Association of polymorphism of the enzyme catechol-o-methyltransferase with fibromialgic syndrome and its clinical repercussions

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Abstract

Background: Fibromyalgia syndrome (FMS) is a clinical condition that mostly affects women of working age, with chronic diffuse pain, physical disability, mood swings, anxiety, fatigue and insomnia. Although its pathophysiology is not fully understood, it is believed that there are genetic contributions to its origin. There is evidence that some single nucleotide polymorphisms (SNPs) can change the function of proteins that participate in pain modulation, increasing the individual's susceptibility to pain processes. Catechol-O-methyltransferase (COMT) is an enzyme responsible for the inactivation of catecholamines in the central nervous system, participating in descending nociceptive inhibitory pathways. Polymorphisms in the gene encoding COMT can impair its formation and, consequently, its function, accentuating painful conditions in FMS patients. This study verified the association of SNPs rs4680, rs6269, rs4633 and rs4818 of the COMT gene with clinical aspects in patients with FMS undergoing treatment in public and private health services in Cuiabá, Mato Grosso Estate, Brazil.

Methods: Forty-seven volunteers who underwent medical follow-up by FMS were selected, in which the Fibromyalgia Impact Questionnaire, the Beck's Depression and Anxiety Inventories, the Insomnia Severity Index and the Mini-Mental State Examination were applied, in order to evaluate the clinical repercussions of the disease. Blood samples were collected for genetic evaluation. The DNA was extracted by salting out and the SNPs were evaluated by real time reverse transcription polymerase chain (RT-PCR). The association between clinic and SNPs was tested by the Fisher's exact test. A 95% CI and p value < 0.05 were adopted.

Results: The results showed that there was no association between such SNPs and the participants' clinic regarding the tests used.

Conclusions: This study showed that, although the disease has an important impact on patients' daily lives, increasing the chances of depression, anxiety, insomnia and cognitive losses, it is not associated with the SNPs researched. Further investigations, with larger samples, are needed to assess these and other associations between genetics factors and FMS.

Background

According to the International Association for the Study of Pain (IASP), “pain can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, caused by an external agent or an internal pathogenic process” (1).

In general, pain can be divided into three categories: nociceptive pain, neuropathic pain, and nociplastic pain. Nociceptive pain fulfills an essential function for survival, as it alerts the individual to some harmful action to the organism. However, in chronic pain conditions, in which the pain remains for long periods, even after the nociceptive event has ended, or even when it is triggered by an innocuous event, the pain loses the function of a symptom and becomes the disease itself. In this situation, it is related to real damage to the central nervous system (CNS), and is now considered neuropathic pain. When this
damage is reversible, allowing some modulation of pain, it is called nociplastic. In all situations, there are important repercussions on the individual's life (1,2).

Some chronic pain syndromes, such as cluster headache, migraine, myofascial pain syndrome and fibromyalgic syndrome (FMS), are considered conditions that represent such a chronification process (3).

FMS is characterized by persistent generalized musculoskeletal pain, of moderate to severe intensity, commonly associated with fatigue, anxiety, depression, non-restorative sleep, decreased cognitive capacity and physical limitation (4,5).

As it is the third most common painful musculoskeletal condition among adults, appearing behind low back pain and osteoarthritis, FMS appears as an important cause of demand for Primary Health Care (PHC) services. The global prevalence of FMS varies between 0.2% and 6.6%, with a predominance of three women for every man in the female population. Although it can be diagnosed in any age group, it is most commonly seen in the world between 30 and 35 years of age (6,2).

In Brazil, FMS has an estimated prevalence of 2.5% in the population. It affects both sexes, of all ethnicities, predominating in the female population in a proportion of nine women for every man, being more common between the third and fifth decades of life (7).

The diagnosis of FMS is eminently clinical and can be considered a challenge, given that it presents a very broad spectrum of signs and symptoms. The American College of Rheumatology (ACR) established the first diagnostic criteria for the disease in 1990. To be considered FMS, the individual should have generalized pain, above and below the waist, for at least three months, and at least 11 tender points, among 18 of them, during the physical examination (8,5).

Due to the difficulty in objectively evaluating tender points, and the fact that the ACR 1990 disregarded some somatic symptoms that are commonly present and that impact as much as the pain itself, in 2010 the entity established new diagnostic criteria, which were revised in 2012 and 2016. From then on, palpation of tender points was excluded from the investigation and two scores were analyzed: Generalized Pain Index (GPI), composed of 19 possibly painful areas to be referred by the patient; and Symptom Severity Scale (SSS), a sum of points that involves fatigue, insomnia, cognitive losses and somatic symptoms (4,9).

Pain pathophysiology

The perception of pain occurs in a few main stages. The first involves a peripheral tissue injury, which triggers a local inflammatory response, stimulating specific pain receptors, which, in turn, emit a signal that will be taken to the spinal cord, and from there to the CNS (10).

