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Investigation of functional gene polymorphisms: *IL-1B*, *IL-6* and *TNFA* in benign migratory glossitis in Brazilian individuals

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BACKGROUND: Benign migratory glossitis (BMG) is a very common immunological oral disease of unknown aetiology.

METHODS AND SUBJECTS: Fifty-three consecutive subjects affected by BMG and 53 age- and sex-matched control subjects were genotyped for *IL-1B*, *IL-6* and *TNFA* polymorphisms. Binary logistic regression models were fitted and values of $P < 0.05$ were considered significant.

RESULTS: A significant difference in the distribution of *IL-1B* genotypes was observed in the group with BMG in univariate analyses ($P = 0.01$). The multivariate analyses showed that the CT genotype of the *IL1-B* gene was significantly associated with a high risk to develop BMG ($P = 0.02$, OR 2.76). The combined presence of IL-1β high and intermediate producers genotypes was also associated with BMG in multivariate analyses ($P = 0.01$, OR 3.05). *IL-6* and *TNFA* polymorphisms were not associated with BMG in the univariate and multivariate analyses.

CONCLUSION: Our findings demonstrate that the polymorphism *+3954 IL-1B* is associated with an increased risk of BMG development and suggest a genetic basis for disease development.

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Keywords: benign migratory glossitis; cytokine; geographic tongue; interleukin; pathogenesis; polymorphism

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Introduction

Benign migratory glossitis (BMG) is a very common oral disease of unknown aetiology. This condition is characteristically described as a multiple, variably sized, well-demarcated, erythematous area usually surrounded by a slightly elevated, yellowish-white, circinate linear border, usually occurring on the anterior two-thirds of the dorsal tongue; reminiscent of land masses and oceans on a map (1, 2). Although BMG is generally asymptomatic, a minority of patients exhibit discomfort ranging from sensitivity to cigarette smoke, spicy foods and fruit (3). BMG is characterized by periods of remission and exacerbation of varied duration. During remission, the condition resolves without residual scar formation. When lesions recur, they tend to occur in new locations, thus, producing the migratory pattern (4). The prevalence of BMG varies according to some studies. This could be explained by a wide range of differences in sampling, diagnosis and type of examination (5).

Some reports in the literature indicate that BMG may relate to hormonal disturbances (6), psychological findings (7) and diabetes mellitus (8). BMG is more prevalent in patients who have a tendency to develop immunological diseases (9–12). The association between human leucocyte antigen (HLA) Cw6, BMG and psoriasis suggests that genetic factors are important on BMG pathogenesis (13). Functional *IL-B*, *IL-6* and *TNFA* polymorphisms are associated with distinct protein expression and have been related to the pathogenesis of some immunological diseases (14–16). Moreover, *IL-B*, and *TNFA* polymorphisms were found to be associated with psoriasis, a similar clinical condition (17, 18). Therefore, the objective of the current study was to investigate for the first time a possible association between the cytokines functional genetic polymorphisms and BMG in a sample of Brazilian patients.

Material and methods

Subjects and sample collection

Fifty-three consecutive subjects affected by BMG (Table 1) and 53 age- and sex-matched control subjects (mean age 31 years; range 8–66 years) were included in this study. There were 18 (34%) males and 35 (66%) females in both groups. The patients were recruited from the Oral Diagnosis Clinic at the Universidade Federal de Minas Gerais. Both the experimental and control groups were of the same geographic area and had identical socio-economic status. The individuals were not stratified in ethnic groups based on skin colour, race and geographic origin due to the strong miscegenation among Brazilians (19).

The diagnosis of BMG was based on accepted clinical criteria (20). The control group was comprised of patients without any history of BMG or systemic diseases. Exclusion criteria for both groups were the presence, apart from dental caries, of any other significant local or systemic diseases, including periodontitis. The study protocol was approved by local Ethics Committee and informed consent was obtained from all patients or from the parents of those patients with less than 18 years.

Oral mucosa swabs were taken once from the subjects on the buccal mucosa. The swabs were performed with sterile plastic tips, placed immediately in Eppendorf microtubes containing 500 μl of Krebs buffer, and the pellet obtained after 5 min of centrifugation at 13 000 *g* was stored at −20°C until processing.

DNA isolation

DNA extraction was carried out as described before (21). We added 450 μl of lyses buffer (6.0 M GuSCN, 65 mM Tris–HCl pH 6.4, 25 mM EDTA, 1.5% TritonX-100) and 20 μl of silica (SiO_2, Sigma S-5631, Sigma-Aldrich, St. Louis, MO, USA) to the microcentrifuge tube containing the oral mucosa swab pellet. The tube was mixed and incubated for 10 min at 56°C, centrifuged at 3000 *g* for 1 min and the supernatant discharged. The pellet with the DNA adsorbed into the silica was washed twice with 450 μl washing buffer (6.0 M GuSCN, 65 mM Tris–HCl), twice with 70% ethanol, once with 450 μl of acetone and then was dried at 56°C for 10 min. Finally, 100 μl of TE buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA) was added and incubated at 56°C for 10 min to elute the DNA. After incubation the solution was vortexed and centrifuged at 5000 *g* for 2 min and approximately 90 μl of supernatant containing DNA was transferred to a new tube.

