




## Polymorphism of Serotonin Transporter SLC6A4 (5-HTTLPR) Gene in Cheilitis Angularis Patients


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Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 01 May 2019 / Accepted: 17 August 2019 / Published: 26 August 2019

### Abstract

**Objective:** To determine the relationship between the Serotonin transporter SLC6A4 (5-HTTLPR) gene polymorphism in cheilitis angularis patients. **Material and Methods:** We conducted a descriptive analysis of 100 DNA samples extracted from the blood serum of 50 patients with cheilitis angularis and 50 patients without cheilitis angularis. Analysis of the Serotonin transporter SLC6A4 (5-HTTLPR) gene polymorphism was observed by carrying out PCR method followed by electrophoresis for the analysis, without the usage of restriction enzyme. The Chi-square test was used for statistical analysis. **Results:** In the cheilitis angularis group there were 24 samples with SS genotype, 23 samples with LS genotype, and 3 samples with LL genotype. Whereas in the non-cheilitis angularis group, there were 5 samples with SS genotype, 18 samples with LS genotype, and 27 samples with LL genotype. In the cheilitis angularis group found 71 S alleles and 29 L alleles, and in the non-cheilitis angularis group 28 S alleles and 72 L alleles were found. A statistically significant difference was found between the groups ( $p < 0.001$ ). **Conclusion:** There were significant differences in the distribution of the Serotonin transporter SLC6A4 (5-HTTLPR) gene polymorphism between patients with and without cheilitis angularis.

**Keywords:** Genes; Polymorphism, Genetic; Cheilitis; Genotyping Techniques.

## Introduction

Cheilitis angularis is a complex disease with multifactorial inheritance affecting approximately 20% of the population. It is believed that cells of the innate and acquired immune system, among them dendritic cells and T-cells, collaborate to produce a pattern of cutaneous angiogenesis, inflammation and epidermal changes that underlie the formation of sharply demarcated erythematous squamous plaques especially in mucous membranes, the typical clinical presentation of cheilitis angularis [1].

Because cheilitis angularis is a chronic, often widespread and stigmatizing condition that may extensively reduce the quality of life of affected patients, the observed increased prevalence of depressive symptoms among patients compared with healthy individuals could be regarded as secondary to the manifestation of a significant medical condition [2]. It is also not excluded that the cheilitis angularis inflammatory process plays a direct role via the constantly increased production of mediators that may also influence mental functions or that cheilitis angularis and depression share common risk factors at the genetic level. On the other hand, it has been suggested that stress can aggravate skin inflammation in patients with cheilitis angularis, indicating another level of possible interactions between psychological symptoms and skin inflammation.

The serotonergic system is a promising candidate for establishing a neuroimmunological link between lesion skin disease and psychological symptoms. In the central nervous system, serotonergic neurons project to almost all regions of the brain and influence many physiological functions such as pain, sleep, motor functions, neuroendocrine circuits and the mood. The main producers of 5-HT are enterochromaffin cells of the gut and the serotonergic neurons of the brain, but 5-HT is stored in many cell types outside the central nervous system including platelets, lymphocytes, monocytes/macrophages and dendritic cells [3]. Recently, it has been shown that lymphocytes also synthesize and excrete 5-HT [4]. The production of 5-HT is increased at sites of inflammation and several functional activities of 5-HT have been revealed that play an important role in chronic inflammatory diseases such as cheilitis angularis including the activation of mast cell migration and adhesion, the stimulation of cytokine release from dendritic cells and the mediation of dendritic cell-T-cell interactions [4-6].

The expression and function of the serotonin transporter (5-HTT), which is responsible for the uptake of 5-HT into cells thereby removing 5-HT from the extracellular space, is a key parameter in the regulation of 5-HT-mediated effects in the central nervous system and the immune system [7]. The expression of 5-HTT is controlled by a repeat length polymorphism in the 5-HTT linked polymorphic region (5-HTTLPR). The long (high activity) allele of this polymorphism is associated with a higher number of 5-HTT molecules on lymphocytes [8]. The biological relevance of the 5-HTTLPR polymorphism is underscored by the recent finding of an association with primary pulmonary hypertension, and the observed influence on the development of depressive symptoms in individuals afflicted by some adverse life event, and on suicidal behavior [9,10].

Serotonin (5-HT) represents a potential target for cytokine-induced regulatory mechanism contributing to the pathophysiological processes underlying depressive illnesses. Indeed, pro-inflammatory cytokines have been implicated in the regulation of serotonergic homeostasis in vivo through the modulation of central serotonin activity and metabolism. A particular polymorphism in the promoter region of the gene encoding 5-HTT, referred to as 5-HTTLPR, is a 27 bp deletion/insertion that generates two alleles of 5-HTTLPR, with the 14-repeat short (S) variant having less transcriptional activity and lower serotonin uptake than the 16-repeat long (L) variant. Researchers have speculated that the differential transcriptional activity caused by this polymorphism would influence complex traits and diseases, including affective disorders [11,12].