After tissue damage, inflammatory and immune factors are released in that region, through which the following can occur: formation of bradycin, by the breakdown of fatty acids; release of arachidonic acid, by direct injury to the cell membrane; activation of macrophages, which release cytokines (IL1, IL6 and
TNF-alpha); release of selectins, integrins, chemotactic factors and nitric oxide (NO); migration of defense cells; tissue repair by the formation of catalases and collagenases; release of chemical mediators that will stimulate pain receptors (11).

Nociceptors are distributed throughout the body. There are three categories of them: mechanoceptors (pressure-sensitive); thermoreceptors (sensitive to temperature changes); and polymodal receptors (sensitive to pressure, temperature and chemicals agents). Mechanoceptors and thermoreceptors are innervated by myelinated nerve fibers of the A-beta and A-delta types, carrying pain information more rapidly to a second neuron in the spinal cord. Polymodal receptors are innervated by type C unmyelinated nerve fibers, which more slowly convey information to the second neuron (12).

In the spinal cord, there are still two possible pathways through which pain information can reach the CNS: neospinothalamic pathway (faster, due to the fact that there are few synapses), responsible for acute, somatic, well-localized pain; and the paleospinothalamic pathway (slower, due to the existence of more synapses), responsible for visceral pain, of less precise location (13).

Centrally, pain processing involves the cortex, hypothalamus, basal ganglia, brainstem and spinal cord, and may have peripheral repercussions on the adrenals. It involves somatosensory areas, the limbic system and even the reward system, and the main related neurotransmitters are dopamine, serotonin and norepinephrine, with greater emphasis on the first in the descending pathway (14,15,16).

After pain is centralized, information is carried down the descending pathway through type C fibers in the spinal cord, communicating with interneurons. These release endogenous opioids (enkephalins, endorphins and dynorphins), which bind to opioid receptors, limiting substance P release and, therefore, controlling pain (17).

In this sense, pain modulation requires a balance mechanism between ascending pro-nociceptive pathways (neuronal growth factor, substance P, excitatory amino acids and prostaglandins) and descending inhibitory nociceptive pathways (catecholamines and endogenous opioids). Peripheral pain processes that ascend to the CNS, where they are interpreted, receive a response in the opposite direction, modulating the pain intensity (18,19,20).

However, neuroplastic changes dictated by heredity, associated with environmental variants, psychosocial factors and physical triggers, lead to neurohormonal changes in the CNS, causing damage to descending inhibitory pathways. Such changes include a decrease in dopamine and serotonin - which predisposes to mood swings, negative reactions to external stimuli and stress, in addition to ineffective sleep; and increased substance P and secretion of interleukins - causing peripheral muscle hypoxia and diffuse pain (21,19).

The relationship between dopamine and pain

In FMS, there is a dysfunction in monoamines, according to which there is an increase in excitatory neurotransmitters (glutamate and substance P), and a concomitant decrease in serotonin and
norepinephrine in the spinal cord, as well as in dopamine and endogenous brain opioids, as previously discussed. (5).

Dopamine is synthesized centrally from the amino acid tyrosine, which is converted to L-dopa, which in turn is decarboxylated. To perform its functions, dopamine binds to its receptors. There are two main families of dopamine receptors: D1 (D1 and D5 receptors), with G protein and adenylcyclase activation; D2 (D2, D3 and D4 receptors), without adenylcyclase stimulation. Among these receptors, D1 and D2 are the most important and most commonly found. D1 is often located in the cerebral cortex and striatum, participating in processes involving behavior and emotions. D2 is also found in the cortex and striatum, but also in the hypothalamus and anterior pituitary, in this region it acts on the secretion of prolactin (22,23).

Dopamine acts on four central pathways: nigrostriatal (responsible for movement); mesolimbic or reward (related to behavior); mesocortical (very much associated with the anterior pathway, therefore also related to emotions, but it is the most important for pain processing); tuberoinfundibular or tuberohypophyseal (related to the control of prolactin secretion) (23).

The importance of the COMT enzyme in FMS

Two enzymes are responsible for metabolizing dopamine: monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT), generating the metabolites hydroxyphenylacetic acid (HDPA) and homovanillic acid (HVA), respectively.

COMT has a very complex molecular structure and is distributed intracellularly or is anchored to the plasma membrane of central neurons, in addition to being expressed in other tissues (liver, kidneys, intestinal tract and heart). Its function is to transform dopamine into HVA, in the central pathways of the nervous system, through an O-methylation reaction using S-adenosyl-L-methionine (SAM) as a methyl group donor to the hydroxyl group of the substrate. HVA is naturally excreted in the urine (24,25).