Table 1 Summary of the clinical data of BMG patients included in the study

Characteristics	*Values*
Age (years)	
Median	29.5
Range	4–75
Patient gender	
Male, *n* (%)	18 (34)
Female, *n* (%)	35 (66)

Genotyping

The polymorphisms were assessed by polymerase chain reaction (PCR) amplification and digestion. The sequences of PCR primers were showed in Table 2, as PCR was carried out in a total volume of 50 μl, containing approximately 400 ng of DNA, primers (20 pmol per reaction) and 25 μl of Pre-mix buffer (Phoneutria Biotecnologia, Belo Horizonte, Brazil). According to the manufacturer the Pre-mix buffer contained 50 mM KCl, 10 mM Tris–HCl pH 8.4, 0.1% Triton X-100, 1.5 mM $MgCl_2$, deoxynucleoside triphosphates and 1.25 units of Taq DNA polymerase. The conditions for amplification consisted of 94°C for 3 min followed by 35 cycles of 94°C for 30 s, 54°C for 35 s and 72°C for 30 s. The run was terminated by final elongation at 72°C for 5 min. In all steps the lid temperature was 103°C. The products were digested with restriction enzyme according to manufacturer's protocols (see Table 2). The visualization was performed in a 6.5% polyacrylamide gel electrophoresis stained with ethidium bromide (0.5 μg⁄ml).

Statistical analysis

Univariate analyses were performed using the Fisher's exact test or the Chi-squared test. To investigate the association between the single nucleotide polymorphisms and risk of BMG, binary logistic regression models were fitted. The associations were expressed by

Table 2 Primers sequences, references and restrictions enzymes used for each polymorphism

Genes	*Primers (references)*	*Restriction enzyme (condition)*	*Genotypes*
IL-1β + 3954 (C/T)			
	5′-CTCAGGTGTCCTCGAAGAAATCAAA-3′	Taq I[a] (65°C⁄4hs)	TT-182 + 12 bp
	5′-GCTTTTTTGCTGTGAGTCCCG-3′		CT-182 + 97 + 85 + 12 bp
	Pociot et al. (16)		CC-97 + 85 + 12 bp
IL-6-174(G/C)			
	5′-CAGAAGAACTCAGATGACCTG-3′	hsp92II[a] (37°C⁄4hs)	CC-229 + 122 + 51 + 29 bp
	5′-GTGGGGCTGATTGGAAACC-3′		CG-229 + 173 + 122 + 51 + 29 bp
	Klein et al. (35)		GG-229 + 173 + 29 bp
TNF-α-308(G/A)			
	5′-AGGCAATAGGTTTTGAGGGCCAT-3′	NcoI[a] (37°C⁄12hs)	AA-107 bp
	5′-TCCTCCCTGCTCCGATTCCG-3′		GA-107 + 87 + 20 bp
	Wilson et al. (15)		GG-87 + 20 bp

[a]Promega, Madison, WI, USA.

Table 3 Distribution of the genotypes in patients with BMG and control subjects

Genotypes	*BMG (n = 53)*	*Control (n = 53)*	*P-value*
IL-1β+3954 (C/T)			
CC	18 (34)	31 (58.5)	
CT	31 (58.5)	22 (41.5)	
TT	4 (7.5)	0 (0)	**0.01**
IL-6-174 (G/C)			
CC	2 (4)	0 (0)	
GC	22 (41.5)	19 (35.8)	
GG	29 (54.5)	34 (64.2)	0.27
TNF-α-308 (G/A)			
GG	40 (75.5)	38 (71.7)	
GA	5 (9.5)	10 (19)	
AA	8 (15)	5 (9.3)	0.30

P-values from chi-squared test. A significance level of $P \leq 0.05$ was used. Values in parentheses are percentages.

odd ratios (OR) and adjusted for age and gender, with the corresponding 95% CI. Values of $P < 0.05$ were considered statistically significant. The genotypes considered as referents were those associated with less cytokine production in accordance with the literature. Multivariate analyses were assessed using SPSS (SPSS Inc.,Chicago, IL, USA), version 14.0, and univariate analyses were performed using BioStat 3.0 software (Optical Digital Optical Technology, Belém, Brazil).

Results

Only *IL-1B+3954* polymorphism showed association with BMG. In univariate analyses, a significant difference in the distribution of *IL-1B* genotypes was observed between case and control groups ($P = 0.01$, Table 3). Multivariate analyses showed that the CT genotype of the *IL1-B* gene was significantly associated with BMG ($P = 0.02$, OR 2.76, Table 4). The combined presence of *IL-1*β high and intermediate producers genotype were also associated with BMG in multivariate analyses ($P = 0.01$, OR 3.05, Table 4). *IL-6* and *TNFA* polymorphisms were not associated with BMG in the univariate and multivariate analyses (Tables 3 and 4).

