PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ

ESCOLA DE CIÊNCIAS DA VIDA

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

ÁREA DE CONCENTRAÇÃO CLÍNICA ODONTOLÓGICA INTEGRADA

CLINICAL ASPECTS AND POLYMORPHISM IN THE *BRINP3* GENE AND BENIGN MIGRATORY GLOSSITIS

LAÍS CRISTINA GIACOBBO

CURITIBA

2021

LAÍS CRISTINA GIACOBBO

CLINICAL ASPECTS AND POLYMORPHISM IN THE *BRINP3* GENE AND BENIGN MIGRATORY GLOSSITIS

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do título de Doutora em Odontologia, área de concentração em Clínica Odontológica Integrada (Ênfase em Ortodontia).

Orientadora: Profª Drª Paula Cristina Trevilatto.

CURITIBA

2021

Dados da Catalogação na Publicação
Pontifícia Universidade Católica do Paraná
Sistema Integrado de Bibliotecas – SIBI/PUCPR
Biblioteca Central
Edilene de Oliveira dos Santos CRB-9/1636

G429c 2021

Giacobbo, Laís Cristina
Clinical aspects and polymorphism in the *BRINP3* gene and benign migratory glossitis / Laís Cristina Giacobbo ; orientadora: Paula Cristina Trevilatto. -- 2021
40 f. : il. ; 30 cm

Tese (doutorado) – Pontifícia Universidade Católica do Paraná, Curitiba, 2021.
Inclui bibliografias

1. Odontologia. 2. Polimorfismo (Genética). 3. Glossite migratória benigna. 4. Estudos de casos e controles. I. Trevilatto, Paula Cristina. II. Pontifícia Universidade Católica do Paraná.
Programa de Pós-Graduação em Odontologia. III. Título

CDD. 20. ed. – 617.6

Pontifícia Universidade Católica do Paraná
Escola de Ciências da Vida
Programa de Pós-Graduação em Odontologia

TERMO DE APROVAÇÃO

LAÍS CRISTINA GIACOBBO

CLINICAL ASPECTS AND POLYMORPHISM IN THE *BRINP3* GENE AND BENIGN MIGRATORY GLOSSITIS

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos parciais para a obtenção do Título de **Doutor em Odontologia**, Área de Concentração em **Clínica Odontológica Integrada (Ortodontia)**.

Orientador (a): Profª Drª Paula Cristina Trevilatto
Programa de Pós-Graduação em Odontologia, PUCPR

Prof. Dr. Orlando M. Tanaka
Programa de Pós-Graduação em Odontologia, PUCPR

Profª Drª Luciana Reis Azevedo Alanis
Programa de Pós-Graduação em Odontologia, PUCPR

Profª Drª Rafaela Scariot
Programa de Pós-Graduação em Odontologia, UFPR

Profª Drª Isabela Almeida Shimizu
Curso de Especialização em Ortodontia, ILAPEO

Curitiba, 23 de abril de 2021.

Dedicatória

Dedico essa conquista aos meus pais, **Leoni Rodrigues Giacobbo** e **Hilário Valdir Giacobbo (*in memoriam*)**. Sem eles, não seria possível chegar até aqui. Vocês são minhas inspirações, as pessoas que eu mais admiro no mundo. Meu pai, meu anjo e eterno herói. Minha mãe, que com sua enorme coragem sempre me incentivou e não mediu esforços para que eu pudesse alcançar esse objetivo.

Obrigada por tudo, de coração! Amo vocês!

AGRADECIMENTO ESPECIAL

À minha querida orientadora, **Profª. Drª. Paula Cristina Trevilatto**. Pela orientação, dedicação e enorme aprendizado desde o Mestrado. Obrigada por toda paciência, educação e incentivo sempre. Serei eternamente grata e levarei para sempre os seus ensinamentos. Foi uma honra ser sua orientada!

AGRADECIMENTOS

A **Deus**, por iluminar meu caminho. Foi o Senhor que com sua atenção e apoio, me deu forças para superar todos os desafios que enfrentei até aqui. Agradeço ao Pai por ser quem sou hoje.

À minha querida e doce irmã, **Heloíse** Angélica Giacobbo que mesmo longe, está sempre torcendo e me incentivando. Obrigada por ser essa irmã incrível!

Ao meu amável noivo, **Ernani** Schenfert Filho por todo apoio e auxílio, inclusive na revisão e tradução do inglês. E principalmente pela paciência e amparo nesses anos de pós-graduação.

À Profª. Drª. **Rafaela** Scariot, por ter coletado e disponibilizado a amostra de glossite migratória benigna para que muitas pesquisas pudessem ser realizadas.

Ao admirável Prof. Dr. **Alexandre** Rezende Vieira, por ter sugerido o gene candidato para que este trabalho pudesse ser realizado, além de toda contribuição e auxílio.

Às queridas companheiras da equipe de genética, **Thais** Munhoz Pereira, **Rita** de Cássia Broker, **Valéria** Kruchelski Huk, **Neblyssa** Schneider Silveira e **Maria** Augusta Andrigo Perin, pela parceria sempre.

Ao Prof. Dr. Orlando Motohiro **Tanaka**, por sempre acreditar em mim, por todo conhecimento compartilhado e por estar ao meu lado em mais essa conquista. Minha eterna gratidão.

À Profª. Drª. **Elisa** Souza Camargo, pelo enorme carinho e contribuições sempre. Seu apoio foi essencial na minha formação.

Ao Prof. Dr. **Odilon** Guariza Filho, pela amizade, simpatia e conhecimento compartilhado nesses anos de Ortodontia.

À Profª. Drª. **Luciana** Azevedo Alanis pelo carinho, educação e contribuições enriquecedoras desde os seminários do Mestrado até o Doutorado.

À Profª. Drª. **Isabela** Almeida Schimizu pelo carinho, participação e apoio durante o Mestrado e agora também no Doutorado.

Aos demais **professores** do Programa de Pós-Graduação em Odontologia (PPGO), por todo apoio e dedicação com o programa.

À **Neide** Reis Borges, secretária do PPGO, pela eficiência, alegria e amizade.

A todos os **colegas** e **amigos** que estiveram ao meu lado todo esse tempo.

Muito obrigada pelo suporte e amizade.

À Pontifícia Universidade Católica do Paraná (**PUCPR**), Universidade Federal do Paraná (**UFPR**) e colaboradores, pela grande parceria para que este trabalho pudesse ser realizado.

Aos **pacientes** que se dispuseram a participar desse trabalho, minha gratidão.

À **Pontifícia Universidade Católica do Paraná**, por sempre me acolher desde a graduação.

“A gratidão é a memória do coração.”
Antístenes.

SUMÁRIO

ARTIGO EM INGLÊS

Title page

CLINICAL ASPECTS AND POLYMORPHISM IN THE *BRINP3* GENE AND BENIGN MIGRATORY GLOSSITIS

Laís Cristina Giacobbo
Pontifícia Universidade Católica do Paraná
School of Life Sciences
Graduate Program in Dentistry
Email: laisgiacobbo@hotmail.com

Corresponding author: Paula Cristina Trevilatto. Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná (PUCPR). Rua Imaculada Conceição, 1155, Prado Velho. Curitiba, PR, Brasil. CEP: 80215-901. Telefone: +55 (41) 3271-1849, email: paula.trevilatto@pucpr.br

Abstract

Objectives The aim of this study was to investigate association of sociodemographic, clinical variables, and genetic polymorphism in the *BRINP3* gene with benign migratory glossitis (BMG).