High levels of dopamine are released from neurons in the ventral tegmental area in the synaptic clefts of the prefrontal cortex, where there are few dopamine transporters. This allows the neurotransmitter to diffuse into the extrasynaptic space, where it can be a substrate for COMT. Low COMT activity will make more dopamine available to bind to its excitatory receptors on glutamatergic pyramidal neurons. The increased stimulation of these neurons then increases the release of glutamate in the nucleus accumbens. In this, tonic dopamine release occurs when neurons in the ventral tegmental area release dopamine at low levels, regulated by glutamatergic afferents such as those in the prefrontal cortex. The increase in glutamate stimulates the release of dopamine into the extrasynaptic space, where it can bind to presynaptic dopaminergic receptors, which inhibit the phasic release of dopamine (triggered by the firing of neurons in the ventral tegmental area that provide high levels of dopamine in the synaptic cleft). Here, dopamine can stimulate postsynaptic receptors, responsible for descending inhibition of pain stimuli. Elevated levels of tonic dopamine inhibit the release of phasic dopamine, consequently reducing
downward inhibition, which increases sensitivity to pain, making the individual more susceptible to it (24,22).

High COMT activity in the prefrontal cortex causes breakdown of extrasynaptic dopamine and therefore less dopamine binding to excitatory receptors on glutamatergic pyramidal neurons. In the nucleus accumbens, the lack of this glutamatergic stimulus leads to low levels of tonic dopamine and less inhibition of phasic dopamine. This increases dopamine levels in the synaptic cleft, stimulating postsynaptic receptors, which inhibit pain sensitivity through the descending pathway, making the individual less susceptible to it (24,26).

In summary: a) COMT enzyme with low activity increases dopamine of tonic production and reduces dopamine of phasic production, decreasing the ability to control pain in the descending axis; b) COMT enzyme with high activity, in contrast, reduces dopamine of tonic production, but increases dopamine of phasic production, increasing the response in the descending axis and controlling pain more effectively (24).

COMT genetic aspects

Respecting the central dogma of molecular biology, according to which a gene is transcribed into an mRNA and this is translated into a protein, if there is any modification in the gene, its protein product may also be different from that transcribed by the wild-type gene, and may present some functional deficit. Some of these possible modifications are single nucleotide polymorphisms (SNPs), in which only one nucleotide of a given gene is replaced by another. SNPs are inherited mutations present on average in 1% of the population and generally do not cause drastic phenotypic changes in the individual, but are associated with increased susceptibility to some characteristics or medical conditions (24,27).

Like any other gene, the COMT gene may have some SNPs. The most frequently studied among patients with FMS are: rs4680, rs6269, rs4633 and rs4818 (28,29).

The COMT gene is located at 22q11.2 and contains six exons. The guanine nucleotide (G) is found at codon 158 of the wild-type allele. The resulting translated protein will have the amino acid valine (Val) in the position corresponding to this codon, having its usual tertiary structure and effective function, as described above. However, when the COMT gene has the adenine (A) nucleotide in the same codon 158, it is considered to have the rs4680 SNP. In this situation, the translated protein will have the amino acid methionine (Met). This minimal change in the gene (G>A) and, therefore, in the enzyme (Val158Met), generates low-activity COMT, which increases the levels of catecholamines, notably epinephrine. As a result, there are persistent pain states, due to the stimulation of beta-2-adrenergic receptors in the central and peripheral nervous systems (24,30,25).

In this sense, when it comes to the rs4680 SNP, an individual can carry one of three genotypic variations: while the wild homozygous Val/Val genotype generates high activity COMT enzymes, the polymorphic homozygous Met/Met genotype produces low activity enzymes, and the heterozygous polymorphic
Val/Met genotype generates enzymes with intermediate activity. In a sample of Brazilian women with FMS, the frequency of Met/Met was 37.25%, while among healthy individuals this variation was equal to 9.8%, indicating that that genotype confers susceptibility to the condition (31,32, 33).

Data from another study with subjects affected by FMS showed that the SNP rs4680 was associated with greater psychological vulnerability (depression, anxiety and catastrophizing), as well as greater difficulty in stopping the use of antidepressants when they were using them, even for a short time, among members of the case group carrying the Met/Met genotype (34,35).

Other COMT polymorphisms have been associated not only with increased pain susceptibility, but also with other conditions, such as Parkinson's disease, depression, schizophrenia and attention deficit disorder, for example (36,37,38).

SNPs rs4680 (G>A), rs6269 (A>G), rs4633 (C>T) and rs4818 (C>G) were analyzed concomitantly in patients with Parkinson's disease. It was suggested that the structure of the haplotype formed by the four SNPs would influence enzymatic activity more intensely than a single SNP alone, intensifying the clinical picture and worsening the individual's therapeutic response. So much so that, when comparing the existence of the four grouped SNPs with the response to treatment with levodopa, it was found that those who had a greater number of SNPs required higher doses of medication (39).

Another analysis, specifically directed at the association between COMT gene polymorphisms and FMS in an Asian population, involved haplotypes of these same four SNPs, revealing that five of them (ACCA, GCGA, ATCA and ACCG) were more frequent among patients with FMS than among healthy controls. Among such haplotypes, ACGA was most strongly associated with disease, as well as accompanying a greater number of tender points on the body and a tendency to score higher on quality of life questionnaires (40).