Discussion

Some studies have observed a higher incidence of BMG in patients with immunological disturbances, including atopy (9), psoriasis (11, 22, 23) and Reiter's syndrome (13). A possible role of a genetic factor in BMG aetiology was suggested in the past (13, 24). In the current study we investigated a possible association of *IL-1B+3954 (C/T)*, *IL-6-174 (G/C)* and *TNFA-308 (G/A)* genetic polymorphisms and BMG.

Functional polymorphisms in cytokines genes may be responsible for changes in cytokine expression and have been associated with various diseases (15, 16, 21, 25, 26). Polymorphisms in the *IL-1B* gene at the position +3954 and -511 could change the production of the cytokine. Considering the +3954 *IL-1B* polymorphism, homozygous individuals for the T allele produce a four-fold higher amount of IL-1β than individuals displaying the C/C genotype. The heterozygous individuals have the intermediary production of IL-1β (16). A significant difference in distribution of IL1B genotypes were observed between control and BMG patients ($P = 0.01$). Heterozygote individuals presented an increased risk to develop BMG (OR 2.76, $P = 0.02$). Despite the low frequency of TT individuals in this population (3.75%), the combined presence of the higher and intermediate IL-1β producers genotypes showed significantly more chance to develop BMG

Table 4 Distribution of the genotypes in patients with BMG and control subjects and analysis by a binary logistic regression

Polymorphism	*BMG (n = 53), n (%)*	*Control (n = 53), n (%)*	*OR*[a]	*95% CI*[a]		*P*
				Min	*Max*	
IL-1B+3954 (C/T)						
CC	18 (34)	31 (58.5)	Referent			
CT	31 (58.5)	22 (41.5)	2.76	1.15	6.62	**0.02**
TT	4 (7.5)	0 (0)	NA	0.00	–	0.99
CC + CT against *TT*	49 (92.5) vs. 4 (7.5)	53 (100) vs. 0 (0)	NA	0.00	–	0.99
CC against *CT + TT*	18 (34) vs. 35 (66)	31 (58.5) vs. 22 (41.5)	3.05	1.30	7.11	**0.01**
IL-6-174 (G/C)						
CC	2 (4)	0 (0)	Referent			
GC	22 (41.5)	19 (35.8)	NA	0.00	–	0.99
GG	29 (54.5)	34 (64.2)	NA	0.00	–	0.99
CC + CG against *GG*	24 (45.5) vs. 29 (54.5)	19 (35.8) vs. 34 (64.2)	1.59	0.66	3.38	0.32
CC against *GC + GG*	2 (4) vs. 51 (96)	0 (0) vs. 53 (100)	NA	0.00	–	0.99
TNFA-308 (G/A)						
GG	40 (75.5)	38 (71.7)	Referent			
GA	5 (9.5)	10 (19)	0.345	0.09	1.33	0.12
AA	8 (15)	5 (9.3)	0.96	0.25	3.78	0.96
GG + GA against *AA*	45 (85) vs. 8 (15)	48 (90.7) vs. 5 (9.3)	1.45	0.40	5.26	0.57
GG against *GA + AA*	13 (24.5) vs. 40 (75.5)	15 (28.3) vs. 38 (71.7)	1.57	0.60	4.10	0.35

A significance level of $P \leq 0.05$ was used.
[a]Adjusted for gender and age.

(OR 3.05, $P = 0.01$). The rarity of the genotype TT was already reported in different populations (21, 27–30).

The pathophysiological characteristics of BMG include a subepithelial polymorphonuclear infiltrates and microabsesses, leukocyte invasion into the epithelial layer, interepithelial oedema, lack of differentiation into keratinized cells, cell necrosis with exfoliation of necrotic epithelial cells and leukocytes in the surface layer (31). These characteristics can be influenced by IL-1β expression. This molecule induces the expression of endothelial cell adhesion molecules that facilitate migration of leukocytes into tissues (32).

Considering that no study has attempted to evaluate the association of genetic polymorphisms or cytokine production in BMG, we compared our data with those reported in psoriasis, which is a similar immunological condition. BMG is not only more prevalent in psoriatic patients (11), but it is also an indicator of psoriasis severity (23). Evidence suggests that IL-1 might play a central pathogenic role in psoriasis (17, 33, 34). Transgenic mice expressing higher levels of the IL-1 family molecules in basal epidermis have presented cutaneous inflammation with histological similarities to psoriatic lesions (33). Moreover, an increased IL-β mRNA level associated with an enhanced IL-1 bioactivity is reported in the lesions of psoriatic patients (34). The IL1β low production genotype at the locus +511 was significantly more common in patients with late psoriasis, a less aggressive form of this disease (17). Although these data suggest that IL1β has an important role in BMG pathogenesis, further studies evaluating the expression and modulation of this cytokine in blood and lesions of patients affected by the disease are necessary.

In the current study no association was observed between *-308TNFA* and *IL-6* polymorphisms and BMG. While in one study, no association was found between the alleles of locus *-308TNFA* and psoriasis (17), in the same report an association between the allele 2 of locus -238 of *TNFA* gene and psoriasis was observed.

In conclusion, our findings demonstrate that the polymorphism *+3954 IL-1B* is associated with an increased risk of BMG development. *IL1*β modulation could be an interesting therapeutic target for BMG.

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