Serotonin receptor (5-HT 2A) and catechol-O-methyltransferase (COMT) gene polymorphisms: Triggers of fibromyalgia?

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RESUMO

Introduction: Fibromyalgia is a rheumatic syndrome characterized by diffuse and chronic pain associated with fatigue, sleep disorders, anxiety, depression, memory loss, and dizziness. Although the physiological mechanisms that control fibromyalgia have not been precisely established, neuroendocrine, genetic or molecular factors may be involved in fibromyalgia. **Objective**: The aim of the present study was to characterize serotonin receptor (5-HT2A) and catechol-O-methyltransferase (COMT) gene polymorphisms in Brazilian patients with fibromyalgia and to evaluate the participation of these polymorphisms in the etiology of the disease. **Material and Methods**: Genomic DNA extracted from 102 blood samples (51 patients, 51 controls) was used for molecular characterization of the 5-HT2A and COMT gene polymorphisms by PCR-RFLP. **Results**: Analysis of the 5-HT2A polymorphism revealed a frequency of 25.49% C/C, 49.02% T/C and 25.49% T/T in patients, and of 17.65% C/C, 62.74% T/C and 19.61% T/T in the control group, with no differences between the two groups. Analysis of the COMT polymorphism in patients showed a frequency of 17.65% and 45.10% for genotypes H/H and L/H, respectively. In the control group the frequency was 29.42% for H/H and 60.78% for L/H, also with no differences between the two groups. However, there was a significant difference in the frequency of the L/L genotype between patients (37.25%) and controls (9.8%), which permitted differentiation between the two groups. **Conclusion**: The L/L genotype was more frequent among fibromyalgia patients. Though considering a polygenic situation and environmental factors, the molecular study of the rs4680 SNP of the COMT gene may be helpful to the identification of susceptible individuals.

Keywords: catechol-O-methyltransferase, fibromyalgia, polymorphism, serotonin receptor gene.

INTRODUCTION

Fibromyalgia (FS) is a rheumatic condition characterized by diffuse and chronic pain, commonly associated with fatigue, sleep disorders, anxiety, morning stiffness, depression, memory loss, dizziness, and generalized muscle tenderness.[1] FS is the result of abnormal changes in the central sensory processing of pain signals, which are thought to arise from a combination of interactions between neurotransmitters, external stressors, behavioral constructs, hormones, and the sympathetic nervous system.[2] Due to its painful and chronic character, the syndrome usually has a negative impact on the quality of life of the patients. The prevalence of fibromyalgia in the general population ranges from 0.66 to 4.4%,[3] with the disease being 10 to 20 times more frequent among women than men[4] and often affecting individuals of productive age (35 to 60 years). In a study conducted in Latin American, Senna *et al*.,[5] in Minas

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Gerais, Brazil, found that FS was the second most frequent rheumatologic disorder, with a prevalence of 2.5%. Therefore, FS can be considered a major health problem among women.[6] Despite the large number of studies, no diagnostic methods or effective treatments are available for the disease.[7] Although the physiological mechanisms that control fibromyalgia have not been precisely established, neuroendocrine factors seem to play an important role. Furthermore, genetic or molecular mechanisms may be involved in fibromyalgia.[8, 9] In addition, several studies have addressed the frequency of the syndrome in families of patients with FS and suggested that genetic and family factors may play a role in its etiopathogenesis.[10]

A decrease in serotonin (5-HT) and other neurotransmitters increases the sensitivity to painful stimuli and might be implicated in the reduced blood flow observed in muscles and superficial tissues of fibromyalgia patients. The serotonin transporter gene has long been implicated to be involved in the pathogenesis of several psychiatric disorders.[11,12] Serotonin may also contribute to the etiology of FS considering the efficacy of 5-HT reuptake inhibitors in the management of chronic pain.[13] Catechol-O-methyltransferase (COMT) is an enzyme that inactivates catecholamines and catechol-containing drugs.[14] There are different single-nucleotide polymorphisms (SNPs) in the COMT gene which induce important functional alterations of the enzyme. The association between gene polymorphism and FS is still not well established, but various authors suggest that mutations in these genes may represent the basis for new pharmacologic treatment in the future.[15] Therefore, the aim of the present study was to evaluate the frequency of the serotonin receptor (5-HT2A) and COMT gene polymorphisms in Brazilian patients with fibromyalgia and to compare these data with a control group of healthy subjects in order to establish the overall frequency of these polymorphisms in our population.

