	0.79	1.34 (0.92 – 1.94)	0.12	1.55 (0.75 – 3.20)	0.23	0.95 (0.51 – 1.75)	0.86
GG	0.98 (0.40- 2.40)	0.97	1.79 (0.97 – 3.32)	0.06	1.22 (0.29 – 5.00)	0.78	**3.21 (1.38 – 7.42)**	**0.006**
rs1531343-*RPSAP52*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
GG	Ref		Ref		Ref		Ref	
GC	1.49 (0.87 – 2.53)	0.14	**1.59 (1.06 – 2.38)**	**0.02**	1.23 (0.59 – 2.77)	0.53	**2.06 (1.16-3.64)**	**0.01**
CC	1.11 (0.30- 4.04)	0.87	1.15 (0.45 – 2.96)	0.76	empty		0.35 (0.38-3.11)	0.34
rs6712932-*GPR45*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref		Ref	
CT	0.61 (0.36 – 1.03)	0.06	0.95 (0.65 – 1.39)	0.79	**0.38 (0.17 – 0.82)**	**0.01**	0.76 (0.42-1.38)	0.38
TT	0.57 (0.24- 1.31)	0.19	0.89 (0.50 – 1.58)	0.69	0.28 (0.07 – 1.05)	0.06	0.89 (0.39-2.03)	0.78
rs352140-*TLR9*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref		Ref	
CT	0.75 (0.43 – 1.33)	0.33	0.73 (0.48 – 1.11)	0.14	0.68 (0.31 – 1.49)	0.34	0.84 (0.45 – 1.55)	0.57
TT	0.68 (0.34 - 1.36)	0.27	0.65 (0.36 – 1.07)	0.09	0.36 (0.13 – 1.99)	0.05	0.60 (0.28 – 1.27)	0.18

rs187238-*IL18*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
CC	Ref		Ref		Ref		Ref	
CG	0.91 (0.55 – 1.50)	0.72	0.89 (0.62 – 1.29)	0.56	1.02 (0.50 – 2.08)	0.95	0.89 (0.51 – 1.54)	0.67
GG	0.41 (0.12- 1.41)	0.16	0.64 (0.29 – 1.39)	0.26	2.24 (0.74 – 6.78)	0.15	1.32 (0.53 – 3.28)	0.54
rs3826782-*EMR1*								
All patients †	Adjusted OR†		Adjusted OR†		Adjusted OR†		Adjusted OR†	
GG	Ref		Ref		Ref		Ref	
GA	0.82 (0.46 – 3.32)	0.47	0.89 (0.54 – 5.67)	0.39	0.36 (002. – 3.53)	0.32	0.76 (0.41 – 4.22)	0.18
AA	1.45 (0.06- 7.87)	0.56	0.43 (0.02 – 6.56)	0.32	0.43 (0.07 – 5.72)	0.46	1.56 (0.12- 5.56)	0.77

Bold font indicates $p < 0.05$; *indicates statistical significance after Bonferroni correction ($p < 0.0042$).

† OR with 95% CI were estimated by multiple logistic regression models after controlling for age, sex and smoking.

HbA1c: Glycated Hemoglobin.

Supplementary Table 1. Alleles and genotype distribution of the SNPs compared among the groups (chi-square test), Minor Allele Frequency (MAF), and Hardy–Weinberg Equilibrium (HWE) data found in each group

	Control n=356	**Periodontitis n= 358**	**Periodontitis+T2DM n=239**
rs5219-*KCNJ11*	n (%)	n (%)	n (%)
Alleles C/T	458 (66.4)/222(32.2)	485 (69.7)/199(28.6)	269 (66.9)/ 101 (25.1)
MAF	0.33	0.29	0.27
Genotypes			
CC	152 (44.06)	174 (50.0)	99 (49.3)
CT	154 (44.6)	137 (39.4)	71 (35.3)
TT	34 (9.9)	31(8.9)	15 (7.5)
HWE	0.62	0.60	0.86
rs5215- *KCNJ11*	n (%)	n (%)	n (%)
Alleles C/A	414 (60.0)/ 190 (27.5)	420 (60.4)/ 194 (27.9)	240 (59.7)/ 160 (46.0)
MAF	0.32	0.31	0.40
Genotypes			
CC	142 (41.2)	144 (41.4)	78 (38.8)
CA	130 (37.7)	132 (37.9)	84 (41.8)
AA	30 (8.7)	31 (8.9)	38 (18.9)
HWE	1.00	0.90	0.04
rs7957197-*HNF1A*	n (%)	n (%)	n (%)
Alleles T/A	442 (64.1)/ 242 (35.1)	457 (65.7)/ 217 (31.2)	214 (53.2)/ 138 (34.3)
MAF	0.36	0.32	0.39
Genotypes			
TT	141 (40.9)	168 (48.3)	65 (32.3)
TA	160 (46.4)	121 (34.8)	84 (41.8)
AA	41 (11.9)	48 (13.8)	27 (13.4)
HWE	0.72	0.001	0.76
rs2943641- *IRS1*	n (%)	n (%)	n (%)
Alleles C/T	586 (84.9)/ 104 (15.1)	557 (80.03)/ 125 (18.0)	314 (78.1)/ 68 (16.9)
MAF	0.16	0.18	0.18
Genotypes			
CC	254 (73.6)	228 (65.5)	125 (62.2)
CT	78 (22.6)	101 (29.02)	64 (31.8)
TT	13 (3.8)	12 (3.5)	2 (1.0)

HWE	0.04	0.86	0.05
rs7903146-*TCF7L2*	n (%)	n (%)	n (%)
Alleles C/T	521 (75.5)/ 153 (22.2)	480 (69.0)/ 197 (28.3)	283 (70.4)/ 89 (23.4)
MAF	0.23	0.29	0.29
Genotypes			
CC	203 (58.8)	173 (49.7)	112 (55.7)
CT	115 (33.3)	134 (38.5)	59 (29.4)
TT	19 (5.5)	32 (9.2)	15 (7.5)
HWE	0.64	0.43	**0.07**
rs7754840-*CDKAL1*	n (%)	n (%)	n (%)
Alleles C/G	460 (66.7)/ 216 (31.3)	454 (65.2)/ 234 (33.6)	233 (58.0)/ 123 (30.6)
MAF	0.32	0.34	0.34
Genotypes			
CC	154 (44.6)	146 (42.0)	88 (43.8)
CG	152 (44.1)	162 (46.6)	57 (28.4)
GG	32 (9.3)	36 (10.3)	33 (16.4)
HWE	0.62	0.40	>0.001
rs7018475-*CDKN2B*	n (%)	n (%)	n (%)
Alleles T/G	494 (71.6)/ 190 (27.5)	468 (67.2)/ 214 (30.7)	213(53.0)/ 125 (31.1)
MAF	0.28	0.32	0.36
Genotypes			
TT	182 (52.8)	163 (46.8)	77 (38.3)
TG	130 (37.7)	142 (40.8)	59 (29.4)
GG	30 (8.7)	36 (10.3)	33 (16.4)
HWE	0.35	0.53	0.001
rs1531343-*RPSAP52*	n (%)	n (%)	n (%)
Alleles G/C	423 (61.3)/ 235 (34.1)	449 (64.5)/ 215 (30.9)	249 (61.9)/ 109 (27.1)
MAF	0.36	0.33	0.29
Genotypes			
GG	137 (39.7)	154 (44.3)	89 (44.3)
GC	149 (43.2)	141 (40.5)	71 (35.3)
CC	43 (12.5)	37 (10.6)	19 (9.5)
HWE	0.81	0.62	0.39
rs6712932-*GPR45*	n (%)	n (%)	n (%)
Alleles C/T	333 (48.3)/ 349(50.6)	375 (53.9)/ 311 (44.7)	209 (52.0)/ 183 (45.5)
MAF	0.49	0.45	0.46
Genotypes			
CC	74(21.5)	105 (30.2)	55 (27.4)
CT	185(53.6)	165(47.4)	99 (49.3)
TT	82 (24.4)	73(21.0)	42 (20.9)
HWE	0.13	0.59	0.89
rs352140-*TLR9*	n (%)	n (%)	n (%)
Alleles C/T	504 (66.8)/ 188 (27.3)	524 (75.3)/ 164 (23.6)	285 (70.9)/ 103 (25.6)
MAF	0.27	0.24	0.26
Genotypes			
CC	185 (53.6)	194 (55.7)	106 (52.7)
CT	134(38.8)	136(39.1)	73 (36.3)
TT	27(7.8)	14(4.02)	15 (7.5)
HWE	0.69	0.14	0.59
rs187238-*IL18*	n (%)	n (%)	n (%)
Alleles T/A	461 (66.8)/ 231 (33.5)	487 (70.0)/ 207 (29.7)	285 (70.9)/ 107 (26.6)
MAF	0.34	0.29	0.27
Genotypes			

TT	151 (43.8)	173 (49.7)	103 (51.2)
TA	159 (46.1)	159 (45.7)	79 (39.3)
AA	36 (10.4)	36 (10.4)	14 (7.0)
HWE	0.63	0.61	0.86
rs3826782-*EMR1*	n (%)	n (%)	n (%)
Alleles G/A	619 (89.7)/ 65 (9.4)	612 (87.9)/ 74 (10.6)	348 (86.6)/ 32 (7.9)
MAF	0.09	0.11	0.08
Genotypes			
GG	282 (81.7)	274 (78.7)	160 (79.6)
GA	55 (15.9)	64 (18.4)	28 (13.9)
AA	5 (1.5)	5 (1.4)	2 (0.9)
HWE	0.20	0.57	0.64

HWE: Hardy Weinberg Equilibrium; MAF: Minor Allele Frequencies

4 DISCUSSÃO

Uma característica importante de doenças complexas, como a periodontite e o DM2, é o fato de serem poligênicas, ou seja, inúmeros genes estão envolvidos na suscetibilidade e gravidade da doença, cada um garantindo uma pequena contribuição. A abundância da variação genética no genoma humano, principalmente dada pela presença de polimorfismos, os tornou alvos para explicar diferenças no risco de doenças comuns[52-54]. Portanto, considerando algumas semelhanças na etiopatogenicidade da periodontite e da DM2, incluindo o fato de serem doenças comuns caracterizadas por uma potente resposta inflamatória a vários estímulos e a crescente incidência de pacientes afetados concomitantemente pelo DM2 e periodontite, sugere-se que ambas as doenças compartilham uma fração de variantes genéticas entre si[55]. Portanto este estudo buscou entender como que polimorfismos "classicamente" investigados na periodontite, e polimorfismos selecionados a partir de estudos de GWAS e bioinformática podem estar associados à suscetibilidade a periodontite e ao DM2 e também a ambas as doenças, como comorbidades.

Este estudo apresentou detalhado perfil clínico periodontal e o perfil metabólico dos pacientes como forte aspecto. Em relação aos parâmetros clínicos periodontais e bioquímicos, observou-se que pacientes com a presença da periodontite e do DM2 apresentaram a pior condição (tanto periodontal como glicêmica e lipídica) em comparação com o grupo controle ou com os pacientes do grupo periodontite, que apresentavam apenas a periodontite (Tabela 2). Esse achado reforça a evidência da associação entre a DM2 e periodontite[55]. Estudos epidemiológicos e ensaios clínicos mostraram que pacientes com diabetes sem adequado controle glicêmico têm maior probabilidade de apresentarem periodontite, principalmente em sua forma mais severa em comparação com pacientes diabéticos com controle glicêmico e pessoas sem DM2. Também é visto que a presença da periodontite dificulta o controle do DM2[55-57]. A inter-relação bi-direcional entre DP e DM tem sido estudada há vários anos [19, 20]. A premissa para tal inter-relação entre DP e DM é a presença de citocinas pró-inflamatórias, bactérias e toxinas na DP que são liberadas localmente no tecido periodontal e adentram na circulação sistêmica, influenciando tecidos e órgãos à distância. Ao mesmo tempo, as citocinas pró-inflamatórias sistemicamente envolvidas no DM infiltram-se nos tecidos periodontais e agravam a condição periodontal, resultando numa relação bidirecional [19, 21].

Como demonstrado no capítulo 1, este foi o primeiro estudo a investigar a potencial associação de SNPs classicamente associados à periodontite, com a suscetibilidade ao DM2

concomitante a periodontite (como comorbidades) na população do sudeste do Brasil. Na literatura, os polimorfismos no gene *IL1* são os mais classicamente investigados em estudos de periodontite. Korman et al., 1997 foram os primeiros a relatar a suscetibilidade do SNP rs114363 no gene *IL1B* (+ 3954) e a presença de um genótipo composto, com a periodontite[58]. Desde então, vários estudos mostraram uma grande variedade de resultados, o que permitiu a desenvolvimento de algumas meta-análises[59, 60]. Uma meta-análise realizada em 2012 que elegeu dez estudos com grande número amostral mostrou OR=1,54 para a associação entre o SNP *IL11B* rs1143634 e a periodontite[60]. Recentemente, outra meta-análise, avaliando 54 estudos de caso-controle, com um total de 4924 pacientes com periodontite crônica e 4452 pacientes saudáveis, mostrou que alelo recessivo do SNP rs1143634 estava associado à periodontite (OR=1,35), enquanto o alelo mais comum estava associado aos indivíduos saudáveis (OR=0,74)[59]. Linhartova et a., 2019, investigaram esse SNP em pacientes com DM2 da República Tcheca com e sem periodontite mas não foram encontradas diferenças significativas na variabilidade do rs1143634 gene *IL1B* entre os grupos doentes e os saudáveis[61]. Nosso estudo não demonstrou associação entre o rs1143634 e a periodontite isoladamente, mas diferente de Linhartova et al 2019[61], observamos proteção contra o desenvolvimento de periodontite em conjunto com o DM2 em pacientes brasileiros portadores do genótipo heterozigoto GA, principalmente após a estratificação da população por sexo (Tabela 4). Curiosamente, Lavu et al. 2017 observaram que pacientes heterozigotos para o rs1143634 apresentaram níveis mais altos de IL-1β no fluido gengival gengival, mas não notaram diferença nos níveis de IL-1β entre portadores do alelo mais raro e mais comum[62]. Portanto, sugerimos que mais estudos investiguem os níveis de IL-1β em relação ao rs1143634-GA, envolvendo uma população maior e também considerando no desenho do estudo uma avaliação mais profunda da influência do sexo. Pode-se notar que, mesmo após décadas de estudos sobre esse SNP e periodontite em diferentes populações étnicas, a literatura ainda mostra uma alta variabilidade de resultados, provavelmente associada ao delineamento experimental, diferenças entre número de amostras, amostras não homogêneas ou não corrigidas para variáveis clássicas como idade, sexo, tabagismo e dependência da localização geográfica. Também há interesse na avaliação do risco genético no grau da periodontite. Um importante estudo de GWAS não mostrou associação significativa entre o polimorfismo rs1143634 e a ocorrência de periodontite moderada ou grave[63], da mesma forma que o presente estudo mostrou na Tabela 5 (capítulo 1).