Methods This is a case-control study with a sample of 174 individuals divided into a case group, with 44 patients who had BMG and a control group, with 130 patients without BMG. The sociodemographic and clinical parameters examined were age, sex, ethnicity, socioeconomic status, systemic diseases, systemic diseases in first-degree relatives, continuous medication, allergies, smoking, alcohol consumption, fissured tongue, anxiety scale, DMFT index, and decayed + filled teeth. The DNA was obtained from oral epithelial cells. The tagSNP rs1342913 was selected based on the SNPinfo Web Server, it is in high linkage disequilibrium with other 67 markers, covering an extensive portion (approximately 50%) of the *BRINP3* gene. Genotyping was performed using the Real Time Polymerase Chain Reaction (PCR) technique. Univariate and multivariate statistical analyses were performed ($p<0.05$).

Results In the univariate analysis fissured tongue ($p=3.7*10^{-15}$; OR=107.5; CI:13.76-839.3), anxiety ($p=0.001$), DMFT index ($p=0.013$; OR=2.388; CI:1.188-4.798), and decayed + filled teeth ($p=0.000$; OR=4.091; CI:1.902-8.800) were associated with BMG. No association was found between the polymorphism rs1342913 of the *BRINP3* and BMG. After multivariate analysis, association was maintained for fissured tongue ($p=1.3*10^{-5}$; OR=131.953; CI:14.672-1186.700), anxiety ($p=0.000$), and decayed + filled teeth ($p=0.003$; OR= 5.212; CI:1.756-15.470).

Conclusion Presence of fissured tongue, higher levels of anxiety, DMFT index, and decayed + filled teeth were associated with BMG. No association of rs1342913 with BMG in the sample studied was observed.

Keywords genetic polymorphism, *BRINP3,* benign migratory glossitis, case-control study.

Introduction

Benign migratory glossitis (BMG), also known as geographic tongue or migratory erythema [1], is a benign inflammatory condition, characterized by areas of depapillation of the filiform papillae and erythema [2]. The erythematous areas are surrounded by irregular whitish borders [3] resulting in an appearance like the contours of a “geographical map” [2] (Fig. 1). In addition, BMG is usually located in the anterior two dorsal thirds of the tongue. However, the lesions can also manifest on the lateral or ventral surface [3].

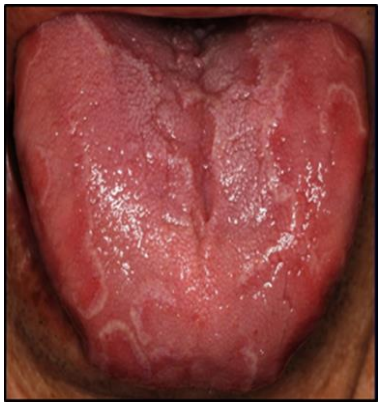

Figure 1. Clinical aspect of benign migratory glossitis: erythematous areas with irregular, whitish edges, with loss of filiform papillae. Source: Ishibashi, M., Tojo, G., Watanabe, M., Tamabuchi, T., Masu, T., & Aiba, S (2010) Geographic tongue treated with topical tacrolimus. J Dermatol Case Rep 4:57-59. https://doi.org/10.3315/jdcr.2010.1058

This benign and inflammatory condition is characterized by periods of remission and exacerbation of different durations [4]. During remission, the lesions heal and when they return, they tend to develop in new places, producing a migratory pattern [5]. Regarding the duration of the injury, it is a fact that shows great variability, with injuries repairing within two weeks and injuries that continue to develop for more than a year [6].

Furthermore, although it is generally asymptomatic, some patients report pain or a burning sensation, especially when eating spicy or acidic foods [7] and even sensitivity to cigarette smoke [8]. These symptoms may impact the quality of life of affected patients [9]. BMG usually does not need treatment, however, to alleviate

symptoms it is recommended to use analgesics, anti-inflammatories, mouthwash, anesthetic ointments, and corticoid remedies [10].

GMB is commonly present in adults, but few cases are reported in children [11]. The prevalence is reported to be approximately 1% to 2.5% of the global population [12] and 7% in Southeast Brazil [13].

Several etiological factors have been suggested such as emotional stress, tobacco consumption, hormonal disorders, fissured tongue, psoriasis, diabetes mellitus, immunological and genetic factors [9].

Previous studies have indicated that BMG may have a genetic background. In the 1960s, the possible influence of a genetic component on its etiology was suggested. A family aggregation study analyzed three generations of a large family and heritability was verified [14]. Currently, other studies have also suggested a genetic component for BMG [13, 15, 16]. A study carried out in a Brazilian population, estimated that 36.6% of the phenotype was explained by genetic factors [13]. The next steps should be to identify which candidate genes are involved in the control of susceptibility to BMG.

The bone morphogenetic protein/specific neural inducible retinoic acid 3 (*BRINP3)* gene, also called *FAM5C*, was originally identified in the mouse brain [17]. It is located on chromosomal region 1q31.1 (Fig. 2) [18, 19], consists of 8 exons, and encodes a protein of 766 amino acids [18]. BRINP3 protein has been reported in several cellular functions, such as proliferation, migration, and programmed cell death [19].

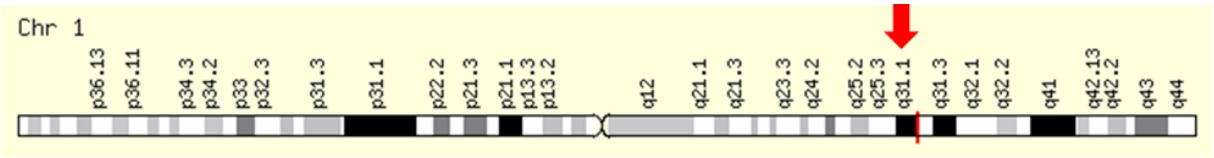

Figure 2. Chromosomal location of the *BRINP3* gene. Source: Gene Cards.

BRINP3 gene is highly expressed in the nervous system until adulthood. But its expression is not limited to neural tissues only. It is also expressed in vascular smooth muscle cells, myoblastic cells, cancer cells, and fibroblast cultures. Nevertheless, the mechanism that regulates the expression of *BRINP3* remains unknown [21].

Interestingly, studies have shown the relationship of *BRINP3* gene with human diseases, such as squamous cell carcinoma of the tongue [22], gastric cancer [23], myocardial infarction [24], and tumors pituitary glands [25].

Genetic polymorphisms are defined as changes in the DNA sequence, which occur in frequency of more than 1% in the population [26]. The high frequency of polymorphisms in the human genome becomes a tool for understanding genetic variability [27].

Therefore, our hypothesis is that, together with sociodemographic and clinical aspects, the polymorphism in the *BRINP3* gene may be associated with the occurrence of BMG. In addition, in terms of relevance, it is important to note that this is the first study investigating *BRINP3* and its association with BMG.

Objectives

General objective

The aim of this study was to investigate the association of sociodemographic, clinical aspects, and polymorphism rs1342913 in the *BRINP3* gene with BMG.

Specific Objectives

a) Investigate sociodemographic aspects involved in the susceptibility to BMG;
b) Examine clinical aspects influencing BMG outcome;
c) Analyze the association of polymorphism tagSNP rs1342913 in the *BRINP3* gene with BMG.

Materials and Methods

Patients' selection

This quantitative, observational, case-control study included 174 unrelated patients of both sexes, aged 18 years or over. The control group consisted of 130 patients who had no history of BMG and the case group consisted of 44 patients who had BMG in its active phase, that is, presenting reddish lesions surrounded by whitish and irregular borders, with loss of filiform papillae at the time of DNA collection.

The mean age of the subjects was 33.7 (±12.5; range 19-61) years for the case group and 32.8 (±12.0; range 18-63) years for the control group. The proportion of cases and controls was 1:3, to increase the statistical power of the study. Patients with syndromes, psoriasis, and continued use of corticoids were not included.

The patient population of both groups was recruited from the Dental Clinics of the Dentistry Program at the Pontifical Catholic University of Paraná (PUCPR) and from the Federal University of Paraná (UFPR), between the years 2012 and 2013.