MATERIAL AND METHODS

Study population

Fifty-one patients with FS admitted to Centro Clínico Electro Bonini- UNAERP between May 2005 and July 2006 were included in the study. The criteria for inclusion of patients was a diagnosis of FS according to the 1990 guidelines of the American College of Rheumatology (ACR)[1] and to be free of any rheumatic disease. Fifty-one unrelated healthy volunteers were also included and constituted the control group. Controls were individuals who considered themselves to be healthy and who did not report any chronic pain. The project was approved by the Ethics Committee on Human Research of UNAERP (number 008/06), and all participants signed an informed consent form. A questionnaire was applied to collect data regarding the etiology of the disease (Chart 1).

Molecular analysis of the 5-HT2A and COMT gene polymorphisms

Genomic DNA was extracted from 5 mL whole blood samples containing EDTA using the Wizard kit (Promega) according to manufacturer instructions. A 352-bp fragment of the 5-HT2A gene was amplified using primers HT2AFWD 5'-CCT CAT CTG CTA CAA GTT CTG GCT T-3' and HT2A-REV 5'-GCA TTC TGC AGC TTT TTC TCT AGG G-3', modified from Gursoy *et al*.[2] The primers described by Gursoy *et al*.[14] were used to amplify a 185-bp fragment of the COMT gene. The reaction mixture for amplification of these two genes contained 100-500 ng DNA, 10 mM of each primer, 100 mM dNTPs, 1 U *Taq* polymerase (Invitrogen), and 1 mM $MgCl_2$ in a final

Chart 1. Questionnaire

1. Age?
2. Profession?
3. Marital status?
4. Children? How many?
5. How long have you been feeling pain?
6. How long have you been diagnosed with fibromyalgia?
7. Have you ever been or are you under treatment for fibromyalgia? Which?
8. Do you take any anti-inflammatory drugs? Which? What dosage? What are the side effects?
9. Are you taking any muscle relaxants? If so, which ones? What dosage? What are the side effects?
10. What other treatments for muscle tension relief have you undergone or are you currently undergoing?
    ( ) Massage ( ) Acupuncture ( ) Heat or cold application
    ( ) Stretching ( ) Tender point injections
    ( ) Other . . . . . .
11. Are you taking any anti-depressants? What dosage? What are the side effects?
12. Are you taking any anxiety control medications? What dosage? What are the side effects?
13. How has the pain affected your work, leisure and/or other activities?
14. What symptoms other than pain do you have (fatigue, sleeplessness, depression, etc.)?
15. Personally, what does it mean to feel pain?
16. Does anyone in your family have fibromyalgia? What is their relationship to you?
17. How often do you feel the need to urinate?
18. Do any of your relatives have a history of depression?

volume of 50 µl. Thermal cycling was carried out in a PTC-100 Thermocycler (MJ Research, Waterdown, MA, USA) under the following conditions: initial denaturation at 95° C for 3 min, followed by 40 cycles at 95° C for 30 s, annealing at 55° C for 30 s and 72° C for 30 s for extension, followed by a final extension step at 72° C for 10 min.