A interleucina-4 inibe a persistência de macrófagos nas lesões da periodontite e regula negativamente o CD14, um dos principais receptores do LPS do periodontopatógeno

Porphyromonas gingivalis, portanto, leva à diminuição da destruição tecidual[64]. Gonzales et al., 2007 demonstraram uma associação entre o genótipo -590 TT (rs2243250 SNP) no gene *IL4* e pacientes alemães com periodontite agressiva[65]. No entanto, nenhuma relação foi observada nesse polimorfismo com periodontite crônica em pacientes turcos[66], caucasianos suecos[67] e populações iranianas[68]. Annovazi et al., 2010 mostraram que indivíduos portadores de pelo menos um alelo T (para SNP-590; OR ajustado=3,04, IC 95%=1,66-5,54) eram mais suscetíveis à periodontite, mesmo após ajuste para as covariàvies idade, sexo, tabagismo para a população brasileira[69]. Um estudo de meta-análise com 37 estudos envolvendo 4.385 pacientes com periodontite e 5.168 indivíduos controles não mostrou associação entre o SNP rs2243250 e a periodontite[70]. Obviamente, as meta-análises têm limitações, sendo a mais relevante a alta heterogeneidade entre os estudos incluídos, principalmente em relação à variabilidade nos parâmetros clínicos selecionados e nas populações etnicamente diferentes. Ao considerar que o SNP como parte de um haplótipo, Annovazi et al,. 2010 identificaram a associação ao menor risco de periodontite. Em um estudo subsequente, o denominado haplótipo de proteção para o gene *IL4* foi associado à alta produção de mRNA de IL-4 e níveis de proteínas e níveis mais altos de citocinas anti-inflamatórias, enquanto o haplótipo suscetível produziu níveis mais altos de citocinas pró-inflamatórias[71]. O presente estudo não mostraram associação desse SNP com a ocorrência de periodontite isolada, mas com o desenvolvimento de periodontite juntamente com o DM2 (P+T2DM) em pacientes portadores do genótipo CT para o rs2243250. Até onde sabemos, este é o primeiro estudo a relatar um papel potencialmente dimórfico de um SNP no gene *IL4* com suscetibilidade a periodontite concomitante ao DM2. Curiosamente, a relação entre o SNP do gene *IL4* (-590) rs2243250 e a suscetibilidade ao DM2 foi demonstrada em diferentes populações, como os egípcios que mostraram associações positivas com a DM2 nos modelos de herança dominante, codominante e dominante[72]. Em contraste, pacientes caucasianos com DM2 apresentaram após análise de regressão logística ajustada para sexo, idade, duração do diabetes e hemoglobina glicada, nenhuma associação entre o rs2243250 e o risco de nefropatia diabética (OR=1,06; IC95%=0,37-3,05; p=0,9)[73].

O SNP rs1800795 no gene *IL6* está associado à suscetibilidade à periodontite crônica nas populações alemã[74] e brasileira[75]. O aumento da expressão de IL-6 tem sido associado à gravidade da doença periodontal em indivíduos brasileiros 16. De acordo com Costa et al., 2010, mulheres brasileiras idosas portadoras do genótipo GG foram mais severamente afetadas pela periodontite crônica[76]. Esse polimorfismo também foi associado ao aumento das chances de detectar *A.actinomycetemcomitans*, *P. gingivalis* e *T. forsythensis* em pacientes

com periodontite agressiva, após ajuste para idade, etnia, tabagismo e periodontite, sustentando a hipótese de que interações complexas entre microbiota e genoma do hospedeiro podem estar na base da suscetibilidade a essa doença[77-79]. Em uma meta-análise com 18 estudos de caso-controle compreendendo 1.616 casos de periodontite e 1.511 controles, sugeriu que o SNP rs1800795 no gene *IL6* pode estar associado a uma proteção contra a periodontite, uma vez que o modelo de herança CC vs. GG reduziu o risco de periodontite geral em 60% (IC95%=0,25-0,65, p <0,01) e 31% (IC95%=0,38-0,97, p=0,04), respectivamente[80]. Em outra meta-análise, para as populações brasileira e caucasiana, o genótipo GG atuou como fator de risco para periodontite[81]. Além disso, o SNP rs1800795 já estava associado ao DM2[82, 83] além da obesidade[84] e muitos tipos de câncer[85, 86]. Aqui, nossa análise do SNP rs1800795 (gene *IL6*) não mostrou resultados significativos para a suscetibilidade à periodontite, mas ao focar a associação de T2DM e periodontite, descobrimos que os homens com genótipo CC eram 88% menos propensos a desenvolver P+T2DM, com efeito real na população entre 98% e 30% (OR ajustado=0,12, IC95%=0,02 - 0,70, p=0,01).

Foi demonstramos neste estudo, pela primeira vez, a associação influenciada pelo sexo dos SNPs rs1143634 no gene *IL1B*, rs2243250 no gene *IL4* e rs1800795 no gene *IL6*, previamente associados à periodontite, com a periodontite concomitante ao DM2. Principalmente na última década, os estudos genéticos têm explorado mais profundamente a interação gene-sexo. Em relação ao risco de periodontite, dez SNPs com forte desequilíbrio de ligação no gene *Neuropeptide Y* (*NPY*), principalmente o rs198712, sugeriram uma interação gene-sexo com periodontite com OR=1,63 em homens e OR=0,69 em mulheres analisando uma população alemã[87]. É necessária uma influência de gênero na transcrição gênica, pois homens e mulheres usam um genoma semelhante para obter fenótipos distintos[88]. A experiência clínica[89], bem como estudos *in vitro*[90] e *in vivo*[91], indicam a influência de hormônios esteróides na inflamação, resposta imune e metabolismo ósseo, demonstrando a existência de uma associação de periodontite com os níveis de hormônios sexuais.

Para o capítulo1, decidimos não aplicar a correção de Bonferroni (BC) aos nossos resultados, pois reduziria o valor α para 0,0056 (0,05/9 SNP), podendo impedir a identificação de efeitos biológicos discretos. O uso de BC aumentaria o erro tipo II[92, 93], o que nos levaria a rejeitar a hipótese de uma verdadeira associação biológica. As associações verdadeiras podem ser perdidas quando a correção para vários testes é implementada[94]. Portanto, nossos resultados devem ser interpretados com cautela, uma vez que alguns dos valores de p menores que 0,05 podem ser devidos ao acaso.

Em relação ao capítulo 2, foi possível validar os rs7957197 no gene *HNF1A* e rs7018475 no gene *CDKN2B* como marcadores genéticos de suscetibilidade a ambas as doenças complexas (P+T2DM), enquanto o rs7754840 no gene *CDKAL1* demonstrou ser um marcador de menor risco para desenvolvê-las. Esses resultados encontrados foram estatisticamente significativos, mesmo após a correção de Bonferroni ($p \leq 0{,}0042$). Outros resultados interessantes foram observados aqui, apesar de não seguir a correção de Bonferroni, como o aumento do risco de periodontite+T2DM associado ao rs2062377 no gene *TCF7L2*, rs1531343 no gene *RPSAP52* e rs187238 no gene *IL18*; enquanto um risco menor de Periodontite+T2DM foi identificado para os rs5219 e rs5215 gene *KCNJ11*, rs2943641 no gene *IRS1*, rs6712932 no gene *GPR45* e rs352140 no gene *TLR9*. Consideramos esses resultados biologicamente relevantes, conforme explicado a seguir, e porque é importante ter em mente que a aplicação da correção de Bonferroni é controversa. Apesar de o BC ser amplamente utilizado na genética de populações, tem sido discutido constantemente, já que o BC pode não garantir um resultado prudente devido a uma hipótese nula irrelevante, relutância em rejeitar a hipótese nula, interpretações diferentes de valores-p idênticos e erro tipo II inflado[95].

Os SNPs rs352140 no gene *TLR9* e rs187238 no gene *IL18* foram primeiramente associados à periodontite pelo método integrativo de classificação de genes, uma ferramenta de bioinformática para priorizar a identificação de genes candidatos[96]. TLR9 é um membro da família TLR. Rojo-Botello et al.[97], 2012 outros relataram que a expressão de TLR9 foi maior em pacientes com periodontite quando comparados a indivíduos saudáveis. Kajita et al.[98], 2007 compararam os níveis de expressão de TLR9 entre pacientes com gengivite e aqueles com periodontite e descobriram que a expressão de TLR9 era significativamente maior em lesões de periodontite do que em lesões de gengivite. Holla et al.[99], 2010 concluíram que os haplótipos no gene *TLR9* podem estar associados à suscetibilidade do paciente à periodontite crônica, enquanto outros estudos com indianos[100] e caucasianos[101] não encontraram associação com a periodontite. O SNP rs352140 (gene *TLR9*) foi investigado em relação ao T2DM, mas não foi encontrada associação[102, 103]. Em nosso estudo, temos um número maior de pacientes e um perfil periodontal e glicêmico mais detalhado que os estudos citados, e aqui podemos associar o rs352140 com menor risco ao desenvolvimento de Periodontite e DM2. A citocina IL-18 tem sido implicada na inflamação e modula a atividade de macrófagos e neutrófilos[104, 105]. Nossos achados foram concordantes com alguns estudos[106, 107], incluindo uma meta-análise com um total de 576 pacientes com periodontite e 458 controles saudáveis, que mostraram a associação com maior risco de periodontite[108].

A proteína codificada pelo gene *KCNJ11 (Potassium inwardly rectifying channel subfamily J member 11*) é uma proteína de membrana integral e um canal de potássio do tipo retificador interno. Os canais de potássio estão presentes na maioria das células de mamíferos, onde participam de uma ampla gama de respostas fisiológicas[109]. Os SNPs rs5219[110, 111] e rs5215[42, 112] no gene *KCNJ11* foram associados previamente ao DM2 por estudos GWAS. Referindo-se ao polimorfismo rs5215, 3 estudos mostraram fortes associações entre essa variante e DM2[113-115], enquanto outros estudos mostraram o contrário, ou seja, nenhuma associação com DM2[116-119]. No presente estudo, associamos o SNP rs5215 com menor risco ao DM2 em nossa população. Para o SN52 rs5219, não validamos aqui os achados anteriores de associação ao DM2[120, 121]. Além disso, não validamos na população brasileira estudada a variante genética rs3826782 no gene *EMR1* como marcador genético de periodontite, conforme previamente identificado por Divaris et al.[63], 2013.

Como membro da família de proteínas do substrato receptor de insulina (IRS), a proteína codificada pelo gene *IRS1* desempenha um papel patogênico essencial na via de sinalização estimulada pela insulina e, em seguida, no desenvolvimento de DM2[122]. A variante genética, rs2943641, localizada adjacente ao gene *IRS1*, foi associada ao DM2, resistência à insulina, hiperinsulinemia e adiposidade por estudos de GWAS[123, 124]. Não há resultados consistentes sobre a associação entre o SNP rs2943641 e o DM2, como demonstrado por uma meta-análise recente que mostrou que o modelo codominante do rs2943641 estava associado ao risco de DM2 em pacientes caucasiano, não na população geral[125]. O que pode ser explicado pelos diferentes antecedentes genéticos e fatores ambientais que podem afetar a sensibilidade a variantes genômicas específicas nas diferentes populações.

Para o SNP rs6712932 no gene *GPR45*, embora estudos GWAS incluindo quatro populações diferentes mostrarem associação com DM2 (The DiaGen Consortium)[126], aqui não encontramos a associação com DM2, mas somos os primeiros a mostrar a associação desse SNP com periodontite. O SNP rs1531343 no gene *RPSAP52* já estava associado ao DM2 nos europeus pelo GWAS[42, 43, 127, 128], mas na população brasileira não validamos esse resultado, pois encontrou-se uma fraca associação entre esse SNP com o P+DM2. Um dos genes de risco mais reprodutíveis para diabetes identificados em diferentes populações étnicas é a *Quinase 5 dependente de ciclina* (*CDK*), que regula a subunidade 1 da proteína 1 homólogo 1 (*CDKAL1*)[129]. Em 2007, usando os resultados obtidos nos estudos de GWAS da Iniciativa Genética para o Diabetes (DGI)[127], Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION)[43], Wellcome Trust Case Control

Consortium (WTCCC), e o United Kingdom Type 2 Diabetes Genetics Consortium (UKT2D) relataram que o polimorfismo rs7754840 GC no gene *CDKAL1* estava fortemente associado ao DM2. Aqui observamos que o genótipo heterozigoto do SNP rs7754840 no *CDKAL1* foi associado a um maior risco para o desenvolvimento de DM2 em todos os pacientes, mas principalmente no sexo feminino. Esse resultado valida na população brasileira os achados anteriores. Uma meta-análise com 70141 indivíduos que apresentaram resultados semelhantes no modelo recessivo para o genótipo homozigoto (OR=1,58, IC 95%=1,20-2,08, p <0,001)[130]. Além disso, existem muitos estudos mostrando a associação deste SNP com diabetes gestacional[131-133]. Até onde sabemos, é a primeira vez que o SNP rs7754840 no gene *CDKAL1* foi associado a menor risco de desenvolver periodontite; na verdade, poderíamos validar sua associação com o DM2 (OR=2,49; IC95%=1,41–4,42; p=0,002).

