

Original

Polymorphism in the promoter region of the gene for 5-HTT in individuals with aggressive periodontitis

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(Received 13 November 2007 and accepted 26 April 2008)

Abstract: Susceptibility to and development of periodontal disease have been associated with psychological conditions. Previous studies have associated the presence of polymorphism in the promoter region of the serotonin transporter with several behavioral traits and psychological conditions such as depression, anxiety, and stress. The short allele S has a reduced transcriptional efficiency and is associated with lowered serotonin expression and uptake. The purpose of the present study was to investigate the association between 5-HTTLPR polymorphism and aggressive periodontitis in a sample of Brazilian individuals. This study involved 61 individuals affected by aggressive periodontitis and 71 without periodontitis. Genomic DNA was obtained from oral swabs, amplified by polymerase chain reaction (PCR), and genotyped at 5-HTTLPR. The Chi-square test and multivariate logistic regression were used for statistical analysis. The aggressive periodontitis group displayed a significantly higher occurrence of genotype SS ($P < 0.01$) and of allele S ($P < 0.01$). After adjustment for gender and age, it was observed that genotype SS occurred 8 times more frequently in this group. Our findings suggest that 5-HTTLPR polymorphism might be associated with aggressive periodontitis in the Brazilian population. (J.

Oral Sci. 50, 193-198, 2008)

Keywords: aggressive periodontitis; periodontal disease; polymorphism; serotonin gene transporter; stress.

Introduction

Periodontitis is a chronic infectious disease associated with pathogenic microorganisms present in the subgingival microbiota. Periodontitis affects the supporting structures of the teeth, leading to their progressive destruction – it represents the main cause of dental mortality in developed countries. Although the presence of gram-negative bacteria is essential for initiating and perpetuating the disease process, it is not enough to explain the susceptibility and progression patterns of the different forms of periodontitis (1-2).

Susceptibility to and progression of periodontitis can be influenced by local and systemic factors that can modify the resistance of the host against periodontal pathogens (3). The importance of risk factors for periodontal disease stemming from behavioral and biological aspects has already been well established (4-6). In addition, prior reports have shown strong evidence of the influence of genetic factors in the susceptibility to and progression of periodontal disease. The presence of genetic traits has also been linked to the familial pattern of periodontitis distribution observed in some forms of the disease, especially aggressive forms (7).

A considerable number of studies have suggested an association between periodontal disease and psychological factors, including anxiety and psychological stress (8-11). Stress can affect the disease process through alterations in both cellular (12,13) and humoral immune responses

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(14), and through behavioral and lifestyle factors such as smoking (15), poor oral hygiene (4,5), and poor compliance with dental care (16).

Various complex behavioral traits and disorders such as depression, bipolar disorders, anxiety, obsessive compulsion, schizophrenia, neurodegenerative disorders, substance abuse, and eating disorders have been associated with deregulation of serotonin transporter (5-HTT) function (17,18). Some studies have also indicated a possible association of 5-HTTLPR genotype with suicidal behavior (19), depression and anxiety (20,21), and alcoholism (22-23).

The serotonin transporter (5-HTT) represents the main mechanism for terminating serotonin transmission via the reuptake of released serotonin from the synaptic cleft. This mechanism for the reuptake of serotonin allows serotonin transmission to be regulated and is the most important means of restoring the pool of neurotransmitters (24).

The gene for 5-HTT (SLC6A4) is located on chromosome 17q12 and consists of a promoter region and 14 exons spanning 31kb (25). Two polymorphic regions of SLC6A4 have been identified; a 44bp insertion-deletion in the promoter region (5-HTTLPR) and a 17bp variable number of tandem repeat (VNTR) in the second intron of the gene (25,26). Two alleles, known as long (L, with 16 repetitions) and short (S, with 14 repetitions) alleles, can be identified in the first polymorphism (of 5-HTTLPR).

Recently, it was demonstrated that the L and S variants of the promoter polymorphism differentially modulate the transcription of SLC6A4. Allele L has been associated with a larger reuptake of serotonin (27), while allele S has been associated with reduced transcriptional efficiency of the 5-HTT (20,26).

