

Article - Human and Animal Health

Relationship between Serotonin-2A Receptor Gene Polymorphism and Wound Healing in Brazilian Patients

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Editor-in-Chief: Paulo Vitor Farago

Associate Editor: Paulo Vitor Farago

Received: 13-Sep-2021; Accepted: 08-Nov-2021.

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HIGHLIGHTS

- The platelets serotonin stands out in obtaining wound healing closure.
- Serotonin receptor gene (5-HTR2A) polymorphisms can cause alterations in the tissue repair.
- 102T-C polymorphism in the 5-HTR2A gene is associated to alterations in wound healing

Abstract: Genetic changes in platelet serotonin receptors (5-HTR2A) impair the initial process of tissue repair, regardless of the triggering factor of the skin wound. Objective was to determine the prevalence of the 102T-C polymorphism in the 5-HTR2A gene in Brazilian patients with and without skin wounds. Cross-sectional case-control study, in which 100 patients were evaluated as Cases Group (subdivided into I-with Chronic Wound and II-with Acute Wound) and 100 individuals as Controls, of both genders. DNA was extracted from leukocytes of peripheral blood and the region that covers the polymorphism was amplified by the molecular techniques Polymerase Chain Reaction/Restriction Fragment Length Polymorphism. The TT genotype was significantly associated with the protective factor against alterations in the healing process of skin wounds (OR: 0.4833; 95%CI: 0.2704-0.8638; $p < 0.05$) in the Control Group. The genotypic analysis between Cases Group (I-Chronic Wound and II-Acute Wound) determined that the TT genotype was significantly associated with the protection factor in Case II (OR: 0.3333; 95%CI: 0.1359-0.8177; $p < .005$) and the CC genotype was significantly associated with the chance to develop chronic ulcers in the Case I (OR: 6.667; 95%CI: 1.801-24.683; $p < 0.05$). Patients with chronic skin wounds have a higher prevalence of the 102T-C polymorphism in the 5-HTR2A gene, which is associated to alterations in the healing process in this population. There are differences, at the molecular level, in patients, with and without these lesions, and the probable role of the serotonergic system in wound healing.

Keywords: blood platelets; genetic polymorphism; serotonin; serotonin 5-HTR2A receptor; wound healing.

INTRODUCTION

In healthy skin, when an injury occurs, the healing process begins, which is divided into four well-defined phases: hemostasis, inflammation, proliferation and tissue remodeling, which are dynamic and overlapping each other. A change, in any one of them, can cause discontinuity of the entire process [1,2].

After the injury has been triggered by events of any kind, a well-coordinated series of repair phases comes into play, featuring the acute healing phase, which usually takes place in three weeks. When other pathological factors are associated, altering the natural healing physiological course, with consequent barrier defect, a chronic wound can form, being defined as that in four to six weeks, there was no reduction in 50% of the injured area or there was no healing in twelve weeks [3,6].

The underlying mechanism in the healing process varies widely, but includes factors that influence blood supply and/or immune function, such as the presence of metabolic diseases, use of medications, previous local tissue damage or genetic changes. External factors, such as pressure, temperature and humidity, also play an important role in wound healing [3,6].

Immediately after the skin has suffered an injury, pro-inflammatory factors and blood cells are added to the site, initiating the coagulation cascade for clot formation and angiogenesis. Platelets activated via 5-HTR2A receptors secrete serotonin (5-HT), which is implicated in the pro-inflammatory response to acute injury, especially in the recruitment of neutrophils and in the proliferation of lymphocytes, thus playing an important role in inflammatory stage of wound healing [7].

The characteristics assumed by the lesion, along the dynamics in the repair process, result from the succession and/or overlap of cellular and tissue events resulting from cell activation by chemical mediators, including via the serotonergic pathway. During the evolution of the repair process, the events that follow are hemostasis, infiltration of neutrophils, macrophages, fibroplasia, deposition of extracellular matrix, angiogenesis, healing and reepithelization. Therefore, serotonin, via signaling of platelet 5-HTR2A receptors, stands out in obtaining wound closure, particularly in the early hemostatic stages [8,9].

Abnormalities in the different stages of the healing process cause its impairment, as they induce changes in the metabolism, in the expression of genes and, consequently, in the phenotype. Thus, genetic alterations, especially in platelet 5-HTR2A receptors, impair the initial processes of tissue repair with a consequent decrease in the inflammatory response to the detriment of the continuity of the healing process, regardless of the triggering factor [9,10].