O rs70184759 no gene *CDKN2B* foi associado ao DM2 por Huang et al.[134], 2012. Mais tarde, Sanghera et al.[135], 2012, abordaram a importância desse polimorfismo para a fisiopatologia do fenótipo complexo do DM2. No presente estudo, validamos na população do sudeste do Brasil a suscetibilidade ao DM2, e mostramos originalmente a associação do rs70184759-GG (*CDKN2*B) com o maior risco de periodontite+DM2 em mulheres e nunca fumantes, bem como com os pacientes afetados por periodontite+DM2 com controle deficiente da glicemia. Esse SNP é diferente dos 51 SNPs de marcação previamente investigados por Schaefer et al.[136], 2011, compreendendo os genes *CDKN2BAS* e *CDKN2A* e *CDKN2B* adjacentes que identificaram associação com periodontite agressiva em diferentes populações europeias. Como o gene *CDKN2B* foi associado a outras doenças inflamatórias, como doença cardíaca coronária[137] e leucemia linfoblástica[138], mais estudos devem ser feitos para entender melhor a inter-relação entre periodontite e doenças complexas sistêmicas.

Em relação ao gene do Fator Nuclear Hepatócito 1α (*HNF1A*), que resulta em concentrações reduzidas de ATP nas células β, levando à menor secreção de insulina[139, 140], está associado a muitos casos de diabetes de maturidade no jovem (MODY), uma forma monogênica hereditária de diabetes[141]. Alguns GWAS identificaram o SNP rs7957197 no gene *HNF1A* como um marcador genético de DM2[42, 129, 142]. O SNP rs7957197 no gene *HNF1A* afeta características glicêmicas *in vivo* e *in vitro* no contexto de DM2[143]. Em nosso estudo, verificamos que o genótipo heterozigoto do rs7957197 estava significativamente associado ao aumento do risco de desenvolver periodontite juntamente com o DM2 (P+DM2). Curiosamente, ao considerar apenas a periodontite, o mesmo genótipo indicou menor risco de periodontite, principalmente para a periodontite severa (Tabela 5 - Capítulo 2).

Este estudo tem algumas limitações. Apesar de serem escolhidos os SNPs baseados na

literatura, sabe-se que os genes investigados aqui têm uma variedade de outros SNPs, e é interessante investigar se outros SNPs e seus possíveis haplótipos são relevantes em indivíduos com periodontite e DM2. Apesar de cada grupo estudado aqui ter poder suficiente (pelo menos 150 ou 159 pacientes) para detectar associações genéticas significativas, aumentando o número de pacientes inscritos, maior seria a confiança nos resultados, além de aumentar a chance de encontrar pacientes afetados por apenas DM2. Um desenho de estudo diferente nos critérios de recrutamento e seleção pode superar a limitação deste estudo sobre a ausência de um grupo com DM2 sem Periodontite como comorbidade. Segundo Parra et al.[144], 2003, não é recomendado agrupar brasileiros em grupos étnicos com base na cor da pele e outras características físicas associadas às divisões raciais. Todos os estudos genéticos que envolvem uma população brasileira devem considerar a natureza de sua origem étnica combinada, já que os brasileiros formam uma das populações mais heterogêneas do mundo, resultante dos 5 séculos de cruzamentos interétnicos entre europeus, africanos, asiáticos e ameríndios[145]. Por esse motivo, não exploramos essa característica em nenhuma de nossas análises genéticas. Ao todo, também não exploramos mais profundamente aqui as razões pelas quais os hábitos sexuais ou de fumar podem influenciar as associações gene-fenótipo.

Por outro lado, como pontos positivos de nosso estudo, destacamos que todos os pacientes tiveram o diagnóstico preciso para fazer parte de um grupo (como os exames periodontais completos) e apresentavam status socioeconômico semelhante e foram selecionados na mesma área geográfica.

Mais estudos são necessários para confirmar esses resultados, incluindo populações mais numerosas e etnicamente diversas. Além disso, é importante investigar o papel funcional desses SNPs na doença periodontal e no contexto do DM2 e verificar a possível influência do sexo nos níveis de transcrição ou tradução das proteínas codificadas.

5 CONCLUSÃO

- Houve associação entre o SNP rs1143634 no gene *IL1B* e menores chances de desenvolver a Periodontite concomitantemente com a DM2, para toda a população avaliada, bem como quando a comparação evidenciou as mulheres. Sugerindo que tal associação pode ser influenciada pelo sexo.
- O SNP rs2243250 (gene *IL4*) demostrou que mulheres com genótipo CT tem maior suscetibilidade à periodontite juntamente com a D2M como comorbidades, sendo também influenciada pelo sexo.
- Para o rs1800795 no gene *IL6*, homens com genótipo CC apresentam menores chances de desenvolver o DM2.
- Houve uma fraca associação entre os rs5219 e rs5215 no gene *KCNJ11*, rs6712932 no gene *GPR45*, rs1531343 no gene *RPSAP52*, rs352140 no gene *TLR9* com apresença da periodontite.
- Os SNPs rs5215 no gene *KCNJ11*, rs7957197 no gene *HNF1A*, rs7903146-*TCF7L2* no gene *TLR9*, e rs7903146 no gene *TCF7L2* foram associados com a presença conjunta da periodontite e da DM2.
- As variantes genéticas rs7957197 (*HNF1A*), rs77544840 (*CDKAL1*) e rs7018475 (*CDKN2B*) foram validadas nos brasileiros como marcadores de risco para DM2.

REFERÊNCIAS*

1. Baelum V, Lopez R. Periodontal epidemiology: towards social science or molecular biology? Community Dent Oral Epidemiol. 2004; 32(4): 239-49.
2. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Clin Periodontol. 2018; 45 Suppl 20: S149-S61.
3. Andriankaja OM, DeNardin E, Dunford R, Dorn J, Trevisan M. The association between metabolic syndrome and periodontal disease. Aust Dent J. 2006; 163(11): S30-S.
4. Haffajee AD, Socransky SS, Patel MR, Song X. Microbial complexes in supragingival plaque. Oral Microbiol Immunol. 2008; 23(3): 196-205.
5. Page RC, Kornman KS. The pathogenesis of human periodontitis: an introduction. Periodontol 2000. 1997; 14: 9-11.
6. Assuma R, Oates T, Cochran D, Amar S, Graves DT. IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. J Immunol. 1998; 160(1): 403-9.
7. Franch-Chillida F, Nibali L, Madden I, Donos N, Brett P. Association between interleukin-6 polymorphisms and periodontitis in Indian non-smokers. J Clin Periodontol. 010; 37(2): 137-44.
8. Mealey BL, Oates TW. Diabetes mellitus anal periodontal diseases. J Periodontol. 2006; 77(8): 1289-303.
9. Susin C, Dalla Vecchia CF, Oppermann RV, Haugejorden O, Albandar JM. Periodontal attachment loss in an urban population of Brazilian adults: Effect of demographic, behavioral, and environmental risk indicators. J Clin Periodontol. 2004; 75(7): 1033-41.
10. Michalowicz BS, Diehl SR, Gunsolley JC, Sparks BS, Brooks CN, Koertge TE, et al. Evidence of a substantial genetic basis for risk of adult periodontitis. J Periodontol. 2000; 71(11): 1699-707.
11. Willemsen G, Ward KJ, Bell CG, Christensen K, Bowden J, Dalgard C, et al. The Concordance and Heritability of Type 2 Diabetes in 34,166 Twin pairs from international twin registers: the discordant twin (DISCOTWIN) consortium. Twin Res Hum Genet. 2015; 18(6): 762-71.
12. King GL, Shiba T, Oliver J, Inoguchi T, Bursell SE. Cellular and molecular abnormalities in the vascular endothelium of diabetes mellitus. Annu Rev Med. 1994; 45: 179-88.
13. Cho, N, Shaw, JE, Karuranga, S, Huang Y, da Rocha Fernandes, Ohlrogge AW, et al. IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018; 138: 271-281.

* De acordo com o Guia de Trabalhos Acadêmicos da FOAr, adaptado das Normas Vancouver. Disponível no site da Biblioteca: http://www.foar.unesp.br/Home/Biblioteca/guia-de-normalizacao-atualizado.pdf

14. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998; 15(7): 539-53.

15. Loe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care. 1993; 16(1): 329-34.

16. Lu HK, Yang PC. Cross-sectional analysis of different variables of patients with non-insulin dependent diabetes and their periodontal status. Int J Periodontics Restorative Dent. 2004; 24(1): 71-9.

17. Mealey BL, Oates TW, American Academy of P. Diabetes mellitus and periodontal diseases. J Periodontol. 2006; 77(8): 1289-303.

18. Papapanou PN. Periodontal diseases: epidemiology. Ann Periodontol. 1996; 1(1): 1-36.

19. Taylor HG, Yeates KO, Wade SL, Drotar D, Stancin T, Burant C. Bidirectional child-family influences on outcomes of traumatic brain injury in children. J Int Neuropsychol Soc. 2001; 7(6): 755-67.

20. Oliveira RN CS, Bastos AS, Orrico SRP, Scarel-Caminaga RM. Doença periodontal em pacientes com Diabetes Mellitus: influência de polimorfismos genéticos? Rev Odontol UNESP. 2011; 40(4): 187-94.

21. Xiao LM, Yan YX, Xie CJ, Fan WH, Xuan DY, Wang CX, et al. Association among interleukin-6 gene polymorphism, diabetes and periodontitis in a Chinese population. Oral Dis. 2009; 15(8): 547-53.

22. Correia D, Alcoforado G, Mascarenhas P. Influência da diabetes mellitus no desenvolvimento da doença periodontal. Rev Port Estomatol Med Dent Cir Maxilofac 2010; 51: 167-76

23. Guzman S, Karima M, Wang HY, Van Dyke TE. Association between interleukin-1 genotype and periodontal disease in a diabetic population. J Periodontol. 2003; 74(8): 1183-90.

24. Struch F, Dau M, Schwahn C, Biffar R, Kocher T, Meisel P. Interleukin-1 gene polymorphism, diabetes, and periodontitis: results from the Study of Health in Pomerania (SHIP). J Periodontol. 2008; 79(3): 501-7.

25. Engebretson SP, Hey-Hadavi J, Ehrhardt FJ, Hsu D, Celenti RS, Grbic JT, et al. Gingival crevicular fluid levels of interleukin-1beta and glycemic control in patients with chronic periodontitis and type 2 diabetes. J Periodontol. 2004; 75(9): 1203-8.

26. Lazenby MG, Crook MA. The innate immune system and diabetes mellitus: the relevance of periodontitis? A hypothesis. Clin Sci (Lond). 2010; 119(10): 423-9.

27. Bascones-Martinez A, Matesanz-Perez P, Escribano-Bermejo M, Gonzalez-Moles MA, Bascones-Ilundain J, Meurman JH. Periodontal disease and diabetes-review of the literature. Med Oral Patol Oral Cir Bucal. 2011; 16(6): e722-9.

28. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers. 2017; 3: 17038.

29. Kinane DF, Bartold PM. Clinical relevance of the host responses of periodontitis. Periodontol 2000. 2007; 43: 278-93.

30. Verardi G, Lupatini A, Beltrame J, Trentin M, Oliveira Da Silva S, De Carli J. Doença periodontal e diabete melito tipo 2. Rev. Odonto. 2009; 17: 93-9.

31. Taylor JJ, Preshaw PM, Donaldson PT. Cytokine gene polymorphism and immunoregulation in periodontal disease. Periodontol 2000. 2004; 35: 158-82.

32. Grigoriadou ME, Koutayas SO, Madianos PN, Strub JR. Interleukin-1 as a genetic marker for periodontitis: review of the literature. Quintessence Int. 2010; 41(6): 517-25.

33. Kim YJ, Viana AC, Curtis KMC, Orrico SRP, Cirelli JA, Scarel-Caminaga RM. Lack of association of a functional polymorphism in the interleukin 8 gene with susceptibility to periodontitis. DNA Cell Biol. 2009; 28(4): 185-90.

34. Lopez NJ, Jara L, Valenzuela CY. Association of interleukin-1 polymorphisms with periodontal disease. J Periodontol. 2005; 76(2): 234-43.

35. Tervonen T, Raunio T, Knuuttila M, Karttunen R. Polymorphisms in the CD14 and IL-6 genes associated with periodontal disease. J Clin Periodontol. 2007; 34(5): 377-83.

36. Kornman KS, Crane A, Wang HY, diGiovine FS, Newman MG, Pirk FW, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol. 1997; 24(1): 72-7.

37. Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature. 2001; 409(6822): 928-33.

38. Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito RB, Line SR. Frequencies of the -330 (T --> G) IL-2 and -590 (T --> C) IL-4 gene polymorphisms in a population from south-eastern Brazil. Eur J Immunogenet. 2002; 29(4): 293-6.