It has been previously postulated that clarification of genetic risk for periodontitis requires investigation of a larger set of gene categories (28). Evidence suggesting that psychological factors are potential risk factors for periodontal disease susceptibility, together with previous evidence showing that 5-HTTLPR polymorphism is associated with depression and anxiety, prompted us to investigate this polymorphism in individuals affected by aggressive periodontitis.

The purpose of the present study was to investigate the association between 5-HTTLPR polymorphism and aggressive periodontitis in a sample of Brazilian individuals.

Materials and Methods

This study was carried out at the Dentistry School of the Universidade Federal de Minas Gerais, Brazil, where the studied population was recruited. People from both genders were grouped as follows: a) aggressive periodontitis

group – 61 individuals, ranging from 15 to 45 years of age, affected by aggressive periodontitis according to the criteria proposed by the American Academy of Periodontology (29); and b) control group – 71 individuals, ranging from 15 to 46 years of age, without periodontitis and clinically healthy. Both groups lived in the same geographic area and had low socio-economic status (annual family income lower than 5,000 US dollars), and were therefore considered a homogeneous sample. It is important to emphasize that individuals were not stratified according to ethnic groups based on skin color, race, or geographic origin due to the strong miscegenation among Brazilians (30).

Periodontal examination was performed under proper light and infection control conditions. When necessary, teeth were cleaned with sterile gauze for an adequate assessment of periodontal parameters. Clinical signs of inflammation and periodontal tissue destruction were then collected, using bleeding on probing (BOP), probing pocket depth (PPD), clinical attachment loss (CAL) and plaque index (PLI).

This study was registered and approved by the local ethics committee and written informed consent was obtained from participants or from the parents of those who were younger than 18 in all cases.

Sample collection and DNA amplification

Swabs from the oral mucosa were obtained from each participant using a sterile plastic spatula. After gentle scraping of the oral mucosa, the tip of the spatula was immediately immersed in 2-ml sterile microtubes containing 1,500 µl of Krebs buffer (NaCl 20 %, KCl 2 %, CaCl₂ 2 %, H₂O 2 %, 0.29 g/L MgSO₄, 5.95 g/L KH₂PO₄, and 1.80 g/L C₆H₁₂O₆). DNA extraction was performed as described previously (31) and modified as follows. A pellet of the swab was obtained by centrifugation at 10,000 g for 5 min. The supernatant was removed and 450 µl of lysis buffer (6.0 M GuSCN, 65 mM Tris-HCl pH 6.4, 25 mM EDTA, and 1.5 % Triton X-100) and 20 µl of silica (SiO₂, Sigma, St Louis, MO, USA) were added to the microtubes. Samples were homogenized using a Vortex and incubated for 30 min at 56°C. Following this incubation, samples centrifuged again at 10,000 g for 1 min and the supernatant was discharged. The pellet obtained (with DNA adsorbed on the silica) was washed twice with 450 µl of washing buffer (6.0 M GuSCN, 65 mM Tris-HCl, pH 6.4), twice with 450 µl of 70% ethanol, once with 450 µl acetone, and then dried at 56°C for 10 min. Finally, 100 µl of TE buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) was added and incubated at 56°C for 12 h to release the DNA. After incubation, the solution was homogenized, centrifuged at 10,000 ×g for 2 min, and the supernatant

containing DNA was then transferred to a new tube and stored at -20°C until processing.

The insertion/deletion in the 5-HTT gene-linked polymorphic region (5-HTTLPR) was amplified with primers 5'-CCGCTCTGAATGCCAGCACCTAAC-3' and 5'-AGAGGGACTGAGCTGGACAACCAC-3' (25). After DNA isolation, approximately 240 ng of DNA was used for polymerase chain reaction (PCR). PCR amplification was performed at 94°C for 2 min, followed by 40 cycles of each of the following conditions: 94°C for 30 s, 68°C for 30 s, and 72°C for 45 s. The run was terminated by a 5 min elongation step at 72°C . PCR was carried out in a 50 μl mixture containing Taq DNA polymerase (1 unit/reaction), 2.5 μl buffer IV (Tris-HCl 100 mM, MgCl_2 20 mM, and KCl 500 mM), deoxynucleoside triphosphates (0.1 mM/reaction of each dNTP), and primers (20 pmol/reaction). All samples were amplified using a DNA thermal cycler (Programmable Thermal Controller, MJ Research Inc., Canada). Allele sizes were determined by comparison of bands with size standards after electrophoresis in a 6.5 % polyacrylamide gel and silver staining. Amplification of 5-HTTLPR yielded two alleles differing by 44 bp (L with 522 bp and S with 478 bp).

