Association of Polymorphism of the Enzyme Catechol-O-Methyltransferase with Fibromialgic Syndrome and its Clinical Repercussions

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Abstract

Objective: *Fibromyalgia syndrome (FMS) is a clinical condition that mostly affects women, with chronic diffuse pain, physical disability, mood swings, anxiety, fatigue and insomnia. There are genetic contributions to its pathophysiology. Some single nucleotide polymorphisms (SNPs) can change the function of proteins that participate in pain modulation. Catechol-O-methyltransferase (COMT) is an enzyme responsible for the inactivation of catecholamines in the central nervous system, participating in descending nociceptive inhibitory pathways. This study verified the association of SNPs rs4680, rs6269, rs4633 and rs4818 of the COMT gene with clinical aspects in patients with FMS in Brazil. Methods: Forty-seven volunteers whith 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. The DNA was extracted by salting out and the SPNs were evaluated by real time reverse transcription polymerase chain (RT-PCR). The association between clinic and SPNs 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.*

Keywords: Fibromyalgia, Chronic Pain, Quality of Life, Catechol-O-Methyltransferase, Genetic Polymorphisms

1. 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].

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 [2,3].

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 [4,5].

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. Due to the difficulty in objectively evaluating tender points, the American College of Rheumatology (ACR) established new diagnostic criteria in 2010. 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 [2].

2. 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 [6].

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 [7].

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 [8-10].

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 [11].

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 [8-10,11].

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 [12].

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 [13,14].

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. 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 [13].

COMT genetic aspect According to the central dogma of molecular biology 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 [13].

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 [15,16].

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. 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 [13,14,17].

3. Methods
3.1 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.

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 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.

4. 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).

The levels of psychiatric impairment, regarding depression and anxiety, were measured using the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI), respectively.

Sleep quality was assessed using the Insomnia Severity Index (ISI).

To analyze the level of cognitive impairment, the Mini-Mental State Examination was used.
All Questionnaires were Applied by the Same Evaluator

5. DNA Extraction and Genotyping
Blood samples were collected via venipuncture in the forearm. The DNA of the samples was extracted by the salting out method. 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 Biosystms (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.

6. 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%.

7. Results
7.1 Socioeconomic Aspects
Of the 47 volunteers participating in the study, 95.74% were female, 48.94% declared themselves mixed race and 31.91% white. The general average age among all participants is 51.25 years, with an average of 51.44 years among women. 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).

Sex	Female	95.74%
	Male	4.26%
Skin color	Mixed race	48.94%
	White	31.91%
	Black	17.02%
	Indian	2.13%
Age (mean)	General	51.25
	Female only	51.44
Marital status	Married	68.09%
	Single	19.15%
	Widower	8.51%
	Divorced	4.26%
Education level	University education	27.66%

		Until high school	72.34%
Work		Yes	68.09%
		Not	31.91%
Health insurance		Yes	19.15%
		Not	80.85%
Practice physical exercises		Yes	10.24%
		Not	89.36%
Overweight/obesity		Yes	78.72%
		Not	21.28%

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

8. 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).

GPI	11.02 (mean)	Moderate/high	89.39%
		Low	10.64%
SSS	8.97 (mean)	Moderate/high	95.74%
		Low	4.26%
GPI - Generalized Pain Index. SSS - Symptom Severity Scale			

Table 2: Means and classification of GPI and SSS in patients with FMS in Cuiabá, Brazil (2021)

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).

FIQ	64.14 (mean)	Low/very low quality of life	85.10%
		Moderate quality of life	14.90%
BDI	19.57 (mean)	Moderate/severe	42.55%
		Minimal/mild	57.45%
BAI	21.29 (mean)	Moderate/severe	53.19%
		Minimal/mild	46.81%
ISI	13.51 (mean)	Moderate/severe	48.94%
		No insomnia/mild	51.06
Mini-Mental	Normal results	74.47%	
	Altered results	25.53%	
FIQ - Fibromyalgia Impact Questionnaire. BDI - Beck Depression Inventory. BAI - Beck Anxiety Inventory. ISI - Insomnia Severity Index. Mini-Mental - Mini-Mental State Examination			

Table 3: Means and classification of FIQ, BDI, BAI, ISI and Mini-Mental in patients with FMS in Cuiabá, Brazil (2021)

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.

9. 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).

rs4680	AA	14.89%
	AG	34.04%
	GG	51.06%
rs6269	GG	23.40%
	AG	42.55%
	AA	34.04%
rs4633	TT	14.89%
	CT	36.17%
	CC	48.94%
rs4818	GG	19.14%
	CG	40.43%
	CC	40.43%
A – adenine. G – guanine. C – cytosine. T- thymine.		

Table 4: Frequency of patients with FMS and homozygotes and heterozygotes for SNPs rs4680, rs6269, rs4633 and rs4818, in Cuiabá, Brazil (2021)

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).

	rs4680	rs6269	rs4633	rs4818
GPI	0.666	0.648	1.000	1.000
SSS	1.000	1.000	1.000	1.000
FIQ	1.000	0.112	0.700	0.417
BDI	0.770	1.000	0.770	0.764
BAI	1.000	0.538	1.000	0.766
ISI	1.000	1.000	1.000	1.000
Mini-Mental	0.739	0.289	0.517	0.147
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				

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)

10. Discussion

Qureshi et al. (2021) indicated other candidate genes for greater predisposition to pain and othersymptoms 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 SPNs 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 [4]. 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 [18].

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 [19].

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 [20].

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 we observed a statistically significant association of this with none of the selected SNPs. Likewise, we did not observe an association between the other symptoms present in the diagnosis of FMS (EGS) with any of the SPNs, 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 (Tables 3 and 5).

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 [21]. 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 (Table 3).

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 [21].

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 [22].

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) [23]. 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 [23]. 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 (Tables 3 and 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 (Tables 3 and 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 [24,25].

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 (Tables 3 and 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 [26].

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 [27].

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 (Tables 3 and 5).

Although the validated tests used show relevant clinical results among patients, there is no association between these and the selected SNPs. Unfortunately, the main limitation of the study concerns the small sample size, resulting from data collection carried out during the most critical phase of the COVID-19 pandemic, between March 2020 and October 2021. New studies with larger samples may better elucidate the associations.

11. Declarations
11.1 Ethics Approval and Consent to Participate
This research was approved by the Ethics Committee in Research with Human Beings of the Faculty of Medicine of the Universidade Federal de Mato Grosso (Brazil), according to document 3,924,907 of March 19, 2020. All methods used were performed in accordance with the guidelines and relevant regulations (Declaration of Helsinki). All volunteers signed the Free and Informed Consent Form before entering the study.

12. Conclusions
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.

Competing Interests
The authors declare that they have no competing interests.

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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