Polymorphisms in the serotonin receptor gene (5-HTR2A) are associated with several platelet disorders as they affect the serotonergic system and, among them, the 102T-C polymorphism can cause molecular changes in the effectiveness of tissue repair control, with implications for the healing of skin wounds [11-13].

Given the above, the objective of the present study was to determine the prevalence of the 102T-C polymorphism, in the 5-HTR2A gene, in Brazilian patients, and its association in the healing process of skin wounds.

MATERIAL AND METHODS

In the period from March to December 2020, a retrospective study was carried out, in which 200 consecutive individuals of both genders were evaluated, attended by spontaneous demand at the Institution's Outpatient Wound Clinic, after signing the Free and Informed Consent Form.

The differential diagnosis between acute and chronic wounds [3,6] was always performed by the researcher herself, a nurse qualified in wound care, characterizing the Cases Group as: Case Group I - with Chronic Wound (52 patients) and Case Group II - with Acute Wound (48 patients), regardless of the triggering factor. Age and gender were documented in all patients in the Cases Group and those with degenerative diseases, drug addicts, alcoholism and somatic and/or neurological and/or psychiatric diseases were excluded.

The Control Group included 100 volunteers of both genders, in the same period described, without any history of acute or chronic wounds, and the age and gender of all included individuals were also documented.

Patients and controls are of the same ethnicity/skin color - Caucasians, and from the same geographical area - State of São Paulo, Brazil.

In order to better characterize the age, patients from both Groups (Cases and Controls) were divided into the following classification: adolescence (11 to 17 years old), young adult (18 to 40 years old), adult (41 to 65 years old) and elderly (> 65 years) [14].

Therefore, this study was characterized as a longitudinal study, in which it was retrospectively evaluated whether cases and controls (independent, unpaired samples) present or not the 102T-C polymorphism, in the 5-HTR2A gene, as an additional factor of alteration in the wound healing process.

Molecular analysis

Genomic DNA was isolated from peripheral blood leukocytes using the Illustra Blood Genomic Prep Mini Spin Kit (GE Healthcare UK Limited™, UK) by the manufacturer's protocol. To detect the 102T-C (GenBank NM_000621.3; rs#6313) polymorphism, nuclear DNA fragments that encompassed the polymorphic site in the HTR2A gene (MIM ID* 182135) were amplified by PCR and digested, by RFLP, with 10 U MspI enzyme (New England Biolab™) for 2.0 h at 37°C.

Polymerase chain reaction conditions

Standard polymerase chain reaction (PCR) was performed in 25 µl, containing 200 ng genomic DNA, 10 pmol of each primer, and Fidelity™ PCR Master Mix (2x) (GE Healthcare UK Limited™, UK), as by the manufacturer's protocol. Published primer sequences were used to analyze the 102T-C polymorphism [15], and the PCR was performed as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min, followed by a final extension for 10 min at 72°C.

The 102T allele was represented by an uncut 342-bp PCR product, and the 102C allele consisted of 2 fragments at 217 bp and 125 bp.

The products from both PCR/RFLP reactions were added to FlashGel™ Loading Dye 5x, analyzed by electrophoresis on a 2.2% agarose FlashGel™ DNA Cassette, and all gels were photodocumented by FlashGel™ Camera (Lonza Group Ltd Muenchensteinerstrasse 38 CH-4002 Basel Switzerland).

To avoid bias in the molecular analysis and final results, all DNA samples were analyzed without knowledge of the patients' clinical characteristics.

Ethical aspects

The ethics and research protocol were approved by the Research Ethics Committee (FAMERP, São Paulo, Brazil) (#3.902.040/2020). Before initiation of any procedure, signed informed consent was obtained from all patients.

Statistical Analysis

The results were previously submitted to descriptive statistics to determine normality. For independent samples, with normal distribution, the unpaired T test and the Mann-Whitney test were used for samples with non-normal distribution. When applicable, Chi-square (χ^2) or Fisher's Exact tests were used to compare the variables. The odds ratio (OR) with a 95% confidence interval (95% CI) was used to determine the odds ratio of alterations in the healing process of skin wounds by the analyzed polymorphism. The level of significance was set at 5%.

All samples were tested for Hardy-Weinberg equilibrium.

The results were expressed as percentages (%), mean (M), median (Med) and standard deviation (SD). Statistical tests were performed using the GraphPad InStat version 3.00 program, GraphPad Software Inc, San Diego California USA, www.graphpad.com.