Statistical analysis

Statistical analyses included descriptive statistics for periodontal clinical parameters assessed at four sites per tooth, for all present teeth excluding third molars. Data were reported as mean, minimum, and maximum values. Statistical significance of differences between experimental and control groups for alleles and genotypes was determined using the Chi-squared test. The observed genotype frequencies were compared with those calculated using the Hardy-Weinberg equilibrium theory. To investigate the association between the occurrence of polymorphisms and risk of aggressive periodontitis, a logistic regression model, adjusted for age (< 32 and ≥ 32 years) and gender, was performed. A significance level of $P \leq 0.05$ was used and all statistical tests were performed using SPSS version 14.0 (Statistical Package for Social Sciences, Inc. Chicago, IL, USA).

Results

In the aggressive periodontitis (AP) group (61 individuals, 21 females and 40 males), age ranged from 15 to 46 years (mean 31.0 ± 8.9). Similarly, in the control group (71 individuals, 29 females and 42 males), age ranged from 15 to 49 years (mean 30.0 ± 8.8). The number of smokers was 8 in the AP group and 6 in the control group. Smoking was not included in the multivariate model analysis due to the small number of smokers in the sample.

Periodontal clinical parameters of the study sample are described in Table 1. Mean proportion of sites with BOP and mean values of PPD and CAL per subject were greater in the AP group when compared to the control group. Mean PLI was higher for the control group when compared to the AP group (Table 1). There was no significant difference in mean PLI between individuals with the polymorphism in the promoter region of 5HTT (PLI = 0.55) and those without the polymorphism (PLI = 0.51).

The distribution of genotype and allele frequency of 5-HTTLPR polymorphism in subjects with aggressive periodontitis and controls is shown in Table 2. The AP group displayed a higher frequency of the genotype SS (63.9 %) and the allele S (76.0 %) when compared to the control

Table 1 Clinical parameters of the sample study

Clinical parameters	Descriptive values		
	mean	minimum	maximum
BOP (% of sites)			
Controls	4.9	0.0	15.3
AP	34.3	7.8	100.0
PPD (mm)			
Controls	1.96	1.49	2.32
AP	4.2	1.92	7.64
CAL (mm)			
Controls	1.89	1.47	2.18
AP	4.52	1.98	8.73
PLI			
Controls	0.65	0.31	0.89
AP	0.49	0.26	0.69

Controls: $n = 71$, and aggressive periodontitis group (AP): $n = 61$
 BOP = bleeding on probing, PPD = probing pocket depth,
 CAL = clinical attachment level; PLI = plaque index.

Table 2 Genotype and allelic frequency in the aggressive periodontitis and control groups

5-HTTLPR	Aggressive periodontitis group ($n = 61$)	Control group ($n = 71$)	<i>P</i> -value
Genotypes			
SS	39 (63.9%)	13 (18.3%)	0.000
LS	15 (24.6%)	39 (54.9%)	
LL	7 (11.5%)	19 (26.8%)	
Alleles			
S	76.0%	17.0%	0.000
L	24.0%	83.0%	

group (genotype SS = 18.3 %, and allele S =17.0 %). After adjustment for gender and age, it was observed that the genotype SS occurred 8 times more frequently in the AP group (Table 3). In contrast, the distribution of the 5-HTTLPR genotype observed in the group with aggressive periodontitis (SS:LS:LL: 39:15:7) was statistically different from that (13:39:19) expected according to the Hardy-Weinberg equilibrium equation ($P < 0.05$).

Discussion

Aggressive periodontitis, in contrast to chronic periodontitis, generally exhibits a marked disproportion between the severity of periodontal tissue destruction and the amount of local bacterial deposits, suggesting a highly susceptible host (2). It has been accepted that differences in host susceptibility can be partially attributed to environmental factors and exposures. Another portion of the variability of the disease has been ultimately attributed to genetic variations (32). Such genetic variations may explain the variation in the prevalence, severity, and extension of periodontal destruction on an individual level (2).