Considering the exposed in this brief review, the hypothesis of this study is that the SNPs rs4680, rs6269, rs4633 and rs4818 of the gene encoding COMT are more frequent among individuals affected by FMS than in people who do not have the syndrome, accentuating the clinical picture and decreasing quality of life, directly proportional to the amount of concomitant polymorphisms in each individual.

**Methods**

Participants and study design

This is a clinical research, of the association type, whose objectives were: a) check the clinical patients of fibromyalgia patients, the impact that the disease has on their daily lives, and the risk of anxiety, anxiety, insomnia and cognitive loss; b) identify the allele frequencies of the SNPs rs4680, rs6269, rs4633 and rs4818 of the COMT gene in FMS patients; c) investigate the possible association between these SNPs and the SFM framework, based on the results obtained in the validated tests.
47 patients affected by FMS were evaluated, undergoing clinical follow-up in public and private health services in Cuiabá, Mato Grosso State, Brazil. The period of recruitment of volunteers was between March 2020 and October 2021, during the COVID-19 pandemic. The study was approved by the Research Ethics Committee with human beings, according to document number 3.924.907 of March 19, 2020, and all volunteers signed an informed consent form (ICF) before joining the study.

The inclusion criteria for this study were: subjects previously diagnosed with FMS, according to the ACR 2012 criteria, with a minimum age of 18 years, even with other rheumatic or orthopedic conditions that evolve with chronic pain (rheumatoid arthritis, osteoarthritis, psoriatic arthritis, spondyloarthritis, systemic lupus erythematosus, gout, pseudogout, polymyalgia rheumatica, and osteoporosis). Exclusion criteria were: people who had, at some point in their lives, or who were undergoing at the time of the research, treatment for any type of malignant neoplasm or pain of oncological origin.

Participants were clinically evaluated (history and physical examination) in order to confirm or exclude the diagnosis of FMS. On that occasion, they were interviewed regarding socioeconomic aspects.

Assessment of quality of life

Quality of life and the degree of impairment that pain can cause in patients were measured using the ACR 2012 criteria, and especially the Fibromyalgia Impact Questionnaire (FIQ). This is a self-reported scoring system that ranges from zero to 100, whose categories are: normal quality of life (from zero to 24 points); moderate quality of life (from 25 to 49 points); low quality of life (from 50 to 74 points); very low quality of life (from 75 to 100 points). For the purpose of statistical analysis, we considered the value 50 as the cut-off point. In the case of the 2012 ACR, we considered the value 7 as the cutoff point for the GPI and the value 6 as the cutoff point for the SSS.

The levels of psychiatric impairment, regarding depression and anxiety, were measured using the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI), respectively. Both are also self-administered questionnaires, whose scores range from zero to 63, indicating the probability that the subject has depression or anxiety.

The BDI categories include: minimal depression (from zero to 13 points); mild depression (from 14 to 19 points); moderate depression (from 20 to 28 points); severe depression (from 29 to 63 points). For statistical analyses, we considered 20 as the cut-off point.

The BAI categories, in turn, comprise: minimum chance of anxiety (from zero to 10 points); mild anxiety (from 11 to 19 points); moderate anxiety (from 20 to 28 points); severe anxiety (from 29 to 63 points). For statistical analyses, we considered 20 as the cut-off point.

Sleep quality was assessed using the Insomnia Severity Index (ISI). It is also a self-administered questionnaire, with a score ranging from zero to 28 points. The ISI categories include: without insomnia (from zero to 7 points); mild insomnia (from 8 to 14 points); moderate insomnia (from 15 to 21 points); severe insomnia (from 22 to 28 points). For statistical analyses, we considered 15 as the cut-off point.
To analyze the level of cognitive impairment, the Mini-Mental State Examination was used, an instrument widely applied in the clinic, which compares the score obtained by the individual with the expected response according to their education. If the patient does not reach the expected score, the exam is considered altered.

All questionnaires were applied by the same evaluator.

**DNA extraction**

Blood samples were collected via venipuncture in the forearm, complying with international biosafety parameters.

The samples were stored in vacuum tubes containing EDTA, numbered according to the order of collection, stored and sent under refrigeration in a styrofoam box to the laboratory. The DNA of the samples was extracted by the salting out method.

**Genotyping**

Genotyping involved allelic discrimination and was performed by amplifying the DNA fragments using the reverse transcription polymerase chain reaction (RT-PCR method), using the TaqMan® system, using assays with four probes corresponding to the SNPs of interest, designed and validated by Applied Biosystems (SNP rs4680, rs6269, rs4633 and rs4818). For the detection of allelic variants, the presence or absence of the SNP was determined from the change in the fluorescence signals of the amplified target sequence and obtained from the fluorescent dyes VIC and FAM attached to the 5’ end of the probe and from a non-fluorescent quencher at the 3’ end.

