

Original Article

Association between serotonin transporter gene polymorphism and recurrent aphthous stomatitis

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ABSTRACT

Background: Anxiety-related traits have been attributed to sequence variability in the genes coding for serotonin transmission in the brain. Two alleles, termed long (L) and short (S) differing by 44 base pairs, are found in a polymorphism identified in the promoter region of serotonin transporter gene. The presence of the short allele and SS and LS genotypes is found to be associated with the reduced expression of this gene decreasing the uptake of serotonin in the brain leading to various anxiety-related traits. Recurrent aphthous stomatitis (RAS) is an oral mucosal disease with varied etiology including the presence of stress, anxiety, and genetic influences. The present study aimed to determine this serotonin transporter gene polymorphism in patients with RAS and compare it with normal individuals.

Materials and Methods: This study included 20 subjects with various forms of RAS and 20 normal healthy age- and gender-matched individuals. Desquamated oral mucosal cells were collected for DNA extraction and subjected to polymerase chain reaction for studying insertion/deletion in the 5-HTT gene-linked polymorphic region. Cross tabulations followed by Chi-square tests were performed to compare the significance of findings, $P < 0.05$ was considered statistically significant.

Results: The LS genotype was the most common genotype found in the subjects with aphthous stomatitis (60%) and controls (40%). The total percentage of LS and SS genotypes and the frequency of S allele were found to be higher in the subjects with aphthous stomatitis as compared to the control group although a statistically significant correlation could not be established, $P = 0.144$ and 0.371 , respectively.

Conclusion: Within the limitations of this study, occurrence of RAS was not found to be associated with polymorphic promoter region in serotonin transporter gene.

Key Words: Recurrent aphthous stomatitis, serotonin (5-HT), serotonin transporter (5-HTT), serotonin transporter polymorphism (5-HTTLPR)

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INTRODUCTION

Recurrent aphthous stomatitis (RAS) is the most common oral mucosal disease known to human beings. Despite much clinical and research attention, the cause of this oral mucosal lesion remains poorly understood. Many local and systemic etiologic factors

have been associated with this condition including the presence of stress and genetic influences.

Serotonin or 5-HT (5-hydroxytryptamine), a neurotransmitter in the brain is critically involved in

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the pathophysiology of mood and anxiety disorders. Studies have shown that stress, anxiety, depression, and impulsive aggression are related to low levels of serotonin (5-HT) transmission. The sequence variability in the genes of serotonin neurotransmission system may be responsible for a portion of the expression of such behavior. In particular, the focus has been on the serotonin transporter (5-HTT). 5-HTT ends the activity of serotonin in the brain after release from serotonergic neurons by re-uptake of 5-HT from the synaptic cleft. The gene for 5-HTT (SLC6A4) is located on chromosome 17q12. A 44 base pair insertion–deletion in the promoter region of this gene known as serotonin transporter linked polymorphic region (5-HTTLPR) has been identified. Two alleles, termed long (L) and short (S) differing by 44 base pairs, are found in this polymorphism. The presence of the short allele is found to be associated with a lower level of expression of the gene, lower levels of serotonin uptake and thus anxiety-related traits. Serotonin transporter activity has been shown to be decreased in the SS and LS genotypes in comparison to activity in the LL genotype.^[1]

The polymorphism of this serotonin transporter gene has been studied in subjects with suicidal behavior,^[2] depression,^[3,4] and anxiety.^[5] A few initial studies have predicted this association in stress-related oral diseases such as oral lichen planus,^[6] although not much has been documented in this respect. Furthermore, limited studies have been carried out to investigate this polymorphism in patients with RAS.^[1] Hence, further investigations in this direction may reveal if an association exists.

As psychological factors have been implicated in the pathogenesis of RAS, the purpose of this study was to investigate the 5-HTTLPR polymorphism of the 5-HTT gene in subjects with a known history of RAS compared to normal subjects.

MATERIALS AND METHODS

Source of data

Forty subjects selected for the study were chosen from the outpatient department of Oral Medicine and Radiology, D. A. Pandu Memorial R. V. Dental College, Bengaluru, India. A detailed case history related to the ulcers was taken and recorded from the subjects and complete oral examination done for the presence of oral ulcers.

The subjects were divided into study group and control group based on the presence of oral ulcers.