In accordance with previous periodontal literature (2,29), subjects in the AP group exhibited higher mean PPD, CAL, and BOP when compared to controls; however, individuals in the AP group demonstrated lower mean PLI when compared to controls. It is interesting to note that mean PLI did not differ significantly according to presence or absence of 5-HTT polymorphism.

In recent years, studies on genetic susceptibility of aggressive periodontitis have received increasing attention (33,34). Nevertheless, studies have indicated that this form of periodontal disease is disproportionately high among certain families, suggesting some familiar aggregation pattern (35). This evidence reinforces the presence of some genetic basis for the disease pathogenesis.

In addition, certain findings have suggested that periodontitis is associated with polymorphisms of genes involved in processes other than inflammation (28). Genes not involved in the inflammatory process may be a novel focus of studies into genetic risk factors for periodontitis.

Some studies have associated presence of polymorphism in the 5-HTT gene with several behavioral traits and psychological conditions (17,36). In vitro studies have also demonstrated an association between allele S and deficiency in the transcription of serotonin, with consequent reduction in its reuptake and expression (20). Moreover, reports have associated stress, anxiety, and depression with periodontal disease (8,11). To date no study has attempted to investigate a genetic factor associated with psychological behavior in a set of aggressive periodontitis patients. The rationale for the present study is the association between 5-HTT polymorphism and stress, anxiety, and depression; disorders that have been suggested as potential risk factors for aggressive periodontitis.

In the present study, an increased frequency of genotype SS (63.9 %) and allele S (76.0 %) was observed in individuals with aggressive periodontitis when compared to the control group. This finding suggests an association between the presence of allele S and a higher susceptibility to aggressive periodontitis. In addition, the distribution of 5-HTT genotypes in the aggressive periodontitis group demonstrated that individuals with aggressive periodontitis were not distributed according to the balance defined by Hardy-Weinberg equilibrium theory.

Our findings support studies which suggest an association between periodontal disease and 5-HTTLPR polymorphism which could be in turn associated with psychological disorders, and can help to explain the familial basis of the aggressive periodontitis (7,35). Similar patterns of distribution of the control group genotype were demonstrated by Lesch et al. (20).

Table 3 Multivariate model for distribution of genotypes in the aggressive periodontitis and control groups (adjusted for gender and age)

Characteristics	AP group		Control group		OR	95% CI		p
	n	%	n	%				
Genotypes								
LL	7	1.5	19	26.8		references		NA
LS	15	4.6	39	54.9	1.08	0.37	3.11	0.89
SS	39	3.9	13	18.3	8.12	2.76	23.87	0.00
Gender								
Females	21	4.4	29.0	40.8		references		NA
Males	40	5.6	42	59.2	0.74	0.87	0.39	1.95
Age								
< 32 years	32	2.5	46	64.8		references		NA
≥ 32 years	29	7.5	25	35.2	1.56	0.71	3.45	

Significance level of $P \leq 0.05$; AP = aggressive periodontitis, NA = not applicable due to reduced sample size.

In addition, the present study supports future research which should investigate associations between periodontal diseases and specific psychological conditions stemming from 5-HTTLPR polymorphism.

However, it is important to realize that complex diseases are associated with variations in multiple genes, each having a small overall contribution and relative risk for the disease process. The number and type of modifying genes for one condition may not be the same for different populations and/or ethnic groups. In addition, modifying genes can also be influenced by environmental and other genetic factors (37,38).

Information on periodontal disease pathogenesis has gained new insights from different types of research in the genetic field. However, it is important to consider studies with larger and different populations, and multivariate risk assessments, considering gene and environmental interactions. This may help to provide a better diagnostic and susceptibility testing system, and, ultimately, to develop better prevention and treatment strategies that address the etiologic basis of disease.

Acknowledgments

This study was supported by grants from the National Council for Scientific and Technological Development (CNPq) and the Foundation for Support of Research in Minas Gerais (FAPEMIG).

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