The runs were performed on the 7500 Fast Real-Time PCR System equipment (Applied Biosystem, CA/USA) in 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds.

**Statistical analysis**

The data obtained were evaluated using the EPI Info software for Windows (version 7.2.4.0). Mean, median, mode and standard deviation were used for the presentation of continuous numerical variables. Frequencies and percentages were calculated for the presentation of categorical variables. Genotype frequencies of all SNPs were compared with FMS susceptibility using the Fisher’s exact test. A p value < 0.05 was considered to indicate statistical significance. Logistic regression analysis was performed in order to calculate the odds ratio (OR) and relative risk (RR), considering a confidence interval (CI) of 95%.

**Results**

**Socioeconomic aspects**

Of the 47 volunteers participating in the study, 95.74% were female, 48.94% declared themselves mixed race, 31.91% white, 17.02% black and 2.13% indian. The general average age among all participants is
51.25 years, with an average of 51.44 years among women. Of the total sample, 68.09% are married, only 27.66% have completed higher education, 68.09% reported having an employment relationship. In addition, 80.85% of the volunteers do not have any type of health plan or insurance, directly needing the services offered by the Brazilian public health service. Regarding lifestyle, 89.36% do not practice any type of physical activity (at least 20 minutes of aerobic or resistance exercise, three times a week). 78.72% of the participants are overweight or obese (Table 1).

### Table 1
Socioeconomic aspects of FMS patients in Cuiabá, Brazil (2021)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Female</th>
<th>95.74%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>4.26%</td>
</tr>
<tr>
<td>Skin color</td>
<td>Mixed race</td>
<td>48.94%</td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>31.91%</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>17.02%</td>
</tr>
<tr>
<td></td>
<td>Indian</td>
<td>2.13%</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>General</td>
<td>51.25</td>
</tr>
<tr>
<td></td>
<td>Female only</td>
<td>51.44</td>
</tr>
<tr>
<td>Marital status</td>
<td>Married</td>
<td>68.09%</td>
</tr>
<tr>
<td></td>
<td>Single</td>
<td>19.15%</td>
</tr>
<tr>
<td></td>
<td>Widower</td>
<td>8.51%</td>
</tr>
<tr>
<td></td>
<td>Divorced</td>
<td>4.26%</td>
</tr>
<tr>
<td>Education level</td>
<td>University education</td>
<td>27.66%</td>
</tr>
<tr>
<td></td>
<td>Until high school</td>
<td>72.34%</td>
</tr>
<tr>
<td>Work</td>
<td>Yes</td>
<td>68.09%</td>
</tr>
<tr>
<td></td>
<td>Not</td>
<td>31.91%</td>
</tr>
<tr>
<td>Health insurance</td>
<td>Yes</td>
<td>19.15%</td>
</tr>
<tr>
<td></td>
<td>Not</td>
<td>80.85%</td>
</tr>
<tr>
<td>Practice physical exercises</td>
<td>Yes</td>
<td>10.24%</td>
</tr>
<tr>
<td></td>
<td>Not</td>
<td>89.36%</td>
</tr>
<tr>
<td>Overweight/obesity</td>
<td>Yes</td>
<td>78.72%</td>
</tr>
<tr>
<td></td>
<td>Not</td>
<td>21.28%</td>
</tr>
</tbody>
</table>
Clinical aspects

As for the diagnostic criteria for FMS, the average GPI was 11.02 points, with 89.36% of the individuals with the index considered moderate or high (equal to or greater than 7 points). The SSS average was 8.97 points, with 95.74% of the volunteers with the scale considered moderate or high (equal to or greater than 6 points) (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>GPI 11.02 (mean)</th>
<th>Moderate/high</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPI</td>
<td>89.39%</td>
<td></td>
<td>10.64%</td>
</tr>
<tr>
<td>SSS 8.97 (mean)</td>
<td>Moderate/high</td>
<td>95.74%</td>
<td>4.26%</td>
</tr>
<tr>
<td>GPI - Generalized Pain Index. SSS - Symptom Severity Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The FIQ average was 64.14 points, with 85.1% of the participants in the range considered to have low or very low quality of life. The BDI mean was 19.57 points, with 42.55% of the volunteers in the range that considered moderate or severe depression. Meanwhile, the BAI average was 21.29 points, with 53.19% of individuals in the range who considered moderate or severe anxiety. The mean ISI observed was 13.51 points, with 48.94% of participants having moderate or severe insomnia. Of the 47 individuals, only 25.53% showed changes in the Mini-Mental State Examination (Table 3).
Table 3
Means and classification of FIQ, BDI, BAI, ISI and Mini-Mental in patients with FMS in Cuiabá, Brazil (2021)