39. Astolfi CM, Shinohara AL, da Silva RA, Santos MCLG, Line SRP, de Souza AP. Genetic polymorphisms in the MMP-1 and MMP-3 gene may contribute to chronic periodontitis in a Brazilian population. J Clin Periodontol. 2006; 33(10): 699-703.

40. Moreira PR, Costa JE, Gomez RS, Gollob KJ, Dutra WO. The IL1A (-889) gene polymorphism is associated with chronic periodontal disease in a sample of Brazilian individuals. J Periodontal Res. 2007; 42(1): 23-30.

41. Pontes CC, Gonzales JR, Novaes AB, Jr., Taba Junior M, Grisi MF, Michel J, et al. 'Interleukin-4 gene polymorphism and its relation to periodontal disease in a Brazilian population of African heritage'. J Dent. 2004; 32(3): 241-6.

42. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science. 2007; 316(5829): 1336-41.

43. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science. 2007; 316(5829): 1341-5.

44. Raunio T, Knuuttila M, Hiltunen L, Karttunen R, Vainio O, Tervonen T. IL-6(-174) genotype associated with the extent of periodontal disease in type 1 diabetic subjects. J Clin Periodontol. 2009; 36(1): 11-7.

45. Stabholz A, Soskolne WA, Shapira L. Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. Periodontol 2000. 2010; 53: 138-53.

46. Holla LI, Fassmann A, Stejskalova A, Znojil V, Vanek J, Vacha J. Analysis of the interleukin-6 gene promoter polymorphisms in Czech patients with chronic periodontitis. J Periodontol. 2004; 75(1): 30-6.

47. Schafer AS, Jepsen S, Loos BG. Periodontal genetics: a decade of genetic association studies mandates better study designs. J Clin Periodontol. 2011; 38(2): 103-7.

48. Vijayalakshmi R, Geetha A, Ramakrishnan T, Emmadi P. Genetic polymorphisms in periodontal diseases: an overview. Indian J Dent Res. 2010; 21(4): 568-74.

49. Kornman KS, di Giovine FS. Genetic variations in cytokine expression: a risk factor for severity of adult periodontitis. Ann Periodontol. 1998; 3(1): 327-38.

50. Reid ME. Transfusion in the age of molecular diagnostics. Hematology Am Soc Hematol Educ Program. 2009 (1): 171-7.

51. Martins FTA, Ramos PZ, Svidnicki MCCM, Castilho AM, Sartorato EL. Optimization of simultaneous screening of the main mutations involved in non-syndromic deafness using the TaqMan (R) OpenArray (TM) Genotyping Platform. BMC Med Genet. 2013; 14: 112

52. Singh MM, Mathur R, Bedi RS. Genetic variants in periodontal diseases: Impact on disease susceptibility and severity. Adv Med Biol. 70: 55-91.

53. Baioni CS, de Souza CM, Ribeiro Braosi AP, Luczyszyn SM, Dias da Silva MA, Ignacio SA, et al. Analysis of the association of polymorphism in the osteoprotegerin gene with susceptibility to chronic kidney disease and periodontitis. J Periodontal Res. 2008; 43(5): 578-84.

54. Kaul N, Ali S. Genes, Genetics, and Environment in Type 2 Diabetes: implication in personalized medicine. DNA Cell Biol. 2016; 35(1): 1-12.

55. Polak D, Shapira L. An update on the evidence for pathogenic mechanisms that may link periodontitis and diabetes. J Clin Periodontol. 2018; 45(2): 150-66.

56. Zhou X, Zhang W, Liu X, Zhang W, Li Y. Interrelationship between diabetes and periodontitis: role of hyperlipidemia. Arch Oral Biol. 2015; 60(4): 667-74.

57. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. Periodontitis and diabetes: a two-way relationship. Diabetologia. 2012; 55(1): 21-31.

58. Kornman KS, Crane A, Wang HY, di Giovine FS, Newman MG, Pirk FW, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol. 1997; 24(1): 72-7.

59. da Silva FRP, Vasconcelos A, de Carvalho Franca LF, Di Lenardo D, Nascimento HMS, Vasconcelos DFP. Association between the rs1143634 polymorphism in interleukin-1B and chronic periodontitis: Results from a meta-analysis composed by 54 case/control studies. Gene. 2018; 668: 97-106.

60. Karimbux NY, Saraiya VM, Elangovan S, Allareddy V, Kinnunen T, Kornman KS, et al. Interleukin-1 gene polymorphisms and chronic periodontitis in adult whites: a systematic review and meta-analysis. J Periodontol. 2012; 83(11): 1407-19.

61. Borilova Linhartova P, Poskerova H, Tomandlova M, Bartova J, Kankova K, Fassmann A, et al. Interleukin-1 gene variability and plasma levels in czech patients with chronic periodontitis and diabetes mellitus. Int J Dent. 2019; 2019: 6802349.

62. Lavu V, Venkatesan V, Venugopal P, Lakkakula BV, Paul SF, Peria K, et al. Clinical relevance of cytokines gene polymorphisms and protein levels in gingival cervical fluid from chronic periodontitis patients. Iran J Immunol. 2017; 14(1): 51-8.

63. Divaris K, Monda KL, North KE, Olshan AF, Reynolds LM, Hsueh WC, et al. Exploring the genetic basis of chronic periodontitis: a genome-wide association study. Hum Mol Genet. 2013; 22(11): 2312-24.

64. Lauener RP, Goyert SM, Geha RS, Vercelli D. Interleukin 4 down-regulates the expression of CD14 in normal human monocytes. Eur J Immunol. 1990; 20(11): 2375-81.

65. Gonzales JR, Mann M, Stelzig J, Bodeker RH, Meyle J. Single-nucleotide polymorphisms in the IL-4 and IL-13 promoter region in aggressive periodontitis. J Clin Periodontol. 2007; 34(6): 473-9.

66. Kara N, Keles GC, Sumer P, Gunes SO, Bagci H, Koprulu H, et al. Association of the polymorphisms in promoter and intron regions of the interleukin-4 gene with chronic periodontitis in a Turkish population. Acta Odontol Scand. 2007; 65(5): 292-7.

67. Donati M, Berglundh T, Hytonen AM, Hahn-Zoric M, Hanson LA, Padyukov L. Association of the -159 CD14 gene polymorphism and lack of association of the -308 TNFA and Q551R IL-4RA polymorphisms with severe chronic periodontitis in Swedish Caucasians. J Clin Periodontol. 2005; 32(5): 474-9.

68. Hooshmand B, Hajilooi M, Rafiei A, Mani-Kashani KH, Ghasemi R. Interleukin-4 (C-590T) and interferon-gamma (G5644A) gene polymorphisms in patients with periodontitis. J Periodontal Res. 2008; 43(1): 111-5.

69. Anovazzi G, Kim YJ, Viana AC, Curtis KM, Orrico SR, Cirelli JA, et al. Polymorphisms and haplotypes in the interleukin-4 gene are associated with chronic periodontitis in a Brazilian population. J Periodontol. 2010; 81(3): 392-402.

70. Jia XW, Yuan YD, Yao ZX, Wu CJ, Chen X, Chen XH, et al. Association between IL-4 and IL-4R polymorphisms and periodontitis: a meta-analysis. Dis Markers. 2017; 2017: 8021279.

71. Anovazzi G, Medeiros MC, Pigossi SC, Finoti LS, Mayer MP, Rossa CJ, et al. Functional haplotypes in interleukin 4 gene associated with periodontitis. PLoS One. 2017; 12(1): e0169870.

72. Alsaid A, El-Missiry M, Hatata el S, Tarabay M, Settin A. Association of IL-4-590 C>T and IL-13-1112 C>T gene polymorphisms with the susceptibility to type 2 diabetes mellitus. Dis Markers. 2013; 35(4): 243-7.

73. Zavrsnik M, Letonja J, Makuc J, Seruga M, Cilensek I, Petrovic D. Interleukin-4 (IL4) -590C/T (rs2243250) gene polymorphism is not associated with diabetic nephropathy (DN) in Caucasians with type 2 diabetes mellitus (T2DM). Bosn J Basic Med Sci. 2018; 18(4): 347-51.

74. Babel N, Cherepnev G, Babel D, Tropmann A, Hammer M, Volk HD, et al. Analysis of tumor necrosis factor-alpha, transforming growth factor-beta, interleukin-10, IL-6, and interferon-gamma gene polymorphisms in patients with chronic periodontitis. J Periodontol. 2006; 77(12): 1978-83.

75. Moreira PR, Lima PM, Sathler KO, Imanishi SA, Costa JE, Gomes RS, et al. Interleukin-6 expression and gene polymorphism are associated with severity of periodontal disease in a sample of Brazilian individuals. Clin Exp Immunol. 2007; 148(1): 119-26.

76. Costa AM, Guimaraes MC, de Souza ER, Nobrega OT, Bezerra AC. Interleukin-6 (G-174C) and tumour necrosis factor-alpha (G-308A) gene polymorphisms in geriatric patients with chronic periodontitis. Gerodontology. 2010; 27(1): 70-5.

77. Nibali L, Ready DR, Parkar M, Brett PM, Wilson M, Tonetti MS, et al. Gene polymorphisms and the prevalence of key periodontal pathogens. J Dent Res. 2007; 86(5): 416-20.

78. Nibali L, Griffiths GS, Donos N, Parkar M, D'Aiuto F, Tonetti MS, et al. Association between interleukin-6 promoter haplotypes and aggressive periodontitis. J Clin Periodontol. 2008; 35(3): 193-8.

79. Nibali L, D'Aiuto F, Donos N, Griffiths GS, Parkar M, Tonetti MS, et al. Association between periodontitis and common variants in the promoter of the interleukin-6 gene. Cytokine. 2009; 45(1): 50-4.

80. Zhao B, Li R. The association between periodontitis and interleukin-6 genetic polymorphism -174 G/C: A meta-analysis. Arch Oral Biol. 2018; 96: 13-20.

81. Zhu J, Guo B, Fu M, Guo W, Yuan Y, Yuan H, et al. Interleukin-6-174G/C Polymorphism contributes to periodontitis susceptibility: an updated meta-analysis of 21 case-control studies. Dis Markers. 2016; 2016: 9612421.

82. Saxena M, Agrawal CG, Srivastava N, Banerjee M. Interleukin-6 (IL-6)-597 A/G (rs1800797) & -174 G/C (rs1800795) gene polymorphisms in type 2 diabetes. Indian J Med Res. 2014; 140(1): 60-8.

83. Plataki MN, Zervou MI, Samonis G, Daraki V, Goulielmos GN, Kofteridis DP. Association of the interleukin-6 rs1800795 polymorphism with Type 2 diabetes mellitus in the population of the island of crete, Greece. Genet Test Mol Biomarkers. 2018; 22(7): 448-52.

84. Gholami M, Sharifi F, Shahriari S, Khoshnevisan K, Larijani B, Amoli MM. Association of interleukin-6 polymorphisms with obesity: a systematic review and meta-analysis. Cytokine. 2019; 123: 154769.

85. Marangon AV, Colli CM, Cardozo DM, Visentainer JEL, Sell AM, Guimaraes F, et al. Impact of SNPs/Haplotypes of IL10 and IFNG on the development of diffuse large B-Cell lymphoma. J Immunol Res. 2019; 2019: 2137538.

86. Banescu C, Tripon F, Trifa AP, Crauciuc AG, Moldovan VG, Boglis A, et al. Cytokine rs361525, rs1800750, rs1800629, rs1800896, rs1800872, rs1800795, rs1800470, and rs2430561 SNPs in relation with prognostic factors in acute myeloid leukemia. Cancer Med. 2019; 8(12): 5492-506.

87. Freitag-Wolf S, Dommisch H, Graetz C, Jockel-Schneider Y, Harks I, Staufenbiel I, et al. Genome-wide exploration identifies sex-specific genetic effects of alleles upstream NPY to increase the risk of severe periodontitis in men. J Clin Periodontol. 2014; 41(12): 1115-21.

88. Rinn JL, Snyder M. Sexual dimorphism in mammalian gene expression. Trends Genet. 2005; 21(5): 298-305.

89. Meisel P, Krause T, Cascorbi I, Schroeder W, Herrmann F, John U, et al. Gender and smoking-related risk reduction of periodontal disease with variant myeloperoxidase alleles. Genes Immun. 2002; 3(2): 102-6.

90. Lee DJ, Wu L, Shimono M, Piao Z, Green DW, Lee JM, et al. Differential mechanism of periodontitis progression in postmenopause. Front Physiol. 2018; 9: 1098.

91. Steffens JP, Wang X, Starr JR, Spolidorio LC, Van Dyke TE, Kantarci A. Associations between sex hormone levels and periodontitis in men: results from NHANES III. J Periodontol. 2015; 86(10): 1116-25.

92. Uhlen MM, Stenhagen KR, Dizak PM, Holme B, Mulic A, Tveit AB, et al. Genetic variation may explain why females are less susceptible to dental erosion. Eur J Oral Sci. 2016; 124(5): 426-32.

93. Vieira AR, Bayram M, Seymen F, Sencak RC, Lippert F, Modesto A. In vitro acid-mediated initial dental enamel loss is associated with genetic variants previously linked to caries experience. Front Physiol. 2017; 8: 104.

94. Vieira AR, McHenry TG, Daack-Hirsch S, Murray JC, Marazita ML. Candidate gene/loci studies in cleft lip/palate and dental anomalies finds novel susceptibility genes for clefts. Genet Med. 2008; 10(9): 668-74.