RESULTS

This cross-sectional case-control study included 200 patients of both genders. The 102T-C polymorphism in the 5-HTR2A gene was genotyped in all of them. The Cases Group was composed of 100 patients (34 male and 66 female) while the other 100 individuals (78 male and 22 female) constituted the Control Group. Age ranged from 20 to 95 years with a median of 50.5 years in Cases Group, and at Control Group the age ranged from 18 to 63 years with a median of 35 years ($p < 0.0001$). All individuals from both groups had white skin color (100%). Demographic data are summarized in Table 1.

The Case Group I - with Chronic Wound was composed of 52 patients (25 male and 27 female) and 48 patients (9 male and 39 female) constituted the Case Group II - with Acute Wound.

Table 1. Distribution of the demographic data of the Cases Group in relation to the Control Group.

Variables	Cases n=100					Controls n=100					p
Gender	n (%)					n (%)					
Male	34 (34)					78 (78)					<0.0001*
Female	66 (66)					22 (22)					
Age rating	n (%)					n (%)					
Young Adult	31 (31)					69 (69)					<0.0001†
Adult	50 (50)					31 (31)					
Elderly	19 (19)					00 (00)					
Total Age -Years	Min	Max	Median	Mean	±SD	Min	Max	Median	Mean	±SD	<0.0001‡
	20	95	50.5	50.8	18.3	18	63	35	34.7	11.6	
Age – Years/Gender	Min	Max	Median	Mean	±SD	Min	Max	Median	Mean	±SD	
Male	20	95	58.5	55.2	20.0	18	63	36	35.4	11.6	<0.0001‡
Female	20	95	44.0	45.5	17.7	19	54	29.5	32.5	11.6	<0.0019§

*Fisher's Exact Test. † χ^2 Test: 37.897; df=2. ‡Mann-Whitney Test. §Unpaired T Test.

Abbreviations: n: number. ±SD: ± Standard Deviation. Min: minimum. Max: maximum. df: degrees of freedom.

Notes: values in bold indicate significant results - $p < 0.05$.

Molecular Results

Table 2 presents the genotypic and allele results, found in the Cases Group and Control Group, related to the 102T-C polymorphism of the 5-HTR2A gene.

Table 2. Genotypic and allelic relationship for the 102T-C polymorphism, in exon 1, of the 5-HTR2A gene, between the Cases and Control Groups.

Gene/Polymorphism	Genotypes/Alleles	Cases	Controls	OR (95% CI)	p*
		n=100 (%) Alleles n=200 (%)	n=100 (%) Alleles n=200 (%)		
5-HTR2A/ 102T-C	TT	30 (30)	47 (47)	0.4833 (0.2704-0.638)	0.0198
	TC	51 (51)	43 (43)	1.380 (0.7902-2.409)	0.3213
	CC	19 (19)	10 (10)	2.111 (0.9273-4.806)	0.1070
	T	111 (55)	137 (68)	0.5735 (0.3812-0.8629)	0.0099
	C	89 (45)	63 (32)	1.744 (1.159-2.623)	0.0099

*Fisher's Exact Test

Abbreviations: n: number. OR - Odds Ratio. CI - Confidence Interval of 95%.

Notes: Polymorphic alleles are in bold type. Bold values indicate significant results - $p < 0.05$.

The wild homozygous TT genotype was found to be more prevalent in the Control Group (47%), indicating a significant no association of alterations in the healing process of skin wounds, which can be considered a protective factor (OR: 0.4833; 95%CI: 0.2704-0.8638; $p < 0.05$). The heterozygous TC genotype was found to be more prevalent in the Cases Group (51%), with no significant association with the Control Group (OR: 1.380; 95%CI: 0.7902-2.409; $p > 0.05$). Likewise, there was no significant association of the homozygous polymorphic CC genotype between both Groups (OR: 2.111; 95%CI: 0.9273-4.806; $p > 0.05$).

The wild T allele was significantly associated to less chance of alterations in the healing process (OR: 0.5735; 95%CI: 0.3812-0.8629; $p < 0.05$). Regarding the polymorphic C allele, it was significantly associated with a greater chance of healing alterations (OR: 1.744; 95%CI: 1.159-2.623; $p < 0.05$).

Both Groups were tested for the Hardy-Weinberg Equilibrium. There was no significant difference between the observed and expected genotypic values both in the Cases Group ($\chi^2 = 0.106$; df=1; $p = 0.7452$) and in the Control Group ($\chi^2 = 0.001$; df=1; $p = 0.9713$) for the analyzed polymorphism, indicating that the study population is in genotypic equilibrium.

Table 3 presents the genotypic and allele results, found in the Case Group I - with Chronic Wound and Case Group II - with Acute Wound, related to the 102T-C polymorphism of the 5-HTR2A gene.

