# Evaluation of ApaI and FokI polymorphism of VDR gene and functional characterization in patients with fibromyalgia

*Avaliação do polimorfismo ApaI e FokI do gene VDR e caracterização funcional em pacientes com fibromialgia*

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## Abstract

**Introduction:** Fibromyalgia (FM) is a syndrome of unknown origin characterized by several symptoms, and although its pathogenesis has not been completely elucidated, it seems to be related to inflammatory pathways and neurochemical changes in the brain. **Objective:** To evaluate the association between BsmI, ApaI and FokI polymorphisms of the vitamin D receptor (VDR) gene, their polymorphisms, and clinical variables in women with and without FM. **Methods:** This is a case-control study composed of a group of 53 women with FM and another with 40 women without the disease. The McGill Pain Questionnaire, Fibromyalgia Impact Questionnaire, Pain Visual Analogue Scale and the sit-up test were applied. Real-time PCR was performed to analyze the ApaI and FokI polymorphism. **Results:** There was a statistical association between race, comorbidity and FM, where 78.4% of the individuals were white and had FM ($p < 0.002$) and 96.1% had some comorbidity ($p < 0.001$). Seventy-six point five percent (76.5%) of patients with FM underperformed in the sit-up test ($p < 0.001$). There was also an association between the genotypic and allele frequencies of the VDR and FM gene ApaI and FokI polymorphisms ($p < 0.001$). In the VDR gene ApaI polymorphism, the CC genotype exhibited a higher frequency in women with FM, the C allele for the ApaI polymorphism was 3.33 times more likely, and the FokI polymorphism was 10.9 times more likely to develop FM ($p < 0,0001$). **Conclusion:** Women with C allele for ApaI polymorphism are 3.33 times more likely to have FM (95%CI = 1.58-7.02; $p = 0.0024$), and in FokI polymorphism, the prevalence of T allele is 10.9 times greater (95% CI = 4.76-25.38; $p < 0.0001$). No significant associations were found in relation to BsmI polymorphism and frequency alleles ($p = 0.062$ and $p = 0.078$, respectively).

**Keywords:** Fibromyalgia. Pain. Polymorphism. Vitamin D receptor.

### *Resumo*

***Introdução:*** *A fibromialgia (FM) é uma síndrome de origem desconhecida caracterizada por diversos sintomas, e embora sua patogênese não tenha sido completamente elucidada, parece estar relacionada às vias inflamatórias e alterações neuroquímicas no cérebro.* ***Objetivo:*** *Avaliar a associação entre os polimorfismos BsmI, ApaI e FokI do gene do receptor da vitamina D (VDR), seus polimorfismos e variáveis clínicas em mulheres com e sem FM.* ***Métodos:*** *Trata-se de um estudo caso-controle composto por um grupo de 53 mulheres com FM e outro com 40 mulheres sem a doença. Foram aplicados o Questionário de Dor de McGill, Questionário de Impacto da Fibromialgia, Escala Visual Analógica da Dor e o teste de sentar. A PCR em tempo real foi realizada para analisar o polimorfismo ApaI e FokI.* ***Resultados:*** *Houve associação estatística entre raça, comorbidade e FM, onde 78,4% dos indivíduos eram brancos e apresentavam FM (p < 0,002) e 96,1% tinham alguma comorbidade (p < 0,001). Setenta e seis vírgula cinco por cento (76,5%) dos pacientes com FM tiveram desempenho inferior no teste de abdominais (p < 0,001). Também houve associação entre as frequências genotípicas e alélicas dos polimorfismos ApaI e FokI do gene VDR e FM (p < 0,001). No polimorfismo ApaI do gene VDR, o genótipo CC apresentou maior frequência em mulheres com FM, o alelo C para o polimorfismo ApaI foi 3,33 vezes mais provável, e o polimorfismo FokI teve 10,9 vezes mais chance de desenvolver FM (p < 0,0001).* ***Conclusão:*** *Mulheres com alelo C para polimorfismo ApaI têm 3,33 vezes mais chance de ter FM (IC 95% = 1,58-7,02; p = 0,0024), e no polimorfismo FokI, a prevalência do alelo T é 10,9 vezes maior (IC 95% = 4,76-25,38; p < 0,0001). Não foram encontradas associações significativas em relação ao polimorfismo BsmI e alelos de frequência (p = 0,062 e p = 0,078, respectivamente).*