95. Ye Z, Wang Z, Hou Y. Does Bonferroni correction "rescue" the deviation from Hardy-Weinberg equilibrium? Forensic Sci Int Genet. 2020; 46: 102254.

96. Zhan Y, Zhang R, Lv H, Song X, Xu X, Chai L, et al. Prioritization of candidate genes for periodontitis using multiple computational tools. J Periodontol. 2014; 85(8): 1059-69.

97. Rojo-Botello NR, Garcia-Hernandez AL, Moreno-Fierros L. Expression of toll-like receptors 2, 4 and 9 is increased in gingival tissue from patients with type 2 diabetes and chronic periodontitis. J Periodontal Res. 2012; 47(1): 62-73.

98. Kajita K, Honda T, Amanuma R, Domon H, Okui T, Ito H, et al. Quantitative messenger RNA expression of Toll-like receptors and interferon-alpha1 in gingivitis and periodontitis. Oral Microbiol Immunol. 2007; 22(6): 398-402.

99. Holla LI, Vokurka J, Hrdlickova B, Augustin P, Fassmann A. Association of Toll-like receptor 9 haplotypes with chronic periodontitis in Czech population. J Clin Periodontol. 2010; 37(2): 152-9.

100. Ashok N, Warad S, Kalburgi NB, Bilichodmath S, Prabhakaran PS, Tarakji B. Toll-like receptor 9 gene polymorphism in chronic and aggressive periodontitis patients. J Indian Soc Periodontol. 2014; 18(6): 723-7.

101. Sahingur SE, Xia XJ, Gunsolley J, Schenkein HA, Genco RJ, De Nardin E. Single nucleotide polymorphisms of pattern recognition receptors and chronic periodontitis. J Periodontal Res. 2011; 46(2): 184-92.

102. Liu F, Lu W, Qian Q, Qi W, Hu J, Feng B. Frequency of TLR 2, 4, and 9 gene polymorphisms in Chinese population and their susceptibility to type 2 diabetes and coronary artery disease. J Biomed Biotechnol. 2012; 2012: 373945.

103. Wifi MA, Assem M, Elsherif RH, El-Azab HA, Saif A. Toll-like receptors-2 and -9 (TLR2 and TLR9) gene polymorphism in patients with type 2 diabetes and diabetic foot. Medicine (Baltimore). 2017; 96(17): e6760.

104. Neighbors M, Xu X, Barrat FJ, Ruuls SR, Churakova T, Debets R, et al. A critical role for interleukin 18 in primary and memory effector responses to Listeria monocytogenes that extends beyond its effects on Interferon gamma production. J Exp Med. 2001; 194(3): 343-54.

105. Leung BP, Culshaw S, Gracie JA, Hunter D, Canetti CA, Campbell C, et al. A role for IL-18 in neutrophil activation. J Immunol. 2001; 167(5): 2879-86.

106. Tanaka K, Miyake Y, Hanioka T, Furukawa S, Miyatake N, Arakawa M. The IL18 promoter polymorphism, rs1946518, is associated with the risk of periodontitis in Japanese Women: the kyushu okinawa maternal and child health study. Tohoku J Exp Med. 2017; 243(3): 159-64.

107. Martelli FS, Mengoni A, Martelli M, Rosati C, Fanti E. IL-18 gene promoter polymorphisms are only moderately associated with periodontal disease in Italian population. Clin Cases Miner Bone Metab. 2012; 9(3): 153-6.

108. Li ZG, Li JJ, Sun CA, Jin Y, Wu WW. Interleukin-18 promoter polymorphisms and plasma levels are associated with increased risk of periodontitis: a meta-analysis. Inflamm Res. 2014; 63(1): 45-52.

109. Haghvirdizadeh P, Mohamed Z, Abdullah NA, Haghvirdizadeh P, Haerian MS, Haerian BS. KCNJ11: genetic polymorphisms and risk of diabetes mellitus. J Diabetes Res. 2015; 2015: 908152.

110. Timpson NJ, Lindgren CM, Weedon MN, Randall J, Ouwehand WH, Strachan DP, et al. Adiposity-related heterogeneity in patterns of type 2 diabetes susceptibility observed in genome-wide association data. Diabetes. 2009; 58(2): 505-10.

111. Suzuki K, Akiyama M, Ishigaki K, Kanai M, Hosoe J, Shojima N, et al. Identification of 28 new susceptibility loci for type 2 diabetes in the Japanese population. Nat Genet. 2019; 51(3): 379-86.

112. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet. 2008; 40(5): 638-45.

113. Chavali S, Mahajan A, Tabassum R, Dwivedi OP, Chauhan G, Ghosh S, et al. Association of variants in genes involved in pancreatic beta-cell development and function with type 2 diabetes in North Indians. J Hum Genet. 2011; 56(10): 695-700.

114. Liu Z, Zhang YW, Feng QP, Li YF, Wu GD, Zuo J, et al. [Association analysis of 30 type 2 diabetes candidate genes in Chinese Han population]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 2006; 28(2): 124-8.

115. Sakamoto Y, Inoue H, Keshavarz P, Miyawaki K, Yamaguchi Y, Moritani M, et al. SNPs in the KCNJ11-ABCC8 gene locus are associated with type 2 diabetes and blood pressure levels in the Japanese population. J Hum Genet. 2007; 52(10): 781-93.

116. Koo BK, Cho YM, Park BL, Cheong HS, Shin HD, Jang HC, et al. Polymorphisms of KCNJ11 (Kir6.2 gene) are associated with Type 2 diabetes and hypertension in the Korean population. Diabet Med. 2007; 24(2): 178-86.

117. Hotta K, Kitamoto A, Kitamoto T, Mizusawa S, Teranishi H, So R, et al. Association between type 2 diabetes genetic susceptibility loci and visceral and subcutaneous fat area as determined by computed tomography. J Hum Genet. 2012; 57(5): 305-10.

118. Odgerel Z, Lee HS, Erdenebileg N, Gandbold S, Luvsanjamba M, Sambuughin N, et al. Genetic variants in potassium channels are associated with type 2 diabetes in a Mongolian population. J Diabetes. 2012; 4(3): 238-42.

119. Heni M, Ketterer C, Thamer C, Herzberg-Schafer SA, Guthoff M, Stefan N, et al. Glycemia determines the effect of type 2 diabetes risk genes on insulin secretion. Diabetes. 2010; 59(12): 3247-52.

120. Abdelhamid I, Lasram K, Meiloud G, Ben Halim N, Kefi R, Samb A, et al. E23K variant in KCNJ11 gene is associated with susceptibility to type 2 diabetes in the Mauritanian population. Prim Care Diabetes. 2014; 8(2): 171-5.

121. Hu C, Zhang R, Wang C, Wang J, Ma X, Lu J, et al. PPARG, KCNJ11, CDKAL1, CDKN2A-CDKN2B, IDE-KIF11-HHEX, IGF2BP2 and SLC30A8 are associated with type 2 diabetes in a Chinese population. PLoS One. 2009; 4(10): e7643.

122. Sesti G, Federici M, Hribal ML, Lauro D, Sbraccia P, Lauro R. Defects of the insulin receptor substrate (IRS) system in human metabolic disorders. FASEB J. 2001; 15(12): 2099-111.

123. Rung J, Cauchi S, Albrechtsen A, Shen L, Rocheleau G, Cavalcanti-Proenca C, et al. Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. Nat Genet. 2009; 41(10): 1110-5.

124. Haghani K, Bakhtiyari S. The study on the relationship between IRS-1 Gly972Arg and IRS-2 Gly1057Asp polymorphisms and type 2 diabetes in the Kurdish ethnic group in West Iran. Genet Test Mol Biomarkers. 2012; 16(11): 1270-6.

125. Li Q, Qiao Y, Wang C, Zhang G, Zhang X, Xu L. Associations between two single-nucleotide polymorphisms (rs1801278 and rs2943641) of insulin receptor substrate 1 gene and type 2 diabetes susceptibility: a meta-analysis. Endocrine. 2016; 51(1): 52-62.

126. Salonen JT, Uimari P, Aalto JM, Pirskanen M, Kaikkonen J, Todorova B, et al. Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. Am J Hum Genet. 2007; 81(2): 338-45.

127. Diabetes Genetics Initiative of Broad Institute of H, Mit LU, Novartis Institutes of BioMedical R, Saxena R, Voight BF, Lyssenko V, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007; 316(5829): 1331-6.

128. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature. 2007; 445(7130): 881-5.

129. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet. 2010; 42(7): 579-89.

130. Tuerxunyiming M, Mohemaiti P, Wufuer H, Tuheti A. Association of rs7754840 G/C polymorphisms in CDKAL1 with type 2 diabetes: a meta-analysis of 70141 subjects. Int J Clin Exp Med. 2015; 8(10): 17392-405.

131. Noury AE, Azmy O, Alsharnoubi J, Salama S, Okasha A, Gouda W. Variants of CDKAL1 rs7754840 (G/C) and CDKN2A/2B rs10811661 (C/T) with gestational diabetes: insignificant association. BMC Res Notes. 2018; 11(1): 181.

132. Guo F, Long W, Zhou W, Zhang B, Liu J, Yu B. FTO, GCKR, CDKAL1 and CDKN2A/B gene polymorphisms and the risk of gestational diabetes mellitus: a meta-analysis. Arch Gynecol Obstet. 2018; 298(4): 705-15.

133. Liang Z, Wang L, Liu H, Chen Y, Zhou T, Heianza Y, et al. Genetic susceptibility, lifestyle intervention and glycemic changes among women with prior gestational diabetes. Clin Nutr. 2019; 39(7): 2144-2150.

134. Huang J, Ellinghaus D, Franke A, Howie B, Li Y. 1000 Genomes-based imputation identifies novel and refined associations for the Wellcome Trust Case Control Consortium phase 1 Data. Eur J Hum Genet. 2012; 20(7): 801-5.

135. Sanghera DK, Blackett PR. type 2 diabetes genetics: beyond GWAS. J Diabetes Metab. 2012; 3(198): 6948

136. Schaefer AS, Richter GM, Dommisch H, Reinartz M, Nothnagel M, Noack B, et al. CDKN2BAS is associated with periodontitis in different European populations and is activated by bacterial infection. J Med Genet. 2011; 48(1): 38-47.

137. Huang KW, Reebye V, Czysz K, Ciriello S, Dorman S, Reccia I, et al. Liver activation of hepatocellular nuclear factor-4alpha by small activating RNA rescues dyslipidemia and improves metabolic profile. Mol Ther Nucleic Acids. 2019; 19: 361-70.

138. Jang W, Park J, Kwon A, Choi H, Kim J, Lee GD, et al. CDKN2B downregulation and other genetic characteristics in T-acute lymphoblastic leukemia. Exp Mol Med. 2019; 51(1): 4.

139. Triggs-Raine BL, Kirkpatrick RD, Kelly SL, Norquay LD, Cattini PA, Yamagata K, et al. HNF-1alpha G319S, a transactivation-deficient mutant, is associated with altered dynamics of diabetes onset in an Oji-Cree community. Proc Natl Acad Sci U S A. 2002; 99(7): 4614-9.

140. Ostoft SH, Bagger JI, Hansen T, Pedersen O, Faber J, Holst JJ, et al. Glucose-lowering effects and low risk of hypoglycemia in patients with maturity-onset diabetes of the young when treated with a GLP-1 receptor agonist: a double-blind, randomized, crossover trial. Diabetes Care. 2014; 37(7): 1797-805.

141. Andersen G, Hansen T, Pedersen O. Genetics of common forms of glycaemia with pathological impact on vascular biology: are we on the right track? Curr Mol Med. 2005; 5(3): 261-74.

142. Saxena R, Elbers CC, Guo Y, Peter I, Gaunt TR, Mega JL, et al. Large-scale gene-centric meta-analysis across 39 studies identifies type 2 diabetes loci. Am J Hum Genet. 2012; 90(3): 410-25.

143. Jonsson A, Ladenvall C, Ahluwalia TS, Kravic J, Krus U, Taneera J, et al. Effects of common genetic variants associated with type 2 diabetes and glycemic traits on alpha- and beta-cell function and insulin action in humans. Diabetes. 2013; 62(8): 2978-83.

144. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A. 2003; 100(1): 177-82.

145. Alves-Silva J, da Silva Santos M, Guimaraes PE, Ferreira AC, Bandelt HJ, Pena SD, et al. The ancestry of Brazilian mtDNA lineages. Am J Hum Genet. 2000; 67(2): 444-61.

146. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. J Periodontol. 2007; 78(7 Suppl): 1387-99.

147. American Diabetes A. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2019. Diabetes Care. 2019; 42(Suppl 1): S13-S28.

148. American Diabetes A. 6. Glycemic targets: standards of medical care in diabetes-2019. Diabetes Care. 2019; 42(Suppl 1): S61-S70.

149. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007; 39(2): 175-91.

150. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J. 1975; 25(4): 229-35.

151. Muhlemann HR, Son S. Gingival sulcus bleeding--a leading symptom in initial gingivitis. Helv Odontol Acta. 1971; 15(2): 107-13.

152. Teumer A, Holtfreter B, Volker U, Petersmann A, Nauck M, Biffar R, et al. Genome-wide association study of chronic periodontitis in a general German population. J Clin Periodontol. 2013; 40(11): 977-85.

153. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points—a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem. 2016; 37(25): 1944-58.

154. Aidar M, Line SR. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. Braz Dent J. 2007; 18(2): 148-52.

155. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2): 263-5.

156. Mao M, Zeng XT, Ma T, He W, Zhang C, Zhou J. Interleukin-1alpha -899 (+4845) C->T polymorphism increases the risk of chronic periodontitis: evidence from a meta-analysis of 23 case-control studies. Gene. 2013; 532(1): 114-9.