Table 3. Genotypic and allelic relationship for the 102T-C polymorphism, in exon 1, of the 5-HTR2A gene, between the Case Group I - with Chronic Wound and Case Group II - with Acute Wound.

Gene / Polymorphism	Genotypes/Alleles	Case I n=52 (%) Alleles n=104 (%)	Case II n=48 (%) Alleles n=96 (%)	OR (95% CI)	p*
5-HTR2A/ 102T-C	TT	10 (19)	20 (42)	0.3333 (0.1359-0.8177)	0.0172
	TC	26 (50)	25 (52)	0.9200 (0.4196-2.017)	0.8443
	CC	16 (31)	3 (6)	6.667 (1.801-24.683)	0.0020
	T	46 (44)	65 (68)	0.3782 (0.2124-0.6735)	0.0010
	C	58 (56)	31 (32)	2.644 (1.485-4.708)	0.0010

*Fisher's Exact Test

Abbreviations: n: number. OR - Odds Ratio. CI - Confidence Interval of 95%.

Notes: Polymorphic alleles are in bold type. Bold values indicate significant results ($p < 0.05$)

The wild homozygous TT genotype was significantly more prevalent in Case Group II (42%), which may indicate a protective factor for progression to chronic ulcers (OR: 0.3333; 95%CI: 0.1359-0.8177; $p < 0.05$). There was no significant association of the heterozygous TC genotype between both groups (OR: 0.9200; 95%CI: 0.4196-2.017; $p < 0.05$). Regarding the polymorphic homozygous CC genotype, there was a significant association between the two groups, indicating that carriers of this genotype have about 6.6 times more likely to develop chronic ulcers (OR: 6.667; 95%CI: 1.801-24.683; $p < 0.05$).

The wild T allele was significantly more associated with a protective factor for progression to chronic ulcers (OR: 0.3782; 95%CI: 0.2124-0.6735; $p < 0.05$). In relation to the polymorphic C allele, it was significantly associated with a greater chance of alterations in the healing process of chronic skin wounds (OR: 2.644; 95%CI: 1.485-4.708; $p < 0.05$).

Both Cases Groups were tested for the Hardy-Weinberg Equilibrium. As there was no significant difference between the observed and expected genotypic values both in the Case Group I - with Chronic Wound ($\chi^2 = 0.009$; $df = 1$; $p = 0.9224$) and in the Case Group II - with Acute Wound ($\chi^2 = 1.752$; $df = 1$; $p = 0.1855$), for the analyzed polymorphism, this population is in genotypic equilibrium.

DISCUSSION

The molecular basis of the healing process of skin wounds is still largely unknown and worthy of scientific investigation. The serotonergic system is widely studied for its action on the central and peripheral neural systems, but it is only recently that its role in wound healing has been gaining more and more evidence [2,9,10,16].

The 5-HTR2A serotonin receptor is an essential component of the serotonergic system, its expression being under gene control. Therefore, serotonin, via signaling of platelet 5-HTR2A receptors, stands out in obtaining wound closure, particularly in the early hemostatic stages [2,8,9,17]. Thus, polymorphisms in the gene encoding the receptor protein can affect its functional state and, in turn, serotonergic activity, causing several disorders, including changes in the preliminary processes of tissue repair [2,8-13,16,18].

To date, there are no data in the literature on the molecular analysis of the 5-HTR2A gene in order to verify the prevalence of 102T-C polymorphism in patients with and without acute and chronic wounds, regardless of the triggering factor, as performed in the present study.

For the evaluation of results of genetic association, it is important that the research groups have the same ethnicity (or skin color) and the same geographical origin, since the genetic bases of diseases, such as the polymorphic configuration, may differ between various regions and between populations [19], which was considered in the present study, because despite the intense Brazilian miscegenation, patients and controls with apparent similarity in skin color - white and from the same geographical area were included.

In the present study, the general prevalence of patients in the Cases Group is female, with 81% among young adults and adults. In this Group, in relation to the presence of acute or chronic wounds, the female gender is the most prevalent in both types of wounds, being, respectively, 98% among young adults and adults, and 87% between adults and the elderly.

Literature data are divergent in terms of relating gender and age rating to the types of wounds, due to differences in methodology and case series used [20-23]. There is an indication of a higher prevalence in females and in adults and the elderly in chronic wounds and, in relation to acute wounds, there is a prevalence of males and young adults, as they present greater thickening of the skin layers, on ultrasound examination, which could give them a degree of protection. The feminization of old age has been described and associated with different reasons, but, despite the fact that women are showing greater longevity, there is no improvement in quality of life, being still dependent and with greater potential for fragility, due to cultural and/or biological factors, such as hormones, pregnancy, prolonged use of oral contraceptives and less muscle mass [20-23].