***Palavras-chave:*** *Fibromialgia. Dor. Polimorfismo. Receptor de vitamina D.*

## Introduction

Fibromyalgia (FM) is a syndrome of unknown origin characterized by several symptoms such as diffuse and chronic pain thorough the body, muscle sensitivity accompanied by intestinal disorder, fatigue, anxiety, depression, sleep disorders and functional disability.[1]

The therapeutic intervention of FM involves an individualized management, with a multiprofessional team that offers treatments with and without drugs. Among the non-pharmacological treatments, physiotherapy stands out for presenting several evidence of the benefits achieved in the patient's well-being and in the prevention of other disorders arising from the pathology in question. After all, with the physiotherapeutic approach it is possible to achieve the promotion of functional gains, the repair of sleep, analgesia, reduction of fatigue, among other results that together culminate in the improvement in the quality of life of the affected individuals.[2-4]

Although its pathogenesis is not very elucidated yet, FM seems to be related to inflammatory pathways and neurochemical alterations in the brain, amplifying the painful sensation with increased and decreased signaling in descending neural pathways. In addition, FM bearing patients present a reduced threshold of other sensory stimuli such as heat, cold, auditory, and electrical stimuli.[1]

Although the clinical results available on the interface between chronic pain and D hypovitaminosis remain limited, a relative scarcity of experimental and pathophysiological evidence demonstrate that vitamin D affects the pain manifestation, playing a role in the etiology and maintenance of chronic pain states and associated comorbidity. Pain pathways associated with cortical, immunological, hormonal, and neuronal changes in chronic pain are also potentially influenced by vitamin D levels.[5]

Recently, the Vitamin D receptor (VDR) location was enlarged and it was discovered that it is present in several regions of the brain where there is an effect on cellular proliferation, differentiation, neurotransmission and various roles in neuroplasticity, also having neurotrophic and neuroprotective effects,[6] secreting serotonin and dopamine, resulting in central amplification of the peripheral pain signs.[7]

Still, the single nucleotide polymorphism is the most common type of variation in the human genome and, although it often does not have a direct relationship between the manifestation of the disease and the SNPs, research has identified several polymorphisms involving the molecular bases of genetic diseases.[8] This condition can directly influence the rehabilitation of the patient, as it allows the choice of the ideal therapy aiming at aspects that involve prevention, promotion or recovery of the patient's global health condition.[2,9]

A study carried out by Marasli et al.[10] analyzed the VDR gene polymorphism in FM and found no significant differences in the frequency distribution of the genotypes FF, Ff and ff (p = 0.056) and F and f alleles (p = 0.932) of the FokI polymorphism in the VDR gene.

It is important to note that there are no reports in the literature of this investigation in the Brazilian population so far. Considering the lack of clear evidence between the interaction of polymorphisms in the VDR gene and its polymorphisms and the development of FM, this study aims to evaluate the association among BsmI, ApaI and FokI polymorphisms of the VDR gene as well as their polymorphisms and clinical variables in women with and without FM.

## Methods

This is an observational case-control study approved by the Ethics Committee n. 3.057.780, with a convenience sample of 53 women with a clinical diagnosis of FM by neurosurgery, assisted by the project "Multidisciplinary Care for Patients with Fibromyalgia" at the Alto da Colina outpatient clinic, in the city of Londrina, in Bazil. The power calculation was performed between groups 1 (48) and 2 (100) based on the odds ratio (24.39), proportions of p2 (0.5) and error parameters (0.05), obtaining the result of 99%.