157. Nikolopoulos GK, Dimou NL, Hamodrakas SJ, Bagos PG. Cytokine gene polymorphisms in periodontal disease: a meta-analysis of 53 studies including 4178 cases and 4590 controls. J Clin Periodontol. 2008; 35(9): 754-67.

158. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. J Periodontal Res. 1993; 28(6 Pt 2): 500-10.

159. Javed F, Al-Hezaimi K, Salameh Z, Almas K, Romanos GE. Proinflammatory cytokines in the crevicular fluid of patients with peri-implantitis. Cytokine. 2011; 53(1): 8-12.

160. Song GG, Choi SJ, Ji JD, Lee YH. Association between tumor necrosis factor-alpha promoter -308 A/G, -238 A/G, interleukin-6 -174 G/C and -572 G/C polymorphisms and periodontal disease: a meta-analysis. Mol Biol Rep. 2013; 40(8): 5191-203.

161. Fonseca JE, Santos MJ, Canhao H, Choy E. Interleukin-6 as a key player in systemic inflammation and joint destruction. Autoimmun Rev. 2009; 8(7): 538-42.

162. de Waal Malefyt R, Yssel H, Roncarolo MG, Spits H, de Vries JE. Interleukin-10. Curr Opin Immunol. 1992; 4(3): 314-20.

163. Albuquerque CM, Cortinhas AJ, Morinha FJ, Leitao JC, Viegas CA, Bastos EM. Association of the IL-10 polymorphisms and periodontitis: a meta-analysis. Mol Biol Rep. 2012; 39(10): 9319-29.

164. Zhong Q, Ding C, Wang M, Sun Y, Xu Y. Interleukin-10 gene polymorphisms and chronic/aggressive periodontitis susceptibility: a meta-analysis based on 14 case-control studies. Cytokine. 2012; 60(1): 47-54.

165. Silva I, Branco JC. Rank/Rankl/opg: literature review. Acta Reumatol Port. 2011; 36(3): 209-18.

166. Vaithilingam RD, Safii SH, Baharuddin NA, Ng CC, Cheong SC, Bartold PM, et al. Moving into a new era of periodontal genetic studies: relevance of large case-control samples using severe phenotypes for genome-wide association studies. J Periodontal Res. 2014; 49(6):683-95.

167. Rhodin K, Divaris K, North KE, Barros SP, Moss K, Beck JD, et al. Chronic periodontitis genome-wide association studies: gene-centric and gene set enrichment analyses. J Dent Res. 2014; 93(9): 882-90.

168. Zhan Y, Zhang R, Lv H, Song X, Xu X, Chai L, et al. Prioritization of candidate genes for periodontitis using multiple computational tools. J Periodontol. 2014; 85(8):1059-69.

169. Okamura H, Tsutsui H, Kashiwamura S, Yoshimoto T, Nakanishi K. Interleukin-18: a novel cytokine that augments both innate and acquired immunity. Adv Immunol. 1998; 70: 281-312.

170. Ozcaka O, Nalbantsoy A, Buduneli N. Interleukin-17 and interleukin-18 levels in saliva and plasma of patients with chronic periodontitis. J Periodontal Res. 2011; 46(5): 592-8.

171. Pomeroy R, Duncan G, Sunar-Reeder B, Ortenberg E, Ketchum M, Wasiluk H, et al. A low-cost, high-throughput, automated single nucleotide polymorphism assay for forensic human DNA applications. Anal Biochem. 2009; 395(1): 61-7.

APÊNDICE – Metodologia

MATERIAL E MÉTODOS

Seleção da amostra

Este estudo tem a aprovação do Comitê de Ética em Pesquisa em Humanos da FOAr-UNESP Protocolo no 50/06 (ANEXO). Após esclarecer a natureza e os objetivos desta pesquisa, os pacientes concordaram em participar assinaram o termo de consentimento, sendo encaminhados para a Clínica de Periodontologia da Faculdade de Odontologia de Araraquara (FOAr-UNESP) para realização do exame periodontal, coleta de dados e amostra biológica.

Considerando os dados do exame periodontal, de acordo com os critérios definidos pela CDC/AAP (*Centers for Disease Control and Prevention/ American Academy of Periodontology*)[146] (Tabela A1), os pacientes foram divididos como: (i) controle: pacientes periodontalmente saudáveis ou com periodontite leve, (i) periodontite: pacientes com periodontite moderada ou severa. Em relação a presença da DM2, o diagnóstico era de responsabilidade médica, e os pacientes foram divididos de acordo com o controle metabólico avaliado a partir dos níveis de HbA1c, sendo considerados pacientes compensados metabolicamente os que apresentavam níveis de HbA1c até 7%, e descompensados se a HbA1c $\geq$7,1% [147, 148].

Os pacientes sem DM2 tiveram seu perfil glicêmico verificados para confirmação da ausência do diabetes, sendo considerado como resultado de glicemia de jejum inferior a 100mg/dl e HbA1c $\leq$6.4%[148]. Todos os pacientes receberam informação dos resultados dos seus perfis glicêmico, lipídico e periodontal.

Portanto, os pacientes foram divididos conforme seus níveis glicêmicos e seu perfil periodontal nos seguintes grupos:

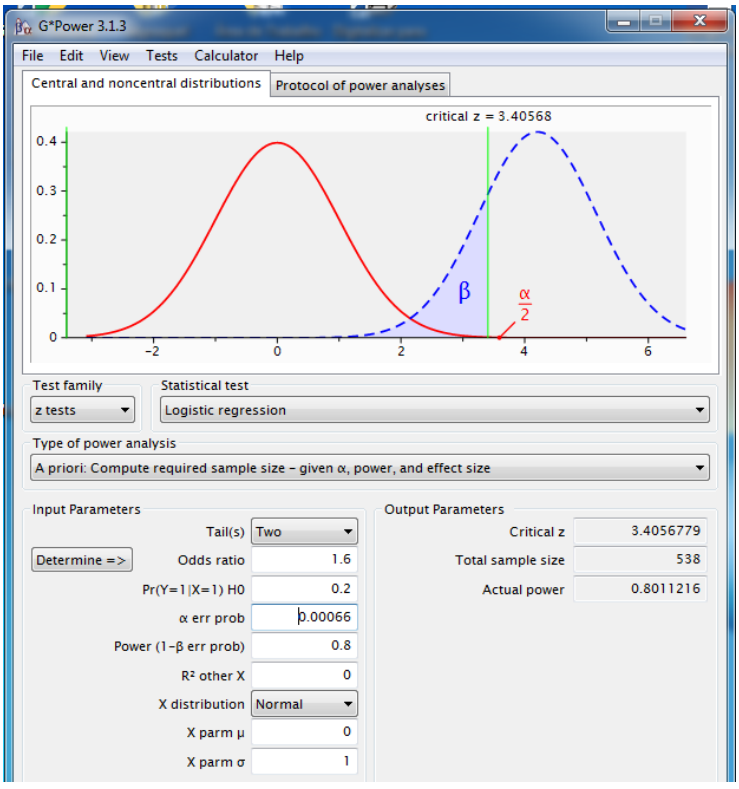
- **DM2+P** (n=239) – Indivíduos com diabetes mellitus tipo 2 e com periodontite moderada ou severa
- **Periodontite** (n=358) – Indivíduos sem diabetes mellitus tipo 2 e com periodontite severa ou moderada
- **Controle/Saudável** (n=356) - Indivíduos sem diabetes mellitus tipo 2 e periodontalmente saudáveis ou com periodontite leve

Cabe observar que o Grupo Periodontite caracteriza-se como um Controle no que se refere ao DM2, enquanto que o Grupo Controle, como um Controle do DM2 e da Periodontite, ou seja, neste grupo os pacientes são sistemicamente e periodontalmente saudáveis.

O número de pacientes com DM2 e PC (n=239), apesar de um pouco menor que os controle, foi obtido a partir do cálculo amostral a seguir:

Para calcular o tamanho da população necessária para que o estudo tivesse poder suficiente de atestar a associação do(s) alelo(s) com a Periodontite foi utilizado o programa G*Power Calculator, versão 3.1[149], considerando os parâmetros: regressão logística; two tail, OR: 1.6; Pr(Y=1/X=1) H0 = 0,2; alfa de 0,00066 e poder de 80%, R2 = 0. É importante salientar que o valor alfa=0,00066 foi obtido de p=0,05 dividido por 75 (60 SNPs + 11 variáveis de ajuste [idade, gênero e tabagismo] e 8 exames bioquímicos). Este cálculo resultou em uma amostra total de 538 pacientes (Figura A1). Com o "Total sample size" de 538, considerando 3 grupos teríamos o valor por grupo de 179,3. Arredondando para 180 pacientes por grupo, a amostra total seria de 540 pacientes. No entanto, como a plataforma de genotipagem OpenArray é fechada para até 960 amostras (pacientes), consideramos adequado utilizar para cada grupo controle (Controle e periodontite) 356 pacientes (total=712), de modo que pudemos incluir até 248 pacientes com DM2 e P (total=960). Deste modo, o nível de confiança do nosso estudo ficou maior que 80%.

Figura A1- Resultado do G*Power utilizando os parâmetros descritos.



Fonte: Figura gerada pelo programa G*Power

Anamnese, Exame Físico e Periodontal

Todos os pacientes foram submetidos à anamnese completa para avaliar o histórico de saúde geral e bucal, inclusive os pacientes advindos com o diagnóstico médico de DM2. Os pacientes foram submetidos também a exame físico para avaliar: circunferência abdominal (cm), proporção cintura/quadril e peso (kg) e altura (m) para obtenção do índice de massa corporal (IMC), em m/kg^2 de cada indivíduo.

O exame periodontal foi realizado com sonda periodontal (tipo Williams - Trinity® – Campo Mourão, Paraná, Brazil), e foi composto de índice de placa visível (IPV), índice de sangramento marginal (ISM), sangramento à sondagem (SS), mensuração da profundidade de sondagem (PS), posição da margem gengival e nível de inserção clínica (NIC) examinados em seis sítios (mésio-vestibular, vestibular, disto-vestibular, mésio-lingual, lingual e disto-lingual) de cada dente.

- Índice de placa visível: presença ou não de placa bacteriana visível a olho nu, após secagem da superfície dentária com jato de ar, em todas as faces de todos os dentes[150];
- Índice de sangramento marginal: presença ou ausência de sangramento marginal após percorrer o espaço do sulco de uma proximal a outra, com a sonda periodontal milimetrada inclinada em 60 graus em relação ao dente[150];
- Posição da margem gengival: distância da margem gengival à junção cemento-esmalte;
- Mensuração da profundidade de sondagem: distância da margem gengival ao fundo do sulco gengival, medida com sonda periodontal milimetrada em 6 sítios por dente: disto-vestibular, vestibular, mesio-vestibular, disto-lingual, lingual e disto-lingual;
- Sangramento à sondagem: presença ou ausência de sangramento, decorrido um tempo de 30 segundos depois de mensurada a profundidade de sondagem[151];
- Avaliação do nível clínico de inserção: corresponde à somatória das medidas da posição da margem gengival e profundidade de sondagem, para cada sítio de cada elemento dentário.

Após o exame periodontal, de acordo com os critérios definidos pela CDC/AAP (*Centers for Disease Control and Prevention/ American Academy of Periodontology*)[146], os indivíduos foram classificados em dois diferentes grupos conforme sua condição periodontal. Tais critérios periodontais já foram utilizados em estudos de polimorfismos genéticos, incluindo estudos do tipo GWAS para Periodontite [63, 152].

Tabela A1- Classificação da Doença Periodontal, de acordo com os critérios definidos pela CDC/AAP

		Definição Clínica		
	Classificação	NI		PS
Grupo Controle	Periodontite leve e Saudáveis	Não se enquadram nos critérios de periodontite severa ou moderada		
Grupo Periodontite	Periodontite Moderada	≥ 2 sítios com NIi ≥ 4mm (em dentes diferentes)	OU	≥ 2 sítios com PSi ≥ 5mm (em dentes diferentes)
	Periodontite Severa	≥ 2 sítios com NIi ≥ 6mm (em dentes diferentes)	E	≥ 1 sítio com PSi ≥ 5mm (em dentes diferentes)

*Exclusão de terceiros molares. PC: periodontite crônica PSi: profundidade de sondagem interproximal; NIi: nível de inserção interproximal.

Fonte: Autoria própria

Critérios de Inclusão e Exclusão para cada grupo estudado

Referente ao Grupo **P+DM2**:

- CRITÉRIOS DE INCLUSÃO: indivíduos com T2DM acima de 30 anos, de ambos os sexos, com pelo menos 10 dentes na cavidade bucal e sem distinção grupo étnico-racial (pois a tentativa de isolar determinadas características raciais é difícil em um país altamente miscigenado). Apresentar periodontite conforme descrito na página anterior.
- CRITÉRIOS DE EXCLUSÃO: indivíduos com história de hepatite e infecção por HIV, com anemia, com pré-medicação antibiótica nos últimos 6 meses ou uso crônico de drogas anti-inflamatórias, além de pacientes grávidas ou em lactação.

Referente ao Grupo **Periodontite:**

- CRITÉRIOS DE INCLUSÃO: indivíduos sem T2DM acima de 30 anos, de ambos os sexos, com pelo menos 10 dentes na cavidade bucal e sem distinção étnico-racial (pois

a tentativa de isolar determinadas características raciais é difícil em um país altamente miscigenado). Apresentar periodontite conforme descrito na página anterior.

- CRITÉRIOS DE EXCLUSÃO: pacientes com história de diabetes, hepatite e infecção por HIV, com pré-medicação antibiótica recente ou uso crônico de drogas anti-inflamatórias, além de pacientes grávidas ou em lactação.