In this study, we describe the genotype and allele frequencies of the 5-HTTLPR gene polymorphism in patients with and without cheilitis angularis.

## Material and Methods

### Study Design and Participants

This laboratory study conducted a descriptive analysis of 100 DNA samples extracted from the blood serum of 50 patients with cheilitis angularis and 50 patients without cheilitis angularis.

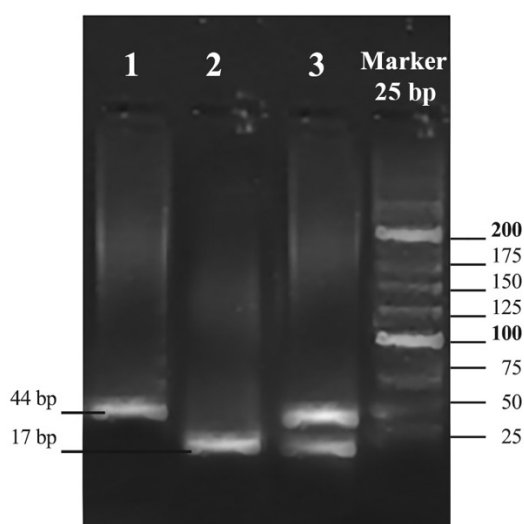
### DNA Isolation

The DNA isolation procedures were applied to 3 mL of peripheral blood taken from the 176 subjects, placed in 15 mL tubes containing 9 mL of red blood lysis solution (1.45M NH<sub>4</sub>Cl, 5mM anhydrous EDTA, and 0.1M KHCO<sub>3</sub>) and incubated at room temperature for 10 min. The sample was then centrifuged at 1500 rpm for 10 min at room temperature, and the supernatant was removed to leave a precipitate of mononuclear leukocytes. These steps were repeated to obtain a white pellet and a supernatant containing no red blood cells. To this pellet, 2 mL of cell lysis solution was added and pipetted until homogeneous and incubated in a water bath at 37°C for 30-60 min until fully homogeneous. Then 1.3 mL of protein precipitation solution (5M ammonium acetate) was added, vortex mixed for 15-20 s and centrifuged at 3000 rpm for 15 min at 4°C, producing a light brown precipitate (proteins) and the supernatant containing DNA.

The supernatant was poured into a new Falcon tube with 2.3 ml of cold isopropanol. The tube was inverted up to 20-30 times until showing a collection of DNA strands. The supernatant was removed and 1.3 mL of 70% ethanol was added for washing, and the DNA solution was centrifuged at 3000 rpm for 5 min at 4°C. After discarding supernatant, the DNA was dried in open air by reversing the tube, then DNA was rehydrated with a solution of 200-300 uL TE (Tris-HCl EDTA) and incubated in a water bath at 37°C for 2 h. The solution was transferred into 1.5 mL sterile microcentrifuge tubes and stored at -20°C in the Oral Biology Laboratory of the Faculty of Dentistry, University of Indonesia [13-15].

### Genotyping

The polymerase chain reaction (PCR)-VNTR method was used to determine the genotypes of the Serotonin transporter SLC6A4 (5-HTTLPR) gene. The primers used in this study were F:5'-CCGCTCTGAATGCCAGCACCTAAC-3' and R:5'-AGAGGGACTGAGCTGGACAACCAC-3' [16]. The PCR reaction was carried out in 20 µl of reaction volume containing 0.3 µl of genomic DNA, 10 µl of Taq polymerase (MyTaq, Bioline Ltd., London, United Kingdom), 0.5 µl of forward primer (IDT), 0.5 µl of reverse primer (IDT), and 8.7 µl of ddH<sub>2</sub>O. Thermal cycling conditions for the fragment containing the XRCC3 gene were as follows: initial denaturation at 95°C for 1 min, followed by 30 cycles of 95°C for 30 s at an annealing temperature of 68°C for 30 s, and at 72°C for 45 s. The final extension was performed at 72°C for 5 min. The PCR products were electrophoresed in 1.5% agarose gel (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) at 60 V, 400 mA, for 40 min with 25 bp DNA ladder and were visualized using Gel Doc. PCR products were visualized on agarose gel containing ethidium bromide. The variants were detected according to their size of fragments: 17 bp (homozygote SS), 44 bp (homozygote LL) or 17bp and 44 bp (heterozygote LS) (Figure 1).



**Figure 1. Representative PCR results of the SERT (5-HTTLPR) polymorphism: lane 2 with 17 bp (homozygote SS), lane 1 with 44 bp (homozygote LL) and lane 3 with 17bp and 44 bp (heterozygote LS); also with 25 bp ladder markers.**

#### Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Science (SPSS) software version 25 (IBM Corp., Armonk, New York, USA). A Chi-square test was used to analyze statistical differences between the control and experimental groups. Fisher's exact test was used to analyze the status of Hardy-Weinberg equilibrium. Statistical significance was set at  $p < 0.05$ .