Inclusion criteria of FM group were female patients, older than 18 years, with a clinical diagnosis of FM according to the 2011 guidelines of the American College of Rheumatology (ACR).[11] The control group consisted of women without fibromyalgia, without any sign of chronic and diffuse muscular pain, and with equivalent age and BMI. Exclusion criteria were considered for both groups: presence of any type of joint inflammatory disease or degenerative joint disease.

A structured and standardized questionnaire was used, consisting of sociodemographic data (gender and age), medications in use and health perception in general, regarding the presence of comorbidities.

For the muscle strength evaluation in the lower limbs, the sitting-rising test was performed, consisting of sitting and rising from a chair with a back height of approximately 45 cm, with the arms crossed over the chest for 30 seconds. Familiarization was performed, and the number of repetitions was recorded. The classification was performed by calculating the 90% percentile, where they were divided into < 9 as low performance, 10 - 12 as normal performance and > 13 as high performance.[12]

Pain Visual Analogue Scale (VAS) was applied, which subjectively measures the patient's pain in a score from 0 to 10, where 0 is total absence of pain and 10 is a very intense pain, almost unbearable.[13]

The McGill Pain Questionnaire (MPQ) and the Fibromyalgia Impact Questionnaire (FIQ) were applied to the participants in the FM group. MPQ is a multi-dimensional instrument to evaluate pain intensity and multiple dimensions of pain experience, representing the four indexes of sensory, affective, evaluative and diverse pain classification.[14] The FIQ is used to evaluate functional status, the disease progression and results, including physical function, occupation, depression, anxiety, sleep, pain, stiffness, fatigue and well-being, evaluating the FM impact on patients in the previous seven days.[15]

For DNA analysis, peripheral blood leukocytes were collected through venipuncture in tubes containing EDTA (0.6%), and DNA extraction was performed using the QIA amp DNA Blood Mini Kit (Qiagen, Germany), following the guidelines provided by the manufacturer. The extracted DNAs were stored in a freezer -80 °C until polymorphism analyzes were performed. DNA quality and quantity were measured by the absorbance analysis in a spectrophotometer (Thermo Scientific NanoDrop 2000) at 260 nm and 280 nm. Then, DNA dilution was performed in ultra-pure water to obtain the final concentration of 30 ng/UL.

In order to analyze the serotonin receptor of a single nucleotide polymorphism (SNP), the DNA fragments amplification technique by polymerase chain reaction was used in real time with the TaqMan® system (Applied Biosystems, Foster City,USA). The reaction consisted of a final volume of 10 µL, namely: 5.25 µl of Taqman® Genotyping Master Mix (1x), 0.5 µL of probe (1x) (Applied Biosystems, Foster City, EUA), 3.25 µL of ultrapure water Milli-Q® and 1µL of ADN (30 ng/uL). The Rotor-Gene Q® real-time thermal cycler (Qiagen, Germany) was used with a cycle of 60 °C for 30 seconds (initial denaturation), 95 °C for 10 minutes for initial denaturation, 50 cycles of 95 °C for 15 seconds (denaturation) and 60 °C for 1 minute and 30 seconds (primers matching and extension), and a final cycle of 30 seconds at 60 °C. For SNP allele discrimination, the Software Gene Rotor Q-Pure Detection version2.0.3 (Qiagen, Germany) was used.

### Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS 21) (Inc., Chicago) statistical program, and a 95% confidence interval and a 5% significance level ($p < 0.05$) were established for all the applied tests.

The Shapiro-Wilk normality test was used to evaluate the data distribution and, considering that the data do not present normal distribution, non-parametric tests were used. The Chi-square test ($X^2$) was used to test the association between FM and genotypic and allele frequencies. Odds ratio was used to verify possible interaction between FM and the genotypic and allele frequencies of the VDR gene.