For the 5-HT2A gene, the PCR products were digested with 3 U *Msp*I (Promega) at 37 °C for 18 h and separated on 1% agarose gel. Allele 1 (T102 allele) corresponded to the undigested 352-bp PCR product, and allele 2 (C102 allele) consisted of two fragments of 220 bp and 132 bp.[2]

For the COMT gene, the PCR products were digested with 3 U *Hsp*92 II (Promega) restriction enzyme at 37 °C for 18 hours. The bands were visualized on 8% polyacrylamide gel after staining with ethidium bromide (10 mg/mL) for 20 min. The COMT-L/L genotype was represented by fragments of 114 bp, 36 bp, and 35 bp, the COMT-H/H genotype by fragments of 96 bp, 35 bp, 36 bp and 18 bp, and the COMT-L/H genotype by fragments of 114 bp, 96 bp, 36 bp, 35 bp and 18 bp.[14]

Data analysis

The results regarding the gene polymorphisms were analyzed by the Tukey test using the SISVAR program.

RESULTS

Patient-related data were obtained by application of a questionnaire (Chart 1). The sample consisted of 102 subjects of the same ethnic and geographic origins, including 51 patients with a diagnosis of FS (mean age of 50 ± 12 years) and 51 healthy controls (45 ± 10 years). There was no significant difference in the characteristics between the two groups (controls and patients) for age, gender and marital status. The two samples are composed for married women with mean age of 45-50 years The mean duration of fibromyalgia was 7 ± 6 years. Regarding the presence of muscle pain, 67% of the patients reported pain for a period of 1-5 years and 4% reported muscle pain for over 20 years (Table 1).

With respect to drug treatment, 58.49% of the patients are using or had used some anti-inflammatory drug to relieve muscle pain, but reported that the use of this kind of medication only brought temporary relief. Muscle relaxants, more specifically cyclobenzaprine, are used or had been used by 69.81% of the patients, but not all patients tolerated this medication and reported side effects during treatment. In addition, 73.58% of the patients are using and had used anti-depressants for the treatment of fibromyalgia. Regarding serotonin reuptake inhibiting antidepressants, patients reported the use of fluoxetine (48.97%), amitriptyline (5.76%), sertraline (5.76%), paroxetine chloride (1.92%) and citalopram (1.92%), as well as the tricyclic antidepressant, imipramine (9.61%). Only 1.96% of patients reported the use of the anti-anxiety drug alprazolam for the treatment of FS. Alternative treatments included physiotherapy (38.46%), water gymnastics (25%), and walking (23.07%).

In the present study, analysis of the 5-HT2A gene polymorphism demonstrated frequencies of 25.49%, 49.02% and 25.49% for the C/C, T/C and T/T genotypes, respectively, in patients with FS, and of 17.65%, 62.74% and 19.61% in the control group, with no significant difference in the three genotypes between the FS and control groups (Table 2).

Analysis of the COMT gene showed a frequency of 17.65%, 45.10% and 37.25% for the H/H, L/H and L/L genotypes in patients with FS. This proportion was 29.42%, 60.78% and 9.80% in the control group, with no significant difference in the H/H or L/H genotype between the two groups. The most interesting result of our study was the significant difference in the frequency of the L/L genotype between patients (37.25%) and controls (9.8%), which permitted differentiation between the two groups (Table 3).

Molecular analysis of the 5-HT2A gene polymorphism in patients demonstrated a prevalence of 25.49% of the wild-type homozygous genotype, 49.02% of the heterozygous genotype, and 25.49% of the recessive homozygous genotype. The COMT gene polymorphism showed a prevalence among patients of 17.65% of the wild-type homozygous genotype, 45.10% of the heterozygous genotype, and 37.25% of the recessive homozygous genotype (with a predominance of the Met-158-Met mutation).

DISCUSSION

Our results showed that molecular analysis of the 5-HT2A showed no relation to the gene polymorphism to FS in the Brazilian population. Similar results have been described by Gursoy *et al*.[2] in Turkish patients.