<table>
<thead>
<tr>
<th>FIQ</th>
<th>64.14 (mean)</th>
<th>Low/very low quality of life</th>
<th>85.10%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Moderate quality of life</td>
<td>14.90%</td>
</tr>
<tr>
<td>BDI</td>
<td>19.57 (mean)</td>
<td>Moderate/severe</td>
<td>42.55%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimal/mild</td>
<td>57.45%</td>
</tr>
<tr>
<td>BAI</td>
<td>21.29 (mean)</td>
<td>Moderate/severe</td>
<td>53.19%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimal/mild</td>
<td>46.81%</td>
</tr>
<tr>
<td>ISI</td>
<td>13.51 (mean)</td>
<td>Moderate/severe</td>
<td>48.94%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No insomnia/mild</td>
<td>51.06</td>
</tr>
<tr>
<td>Mini-Mental</td>
<td>Normal results</td>
<td></td>
<td>74.47%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Altered results</td>
<td>25.53%</td>
</tr>
</tbody>
</table>

FIQ - Fibromyalgia Impact Questionnaire. BDI - Beck Depression Inventory. BAI - Beck Anxiety Inventory. ISI - Insomnia Severity Index. Mini-Mental - Mini-Mental State Examination

All volunteers reported repeatedly using some type of non-hormonal or hormonal analgesic or anti-inflammatory, and 34.04% reported using opioids. Of the total, 48.94% use some type of tricyclic antidepressant, 31.91% use some selective or non-selective serotonin reuptake inhibitor, and 17.02% use an anticonvulsant or gabapentinoid. In addition, 12.77% of the volunteers reported using benzodiazepines chronically.

Genetic aspects

Regarding the rs4680 SNP, 14.89% of the individuals are homozygous (AA), and 34.04% are heterozygous (AG) for the mutation. 23.40% are homozygous (GG), while 42.55% are heterozygous (GA) for SNP rs6269. Considering the rs4633 SNP, 14.89% of the volunteers are homozygous (TT), and 36.17% are heterozygous (TC) for the mutation. Furthermore, 19.14% are homozygous (GG) and 40.43% are heterozygous (GC) for the rs4818 SNP (Table 4).
Table 4
Frequency of patients with FMS and homozygotes and heterozygotes for SNPs rs4680, rs6269, rs4633 and rs4818, in Cuiabá, Brazil (2021)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4680</td>
<td>AA</td>
<td>14.89%</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>34.04%</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>51.06%</td>
</tr>
<tr>
<td>rs6269</td>
<td>GG</td>
<td>23.40%</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>42.55%</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>34.04%</td>
</tr>
<tr>
<td>rs4633</td>
<td>TT</td>
<td>14.89%</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>36.17%</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>48.94%</td>
</tr>
<tr>
<td>rs4818</td>
<td>GG</td>
<td>19.14%</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>40.43%</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>40.43%</td>
</tr>
</tbody>
</table>


Although proportionally there was a greater number of individuals carrying at least one of the alleles of each of the SNPs, there was no statistically significant association between any of them with the evaluated clinical aspects - GDI, EGS, FIQ, BDI, BAI, ISI and Mini-Mental State Examination. In all tests performed, the p-value obtained was greater than 0.05 (Table 5).
Table 5
P-values resulting from the association between SNPs and the results of clinical tests applied to patients with FMS in Cuiabá, Brazil (2021)

<table>
<thead>
<tr>
<th></th>
<th>rs4680</th>
<th>rs6269</th>
<th>rs4633</th>
<th>rs4818</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPI</td>
<td>0.666</td>
<td>0.648</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>SSS</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>FIQ</td>
<td>1.000</td>
<td>0.112</td>
<td>0.700</td>
<td>0.417</td>
</tr>
<tr>
<td>BDI</td>
<td>0.770</td>
<td>1.000</td>
<td>0.770</td>
<td>0.764</td>
</tr>
<tr>
<td>BAI</td>
<td>1.000</td>
<td>0.538</td>
<td>1.000</td>
<td>0.766</td>
</tr>
<tr>
<td>ISI</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Mini-Mental</td>
<td>0.739</td>
<td>0.289</td>
<td>0.517</td>
<td>0.147</td>
</tr>
</tbody>
</table>

GPI - Generalized Pain Index. SSS - Symptom Severity Scale. FIQ - Fibromyalgia Impact Questionnaire. BDI - Beck Depression Inventory. BAI - Beck Anxiety Inventory. ISI - Insomnia Severity Index. Mini-Mental - Mini-Mental State Examination

Discussion

Although the sample is considerably small for the type of study, it is in accordance with epidemiological reviews on FMS, considering the predominance of women among those who have the disease (6, 2).

Regarding the age group, it is observed that it also corroborates other local studies, as it shows a higher prevalence among women in the age group between 50 and 60 years. However, such data may differ from those reported in other regions, where FMS prevails on average up to 35 years of age (7, 5).

In a large survey, involving more than 2700 American individuals diagnosed with FMS, it was observed that non-whites were at greater risk of developing the disease, with African-Americans being 1.52 times more likely to have the diagnosis, in compared to whites (41). It is observed that the sample of our study, gathered in the municipality of Cuiabá, agrees with this reality, since almost 50% of it is made up of browns, and just over 30% of whites, with a predominance of the former over all other colors of skin.