Study group: Twenty subjects giving a history of recurring oral ulcers and presenting with lesions suggestive of RAS.^[7] **Control group:** Twenty subjects with healthy oral cavity and no history of any ulcers in the mouth.

Both study group and controls were chosen from the age group of 18 and 69 years. They belonged to the same geographic area and were age- and gender-matched. Pregnant women were excluded from the study.

Method of collection of data

The patient was made to rinse his/her mouth thoroughly with water. Desquamated oral mucosal cells were collected by rolling the sterile cotton swabs firmly against the normal areas of the buccal mucosa on each side in all subjects. The swabs were air-dried for 10 min and placed in a sterile plastic container. These swabs could be stored at room temperature for 1 week and were transported within 1 week for further genetic investigation.

Genomic DNA isolation was performed using a commercially available kit, the Buccal Amp DNA Extraction Kit (Epicentre Biotechnologies, 5602 Research Park Blvd #200, Madison, WI 53719, United States.). The insertion/deletion in the 5-HTT gene-linked polymorphic region (5-HTTLPR) was amplified with primers–

Forward: 5°-TCCTCCGCTTTGGCGCCTCTTCC-3°,

Reverse: 5°-TGGGGGTTGCAGGGGAGATCCTG-3°.^[8]

Polymerase chain reaction was performed to amplify the required genomic fragment.

Interpreting the results

Allele sizes were determined by comparison of bands with size standards on gel electrophoresis. Amplification of 5-HTTLPR gave two alleles differing by 44 base pairs (L and S) [Figure 1]. Homozygotes for L allele (LL) showed a band corresponding to 528 base pair level whereas homozygotes for S allele (SS) showed a band at 484 base pair level. The heterozygotes (LS) showed two different bands instead of a single band corresponding to both 528 and 484 base pair level.

Method of statistical analysis

Cross tabulations followed by Chi-square tests were performed to compare the significance of categorical findings wherever applicable.

RESULTS

In the study group, five subjects had the SS genotype. Twelve subjects had the LS genotype, and three subjects were found to have the LL genotype. In the control group again, five subjects had the SS genotype but the number of subjects with LL and LS genotypes differed from the study group. Eight subjects had the LS genotype and seven subjects had the LL genotype.

The three genotypes were compared between the study group and control group. Although the genotype LS was found to be the highest in the study group (60%) compared to control group (40%), no statistically significant association was found between the presence of a particular genotype and occurrence of recurrent aphthous ulcers as per Chi-square test ($P > 0.05$).

In the study group, 17 subjects were found to have stress-related genotypes, that is, SS or LS, whereas in the control group 13 subjects were found to have these genotypes. However, the presence of these

genotypes in the study group as compared to the controls was not found to be statistically significant as per Chi-square tests ($P > 0.05$).

The frequency of S and L allele was calculated in both the study and the control groups. The frequency of S allele was found to be 55% in the study group and 45% in the control group whereas that of L allele was 45% in the study group and 55% in the control group. Although the frequency of S allele was higher in study subjects than controls, the association was not found to be statistically significant as per Chi-square test ($P > 0.05$) [Table 1].

DISCUSSION

The occurrence of RAS has been attributed to various local and systemic agents, and there is evidence that there may be a genetic and immunopathologic basis for recurrent aphthous ulceration. However, the exact cause has not been identified and the condition is often described as related to a multifactorial etiology.

Emotional factors have also been implicated in the etiology of RAS as early as the 1800s when this condition was referred to as “neurotic ulcers of the mouth.”^[9]

Studies using various questionnaires have found a significant association between aphthous stomatitis and stress.^[10-12]

Stressors, including both psychological as well as physiological, have been demonstrated to influence the immune response, presumably through activation of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system.^[13]

Emotional stress has also been shown to lead to certain parafunctional habits such as bruxism and

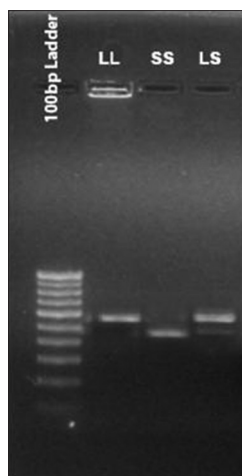


Figure 1: Representation of the three genotypes.