#### Ethical Aspects

This study was approved by the Ethical Committee of the University of Indonesia (Protocol No. 011121018)

## Results

The study consisted of 100 participants from the Indonesian population, one half with cheilitis angularis and the other half without cheilitis angularis. The results showed significant differences in the genotype and allele distributions between the groups with and without cheilitis angularis for the SERT (5-HTTLPR) polymorphism ( $p < 0.001$ ) (Table 1). In particular, the SS genotype and S allele were much more common in the group with cheilitis angularis than in the control group, 48% and 71%, respectively.

**Table 1. Genotype and allele distributions between those with and without cheilitis angularis for the SERT (5-HTTLPR) polymorphism.**

Variables	Cheilitis Angularis				p-value
	Yes		No		
	N	%	N	%	
Genotype					
LL	3	6.0	27	54.0	0.001
LS	23	46.0	18	36.0	
SS	24	48.0	5	10.0	
Total	50		50		
Allele					
L	29	29.0	72	72.0	0.001
S	71	71.0	28	28.0	
Total	100	100.0	100	100.0	

## Discussion

To the best of our knowledge, this is the first study to investigate a possible relationship between a functionally relevant polymorphism in the promoter of the gene encoding the serotonin transporter and an inflammatory skin disorder. The control group showed genotype frequencies of the 5-HTTLPR polymorphism similar to those reported in an earlier study with healthy German individuals [17].

Depressive symptoms have been reported to be more prevalent among psoriatic patients than among healthy individuals [2]. However, it has not been established whether depression is exclusively a consequence of the impaired quality of life and stigmatization of the disease or whether neuroimmunological mechanisms may also contribute to depressive symptoms. The observation of elevated levels of proinflammatory cytokines in depressive patients and the relatively frequent induction of depressive symptoms during the therapeutic application of interferon- $\alpha$  point to a possible role of proinflammatory cytokines in the development of depressive disorders [17]. The reduction of depressive symptoms in patients with cheilitis angularis treated with the TNF-antagonist etanercept parallel to the improvement of skin symptoms could, therefore, be interpreted not only as an indirect effect but also as a direct effect related to the modulation of peripheral or central neurological functions [18].

Both depression and cheilitis angularis are complex diseases. Carriage of the 'S' allele of the 5-HTTLPR polymorphism has been shown to influence susceptibility to develop depressive

symptoms after stressful life events but also in association with other diseases such as Parkinson's disease [19-21].

The serotonin/serotonin transporter system is a possible link between neuropsychological and immunological functions that may particularly be relevant in diseases such as psoriasis that are characterized by stress-induced chronic inflammation and an increased prevalence of depression. In this pilot study, we could to demonstrate an association between psoriasis and the functionally relevant 5-HTTLPR polymorphisms of the serotonin transporter. This includes a small contribution of the 5-HTTLPR polymorphism to cheilitis susceptibility or a modulatory effect on the development of depressive symptoms in patients affected by the disease. The specific limitation of the study related to the small number of individuals enrolled is underlined by recent studies indicating that analysis of several thousand individuals may be necessary to detect associations between SNPs of genes involved in cheilitis pathophysiology and the disease [22].

However, smaller studies with hundreds of patients have for example identified an association between the 5-HTTLPR polymorphism and anxiety-related personality traits. This is in line with the findings from the present investigation that at least support a major role of the 5-HTTLPR polymorphism in oral lesion, especially cheilitis angularis [23,24]. In addition, it may be necessary to analyze genetic influences on depression separately in male and female patients with psoriasis, as depressive symptoms tend to be more severe in women and genetic effects of the 5-HTTLPR may be superimposed by gender effects.

Finally, other functionally relevant genetic variations of the serotonergic system have been identified including a variable number of tandem repeat polymorphisms in intron 2 of the 5-HTT gene and polymorphism of serotonin receptors that could play a role in the pathogenesis of cheilitis angularis and depressive symptoms in patients with oral lesions [24]. Wider prospective trials are needed to evaluate the genetic basis of the complex interaction of oral lesions, environmental stressors, gender and development of depressive symptoms.

## Conclusion

The 5-HTTLPR might be a susceptible high-risk in cheilitis angularis or other oral lesions by affecting the functional immune system.

**Authors' Contributions:** L contributed to performing the experiment, analysis, and writing the manuscript, RII, FBP, and AWS contributed to conception and critically revising the manuscript, EIA designed the study, contributed to analysis of results, and critically revised the manuscript. All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

**Financial Support:** Indonesian Ministry of Research, Technology and Higher Education through the University of Indonesia (EIA, UI-Grant No. NKB-0409/UN2.R3.1/HKP.05.00/2019).

**Conflict of Interest:** The authors declare no conflicts of interest.

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