## Results

No statistically significant differences were found in relation to body mass index between the control and FM groups ($p > 0.368$). However, a statistically significant association may be observed among race, comorbidity and FM, where 78.4% of the individuals analyzed were white and presented FM ($p < 0.002$), and 96.1% presented some comorbidity ($p < 0.001$). The sitting-rising test showed that 76.5% of the patients with FM presented low performance that may indicate lower limb weakness (MMII) ($p < 0.001$) (Table1).

In the genotype analysis, an association between genotypic and allele frequencies of ApaI and FokI polymorphisms of the VDR gene and FM ($p < 0.001$) can be observed, which was not evidenced for the BsmI polymorphism ($p = 0.062$). A larger portion (28.6%) of women with FM presented the CC genotype for the ApaI polymorphism of the VDR gene compared to women without FM (6.1%) ($p < 0.001$). Most patients (57.7%) with the TT genotype for FokI polymorphism presented FM ($p < 0.001$) (Table1).

Regarding the allele frequency, it was observed that women that have the C allele for ApaI polymorphism exhibit 3.33 times more chances of having FM compared to women without the disease (CI95% = 1.58-7.02; $p = 0.0024$). Whereas for FokI polymorphism, T-allele bearing women have 10.9 times more chances of developing FM when compared to women without FM (CI95% = 4.76-5,38; $p < 0.0001$).

**Table 1** - Distribution of the variables race, body mass index (BMI), comorbidities, sitting-rising test, genotypic and allele frequencies of ApaI, BsmI and FokI of the VDR gene in women with (FM Yes) and without fibromyalgia (FM No)

| Variables | FM Yes n (%) | FM No n (%) | p-value |
|---|---|---|---|
| **Race** | | | |
| White | 40 (78.4) | 53 (53.5) | 0.002* |
| Non-White | 11 (21.6) | 46 (46.5) | |
| **BMI** | | | |
| Low Weight | 0 (0.0) | 1 (1.0) | |
| Eutrophic | 16 (32.7) | 24 (24.2) | 0.368 |
| Overweight | 20 (40.8) | 35 (35.4) | |
| Obesity | 13 (26.5) | 39 (39.4) | |
| **Comorbidities** | | | |
| No | 2 (3.9) | 30 (30.3) | 0.001* |
| Yes | 49 (96.1) | 69 (69.7) | |
| **Sitting/rising performance** | | | |
| Low | 39 (76.5) | 13 (26.5) | |
| Normal | 13 (26.5) | 13 (26.5) | 0.001* |
| High | 13 (26.5) | 13 (26.5) | |
| **APAI genotype** | | | |
| CC | 8 (28.6) | 2 (6.1) | 0.001* |
| AA | 1 (3.6) | 13 (39.4) | |
| AC | 19 (67.9) | 18 (54.5) | |
| **FOKI genotype** | | | |
| CC | 0 (0.0) | 17 (50.0) | |
| TT | 15 (57.7) | 3 (8.8) | 0.001* |
| CT | 11 (42.3) | 14 (41.2) | |
| **BSMI genotype** | | | |
| CC | 1 (6.7) | 12 (26.5) | |
| TT | 2 (13.3) | 1 (6.1) | 0.062 |
| CT | 12 (80.0) | 33 (67.3) | |
| **BSMI alleles** | | | |
| C | 14 (46.7) | 45 (66.2) | 0.078 |
| A | 16 (53.3) | 23 (33.8) | |
| **APAI alleles** | | | |
| C | 35 (62.5) | 22 (33.3) | 0.002* |
| A | 21 (37.5) | 44 (67.7) | |
| **FOKI alleles** | | | |
| C | 11 (21.1) | 59 (74.7) | 0.001* |
| T | 41 (78.9) | 20 (25.3) | |

Note: *Statistically significant.