In addition, among the skin changes inherent to aging, such as the decrease in skin thickness, the reduction of the inflammatory response, collagen synthesis and neoangiogenesis can be considered as consequences of this process. In parallel, there is an increase in capillary fragility and epithelization time resulting in a delay in the healing phases [22,24,25]. This delay, which may be more accentuated, when associated with genetic alterations, in platelet serotonergic activity [2,8-13,16-18], such as the 102T-C polymorphism, in the 5-HTR2A gene, investigated in the present study.

Currently, genetic tests are being carried out to investigate the most diverse diseases, not only because they are considered non-invasive, but also because of the high sensitivity and specificity for molecular study, as performed in the present study. Thus, statistical comparisons of genotypic and allele frequencies were performed between groups of patients and controls of the 102T-C polymorphism, in the 5-HTR2A gene. Both genotypes, TT (wild homozygote) in the Control Group and CC (polymorphic homozygote) in the Cases Group, were more prevalent in their groups, with statistical significance, considering that they are associated, respectively, with the protection factor and the greatest chance of alterations in the healing process in skin wounds. However, there are no data in the literature, so far, for comparative analysis of the results found.

Likewise, statistical comparisons of genotype and allele frequencies between the Case Group with Chronic Wound and Case Group with Acute Wound were also performed. The genotype CC (polymorphic homozygote) was significantly more prevalent in the patients in the Case Group with Chronic Wound and the TT genotype (wild homozygote) was more prevalent in those in the Case Group with Acute Wound, also with statistical significance. Thus, the CC genotype (polymorphic homozygote) can be associated with greater chances of alterations in the healing process with progression to chronic ulcers and the TT genotype (wild homozygote) associated with the protection factor, without progression to chronic ulcers, both with statistical significance. And, also, there are no data in the literature, until the present moment, for comparative analyzes of these results found.

The variant genotypes of this gene, because they have different levels of activity, can affect or modify serotonergic activity, by decreasing the number of receptors, especially at the platelets, resulting in a lower concentration of serotonin in the extracellular space, that is, of active serotonin. These low serotonergic levels are not, therefore, sufficient to provide adequate platelet activation, thus triggering less aggregation of the same, with damage in the formation of the clot that will prevent the approximation of the edges of the wound, the aggregation of fibronectin, fibroblasts, endothelial cells and keratinocytes [2,8-10,16-18,26,27].

Consequently, there will be a loss of interaction between platelet granule proteins and extracellular matrix proteins and platelet bodies, as they are not aggregated. This entire altered process will not allow the stabilization and formation of the preliminary matrix, in addition to the impairment in the migration of the cells responsible for triggering and continuing the repair process, which will be more prolonged [2,8-10,16-18,26,27].

Thus, these genotypic variants can have different molecular functions in the healing process of skin wounds, either because they are involved in its pathogenesis itself or because they determine genetic predisposition. The genetic association with changes in the healing process emphasizes the hypothesis of dysfunction of the serotonergic system when considering its etiopathogenesis [2,9,10].

Despite the positive results found in the present study, they must be interpreted with caution and need to be corroborated by independent and/or multicentric studies to determine the real prevalence of the 102T-C polymorphism in the 5-HTR2A gene and its association with the healing of cutaneous wounds in the general population and also in the Brazilian population.

Greater knowledge of this relationship of the serotonergic system is not only important for understanding the physiology and pathophysiology of healing, but also to assist in the discovery of its molecular causes. This will provide new perspectives and direction of humanized and personalized protocols for an early intervention in clinical practice, through specific pharmacological therapies/therapeutic schemes and wound

closure techniques, which provide the stimulation of the healing process in its different phases, even in the face of these genetic changes.

In addition, it aims to stimulate and reinforce continuity in scientific research in order to investigate the serotonergic influence on the healing process of skin wounds.

CONCLUSION

Patients with chronic skin wounds have a higher prevalence of the 102T-C polymorphism in the 5-HTR2A gene, which is associated with changes in the healing process in this population. There are differences, at the molecular level, in patients, with and without these lesions, and the probable role of the serotonergic system in wound healing.

Funding: This research received no external funding.

Acknowledgments: The authors thank the patients who participated in this research. Their contribution has been extremely important in improving Brazilian research.

Conflicts of Interest: The authors declare no conflict of interest.

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