In a systematic review, Alciati et al. (2021) indicated other variables possibly associated with the disease, in addition to the already known female gender: low socioeconomic levels and obesity are risk factors for FMS. In our study, only a third of the participants reported having completed higher education or reported being attending this level of education, which can directly impact income, as almost 90% of them receive a maximum of up to three minimum wages. Education level can define the possibility of accessing more specific health plans and treatments. As shown by the data mentioned above, more than 80% of the participants do not have access to such services, requiring almost exclusively those provided by the Brazilian public health service (4).
In another study, which evaluated 274 women with FMS, a statistically significant association was observed between physical inactivity and symptom severity with the rs4680 SNP. Regarding lifestyle, more than 75% of the sample of our study is in the overweight or obese range, and almost 90% do not perform any type of physical activity (42).

Qureshi et al. (2021) indicated other candidate genes for greater predisposition to pain and other symptoms in FMS, in addition to the COMT gene. The S/S genotype of the serotonin gene appears to be more prevalent among fibromyalgic women with depression and anxiety than among controls with FMS alone. Mutations in the serotonin transporter gene (SLC64A4) and the serotonin receptor SNP rs6313 (HTR2A) are also associated with increased susceptibility to the disease. The SNPs rs10799897, rs2842003 and rs2805050 of the G protein signal regulator gene (RGS4), responsible for decreasing the inhibition of pain perception, also seem to be present among FMS patients. Likewise, mutations in the dopamine D4 receptor and μ1 opioid receptors have also been associated with the disease (6). Tour et al. (2017) also analyzed the interaction between different genes in the modulation of pain processes between FMS patients and healthy controls, identifying a certain pattern of antagonism between genes involved in the production of serotonin and endogenous opioids (43).

In Brazil, Matsuda et al. (2010) tested the association between two polymorphisms in different genes with FMS, concluding that there is no relationship between the disease and molecular alterations of the serotonin gene (5-HT2A), but there is in relation to the COMT rs4680 polymorphism. The frequency of the genotype corresponding to low enzyme activity (Met/Met) was 37.25% among the individuals in the case group, and only 9.8% among the control group, demonstrating a statistical difference (33).

In a case-control study involving an Asian population, Park et al. (2015) noted that, among the SNPs rs4680, rs6269, rs4633 and rs4818, only the latter was associated with susceptibility to FMS (40).

Vargas-Alarcón et al. (2007) compared six COMT SNPs (rs4680, rs6269, rs4633, rs4818, rs2097903 and rs165599) in a group of Spanish patients, finding an association between SNPs rs4680, rs6269 and rs4818 with FMS. In this same sample, it was also observed that the ACCG haplotype was associated with worse FIQ results (44).

In our research, although data related to pain perception (GPI) show an average value of 11.02 points, with almost 90% of individuals in the pain range considered moderate or high, there was no observed a statistically significant association of this with none of the selected SNPs (Table 5). Likewise, we did not observe an association between the other symptoms present in the diagnosis of FMS (EGS) with any of the SNPs (Table 5), even though the results showed a mean EGS of 8.97 points, with more than 95% of the sample in the range considering moderate or severe symptoms.

Desmeules et al. (2012) compared data from two subgroups of fibromyalgia patients with a control group. One of them was considered capable of interrupting the medications they were using and the other unable to do so. In both groups of patients, the results in the psychological and functional tests were worse compared to the control group, and, among the two groups of patients, the BDI and FIQ had worse
scores in the group that could not withhold treatment. In this same group, the FIQ average obtained was 54.5 points. The FIQ results of our study, in turn, showed a mean equal to 64 points, and more than 85% of the volunteers belonging to the group had low or very low quality of life (31).

Desmeules et al. (2012) also noted that there was a statistically significant association between the COMT SNP rs4680 and the group that could not stop the medication, compared to the group that could (31).

In a case-control study involving Brazilian fibromyalgia patients, a significantly higher frequency of SNPs rs4680 and rs4818 was observed, in addition to worse scores on the FIQ, among those affected by the disease than in healthy controls (45).

Although Fernández-de-las-Peñas et al. (2012) observed no association between the intensity of generalized pain (assessed using a numerical scale) and the rs4680 SNP in FMS patients, warned that there was an association between the homozygous genotype for this polymorphism (Met/Met) and the degree of functional capacity (assessed by the FIQ), probability of depression (assessed by the BDI), as well as anxiety (measured by the HADS - Hospital Anxiety and Depression Scale), compared with heterozygous genotypes (Val/ Met) and wild-type homozygote (Val/Val) (32). However, in our study, there was no statistically significant association between the FIQ parameter and any of the SNPs evaluated, including rs4680, the most widely investigated polymorphism in the disease (Table 5).