All patients signed an informed consent form. The project was approved by the Research Ethics Committee of PUCPR and UFPR, under protocol numbers 01328412.5.0000.0020 and 1002.127.10.09, respectively.

Clinical Evaluation

Information of the universe of patients in the sample was collected from questionnaires prepared on personal, medical, and dental history. The sociodemographic and clinical parameters examined were age, sex, ethnicity, socioeconomic status, systemic diseases, systemic diseases in first-degree relatives, continuous medication, allergies, smoking, alcohol consumption, fissured tongue, anxiety scale, decayed, missing, and filled teeth (DMFT) index, and decayed + filled teeth.

For the evaluation of the socioeconomic classification, the ABEP [28] evaluation instrument was used. This comprises a set of specific indicators, such as the number of family cars, number of bathrooms, number of full-time domestic employees, possession of household items such as televisions, radio, vacuum cleaners, washing machine, refrigerator, freezer, and the level of education of the head of the family. Points were attributed to these indicators and the final score allowed to classify patients

in socioeconomic groups A1, A2, B1, B2, C, D, and E. It was decided to work with this variable since some oral health conditions are associated with the individual's socioeconomic condition.

The following systemic diseases were analyzed: diabetes, hepatitis, HIV, syphilis, rheumatic fever, tuberculosis, hypertension, and kidney, respiratory, heart, gastrointestinal, rheumatic, blood, and autoimmune diseases.

For the diagnosis of the fissured tongue, the used criterion was the presence of three or more fissures.

The patients' state of anxiety was calculated using the Hamilton scale [29]. This scale consists of 14 items, which were measured using an intensity scale, ranging from 0 to 4 (0=absent; 1=mild; 2=medium; 3=strong; 4=maximum) for each question in the questionnaire. The points were added up, causing the score to vary from 0 to 31 points.

The DMFT index, formulated by Klein and Palmer, in 1937 [30], was also analyzed. It is used by the World Health Organization to assess the prevalence of dental caries in several countries and the acronym DMF stands for the words "decayed", "missing" and "filled", and T stands for teeth. The minimum score obtained in this study was 0 and the maximum was 28.

Importantly, all the data was accessed by only one examiner (R.S).

DNA Collection and Purification

Cells were obtained by rinsing with a 3% glucose solution for 1 minute and scraping the cheek mucosa with a sterile wooden spatula [31]. The tip of the spatula was then stirred into the mouthwash solution. The oral epithelial cells were pelleted by centrifugation at 706 g for 10 minutes. The supernatant was discarded, and the cell pellet resuspended in 1.300 µl of extraction buffer [10 mM Tris-HCl (pH 7.8), 5 mM EDTA, 0.5% SDS]. Ten µl of proteinase K (20 mg/ml), left overnight at 65°C, were added to the solution. The DNA was purified by adding 10 M ammonium acetate, precipitated with isopropanol, and resuspended with 50 µl of extraction solution [10 mM Tris (pH 7.6) and 1 mM EDTA] [32].

Subsequently, to analyze the DNA concentration obtained by extraction, the genetic material was subjected to spectrophotometric reading on the Nanodrop 2000® equipment (Thermo Fisher Scientific). This method uses the principle of selective

absorption of ultraviolet light by DNA molecules at 260 nm, while proteins do this absorption at 280 nm. In this way, it is possible to quantify the DNA and proteins that make up the extracted sample by reading these two wavelengths. To calculate the DNA concentration, the absorbance value found at 260 nm was used and the 260/280 ratio indicated the purity of the sample. DNA samples with 260/280 nm ranging from 1.5 to 2.0 were considered adequate [33]. Three µl of the extracted DNA was used for the polymerase chain reaction (PCR) process.

Analysis of the *BRINP3* gene tagSNP rs1342913

Nearby SNPs are often correlated in terms of linkage disequilibrium (LD). When these SNPs are in strong LD, they can capture information from other SNPs in the same block (or bin) and are called tagSNPs. Genotyping tagSNPs excludes the need to genotype all SNPs of a given gene, reducing research costs and time [34].

In view of this, an extensive search in the literature was performed to identify a tagSNP that captured the largest possible extension of the gene. Thus, the tagSNP rs1342913 of the candidate gene *BRINP3* was selected, according to information available on the SNPinfo Web Server website (http://snpinfo.niehs.nih.gov), in 2019.

The minimum allele frequency (FAM) of the chosen marker was 0.25 in the CEU population (Utah residents with northern and European ancestry) and was in high LD ($r^2 \geq 0.8$) with another 67 markers. Thus, the choice of rs1342913 made it possible to cover an extensive portion (approximately 50%) of the *BRINP3* gene (Fig. 3).

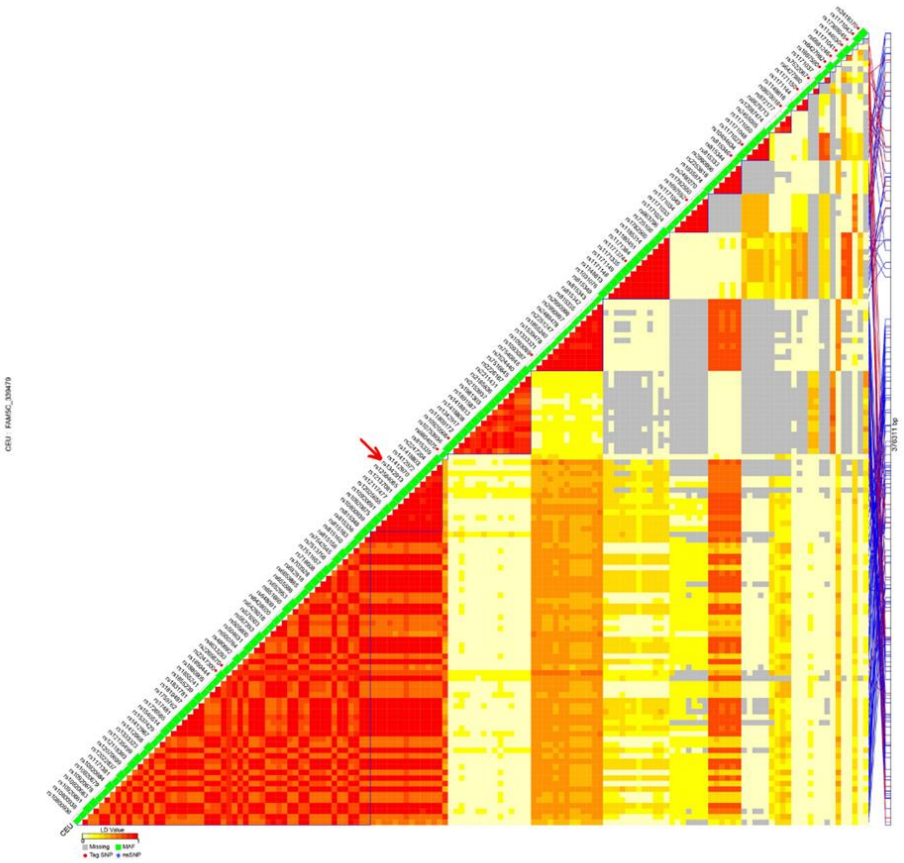

Figure 3. Location of rs1342913 of the *BRINP3* gene. Minimal allele frequency of 0.25 in the CEU population. High LD ($r^2 \geq 0.8$) with the other 67 markers (coverage of approximately 50% of the gene). Source: SNPinfo Web Server (http://snpinfo.niehs.nih.gov)

Patients were genotyped for the tagSNP rs1342913 by the Real Time Polymerase Chain Reaction Technique (Applied Biosystems 7500 Real Time PCR System; Applied Biosystems, Foster City, CA, USA), using TaqMan™ Genotyping Max Mister technology (Applied Biosystems). Negative control was used in the genotyping performed.

Statistical analysis

Statistical analyzes were performed using the SPSS program, version 20.0, and the Hardy-Weinberg equilibrium calculated in Haploview 4.1.