## Discussion

In this study, the relation between FM and the frequency of ApaI, FokI and BsmI polymorphism of the VDR gene was analyzed. It was verified that there were differences in ApaI and FokI polymorphism of the VDR gene, not showing differences in the BsmI in female patients with FM. However, Marasli et al.[10] carried out a study analyzing the VDR gene polymorphism in individuals with FM and did not find differences in the FokI polymorphism of the VDR gene in 100 female patients with FM and 100 healthy women of the control group, all with similar serum vitamin D levels. According Marasli, significant differences were also not found in the frequency distribution of both genotypes (FF, Ff and ff) and alleles (F and f) of the FokI polymorphism in the VDR gene ($p > 0.05$) between groups of patients with FM and control group in the turquoise population.

In the present study no significant differences were observed in the distribution of the genotypes and alleles frequencies of the FokI polymorphism in the VDR gene between the two groups, suggesting genetic heterogeneity of VDR.

Genetic polymorphisms in the serotonin, dopaminergic and catecholaminergic systems play a role in the FM etiology.[16] Studies have shown in their results that the abnormal mechanisms of peripheral or central pain, or both, together with genetic factors, play an important role in generalized chronic pain. Thus, genetically predisposed individuals can trigger the development of FM due to the role of neuroendocrine and autonomous dysfunctions in its pathogenesis and environmental factors contributors.[17]

Vitamin D is a fundamental liposoluble vitamin in skeletal homeostasis and calcium, including cell growth modulation, neuromuscular actions and possible anti-inflammatory properties derived mainly from skin synthesis by ultraviolet radiation (UVB), recognized for manifesting an infinity of extra-skeletal actions, in addition to its role.[18]

Its modulating effects act on gene expression after binding to VDR. There seems to be potential genetic polymorphisms in specific genes that can influence bioavailability, transfer and distribution in lipid reservoirs, metabolism and vitamin D action.[19] Nagpal et al.[20] showed that vitamin D has functions such as cell proliferation regulation, differentiation, apoptosis and angiogenesis.

Shipton and Shipton[21] showed that the VDR gene is present in neuronal and glial cells, along with the protein complex VDR and 1-α-hydroxylase, which foster the development of central sensitization.

VDR and 1-α-hydroxylase proteins are located in several areas of the brain, such as prefrontal cortex, raphe, gelatinous substance, cerebellum, hippocampus, cingulate cortex, substantia nigra and thalamus.[22] Evidences indicate that hypothalamic dysfunction occurs in FM, involving the hypothalamic-pituitary-adrenal axis in particular, causing responses to stressors, increasing neurotrophins levels, suggesting, therefore, that this disorder is of central origin.[23]

It is also suggested that the binding protein of VDR, 1-α-hydroxylase and vitamin D found in the hypothalamus may be associated with a physio pathological role in primary headache, which is recurrent in cases of vitamin D deficiency and migraine. It is also suggested that VDR, due to its presence in neuronal and glial cells, may also play a role in the progression of chronic painful diseases caused by central hypersensitivity, such as FM.[24]

Other authors have been associating the VDR gene in postmenopausal women with type I diabetes and several autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, grave diseases, systemic lupus and thyroid, and even with prostate cancer,[25,26] however, there was no association among the studies.

According to La Marra et al.[27] the low VDR gene expression in carriers of the AATT combination genotype is a discovery that deserves to be studied more deeply, so that the VDR expression possibly implies supplementation of vitamin D of melanoma. The study was carried out at University de Udine (Italy) and evaluated 74 patients (39 men and 35 women, 29-82 years) who performed a surgical excision of primary cutaneous melanoma. When comparing the group with and without the VDR gene expression, the combined genotype ApaI-TaqI AATT ($p = 0.025$) showed a higher frequency in the group with melanoma without VDR expression when compared to other patients.