Another mechanism related to the physiopathology of FS might be malfunction of the gene encoding the COMT enzyme, which inactivates catecholamines, including dopamine and dopamine-containing drugs.[16] This enzyme is responsible for neuroendocrine disorders characterized by an abnormal function of the hypothalamus-pituitary-adrenal axis.[14] There are different single nucleotide polymorphisms (SNPs) that induce important functional alterations in the enzyme. The best studied SNP (rs4680) occurs in codon 158, resulting in a

Table 1
Characteristics of patients with fibromyalgia and controls

	Patients (n = 51)	Controls (n = 51)
Age in years (mean ± SD)	50 ± 12	45 ± 10
Disease duration in years (mean ± SD)	7 ± 6	—
Percentage of patients reporting the presence of muscle pain (duration in years)	T (1-5) = 67%	—
	T (5-10) = 15%	—
	T (10-15) = 8%	—
	T (15-20) = 6%	—
	T (> 20) = 4%	—
Gender (female/male)	49/2	48/3
Married	33 (65 %)	31 (60%)

Table 2
Frequency of serotonin receptor (5-HT 2A) gene polymorphisms in fibromyalgia patients and controls

Genotype	Patients (%)	Controls (%)
C/C	25.49[a]	17.65[a]
T/C	49.02[a]	62.74[a]
T/T	25.49[a]	19.61[a]

Mean values in the same row followed by different superscript letters differed significantly (p=0.05, Tukey test).

Table 3
Frequency of catechol-O-methyltransferase (COMT) gene polymorphisms in fibromyalgia patients and controls

Genotype	Patients (%)	Controls (%)
H/H	17.65[a]	29.42[a]
L/H	45.10[a]	60.78[a]
L/L	37.25[a]	9.80[b]

Mean values in the same row followed by different superscript letters differed significantly (p=0.05, Tukey test).

valine to methionine transition (Val-158-Met). The H/H (Val-158-Val) genotype gives rise to an effective enzyme, whereas the L/L (Met-158-Met) genotype produces a defective enzyme, which is unable to effectively clear catecholamines from the system.[16] In the present study, analysis of the SNP (rs4680) of the COMT gene demonstrated a frequency of the LL genotype (Met-158-Met) of 37.25% in patients and of 9.8% in controls, showing a large statistical difference able to distinguish both groups. Gursoy *et al*.[14] observed an association between FS and the COMT genotypes in Turkish patients, with 73.85% of the patients showing low or intermediate enzyme activity (L/L or L/H) and 26.2% presenting high enzyme activity (H/H). The L/L genotype was more frequent among the patients studied. However, this genotype might only be another factor associated with fibromyalgia.

Vargas-Alarcon *et al*.[6] compared six COMT SNPs (rs 2097903, rs6269, rs4633, rs4818, rs4680, and rs165599) in patients with FS from Spain and Mexico. In the group of Spanish patients, there was an association between FS and the COMT haplotype previously associated with pain sensitivity, but this association was not observed in Mexican patients. Zubieta *et al*.[17] demonstrated that healthy individuals with the COMT Val-158-Val genotype are pain resistant. The opposite is observed in subjects with the COMT Met-158-Met polymorphism.[9] In Spain, García-Fructuoso *et al*.[18] found that FS patients with the Met-158-Met genotype have a more severe form of the disease when compared to fibromyalgia patients carrying the Val-158-Val genotype. However, Tander *et al*.[15] observed no association between polymorphisms in two 5-HT2A genes and COMT genes and FS, but it is likely that multiple interactions of several different neurotransmitter systems and pathways are involved in FS.

In addition to the association of genotypes with diseases, the molecular characterization of polymorphisms in genes encoding enzymes, transporters and receptors for drug metabolization, as well as factors such as age, gender, nutrition and genetic factors, may contribute to the variety of responses to a drug.[19] Sadée and Dai[20] associated polymorphisms in the HTR2A and COMT genes, among others, with the response to certain drugs. Future studies in this area may help establish a correlation between these polymorphisms and the response to treatment in FS.

In conclusion, for the Brazilian population studied, the serotonin receptor polymorphism does not seem to be directly involved in the mechanism of FS. On the other hand, compared to control individuals, the L/L genotype was more frequent in FS. Therefore, carriers of the L/L genotype may be more susceptible to the development of this syndrome. Though considering a polygenic situation and environmental factors, the molecular study of the rs4680 SNP of the COMT gene may be helpful to the identification of susceptible individuals.

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