Continuous variables were described as mean and standard deviation. Categorical variables were expressed by frequencies and percentages. The following genetic models were analyzed: dominant and recessive, evaluated by Pearson's chi-

square test, and additive model, performed by binary logistic regression. The association between continuous variables was estimated by the Student's *t*-test for independent samples.

For multivariate analysis, the binary logistic regression model was used, including independent variables with values of $p<0.20$ in the univariate analysis and the rs1342913 polymorphism using the backward method. Results with a $p<0.05$ were considered statistically significant.

The risk estimates were accessed by calculating Odds Ratio (OR) with a 95% Confidence Interval (CI).

Sample Power

The sample power calculation was performed using the Genetic Power Calculator Tool [35].

Results

Sociodemographic and Clinical Parameters

The clinical parameters that were associated with BMG were: fissured tongue (p=3.7*10^{-15}), anxiety (p=0.001), DMFT index (p=0.013), and decayed + filled teeth (p=0.000) (Table 1).

No association was found between BMG and age, sex, ethnicity, socioeconomic status, systemic diseases, systemic diseases in first-degree relatives, continuous medication, allergies, smoking and, alcohol consumption (p>0.05) (Table 1).

Table 1. Results of the univariate analysis, considering the clinical and sociodemographic variables for the case (n=44) and control (n=130) groups.

Variable	Groups		*p*-value	OR (CI 95%)
	Case	Control		
Age [a]	33.7 (12.5)	32.8 (12.0)	0.694	-
Sex [b]				
Male	19 (43.2)	58 (44.6)	0.869	0.94 (0.47 - 1.88)
Female	25 (56.8)	72 (55.4)		
Ethnicity [b]				
Caucasian	38 (86.4)	114 (87.7)	0.819	1.12 (0.41 - 3.08)
Non-Caucasian	6 (13.6)	16 (12.3)		
Socioeconomic status [b]				
A1/A2/B1/B2	9 (20.5)	35 (26.9)	0.394	1.43 (0.62 - 3.28)
C1/C2/D/E	35 (79.5)	95 (73.1)		
Systemic diseases [b]				
No	21 (47.7)	69 (53.1)	0.539	1.23 (0.62 - 2.45)
Yes	23 (52.3)	61 (46.9)		
Systemic diseases in first-degree relatives [b]				
No	8 (18.2)	33 (25.4)	0.331	1.53 (0.64 - 3.62)
Yes	36 (81.8)	97 (74.6)		
Continuous medication [b]				
No	19 (43.2)	65 (50.0)	0.434	1.31 (0.66 - 2.61)
Yes	25 (56.8)	65 (50.0)		
Allergies [b]				
No	32 (72.7)	95 (73.1)	0.964	1.01 (0.47 - 2.19)
Yes	12 (27.3)	35 (26.9)		
Smoking [b]				
No	39 (88.6)	122 (93.8)	0.256	1.95 (0.60 - 6.32)
Yes	5 (11.4)	8 (6.2)		
Alcohol consumption [b]				
No	24 (54.5)	66 (50.8)	0.665	0.85 (0.43 - 1.70)
Yes/Socially	20 (45.5)	64 (49.2)		
Fissured tongue [b]				
No	24 (54.5)	129 (99.2)	**$3.7*10^{-15}$**	107.5 (13.76 - 839.3)
Yes	20 (45.5)	1 (0.8)		
Anxiety scale [a]	13.9 (8.6)	9.0 (6.1)	**0.001**	-
DMFT index [b]				
0 to 8	18 (40.9)	81 (62.3)	**0.013**	2.388 (1.188 - 4.798)
9 or more	26 (59.1)	49 (37.7)		
Decayed + Filled teeth [b]				
0 to 5	11 (25.0)	75 (57.7)	**0.000**	4.091 (1.902 - 8.800)
6 or more	33 (75.0)	55 (42.3)		

[a]Teste t de Student

[b]Teste Qui-quadrado de Pearson

OR: Odds Ratio

CI: Confidence Interval

Genetic analysis

The distribution of the *BRINP3* genotypes is in Hardy-Weinberg equilibrium for the control group, suggesting the genotyping is reliable.

The rs1342913 tagSNP showed no association with BMG in any of the studied genetic models $p>0.05$ (Table 2).

Table 2. Results of the univariate analysis of gene *BRINP3* tagSNP for the dominant and recessive models and additive model for the control (n=130) and case (n=44) groups.

Gene	dbSNP[a]	Genetic Model[&]	Genotypes	Groups - n (%)		*p*-value	OR (CI 95%)
				Control	Case		
BRINP3	**rs1342913**	Additive n=171[#]	GG	44 (34.4)	13 (30.2)	0.840[‡]	0.903 (0.334 - 2.442)
			AG	54 (42.2)	22 (51.2)	0.369[‡]	0.655 (0.260 - 1.649)
			AA	30 (23.4)	8 (18.6)	0.582[‡]	
		Dominant G n=171[#]	GG + AG	98 (76.6)	35 (81.4)	0.510*	1.339 (0.561 - 3.197)
			AA	30 (23.4)	8 (18.6)		
		Recessive G n=171[#]	AA + AG	84 (65.6)	30 (69.8)	0.618*	1.209 (0.573 - 2.549)
			GG	44 (34.4)	13 (30.2)		

[a]SNP identifier based on the NCBI dbSNP
[&]The allele 1 is more frequent in the case group
[‡]Binary logistic regression
*Pearson chi-square test
[#]n=171 because 3 samples did not amplify
OR: Odds Ratio
CI: Confidence Interval

Multivariate Analysis

Multivariate analysis is a tool used to study the behavior of multiple variables simultaneously [36]. In the specific case, variables that obtained a value of $p<0.20$ in the univariate analysis were considered: fissured tongue ($p=3.7*10^{-15}$), anxiety ($p=0.001$), DMFT index ($p=0.013$), decayed + filled teeth ($p=0.000$) (Table 1), and polymorphism rs1342913 (Table 2).

The variables that remained associated with BMG after the multivariate analysis were: fissured tongue ($p=1.3*10^{-5}$; OR=131.953; CI:14.672-1186.700), anxiety

(*p*=0.000), and decayed + filled teeth (*p*=0.003; OR= 5.212; CI: 1.756-15.470) (Table 3), strengthening the association.

Table 3. Results of the multivariate analysis for the control (n=130) and case (n=44) groups.

Variable	Groups		*p*-value*	OR (CI 95%)
	Case	Control		
Fissured tongue [a]				
No	24 (54.5)	129 (99.2)	**$1.3*10^{-5}$**	131.953 (14.672 - 1186.700)
Yes	20 (45.5)	1 (0.8)		
Anxiety scale [b]	13.9 (8.6)	9.0 (6.1)	**0.000**	-
Decayed + Filled teeth [a]				
0 to 5	11 (25.0)	75 (57.7)	**0.003**	5.212 (1.756 - 15.470)
6 or more	33 (75.0)	55 (42.3)		

*Binary logistic regression
[a]n (%)
[b]Mean (Standard Deviation)
OR: Odds Ratio
CI: Confidence Interval

Sample Power

The sample power was 83%, considering the frequency of the rare allele of 44.5% (Table 4).

Table 4. Calculation of the sample power, using the Genetic Power Calculator tool [35], setting as parameters: prevalence of BMG 7% [13], effect size from 3.0 for carrying one copy of the risk allele and 6.0 carrying two copies of the risk allele, D'=1, frequency of the rare allele of 44.5%, 1:3 case and control ratio and level of significance of 0.05.

	rs1342913	
Allele frequency	**Power**	**n cases for 80% power**
0.05	0.05	5321
0.1	0.11	615
0.2	0.36	131
0.3	0.83	40

Frequency of the rare allele of the polymorphism in the study sample: rs1342913 - 44.5%

Discussion

First described by Rayer in 1831 [37], BMG is an inflammatory condition [38], multifactorial [13], and is usually discovered casually through a clinical examination routine [3]. Despite being very studied, it is characterized as a complex condition with etiological aspects still inconclusive, which attracts the attention of researchers who seek its treatment [39].