Whereas Ruzzon[28] identified that elderly people bearing bAT haplotype (BsmI, ApaI and TaqI) were twice as likely to develop the disease and BaT haplotype bearers were less likely to develop osteoporosis ($p = 0.006$), compared to the BAt reference haplotype. However, no statistically significant association was found between osteoporosis and BsmI, ApaI, TaqI and FokI polymorphisms of the VDR gene.

A study carried out by Rasoul et al.[29] demonstrated a significant effect of two VDR gene polymorphisms (FokI and TaqI) in the homozygous genotype ff ($p < 0.0001$) and the allele f ($p < 0.0001$) among patients with type 1 diabetes mellitus and controls in co-dominant and dominant models, respectively, in the Arab population of Kuwait.

Some evidence suggests that the genotypes FF, BB, tt, and the combination genotype BBAAtt may be associated with increased VDR expression, which in turn regulates vitamin D;[30] however, further studies are necessary to confirm these findings.

Walitt et al.[31] found an association between FM and Caucasian ethnicity corroborating our findings, where 78.4% of the individuals analyzed with FM were white and 96.1% of them had some comorbidity. Cabo-Meseguer et al.[32] carried out a study of epidemiological characteristics in patients with FM and pointed out that women between 46 and 65 years are the most affected by the syndrome, and that more than half have some associated psychological disorder and diagnosis of other comorbidities such as chronic fatigue.

Corroborating with this study, Cardoso et al.,[33] who demonstrated that women with FM had reduced functional capacity, severe pain and worsening of the general health status ($p < 0.05$), the present study found an association between FM and the sitting-rising test, showing that women with FM have lower muscle strength in lower limbs than those in the control group ($p = 0.001$).

When analyzing functional capacity, muscle strength and the risk of falls in women with FM through functional tests, Góes et al.[34] showed that FM patients present deficits in muscle strength in lower limbs, balance and agility, showing a high prevalence of falls in this population, explained by the symptoms and pain caused by the disease.

Studies indicate that the decrease in muscle strength in patients with fibromyalgia is directly related to muscle fatigue and chronic pain.[35-37] Therefore, we think that physical activity programs can help patients with FM by improving their physical conditioning through endogenous analgesic pathways, improving the feeling of well-being and quality of life.[38]

The limitation of this study was the evaluation of only ApaI, FokI and BsmI polymorphisms in the VDR gene. Due to the high cost, other polymorphisms could not be investigated. Investigating ApaI, FokI and BsmI polymorphisms in a larger group in the future will be useful in determining the relation between FM and VDR gene polymorphism.

We believe that future studies with other polymorphisms and a greater number of individuals are necessary, therefore more accurate results can be obtained in relation to VDR gene polymorphisms and FM.

## Conclusion

The association between ApaI and FokI polymorphisms of the VDR gene in Brazilian women with FM could be determined in our study, considering that women presenting C allele for ApaI polymorphism exhibit 3.33 more chances to have FM when compared to women without the disease (CI95% = 1.58-7.02; $p = 0.0024$). Whereas for FokI polymorphism, T-allele bearing women have 10.9 times more chances of developing FM when compared to women without FM (CI95% = 4.76-25,38; $p < 0.0001$). No significant associations were found in relation to BsmI polymorphism and frequency alleles ($p = 0.062$ and $p = 0.078$, respectively).

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## Authors' contributions

Each author contributed individually and significantly to the development of the manuscript. SKFSS began to approach the research problem and participated in all stages: project construction, bibliographic research, data collection, data analysis, discussion of results, conclusion and writing of the manuscript. KBPF and CAMZ participated in all stages, from project construction, data collection, data analysis, discussion of results, writing and critical review of the manuscript. AVS and PDOP participated in data collection and analysis. RCPF participated in guiding

all steps, from project construction, data collection, data analysis, discussion of results, manuscript writing and final review. All authors contributed strongly to the study, so all authors were responsible for all aspects of the work and have approved the final version of the article.

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