Referente ao Grupo **Controle/Saudável**:

- CRITÉRIOS DE INCLUSÃO: indivíduos sem T2DM acima de 30 anos, de ambos os sexos, com pelo menos 10 dentes na cavidade bucal e sem distinção étnico-racial (pois a tentativa de isolar determinadas características raciais é difícil em um país altamente miscigenado). Sem presença de periodontite crônica conforme descrito na página anterior.
- CRITÉRIOS DE EXCLUSÃO: pacientes com hepatite e infecção por HIV, com pré-medicação antibiótica recente ou uso crônico de drogas anti-inflamatórias, além de pacientes grávidas ou em lactação.

Avaliação Exames Bioquímicos

Os pacientes foram encaminhados ao Laboratório de Análises Clínicas para coleta do sangue por punção venosa para realização dos exames: Hemograma (ABX Micros 60), Insulina (método quimioluminescência), Glicemia de Jejum (método Bondar e Mead modificado) (Kit Labtest), HbA_{1c} (método de imunoensaio de inibição turbidimétrica) (Kit Roche), Triglicérides (método enzimático-Trinder) (Kit Labtest), Colesterol Total (método enzimático-Trinder) (Kit Labtest) e HDL-colesterol (método enzimático) (Kit Labtest). Está sendo utilizado o equipamento COBAS MIRA plus.

As frações do VLDL e LDL-colesterol foram calculadas segundo a equação de Friedewald (válida se Triglicérides <400mg/dl) (Sociedade Brasileira de Cardiologia):

- *Colesterol VLDL = triglicérides / 5*
- *Colesterol LDL= Colesterol Total – (HDL+VLDL)*

Para os resultados do perfil lipídico, são considerados como dislipidêmicos os pacientes que apresentarem alterados pelo menos um dos seguintes parâmetros clínicos[153]:

- Colesterol Total (CT): Considerado alto para valores ≥ 240 mg/dl.
- LDL (Low density lipoprotein): Considerado alto para valores ≥ 160 mg/dl.
- HDL (High density lipoprotein): Considerado alto para valores ≤ 40 mg/dl.
- Triglicérides (TG): Considerado alto para valores ≥ 200 mg/dl.

Coleta de material biológico e extração de DNA

Foi solicitado a cada indivíduo que realizasse um bochecho com solução de dextrose a 3% autoclavada por 2 min. Os tubos contendo o bochecho foram mantidos resfriados (em caixa de isopor com gelo) do local da coleta até o momento do processamento em laboratório. Cada tubo será centrifugado a 2000 rpm por 10 min para separar o *pellet* de células que se despregam da mucosa oral. A este *pellet* de células será adicionado uma solução Tampão (TrisHCl 10mM, EDTA 0,1 M, SDS 0,5% - pH 8.0), sendo armazenado em freezer (–80°C) até a finalização das coletas de todos os indivíduos.

Para extração do DNA, como foi um grande número de amostras, e é necessária alta qualidade do DNA, utilizamos o método do acetato de amônia a 8M [154]. Nesta técnica as amostras foram descongeladas, adicionado 5 μL de proteinase K e incubado a 55ºC por 2h. Em seguida adicionou-se 500μL de acetato de amônio 8M e centrifugou-se a amostra a 14.000 rpm por 10 mim a 25ºC. Ao sobrenadante foi adicionado 540 μL de isopropanol e posteriormente centrifugado a 14.000 rpm por 5 mim. Foram feitas 2 lavagens do pellet obtido com etanol 70% para remover o isopropanol e centrifugou-se a 14.000 rpm por 5 mim. O pellet foi seco à temperatura ambiente e ressuspendido em 50 μL de tampão Tris-EDTA (TE).

O DNA genômico de cada amostra foi avaliado quanto à concentração (ng/μl) e pureza (260/280nm; 260/230nm) no aparelho NanoDrop™ 2000/2000c Spectrophotometer (Thermo Fisher Scientific, USA)**,** e foi armazenado a -20°C até o momento da genotipagem.

O protocolo de genotipagem por OpenArray® necessita também da quantificação prévia do DNA de cada amostra utilizando o sistema de fluorescência Qubit 2.0 (Thermo-Fisher). Para isso, as amostras de DNA foram selecionadas de acordo com: (i) a concentração maior ou igual a 50 ng/mL por ser a concentração mínima necessária para utilização na plataforma de genotipagem TaqMan® OpenArray® (Life Technologies); (ii) razões de pureza λ (260/280 nm) e λ (260/230 nm) entre 1,7 e 2,1 (principalmente a razão 260/280).

Para as amostras que não apresentaram concentração igual ou maior que 50 ng/μl e pureza λ (260/280nm) entre 1,7 e 2,1 no aparelho NanoDrop®, foi realizada a precipitação do DNA para purificar e/ou para concentrar as amostras, para torná-las ideais para genotipagem pelo OpenArray® Para realização desta técnica, adicionou-se à amostra de DNA ressuspendida em TE, 1/20 (do volume da amostra de DNA) de acetato de sódio 3M (pH=5,2) à temperatura ambiente. Estimou-se o volume total e adicionou-se o dobro do volume de etanol absoluto gelado. A amostra foi homogeneizada levemente até evidenciar a “nuvem” de

DNA, deixando-se no freezer ao longo de uma noite. No dia seguinte, retirou-se a amostra do freezer para centrifugar a 14.000 rpm por 15min a 4ºC. O pellet obtido foi lavado com etanol 70%, seguido de nova centrifugação a 14.000rpm por 15min a 4ºC. Ao fim, deixou-se secar o pellet à temperatura ambiente. O pellet foi então ressuspendido em 20 μL de TE, objetivando aumentar a concentração do DNA.

5. Seleção e Genotipagem dos SNPs

Para a seleção dos SNPs foi utilizado o programa HAPLOVIEW[155] a partir de dados do banco do *International HapMap Project* (www.hapmap.org), um consórcio internacional contendo informações de milhares de polimorfismos de indivíduos de diferentes etnias. Utilizamos dados de SNPs validados nos blocos haplotípicos considerando as populações CEU+TSI (combinação de populações caucasianas: [CEU = indivíduos residentes de Utah com ascendência Europeia Ocidental e Norte] e [TSI = indivíduos da Toscana na Itália]) do Projeto HapMap. Por exemplo, verificamos nesse banco de dados a frequência do alelo mais raro (*Minimum Allele Frequency* – MAF) de cada SNP de interesse, de modo que fosse pelo menos 0,05 de preferência (equivale a 5%). Foram investigados 21 SNPs distribuídos nos genes presentes na Tabela A2.

Tabela A2- Polimorfismos em genes candidatos selecionados para investigação no presente estudo

Genes de Investigação Clássica para DP					
Gene	**SNP RefSeq (rs)***	**Ensaio TaqMan**	**MAF**		
			Alelos	**CEU (branco)**	**TSI (italianos)**
IL1B	rs1143634	C___9546517_10	A|G	A|A: 0.05	A|A: 0.06
IL1A	rs1800587	C___9546481_20	A|G	A|A: 0.071	A|A: 0.103
IL4	rs2243250	C__16176216_10	C|T	T|T: 0.030	T|T: 0.037
TNFA	rs1800629	C___7514879_10	A|G	A|A: 0.020	A|G: 0.187
IL6	rs1800795	C___1839697_20	C|G	G|G: 0.273	C|C: 0.131
IL10	rs1800871	C___1747362_10	A|G	A|A: 0.051	A|A: 0.084
TNFRSF11B (alias OPG)	rs2062377	C___1970989_10	T|A	T|T: 0.192	T|T: 0.140
upstream TNFSF11(alias *RANKL)*	rs9533090	C___1444411_10	C|T	C|C: 0.283	C|C: 0.271
TNFRSF11A (alias *RANK*)	rs884205	C__30370382_10	A| C	A|A: 0.051	A|A: 0.037
Estudos de GWAS ou de Bioinformática para DP					
Gene	**SNP RefSeq (rs)***	**Ensaio TaqMan**	**MAF**		
			Alelos	**CEU (branco)**	**TSI (italianos)**
close EMR1	rs3826782	C__27506722_10	A|G	A|G: 0.162	A|A: 0.009
TLR9	rs352140	C___2301954_20	C|T	T|T: 0.232	C|C: 0.112
IL18	rs187238	C___2408543_10	C|G	G|G: 0.101	G|G: 0.047
Genes relacionados ao metabolismo glicêmico e estudos de GWAS					
Gene	**SNP RefSeq (rs)***	**Ensaio TaqMan**	**MAF**		
			Alelos	**CEU (branco)**	**TSI (italianos)**
KCNJ11	rs5219	C__11654065_10	C|T	T|T: 0.101	T|T: 0.084
KCNJ11	rs5215	C___2991148_10	C|T	C|C: 0.101	C|C: 0.084
GPR45	rs6712932	C___2099176_10	C|T	C|C: 0.081	C|C: 0.112
CDKN2B	rs7018475	C__11259127_20	G|T	G|G: 0.061	G|G: 0.103
CDKAL1	rs7754840	C__29246232_10	C|G	C|C: 0.101	C|C: 0.093
LOC64673, IRS1	rs2943641	C___1533178_10	CT	T|T: 0.121	T|T: 0.150
HNF1A	rs7957197	C__29094181_10	A|T	A|A: 0.020	A|A: 0.019
HMGA2, RPSAP52	rs1531343	C___1692218_10	C|G	C|C: 0.010	C|C: 0.028
TCF7L2	rs7903146	C__29347861_10	C|T	T|T: 0.152	T|T: 0.168

rs= (Reference SNP cluster ID) número que identifica cada SNP no Banco de Dados dbSNP (https://www.ncbi.nlm.nih.gov/SNP). MAF= (Minimum Allele Frequency) frequência do alelo mais raro.

Fonte: Elaboração própria

A primeira categoria de genes da Tabela A2 é: **Genes de Investigação Clássica para DP (com meta-análise realizada para DP).** Como mencionado, desde que Kornman et al.[36], detectaram uma associação dos polimorfismos dos genes Interleucina 1 alfa (*IL1A* -889) e beta (*IL1B* +3954) com o aumento da severidade da DP, muitos estudos foram realizados para explorar esta associação em diversas etnias, incluindo a população brasileira. Isso possibilitou a realização de algumas meta-análises que detectaram uma associação positiva destes polimorfismos citados nos genes *IL1A* e *IL1B*, além do polimorfismo *IL1B* -511, com a DP [60, 156, 157]. Cabe lembrar que justifica-se biologicamente a investigação do cluster *IL1* pelo fato da citocina IL-1 ter ação pró-inflamatória, potente estimulação da reabsorção óssea, produção de proteases e síntese de ácido araquidônico, substâncias diretamente relacionadas à destruição do tecido periodontal[158]. A interleucina 4 (IL-4) é um potente regulador negative da função de macrófagos que inibe a secreção de citocinas pró-inflamatórias como IL-1, IL-6 e fator de necrose tumoral alfa (TNF-α). Polimorfismos no gene *IL4* (-590 e +33) foram associados à DP crônica na população Brasileira[157], além de terem sido investigados em outras populações possibilitando uma meta-análise que demonstrou associação com DP para caucasianos[63]. Outros genes classicamente investigados que possibilitaram realização de meta-análises comprovando a associação destes com a DP são: (i) *TNFA* que codifica uma citocina pró-inflamatória com importante papel na resposta inflamatória e imune, incluindo a DP [159]. Estudo de meta-análise, incluindo população Brasileira, confirmou que o polimorfismo *TNFA* -308 confere susceptibilidade à periodontite [160]; (ii) A interleucina-6 (IL-6) é uma importante citocina multifuncional envolvida na modulação de resposta imune [161]. Em uma meta-análise, incluindo população Brasileira, o polimorfismo *IL6* -174 conferiu suscetibilidade à DP [160]; (iii) A IL-10 desempenha um papel importante na supressão da resposta imunológica, regulando o processo pró-inflamatório mediado por monócitos e macrófagos [162]. Em meta-análises, incluindo população Brasileira, os polimorfismos *IL10* -819 e -592 foram associados com DP, especialmente entre pacientes brancos[163,164]. A remodelação óssea resulta do equilíbrio entre a atividade osteoblástica e osteoclástica[165]. Nesse contexto, a principal via de sinalização molecular estudada em relação à periodontite envolve a ação das proteínas RANK, RANKL e OPG, que são codificadas pelos genes *TNFRSF11A, TNFSF11 e TNFRSF11B,* respectivamente. Entende-se ser importante investigar esses polimorfismos considerados clássicos na nova população que teve o exame clínico periodontal detalhado, devido à importância de replicar resultados, para que algum desses polimorfismos possa ser considerado futuramente um marcador genético para DP na nossa população. Somente com vasta e segura comprovação da associação de um SNP para DP, é que ele se tornaria um

marcador genético, e não mais um gene candidato à suscetibilidade à DP.

A segunda categoria de genes da Tabela A2 é: **Estudos de GWAS ou de Bioinformática para DP.** *Genome-Wide Association Study* (GWAS) são estudos de associação do tipo caso-controle envolvendo uma grande amostra populacional que, ao invés da abordagem de gene candidato, investigam simultaneamente milhares de polimorfismos genéticos para identificar variantes genéticas associadas à uma doença. Assim, GWAS permitem uma análise em larga escala e imparcial do genoma, que deve envolver grande número de indivíduos para que o estudo obtenha poder estatístico[166]. Com base nos resultados de GWAS, algumas variantes genéticas foram associadas com suscetibilidade à periodontite, porém estes resultados ainda não foram replicados na população Brasileira. Dentre estes genes, podemos destacar: o SNP na região próxima ao gene *EMR1* que foi associado à DP moderada e severa em dois diferentes estudos de GWAS[167] de populações caucasianas.