According to the literature, BMG is a common disease in patients who have a fissured tongue [40, 41]. Eidelman et al. suggested that the same genes could be responsible for both conditions [42]. In our study, there was an association between these two variables, therefore, patients who presented BMG also showed a high prevalence of fissured tongue. A hypothesis that could explain such a situation would be the fact that the fissures can act as areas of stagnation on the surface of the tongue in which BMG can start [40, 43].

Psychosomatic factors such as stress and anxiety appear to play a significant role in the etiology of BMG [44]. Although this condition is generally asymptomatic, for some individuals even painless injuries represent a source of anxiety [45]. Stress is associated with BMG and its reduction can help in the healing of injuries [46]. In this study, the Hamilton Anxiety Scale [29] was used and the mean value of anxiety was higher in the case group than in the control group, reinforcing this association. In addition, these individuals may neglect their self care, which may result in poor hygiene and oral diseases such as caries [47]. It is important to note that anxious patients can sometimes consume a greater amount of food [15]. Moreover, patients with a high degree of stress may experience reduced salivary flow [15], and this reduction in the amount of saliva might intensify the risk to oral disorders [48]. In this context, these same factors might be associated with the DMFT index and decayed + filled teeth. Our research group was the first to evidence the association of BMG with DMFT [15]. In this study, after the multivariate analysis, caries elements remained associated with BMG suggesting that these DMFT components were indeed associated with the outcome.

Regarding the other sociodemographic and clinical variables analyzed, no signs of association with BMG were found. In the literature, the relationship between BMG

and sex varies according to different studies. However, most investigations did not show a predilection for sex [40, 49-50]. The influence of ethnicity is not yet clear [4]. Additionally, the prevalence of BMG seems to decrease with age [51]. According to Jainkittivong et al. (2005), the highest incidence of BMG occurred in individuals aged between 20 and 29 years old [4], very similar to the study by Miloğlu et al. (2009), in which adults aged 21 to 30 years presented higher prevalence of BMG [52].

BRINP3 is located in the mitochondria and its overexpression leads to increased proliferation and cell migration [25]. It is worth mentioning that the main features commonly attributed to mitochondria are the regulation of cell proliferation, ATP generation, cell death, and metabolism [53]. Moreover, it has also been reported that mitochondria have the capacity to perceive signs of inflammation, activating and managing the immune system [54] important phenomena in the pathogenesis of BMG.

A previous case report investigated 5-year-old monozygotic twins with BMG suggests a genetic role in the manifestation of BMG [55]. Monozygotic twins share 100% of their genes, whose phenotype is probably totally explained by their genes, and not by the environment [56]. Case-control studies have evaluated the association of polymorphisms in the *IL6* [14] *IL1B*, *TNFA* [5], *COMT,* and *5HTT* [15] genes with BMG.

The genetic results obtained in this study showed no association of *BRINP3* polymorphism rs1342913 with BMG. However, it is worth noting that the strategy used to select the rs1342913 tagSNP was quite interesting, since this “target” polymorphism captures the information of 67 other SNPs by high LD ($r^2 \geq 0.8$). This tagSNP is located on chromosome 1, base pair 190121025, in the intron region [20]. Therefore, it can capture information from functional polymorphisms or interfere in the splicing of exons, which might modify the structure of the primary protein and consequently its function [57]. Additionally, the rs1342913 polymorphism was previously associated with fracture healing [58], aggressive periodontitis [18] and, peri-implantitis [20]. Therefore, *BRINP3* could be suggested as a candidate gene for immunoinflammatory conditions, such as BMG.

The present study has some limitations. In this study, a tagSNP from the CEU population was selected because the southern region of Brazil mostly presents European descent. Nevertheless, the Brazilian population is “not a pure” Caucasian

population, considered to be miscegenated. In addition, although the rs1342913 tagSNP represents other 67 markers, covering about 50% of the gene sequence, there are other 50% of the gene to be investigated.

Finally, understand which factors are associated with BMG could help improve scientific knowledge about this complex condition, increasing the chances of prevention and treatment.

Conclusions

It was found that fissured tongue, anxiety, DMFT index, and decayed + filled teeth were associated with BMG in the univariate analysis.

No association was found between the polymorphism in the *BRINP3* gene (rs1342913) and BMG.

The variables that remained associated with BMG after the multivariate analysis were fissured tongue, anxiety, and decayed + filled teeth.

Additional studies are recommended to replicate the findings preferably in other samples and populations, which may help elucidate the participation of this gene in the etiology of BMG.

References

1. Al Qahtani NA, Deepthi A, Alhussain NM, Al Shahrani BA, Alshehri H, Alhefzi A, Joseph B (2019) Association of geographic tongue and fissured tongue with ABO blood group among adult psoriasis patients: a novel study from a tertiary care hospital in Saudi Arabia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 127:490-497. https://doi.org/10.1016/j.oooo.2019.01.080

2. Stoopler ET, France K, Ojeda D, Sollecito TP (2018) Benign migratory glossitis. J Emerg Med 54:e9-e10. https://doi.org/10.1016/j.jemermed.2017.09.035

3. Madani FM, Kuperstein AS (2014) Normal variations of oral anatomy and common oral soft tissue lesions: evaluation and management. Med Clin North Am 98:1281-1298. https://doi.org/10.1016/j.mcna.2014.08.004

4. Jainkittivong A, Langlais RP (2005) Geographic tongue: clinical characteristics of 188 cases. J Contemp Dent Pract 6:123-135. https://doi.org/10.5005/jcdp-6-1-123

5. Guimarães, A. L. S., Correia-Silva, J. D. F., Diniz, M. G., Xavier, G. M., Horta, M. C. R., & Gomez, R. S (2007) Investigation of functional gene polymorphisms: IL-1B, IL-6 and TNFA in benign migratory glossitis in Brazilian individuals. J Oral Pathol Med 36:533-537. https://doi.org/10.1111/j.1600-0714.2007.00559.x

6. Vieira, I., Fernandes, Â., Céspedes, J. M. A., Machado, M. Â. N., Brancher, J. A., & de Lima, A. A. S.Taste (2011) Taste evaluation in adolescents and pediatric patients with benign migratory glossitis. Int J Pediatr Otorhinolaryngol 75:1230-1233. https://doi.org/10.1016/j.ijporl.2011.06.020

7. Picciani BL, Domingos TA, Teixeira-Souza T, Santos VD, Gonzaga HF, Cardoso-Oliveira J, Gripp AC, Dias EP, Carneiro S (2016) Geographic tongue and psoriasis: clinical, histopathological, immunohistochemical and genetic correlation-a

literature review. An Bras Dermatol 91:410-421. https://doi.org/10.1590/abd1806-4841.20164288

8. Hume WJ (1975) Geographic stomatitis: a critical review. J Dent 3:25-43. https://doi.org/10.1016/0300-5712(75)90020-2

9. Oyetola EO, Oluwande A, Agho ET (2018) Geographic tongue: pattern of presentation in a south western Nigerian teaching hospital. Ann Ib Postgrad Med 16:131-135.