Table 1: Genotype and allele distribution in study and control group, comparison of various genotypes, stress-related genotypes and allele frequency between study and control groups

Genotype	Study subjects (n=20) (%)	Controls (n=20) (%)	Comparison of distribution of various genotypes		Comparison of stress-related genotypes		Comparison of allele frequency	
			χ^2	P	χ^2	P	χ^2	P
Nonstress related								
LL	3 (15)	7 (35)	2.4	0.301	2.13	0.144	0.80	0.371
Stress related								
LS	12 (60)	8 (40)						
SS	5 (25)	5 (25)						
Alleles								
L	18 (45)	22 (55)						
S	22 (55)	18 (45)						

cheek or lip biting. The physical trauma to the oral mucosa associated with these habits may also initiate the ulcerative process in genetically susceptible individuals.^[12]

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter in brain that has enormous influence over many brain functions. The human serotonin transporter (5-HTT) plays a key role in mediating regulation of the availability of serotonin to the receptors of serotonergic system by terminating the action of serotonin and recycling it in the synaptic clefts. Sequence variability in the gene coding for this serotonin transporter may be responsible for a portion of the expression of externalizing behaviors mediated by this system in the central nervous system.^[14]

Polymorphism of this serotonin transporter gene (SLC6A4) wherein a 44 base pair insertion-deletion in the promoter region (5-HTTLPR) representing the LL, LS, and SS genotypes has been found. The S allele is associated with decreased transcriptional activity compared with the L allele. Serotonin transporter activity has been shown to be decreased in the SS and LS genotypes in comparison to activity in the LL genotype.^[15]

The possible involvement of this serotonin transporter gene polymorphism (5-HTTLPR) has been studied in various stress-related conditions such as suicidal behavior,^[2] depression^[3,4] anxiety,^[5] and habits such as smoking^[16,17] and alcoholism.^[18] The presence of this S allele and SS and LS genotypes has been studied in various stress-related conditions.

In this study, subjects with RAS when evaluated for the genotype, 85% were found to have LS or SS genotypes which have been shown to be associated with various stress-related conditions. In the control group, 65% subjects had these stress-related genotypes. When the three genotypes were considered individually, namely LL, LS, and SS, a statistically significant association between any particular genotype and the presence of recurrent aphthous ulcers could not be established. This is in contrary to the only other similar study carried out where the association between serotonin transporter gene polymorphism and RAS in a Brazilian population was found to be significant.^[1]

A similar study done to determine the association of oral lichen planus with this serotonin transporter gene polymorphism again in Brazilian population failed to establish any relation.^[6]

Furthermore, the study conducted to determine the association between serotonin transporter gene polymorphism in patients of oral squamous cell carcinoma owing to an increase consumption of tobacco and alcohol in the same ethnic population failed to show any statistically significant correlation.^[19]

In a study conducted to determine the association between serotonin transporter polymorphisms in patients with temporomandibular joint disorders in a Japanese population, a contradictory result showing a statistically significant increase in L allele and LL genotype was found in patients with temporomandibular disorders associated with stress.^[20]

Studies on an Indian population relating these genotypes to some certain conditions such as schizophrenia, obsessive compulsive disorder, and autism gained conflicting results. A study conducted on an Indian population to determine the association between serotonin transporter gene polymorphism and subjects with obsessive compulsive disorder did not reveal any statistically significant association.^[21]

The lack of accordance of the present study to the previous study conducted in RAS subjects may be indeed due to the difference in ethnicity and thus the different genetic makeup across different populations. Furthermore, the lack of a statistically significant association could be attributed to a small sample size. However, the percentages of subjects with LS and SS genotypes and the frequency of S allele were higher in the study group in the present study.

Stress symptoms are controlled by various genes in the body^[22,23] and other polymorphisms within the serotonin transporter gene itself have also been described.^[5] Therefore, it is possible that some other genetic polymorphisms may be present in individuals under stress who are predisposed to the appearance of recurrent aphthous ulcers. Ethnic variations in genotypes may also influence the result of such studies.

CONCLUSION

Within the limitations of this study, occurrence of RAS was not found to be associated with polymorphic promoter region in serotonin transporter gene.

Further studies involving a larger population and varying cross sections of study subjects are recommended.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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