Tem-se expandido o número de publicações utilizando bancos de dados genéticos e métodos de bioinformática para identificar genes candidatos relacionados a doenças. Apenas uma publicação foi reportada na literatura utilizando bioinformática para identificação dos genes candidatos mais promissores para DP [168]. Por meio deste método integrativo de ranqueamento de genes, alguns destacam-se: (i) a interleucina 18 (*IL18*) que apresenta um papel importante na regulação da resposta imunoinflamatória, modulando atividade de macrófagos e neutrófilos [169]. A proteína codificada por este gene encontra-se em concentração aumentada na saliva de pacientes com Periodontite Crônica [170]; (ii) *TLR9* é um membro da família de receptores Toll-like (TLR), cuja expressão é aumentada em pacientes com periodontite quando comparado a pacientes com gengivite e pacientes saudáveis [98].

A terceira categoria de genes da Tabela A2 é: **Genes relacionados ao metabolismo glicêmico ou do prévio Transcriptoma de DM2**. Os genes *TCF7L2* e *KCNJ11* são classicamente associados ao metabolismo glicêmico e ao DM2 mediante estudos com diferentes populações. A inclusão dos genes *TCF7L2* e *KCNJ11* neste estudo é devido à importância de buscar replicar a associação com DM2 na nossa população. O gene *KCNJ*11 (*Potassium channel, in wardlyrectifying, subfamily J, member 11*, OMIM 600937) codifica a proteína Kir6.2, um dos componentes do canal de potássio ATP-dependente na célula Beta, com papel crítico na secreção de insulina. Mutações nesse gene estão associados ao DM2.

Quanto aos demais genes dessa categoria foram selecionados SNPs de acordo com resultados de estudos de GWAS para DM2. Foi utilizado o GWAS Catalog (https://www.ebi.ac.uk/gwas/home) e selecionado SNPs em população europeia ou caucasiana

com resultados significativos e maiores *Odds Ratios* (OR, ou razão das chances) para DM2. Destes SNPs foram então selecionados aqueles que apresentavam melhores MAF, de preferência, superiores a 5%.

Como membro da família de proteínas do substrato receptor de insulina (IRS), este tem grande importância na via do sinalização estimulado pela insulina[122]. Muitos estudos identificaram que o IRS1 desempenha um papel patogênico no desenvolvimento de T2DM. Como uma variante genética comum, o rs2943641 localizado adjacente ao gene *IRS1* foi associado a DM2, resistência à insulina, hiperinsulinemia e adiposidade em GWAS[123, 124]. Para o SNP rs6712932 no gene *GPR45*, estudos GWAS incluindo quatro populações diferentes mostrarem associação com DM2 (The DiaGen Consortium)[126]. O SNP rs1531343 no gene *RPSAP52* também foi previamente associado ao DM2 nos europeus por estudos de GWAS[42, 43, 127, 128]. Um dos genes de risco mais reprodutíveis para diabetes identificados em diferentes populações étnicas é a *Quinase 5 dependente de ciclina* (*CDK*), que regula a subunidade 1 da proteína 1 homólogo 1 (*CDKAL1*)[129]. Em 2007, usando os resultados obtidos nos estudos de GWAS da Iniciativa Genética para o Diabetes (DGI)[127], Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION)[43], Wellcome Trust Case Control Consortium (WTCCC), e o United Kingdom Type 2 Diabetes Genetics Consortium (UKT2D) relataram que o polimorfismo rs7754840 GC no gene *CDKAL1* estava fortemente associado ao DM2.

O rs70184759 no gene *CDKN2B* foi associado ao DM2 por Huang et al,. 2012[134]. Mais tarde, Sanghera et al., 2012, abordaram a importância desse polimorfismo para a fisiopatologia do fenótipo complexo do DM2[135]. Como o gene *CDKN2B* foi associado a outras doenças inflamatórias, como doença cardíaca coronária[137] e leucemia linfoblástica[138], mais estudos devem ser feitos para entender melhor a inter-relação entre periodontite e doenças complexas sistêmicas.

Em relação ao gene do Fator Nuclear Hepatócito 1α (*HNF1A*), que resulta em concentrações reduzidas de ATP nas células β, levando à menor secreção de insulina[139, 140], está associado a muitos casos de diabetes de maturidade no jovem (MODY), uma forma monogênica hereditária de diabetes[141]. Alguns GWAS identificaram o SNP rs7957197 no gene *HNF1A* como um marcador genético de DM2[42, 129, 142].

Genotipagem

Os 21 SNPs foram investigados por meio de uma lâmina "customizada" para utilização no sistema de genotipagem TaqMan® OpenArray® (Life Technologies, Foster City, CA, EUA). A lâmina "customizada" apresentava nano-cavidades contendo cada uma o ensaio específico para genotipagem de cada SNP por meio de reações de PCR (*Polimerase Chain Reaction*) em Tempo Real.

Cada poço possui primers e sondas específicas para cada alelo marcadas com os fluoróforos VIC e FAM, no qual, o alelo 1 é marcado com VIC e o alelo 2 é marcado com FAM. Assim, temos os resultados em VIC/VIC, VIC/FAM ou FAM/FAM. Desta forma, um indivíduo homozigoto para um alelo tem a emissão de somente um comprimento de onda (referente à sonda que se anelou no gene alvo), enquanto para um indivíduo heterozigoto há a emissão de ambos os fluoróforos das duas sondas empregadas para detecção do polimorfismo.

Esse método baseia-se na atividade 5'- endonuclease da *Taq* DNA Polimerase para clivar uma sonda anelada durante a extensão do DNA. O sinal de fluorescência é detectado quando as subunidades "*quencher*" e "*reporter*" da sonda se separam. A vantagem dos ensaios com TaqMan é a facilidade, rapidez na obtenção e confiabilidade dos resultados.

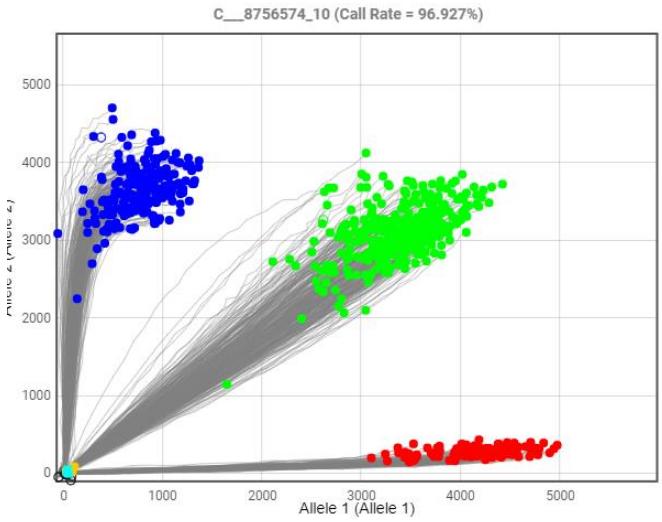
O DNA foi misturado ao TaqMan Genotyping Master Mix (Life Technologies, Foster City, CA, EUA) em uma placa de 384 poços (Life Technologies, Foster City, CA, EUA) e, em seguida, esse conteúdo foi transferido para a lâmina, de forma automatizada, pelo equipamento AccuFill™ (Life Technologies, Foster City, CA, EUA), de acordo com as recomendações do fabricante. A superfície da lâmina era hidrofóbica e o interior dos poços era hidrofílico e biocompatível, permitindo o carregamento e a retenção das amostras com um volume de 33nL em cada poço[171].

Após o carregamento, a lâmina foi transferida para um estojo de acrílico preenchido com um fluido de imersão e selado com cola para seguir com a reação de PCR, no termociclador GeneAmp PCR System 9700 (Life Technologies, Foster City, CA, EUA), com o seguinte ciclo de PCR: um passo inicial a 93ºC, durante 10 minutos; seguido por 50 ciclos de 45 segundos a 95ºC, 13 segundos a 94ºC e 2 minutos e 14 segundos a 53ºC, seguido de uma etapa final, durante 2 minutos a 25ºC e infinito a 4ºC.

Os resultados de fluorescência foram lidos no "*endpoint*" no BioTrove OpenArray SNP plataforma de genotipagem (Life Technologies, Foster City, CA, EUA). A análise da genotipagem foi feita com o software versão TaqMan® Genotyper 1.3 (disponível em

http://www.lifetechnologies.com/br/en/home/global/forms/taqman-genotyper-software download- reg.html), utilizando "*autocalling"* como o método de interpretação automática dos resultados. Os resultados foram considerados válidos quando a taxa chamada de "*Call Rate*" que é determinada pela intensidade do sinal de fluorescência foi maior ou igual a 90%. O software de interpretação dos resultados agrupa as amostras homozigotas para FAM (alelo 2) no eixo Y do gráfico (em azul), as amostras homozigotas para VIC estarão agrupadas no eixo X do gráfico (em vermelho) e as amostras heterozigotas ficaram agrupadas entre os dois eixos (em verde). Um controle branco (NTC) foi utilizado em todas as lâminas, sinalizado em amarelo, conforme mostra a Figura A2.

Figura A2 - Representação esquemática dos resultados no software TaqMan® Genotyper 1.3. Em azul estão as amostras homozigotas para o alelo 2 (FAM/FAM); em vermelho as amostras homozigotas para o alelo 1 (VIC/VIC) e, em verde, estão as amostras heterozigotas (VIC/FAM). Em amarelo, está demonstrado o branco (NTC) plotado em forma de quadrados representando o controle do ensaio.



Fonte: Elaboração própria

7. Análise estatística dos dados

Os dados demográficos e clínicos foram submetidos a análise estatística descritiva e analítica pelo programa IBM SPSS Statistics 20.0 ou com o auxílio do programa GraphPad Prism 6.0. As variáveis demográficas entre casos e controles foram comparadas pelo teste t de Student (idade e parâmetros periodontais) para variáveis contínuas, e pelo teste do $\chi 2$ para variáveis categóricas (sexo e tabagismo). O teste de ANOVA com pós teste de Tukey (número de dentes, placa visível, sangramento marginal, sangramento à sondagem,

profundidade de bolsas periodontais e perda de inserção clínica interproximal e dados bioquimicos) foi usados para avaliar a associação entre as variáveis continuas.

A fórmula de equilíbrio de Hardy-Weinberg (HWE) foi aplicada às frequências genotípicas entre os sujeitos de controle para testar quaisquer desvios do equilíbrio genotípico esperado usando a adequação do qui-quadrado.

As diferentes variáveis independentes binárias (Grupo Controle vs. Grupo PC; Grupo Controle vs. Grupo PC severa; Grupo Controle vs. Grupo moderada) foram usadas para determinar a associação entre cada SNP e o diagnóstico de periodontite crônica e da DM2. Todas as análises de associação de caso-controle foram realizadas, as quais incluíram associações alélicas e genotípicas. Os genótipos foram categorizados em três grupos (alelo homozigoto principal - 11. heterozigoto - 12. e variante homozigoto - 22). Diferentes graus de gravidade da doença (moderada ou grave) foram avaliados pelo teste do qui-quadrado em mo modelo aditivo (genótipo 11 versus 12 versus 22) .

A associação entre cada SNP com doença periodontal (moderada e severa) ou DM2 (compensada ou descompensada) foi estimada como odds ratio (OR) e intervalo de confiança (IC) de 95% por meio de regressão logística múltipla ajustada por idade, sexo e tabagismo. Além disso, os polimorfismos genéticos foram avaliados, através do modelo de regressão logística, separadamente pelo sexo dos pacientes, para investigar a suscetibilidade à PC, ajustada por idade e tabagismo.

O software PLINK foi usado para detectar diferenças nas distribuições para cada SNP entre os grupos. Todas as outras análises foram realizadas pelo software STATA, versão 12.0 para Windowns (Statistics / Data Analysis. Stata Corporation. College Station, Texas, EUA.

ANEXO – Certificado Comitê de Ética

UNIVERSIDADE ESTADUAL PAULISTA " JÚLIO DE MESQUITA FILHO"

FACULDADE DE ODONTOLOGIA DE ARARAQUARA

Comitê de Ética em Pesquisa

Certificado

Certificamos que o projeto de pesquisa intitulado *"DIABETES MELLITUS E DOENÇA PERIODONTAL: ANÁLISE CLÍNICA, GENÉTICA, MICROBIOLÓGICA, IMUNOLÓGICA E BIOQUÍMICA"*, sob o protocolo nº **50/06**, de responsabilidade do Pesquisador (a) *SILVANA REGINA PEREZ ORRICO*, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa–FOAr, com validade de 04 (quatro) anos, quando será avaliado o relatório final da pesquisa.

Certify that the research project titled *"DIABETES MELLITUS AND PERIODONTAL DISEASE: CLINICAL, GENETICAL, MICROBIOLOGICAL, IMMUNOLOGICAL AND BIOCHEMICAL ANALYSIS"*, protocol number **50/06**, under Dr *SILVANA REGINA PEREZ ORRICO*, responsability, is under the terms of Conselho Nacional de Saúde/MS resolution # 196/96, published on May 10, 1996. This research has been approved by Research Ethic Committee, FOAr-UNESP. Approval is granted for 04 (four) years when the final review of this study will occur.

Araraquara, 23 de abril de 2007.

Profª Drª *Miriam Aparecida Onofre*
Coordenadora

Não autorizo a publicação deste trabalho pelo prazo de 24 de setembro de 2022.

(Direitos de publicação reservado ao autor)

Araraquara, 24 de setembro de 2020.

Thamiris Cirelli