10. Pinna R, Cocco F, Campus G, Conti G, Milia E, Sardella A, Cagetti MG (2019) Genetic and developmental disorders of the oral mucosa: Epidemiology; molecular mechanisms; diagnostic criteria; management. Periodontol 2000 80:12-27. https://doi.org/10.1111/prd.12261

11. Nandini, D. B., Bhavana, S. B., Deepak, B. S., & Ashwini, R. (2016) Paediatric geographic tongue: a case report, review and recent updates. J Clin Diagn Res 10:ZE05-ZE09. https://doi.org/10.7860/JCDR/2016/16452.7191

12. Campana F, Vigarios E, Fricain JC, Sibaud V (2019) Geographic stomatitis with palate involvement. An Bras Dermatol 94:449-451. https://doi.org/10.1590/abd1806-4841.20197774

13. Jorge, M. A., Gonzaga, H. F. D. S., Tomimori, J., Picciani, B. L. S., & Barbosa, C. A (2017) Prevalence and heritability of psoriasis and benign migratory glossitis in one Brazilian population. An Bras Dermatol 92:816-819. https://doi.org/10.1590/abd1806-4841.20176389

14. Dawson, T. A. J., & Pielou, W. D (1967) Geographical tongue in three generations. Br J Dermatol 79:678-681. https://doi.org/10.1111/j.1365-2133.1967.tb11436.x

15. Scariot, R., Batista, T. B. D., Olandoski, M., Souza, C. M., Souza, P. H. C., Lima, A. A. S., & Trevilatto, P. C (2017) Host and clinical aspects in patients with benign migratory glossitis. Arch Oral Biol 73:259-268 https://doi.org/10.1016/j.archoralbio.2016.10.027

16. Scariot R, Sebastiani AM, Meger MN, Broska VT, Souza JF, Lima AA, Brancher JA, de Souza CM, Souza PHC, Trevillato PC (2019) Anxiety and genetic polymorphisms in catechol-O-methyltransferase (COMT) and serotonin transportation gene (5HTT) are associated with benign migratory glossitis. Oral Surg Oral Med Oral Pathol Oral Radiol 127:218-224. https://doi.org/10.1016/j.oooo.2018.10.011

17. Ribeiro FV, Santos VR, Bastos MF, De Miranda TS, Vieira AR, De Figueiredo LC, Duarte PM (2012) A preliminary study on the FAM5C expression in generalized chronic periodontitis. Oral Dis. 18:147-152. https://doi.org/10.1111/j.1601-0825.2011.01855.x

18. Carvalho FM, Tinoco EM, Deeley K, Duarte PM, Faveri M, Marques MR, Mendonça AC, Wang X, Cuenco K, Menezes R, Garlet GP (2010) FAM5C contributes to aggressive periodontitis. PLoS One 5:e10053. https://doi.org/10.1371/journal.pone.0010053

19. Tanaka KI, Matsumoto E, Higashimaki Y, Sugimoto T, Seino S, Kaji H (2012) FAM5C is a soluble osteoblast differentiation factor linking muscle to bone. Biochem Biophys Res Commun 418:134-139. https://doi.org/10.1016/j.bbrc.2011.12.147

20. Casado PL, Aguiar DP, Costa LC, Fonseca MA, Vieira TC, Alvim-Pereira CC, Alvim-Pereira F, Deeley K, Granjeiro JM, Trevilatto PC, Vieira AR (2015) Different contribution of BRINP3 gene in chronic periodontitis and peri-implantitis: a cross-sectional study. BMC Oral Health 15:33. https://doi.org/10.1186/s12903-015-0018-6

21. Sato J, Kinugasa M, Satomi-Kobayashi S, Hatakeyama K, Knox AJ, Asada Y, Wierman ME, Hirata K, Rikitake Y (2014) Family with sequence similarity 5, member

C (FAM5C) increases leukocyte adhesion molecules in vascular endothelial cells: Implication in vascular inflammation. PLoS One 9:e107236. https://doi.org/10.1371/journal.pone.0107236

22. Kuroiwa T, Yamamoto N, Onda T, Shibahara T (2009) Expression of the FAM5C in tongue squamous cell carcinoma. Oncol Rep 22:1005-1011. https://doi.org/10.3892/or_00000528

23. Chen L, Su L, Li J, Zheng Y, Yu B, Yu Y, Yan M, Gu Q, Zhu Z, Liu B (2012) Hypermethylated FAM5C and MYLK in serum as diagnosis and pre-warning markers for gastric cancer. Dis Markers 32:195-202. https://doi.org/10.3233/DMA-2011-0877

24. Connelly JJ, Shah SH, Doss JF, Gadson S, Nelson S, Crosslin DR, Hale AB, Lou X, Wang T, Haynes C, Seo D (2008) Genetic and functional association of FAM5C with myocardial infarction. BMC Med Genet 9:33. https://doi.org/10.1186/1471-2350-9-33

25. Shorts-Cary L, Xu M, Ertel J, Kleinschmidt-Demaster BT, Lillehei K, Matsuoka I, Nielsen-Preiss S, Wierman ME (2007) Bone morphogenetic protein and retinoic acid-inducible neural specific protein-3 is expressed in gonadotropic cell pituitary adenomas and induces proliferation, migration, and invasion. Endocrinology 148:967-975. https://doi.org/10.1210/en.2006-0905

26. Brookes AJ (1999) The essence of SNPs. Gene 234:177-186. https://doi.org/10.1016/s0378-1119(99)00219-x

27. Aerts J, Wetzels Y, Cohen N, Aerssens J (2002) Data mining of public SNP databases for the selection of intragenic SNPs. Hum Mutat 20:162-73. https://doi.org/10.1002/humu.10107

28. ABEP (2008) Critério de classificação econômica Brasil (CCEB). Associação Brasileira de Empresas de Pesquisa (ABEP).

29. Hamilton (1959) The assessment of anxiety states by rating. Br J Med Psychol 32:50-55. https://doi.org/10.1111/j.2044-8341.1959.tb00467.x

30. Chaves MM (1986) Odontologia Social. Artes Médicas, São Paulo, pp. 448-448

31. Trevilatto PC, Line SR (2000) Use of buccal epithelial cells for PCR amplification of large DNA fragments. J Forensic Odontostomatol 18:6-9.

32. Aidar M, Line SR (2007) A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. Braz Dent J 18:148-152.

33. Morey M, Fernandez-Marmiesse A, Castineiras D, Fraga JM, Couce ML, Cocho JÁ (2013) A glimpse into past, present, and future DNA sequencing. Mol Genet Metab 110:3-24. https://doi.org/10.1016/j.ymgme.2013.04.024

34. Giacobbo LC, Perin MAA, Pereira T. M, Garmendia MO, Reichow A, Melo AC, Castilhos BB, Trevilatto PC (2020) RANK/RANKL/OPG gene polymorphisms and loss of orthodontic mini-implants. Orthod Craniofac Res 3(2):210-222. https://doi.org/10.1111/ocr.12360

35. Purcell, S., Cherny, S. S., & Sham, P. C (2003) Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19:149-150. https://doi.org/10.1093/bioinformatics/19.1.149

36. Carvalho, M. D. J. D. S. D., Souza, A. D. S., Santos, E. B., Soares Filho, W. D. S., Ledo, C. A. D. S., & Souza, F. V. D (2016) Univariate and multivariate statistical tools for in vitro conservation of citrus genotypes. Acta Sci Agron 38:129-137. https://doi.org/10.4025/actasciagron.v38i1.26433

37. Prinz H (1927) Wandering rash of the tongue (geographic tongue). Dent Cosmos 69:272-275.

38. Abe M, Sogabe Y, Syuto T, Ishibuchi H, Yokoyama Y, Ishikawa O (2007) Successful treatment with cyclosporin administration for persistent benign migratory glossitis. J Dermatol 34:340-343. https://doi.org/10.1111/j.1346-8138.2007.00284.x

39. Banakar, M (2019) What are the best treatments for benign migratory glossitis? Evid Based Dent 20:40-41. https://doi.org/10.1038/s41432-019-0024-7

40. Goswami, M., Verma, A., & Verma, M (2012) Benign migratory glossitis with fissured tongue. J Indian Soc Pedod Prev Dent 30:173-175. https://doi.org/10.4103/0970-4388.100008