Considering the index that assesses the probability of the patient developing depression used in our study, the overall mean of the BDI was 19.57 points, a value higher than that found by Fernández-de-las-Peñas et al. (2012), of 16.8 points (32). Although 42.55% of our sample belong to the category of moderate or severe depression, there was no association between this parameter with any of the SNPs surveyed (Table 5). Also, taking into account the index that assesses the chance of developing anxiety used in our study, we identified a BAI average of 21.29 points, and more than half of the sample in the range that considers moderate or severe anxiety. However, there is also no association between this parameter and any of the analyzed SNPs (Table 5).

As already mentioned, one of the items included in the diagnosis of FMS by the ACR 2012 is sleep disorder. The prevalence of insomnia can reach up to one third of the general population. However, it tends to be different when comparing different groups of people, possibly due to influences of different physiological, physical, social or environmental factors. It can affect between 30% and 48% of the elderly population. For some authors, therefore, advanced age can be considered a risk factor for insomnia. A strong relationship has been observed between chronic pain syndromes and sleep onset or maintenance disorders, as if one were a risk factor for the other and vice versa. More than 40% of patients with insomnia complain of chronic pain and about 90% of patients with FMS report sleep disturbances (46, 47).

The ISI, the index adopted in our study to categorize the level of insomnia among the volunteers, resulted in an average of 13.51 points, and almost 50% of the sample was in the range that considered moderate
or severe insomnia. However, there was no association between this index and any of the selected SNPs (Table 5).

As well as sleep disorders, cognitive changes are also frequent among patients with FMS. The same neurotransmitters that mediate pain transmission are also related to mood, fatigue, sleep and memory. In addition to difficulty concentrating and memory failure, catastrophizing, a frequent symptom among patients with the syndrome, from which the individual views life negatively, demonstrates how their cognition can be affected (48).

Gil-Ugidos et al. (2021), using tests that assess working memory capacity, demonstrated that patients with FMS are deficient in tasks that require short-term memory, divided attention, and information processing ability (49).

In our work, we chose to assess patients’ cognition using the Mini-Mental State Examination. We concluded that only a quarter of the volunteers had an altered result in the exam tests, that is, they did not reach the expected score for their respective level of education. There was no association of this test with any of the chosen SNPs (Table 5).

**Conclusions**

In the present study, although the validated tests used show relevant clinical results among patients, there is no association of these with the selected SNPs. As the main cause for such implications, the small sample size is pointed out, resulting from a data collection carried out during the most critical phase of the COVID-19 pandemic, between March 2020 and October 2021.

Although the physiological mechanisms involved with persistent states of diffuse pain are common understanding, there are still many doubts about the etiology of FMS. Due to the complexity of the topic, it is necessary to expand investigations, using larger samples of volunteers, of multiple ethnicities and ages, aiming to evaluate other single nucleotide polymorphisms, not only in the COMT gene itself. In this regard, it is also essential to assess the interrelationships between these SNPs for increased susceptibility to pain, fatigue, depression, anxiety, insomnia and decognition in the patient.

Still, in addition to the genetic causes possibly involved with the disease, which can only predispose a person to the development or accentuation of the syndrome, environmental, social, economic, nutritional and psycho-emotional factors are also part of the scope of items to be further investigated depth, as they seem to be closely related to it.

Therefore, it is believed that, based on new studies, it will be possible to better elucidate the pathophysiology of FMS, which provides a substrate for thinking and developing new therapeutic approaches, perhaps with new tools that can guarantee a more effective control of the disease symptoms, especially pain.
Abbreviations

CNS – Central nervous system
FMS – Fibromyalgia syndrome
ACR – American College of Rheumatology
GPI – Generalized Pain Index
SSS – Symptom Severity Scale
COMT – Catechol-O-methyltransferase
SNP – Single nucleotide polymorphisms
G – Guanine
Val – Valine
A – Adenine
Met – Methionine
C – Cytosine
T – Thymine
FIQ – Fibromyalgia Impact Questionnaire
BDI – Beck Depression Inventory
BAI – Beck Anxiety Inventory
ISI – Insomnia Severity Index
RT-PCR – real time reverse transcription polymerase chain

Declarations

Ethics approval and consent to participate

This research was approved by the Ethics Committee in Research with Human Beings of the Faculdade de Medicina of the Universidade Federal de Mato Grosso (Brazil), according to document 3.924.907 of March 19, 2020, and all volunteers signed an informed consent form, before entering the study.

Consent for publication
Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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This research did not receive funding from any funding agency, only resources from the university where it was carried out.

Authors' contributions

JACJ responsible for the research design, carried out the interviews, collection of clinical data and biological material, statistical analysis, interpretation of results and writing of the article.

JAMS performed DNA extraction and RT-PCR of the samples.

GLAB performed DNA extraction and RT-PCR of the samples.

LRCAP provided patients for the research and reviewed the article.

FRS contributed to the research design.

BBG contributed to the research design and reviewed the article.

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References