41. Dafar, A., Çevik-Aras, H., Robledo-Sierra, J., Mattsson, U., & Jontell, M (2016) Factors associated with geographic tongue and fissured tongue. Acta Odontol Scand 74:210-216. https://doi.org/10.3109/00016357.2015.1087046

42. Eidelman, E., Chosack, A., & Cohen, T (1976) Scrotal tongue and geographic tongue: polygenic and associated traits. Oral Surg Oral Med Oral Pathol 42:591-596. https://doi.org/10.1016/0030-4220(76)90210-3

43. Nandini, D. B., Bhavana, S. B., Deepak, B. S., & Ashwini, R (2016) Paediatric geographic tongue: a case report, review and recent updates. J Clin Diagn Res 10: ZE05-ZE09. https://doi.org/10.7860/JCDR/2016/16452.7191

44. Redman, RS (1970) Prevalence of geographic tongue, fissured tongue, median rhomboid glossitis, and hairy tongue among 3,611 Minnesota schoolchildren. Oral Surg Oral Med Oral Pathol 30:390-395. https://doi.org/10.1016/0030-4220(70)90320-8

45. Redman, R. S., Vance, F. L., Gorlin, R. J., Peagler, F. D., & Meskin, L. H (1965) A psychological component in the etiology of geographic tongue. Am J Psychiatry 121:805-806 https://doi.org/10.1176/ajp.121.8.805

46. Ebrahimi, H., Pourshahidi, S., Andisheh, T. A., & Shyan, S. B (2010) The relationship between geographic tongue and stress. Iranian Red Crescent Medical Journal 12:313-315.

47. Torales, J., Barrios, I., & González, I (2017) Oral and dental health issues in people with mental disorders. Medwave 17:e7045. https://doi.org/10.5867/medwave.2017.08.7045

48. Scarabelot, V. L., Munerato, M. C., Medeiros, L. F., Oliveira, M. G. D., Chaves, A. C. M., Souza, A. D., & Torres, I. L. D. S (2014) Factors associated to salivary flow alterations in dry mouth female patients. Rev Dor 15:186-190. http://doi.org/10.5935/1806-0013.20140041

49. Yarom, N., Cantony, U., & Gorsky, M (2004) Prevalence of fissured tongue, geographic tongue and median rhomboid glossitis among Israeli adults of different ethnic origins. Dermatology 209:88-94. https://doi.org/10.1159/000079590

50. Honarmand, M., Mollashahi, L. F., Shirzaiy, M., & Sehhatpour, M (2013) Geographic tongue and associated risk factors among Iranian dental patients. Iran J Public Health 42:215-219

51. Shulman, J. D., & Carpenter, W. M (2006) Prevalence and risk factors associated with geographic tongue among US adults. Oral Dis 12:381-386. https://doi.org/10.1111/j.1601-0825.2005.01208.x

52. Miloğlu, Ö., Göregen, M., Akgül, H. M., & Acemoğlu, H (2009) The prevalence and risk factors associated with benign migratory glossitis lesions in 7619 Turkish dental outpatients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 107:e29-e33. https://doi.org/10.1016/j.tripleo.2008.10.015

53. Missiroli, S., Genovese, I., Perrone, M., Vezzani, B., Vitto, V. A., & Giorgi, C (2020) The role of mitochondria in inflammation: from cancer to neurodegenerative disorders. J Clin Med 9:740. https://doi.org/10.3390/jcm9030740

54. Kolmychkova, K. I., Zhelankin, A. V., Karagodin, V. P., & Orekhov, A. N (2016) Mitochondria and inflammation. Patol Fiziol Eksp 60:114-121.

55. Shekhar, M. G (2014) Geographic tongue in monozygotic twins. J Clin Diagn Res 8:ZD01-ZD02. https://doi.org/10.7860/JCDR/2014/7512.4210

56. Sahu, M., & Prasuna, J. G (2016) Twin studies: A unique epidemiological tool. Indian J Community Med 41:177-182. https://doi.org/10.4103/0970-0218.183593

57. Ramírez-Bello, J., Vargas-Alarcón, G., Tovilla-Zárate, C., & Fragoso, J. M (2013) Polimorfismos de un solo nucleótido (SNP): implicaciones funcionales de los SNP reguladores (rSNP) y de los SNP-ARN estructurales (srSNP) en enfermedades complejas. Gac Med Mex 149:220-228.

58. Guimarães JM, Guimarães IC, Duarte ME, Vieira T, Vianna VF, Fernandes MB, Vieira AR, Casado PL (2013) Polymorphisms in BMP4 and FGFR1 genes are associated with fracture non-union. J Orthop Res 31:1971-1979. https://doi.org/10.1002/jor.22455

ANEXOS

Lista de abreviatura e siglas

°C	Celsius degrees
µl	Microliter
bin	Block of linkage disequilibrium
BMG	Benign migratory glossitis
BRINP3	Bone morphogenetic protein/specific neural inducible retinoic acid 3
BRINP3	Gene that encodes BRINP3 protein
CEU	Utah residents with northern and European ancestry
CI	Confidence Interval
dbSNP	SNPs database
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine Tetra-Acetic Acid
et al.	And collaborators
FAM	Minimum allele frequency
FAM5C	Family with Sequence Similarity 5, Member C
HCL	Hydrochloric acid
LD	Linkage disequilibrium
M	Molar
mM	Millimolar
NCBI	National Center for Biotechnology Information
OR	Odds Ratio
PCR	Real Time Polymerase Chain Reaction
pH	Hydrogen potential
rs	reference SNP
SDS	Sodium dodecyl sulfate
SNPs	Single Nucleotide Polymorphism
tagSNP	Representative SNP of gene
Tris	Hydroxymethyl aminomethane

1 Parecer do Comitê de Ética

2

UNIVERSIDADE FEDERAL DO PARANÁ - SETOR DE CIÊNCIAS DA SAÚDE/ SCS -

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ANÁLISE CLÍNICA, CITOLÓGICA E GENÉTICA EM PORTADORES DE GLOSSITE MIGRATÓRIA BENIGNA E DE LÍQUEN PLANO

Pesquisador: Paula Cristina Trevilatto
Área Temática: Área 9. A critério do CEP.
Versão: 3
CAAE: 01328412.5.0000.0020
Instituição Proponente: Pontifícia Universidade Católica do Parana - PUCPR

DADOS DO PARECER

Número do Parecer: 150.486
Data da Relatoria: 26/09/2012

Apresentação do Projeto:
Pesquisa apresentada pela Dra. Paula Cristina Trevilatto, professora titular da PUC do Paraná e que foi aprovado pela instituição proponente.

Objetivo da Pesquisa:
Investigar eventuais associações comuns entre o processo inflamatório e as alterações citológicas e genéticas encontradas em pacientes portadores de glossite migratória benigna e de líquen plano.

Avaliação dos Riscos e Benefícios:
Os riscos associados à coleta de material não são maiores do que uma rotina de escovação normal da boca que podem, no máximo, trazer algum desconforto, informado no TCLE. Os benefícios estão propostos como de longo prazo, na forma de uma melhor compreensão das duas moléstias.

Comentários e Considerações sobre a Pesquisa:
A pesquisa é pertinente e bem descrita.

Considerações sobre os Termos de apresentação obrigatória:
Os termos foram apresentados.

Recomendações:
Não há.

Endereço: Rua Padre Camargo, 280
Bairro: 2ª andar **CEP:** 80.060-240
UF: PR **Município:** CURITIBA
Telefone: (41)3360-7259 **E-mail:** cometica.saude@ufpr.br

Conclusões ou Pendências e Lista de Inadequações:

Projeto aprovado

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

CURITIBA, 21 de Novembro de 2012

Assinador por:
Claudia Seely Rocco
(Coordenador)

Endereço: Rua Padre Camargo, 280
Bairro: 2ª andar **CEP:** 80.060-240
UF: PR **Município:** CURITIBA
Telefone: (41)3360-7259 **E-mail:** cometica.saude@ufpr.br

PROJETO DE PESQUISA

Título: ANÁLISE CLÍNICA, CITOLÓGICA E GENÉTICA EM PORTADORES DE GLOSSITE MIGRATÓRIA BENIGNA E DE LÍQUEN PLANO

Área Temática:

Versão: 3

CAAE: 01328412.5.0000.0020

Pesquisador: Paula Cristina Trevilatto

Instituição: Pontifícia Universidade Católica do Parana - PUCPR

PARECER CONSUBSTANCIADO DO CEP

Número do Parecer: 105.800

Data da Relatoria: 24/08/2012

Apresentação do Projeto:

Pesquisa apresentada pela Dra. Paula Cristina Trevilatto, professora titular da PUC do Paraná que, segundo se depreende, fará parte de uma pesquisa para obtenção de grau de doutorado (no cronograma há referência a defesa de tese). O objetivo da pesquisa é estudar duas moléstias relativamente benignas:1- a estomatite geográfica, caracterizada pela perda das papilas filiformes do dorso da língua e pelo surgimento de um edema inflamatório associado, e 2- o líquen plano, enfermidade sistêmica que pode acometer pele e mucosa bucal caracterizada por reação inflamatória crônica, mucocutânea e de fundo imunológico

Objetivo da Pesquisa:

Investigar eventuais associações comuns entre o processo inflamatório e as alterações citológicas e genéticas encontradas em pacientes portadores de glossite migratória benigna e de líquen plano.

Avaliação dos Riscos e Benefícios:

Os riscos associados à coleta de material não são maiores do que uma rotina de escovação normal da boca podendo trazer no máximo algum desconforto, colocado no TCLE. Os benefícios são de longo prazo, na forma de uma melhor compreensão das duas moléstias.

Comentários e Considerações sobre a Pesquisa:

A pesquisa é pertinente e bem desenhada não cabendo qualquer reparo.

Considerações sobre os Termos de apresentação obrigatória:

Folha de rosto de acordo. A ficha de anamnese é bastante completa e bem estruturada.

Recomendações:

É obrigatório trazer ao CEP/HC uma cópia do Termo de Consentimento Livre e Esclarecido que foi aprovado, para assinatura e rubrica. Após, xerocar este TCLE em duas vias, uma ficará com o pesquisador e uma para o participante da pesquisa.

Endereço: Rua Gal. Carneiro, 181
Bairro: Alto da Glória **CEP:** 80.060-900
UF: PR **Município:** CURITIBA
Telefone: (41)3360-1041 **Fax:** (41)3360-1041 **E-mail:** cep@hc.ufpr.br

Conclusões ou Pendências e Lista de Inadequações:

Pendências atendidas, projeto poderá ser considerado aprovado.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Diante do exposto, o Comitê de Ética em Pesquisa em Seres Humanos do HC-UFPR, de acordo com as atribuições definidas na Resolução CNS 196/96, manifesta-se pela aprovação do projeto conforme proposto para início da Pesquisa. Solicitamos que sejam apresentados a este CEP, relatórios semestrais sobre o andamento da pesquisa, bem como informações relativas às modificações do protocolo, cancelamento, encerramento e destino dos conhecimentos obtidos.
É obrigatório trazer ao CEP/HC uma cópia do Termo de Consentimento Livre e Esclarecido que foi aprovado, para assinatura e rubrica. Após, xerocar este TCLE em duas vias, uma ficará com o pesquisador e uma para o participante da pesquisa.

CURITIBA, 25 de Setembro de 2012

Assinado por:
Renato Tambara Filho

Endereço: Rua Gal. Carneiro, 181
Bairro: Alto da Glória **CEP:** 80.060-900
UF: PR **Município:** CURITIBA
Telefone: (41)3360-1041 **Fax:** (41)3360-1041 **E-mail:** cep@hc.ufpr.br

Normas para publicação – Clinical Oral Investigations

A1, Q1. Impact factor: **2.812**

Submit Original Articles via SPRINGER: https://www.springer.com/journal/784

Title Page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

- Objectives (stating the main purposes and research question)
- Materials and Methods
- Results
- Conclusions
- Clinical Relevance

These headings must appear in the abstract.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.

- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].

2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

The entries in the list should be numbered consecutively.

- Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations.

If you are unsure, please use the full journal title.

Tables

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Color Art

Color art is free of charge for online publication.

If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.

If the figures will be printed in black and white, do not refer to color in the captions.

Color illustrations should be submitted as RGB (8 bits per channel).

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)

Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)

Any figure lettering has a contrast ratio of at least 4.5:1

Atividades complementares - Doutorado

- Participação na reunião da SBPqO 2020 com apresentação de painel**:** Análise de variáveis clínicas e polimorfismo no gene *BRINP3* e a suscetibilidade à Glossite Migratória Benigna.

- Orientação em andamento de uma aluna de PIBIC Vigência 2020 - 2021**:** Giovana Frech Mulezini

- Aprovação no teste de suficiência: Inglês (2019) e Espanhol (2020).

- Primeira autora em artigo publicado em revista A1/Q2**: Giacobbo, Laís Cristina**; Perin, Maria Augusta Andrigo; Pereira, Thaís Munhoz; Garmendia, Mariana Oliveira; Reichow, Alexandre; Melo, Ana Cláudia; De Castilhos, Bruno Borges; Trevilatto, Paula Cristina. *RANK/RANKL/OPG* gene polymorphisms and loss of orthodontic mini-implants. Orthodontics & Craniofacial Research, v. 1, p. 1, 2019.

- Coautora em artigo submetido em revista A1/Q1**:** "A case-control study suggests *TNFAIP3* as a new genetic risk factor for Benign Migratory Glossitis". Thaís Munhoz Pereira; Rafaela Scariot de Moraes; **Laís Cristina Giacobbo**; Laysa Toschi Martins; Marcelo Távora Mira; Paula Cristina Trevilatto.

- Coautora em artigo publicado na revista Orthodontic Science and Practice**:** Camargo, Elisa Souza; **Giacobbo, Laís Cristina**; Schappo, Cláudia; Schneider, Neblyssa Agatha; Pereira, Thais Munhoz; Oppitz, Layza; Guariza Filho, Odilon. Como desimpactar o 1.º molar superior permanente com erupção ectópica?. Ortho Science: Orthodontic Science and Practice, v. 12, p. 41-45, 2019.

- Primeira autora em artigo publicado na revista Case Reports in Dentistry**: Giacobbo, Laís C**; Guimarães, Lara Karolina; Fornazari, Isabelle Adad; Meda, Eduardo Monteiro; Tanaka, Orlando Motohiro . Achieving Better Function through Combining Orthodontics and Restorative Dentistry in the Case of Dental Abrasions. Case Reports in Dentistry (print), v. 2019, p. 1-5, 2019.

- Participação na reunião da SBPqO 2019 com apresentação de painel**:** Estudo de associação de polimorfismos nos genes *RANK/RANKL/OPG* com a perda de mini-implantes ortodônticos.

- Participação no CIOPAR 2019 com apresentação de painel**:** Análise de associação de polimorfismos nos genes *RANK/RANKL/OPG* com a perda de mini-implantes ortodônticos.

- Participação no projeto Cientistas na Escola 2019 com apresentação da palestra**:** Oficina do cientista aprendiz.

- Participou como avaliadora de pôsteres durante a XIV SAOLP 2019.

- Atualmente é Secretária da Comissão Organizadora da Associação dos Ex-alunos Pós-Graduados em Ortodontia da PUCPR (AEPO-PUCPR).

- Ministrou juntamente com 2 colegas a Oficina de Bioestatística 2019**:** Tabulação de dados de pesquisa quantitativa para iniciantes.

- Participação e auxílio nas teses de colegas da equipe.