# Capítulo 86

## NFkB1 gene polymorphism increases the risk for occurrence of fatigue and sleep disorder in patients with sporadic PD

## O polimorfismo do gene NFkB1 aumenta o risco de ocorrência de fadiga e distúrbio do sono em pacientes com DP esporádica

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**ABSTRACT**

Objectives The objective of this study was to investigate a possible relationship between genetic variants in the *TNFα and NFKB1* genes with the occurrence of fatigue and sleep disorder in patients with Parkinson's disease (PD).
Methods A total of 180 Brazilian patients from the Pro-Parkinson service at the Clinical Hospital of the Federal University of Pernambuco, diagnosed with sporadic PD were enrolled. The sequence variants for *TNFα and NFKB1* were determined through the PCR-RFLP technique. Logistic regression was performed to assess the association of polymorphisms with the occurrence of fatigue and sleep disorder in PD patients.
Key findings Multivariate analysis revealed increased risk for the occurrence of fatigue in individuals carrying *NFKB* D/W genotype (Prevalence Ratio: PR=2.091; 95%CI, 1.155-3.784; p=0.015) and D/D genotype (PR= 2.237; 95%CI, 1.078-4.643; p=0.031). In addition, it was observed an increased risk to the occurrence of fatigue in PD patients with sleep disorder (PR= 5.226; 95%CI, 2.934-9.307; p= 0.000). Moreover, the therapy with pramipexole was significantly associated with the development of sleep disorder (p = 0.020).
Conclusion *NFKB1* variants and use of the pramipexole may have a significant influence on increased risk for the occurrence of fatigue and sleep disorder, respectively in a Brazilian PD population.

**Keywords:** Parkinson's disease, Inflamation, Fatigue, Sleep disorder, Polymorphism.

**RESUMO**

Objetivos O objetivo deste estudo foi investigar uma possível relação entre as variantes genéticas dos genes TNFα e NFKB1 com a ocorrência de fadiga e distúrbio do sono em pacientes com doença de Parkinson (DP).

Métodos Foram inscritos 180 pacientes brasileiros do serviço Pró-Parkinson do Hospital Clínico da Universidade Federal de Pernambuco, diagnosticados com DP esporádica. As variantes da seqüência para TNFα e NFKB1 foram determinadas através da técnica PCR-RFLP. A regressão logística foi realizada para avaliar a associação de polimorfismos com a ocorrência de fadiga e distúrbio do sono em pacientes com DP.
Principais conclusões A análise multivariada revelou um risco maior para a ocorrência de fadiga em indivíduos portadores do genótipo NFKB D/W (Razão de prevalência: PR=2,091; 95%CI, 1,155-3,784; p=0,015) e genótipo D/D (PR= 2,237; 95%CI, 1,078-4,643; p=0,031). Além disso, foi observado um risco maior de ocorrência de fadiga em pacientes com distúrbio do sono (PR= 5.226; IC95%, 2.934-9.307; p= 0.000). Além disso, a terapia com pramipexole foi significativamente associada ao desenvolvimento do distúrbio do sono (p = 0,020).
Conclusão As variantes do NFKB1 e o uso do pramipexole podem ter uma influência significativa no aumento do risco de ocorrência de fadiga e distúrbio do sono, respectivamente em uma população de DP brasileira.

**Palavras-chave:** Doença de Parkinson, Inflamação, Fadiga, Transtorno do sono, Polimorfismo.

## 1 INTRODUCTION

Parkinson's disease (PD) is considered the second neurodegenerative disease most common in the elderly population over 60 years old [1]. It is characterized by the loss progressive of dopaminergic neurons in the substantia nigra region of the midbrain [2]. Approximately 1% of the worldwide population and 3.3% of the Brazilian population is affected by this disorder [1]. The etiology of PD is still complex, involving immunologic, genetic and environmental factors [3]. The inflammation process may be considered a hallmark of PD since that were reported levels increased of inflammatory molecules in the brain and cerebrospinal fluid of PD patients [4]. Furthermore, Ghosh et al. showed that NF-kB1, activation a transcription regulator of the κ immunoglobulin light chain (IgK), is induced in the CNS of patients with PD[5].

The proinflammatory cytokines released by activated microglia, including tumor necrosis factor α (TNF-α), interleukins IL-6 and IL-1β, are over-regulated in the brain and cerebrospinal fluid of patients with PD. TNF-α, in particular, can activate neuroinflammation and the molecular mechanisms that lead to neurotoxicity and neuronal death both in vitro and in animal models of PD [6]. In animal models, TNF-α and other proinflammatory cytokines can induce the loss of dopaminergic neurons [7]. Post-mortem studies in patients with PD identified immunoreactive glial cells to TNF-α in the substantia nigra, high levels of TNF-α in nigrostriatal dopaminergic regions and cerebrospinal fluid, and still indicated release of TNF-α from activated glial cells in the substantia nigra [8].

Fatigue and sleep disorder are non-motor symptoms of PD and may cause a great impact on the quality of life of these patients [9]. Some studies on neural-immunological signaling have shown that pro-inflammatory molecules can signal the Central Nervous System (CNS), triggering fatigue and other behavioral changes. These findings raise the hypothesis that fatigue and sleep disorder would be related to the inflammatory process [10,11].

In recent years, genetic mechanisms such as changes in DNA base sequences have begun to be revealed as potential factors in the pathogenesis of PD [12]. Such alterations, known as genetic polymorphisms, play an important role in the regulation of gene expression and in the silencing of repetitive elements of the genome. Thus, genetic variations such as the occurrence of polymorphisms in the NF-kB1 and TNF-α genes may be able to silence, increase or reduce gene expression, contributing to the pathogenesis and progression of neurodegenerative diseases [13,14].

This way, the purpose of this study was to verify a possible association between variants in the TNF-α and NF-kB1 genes with the occurrence of fatigue and sleep disorder in the Parkinson's disease (PD) patients.

## 2 SUBJECTS AND METHODS

### Study Population

A total of 180 PD patients from northeastern Brazil were enrolled in this study. The patients were attended at the PRO-PARKINSON service of the Clinical Hospital of the Federal University of Pernambuco in the period from January 2016 to December 2017. The diagnosis of idiopathic PD was based on the United Kingdom Parkinson's Disease Society Brain Bank criteria [15] and reviewed by an experienced neurologist.

Relevant clinical and demographic features of the study population, such as, sex, age of onset, duration of disease, therapy used, severity of the disease (based on their Hoehn-Yahr score), use of tobacco, alcohol and contact with pesticides, were obtained through medical records and in a face-to-face interview with the patients and their family members. This study was previously evaluated and approved by the ethics committee of the Health Ministry of Brazil (CAAE: 45614415.0.0000.5208) and patients were enrolled after having read and signed an informed consent form.

The occurrence of fatigue was detected when rates were reported for different cut-off points (e.g., ≥ 3.3 and ≥8 points on the Parkinson Fatigue Scale [PFS])16 or was reported according to several recommended measurement scales (e.g., PFS and Fatigue Severity Scale [FSS]) [17]. The sleep disorder score was performed as described by Ray Chaudhuri K et al. [18]. It is a visual scale that addresses 15 symptoms associated with sleep disorder. The maximum cumulative score is 150 (the patient is free of all symptoms).

### DNA extraction and genotyping

Genomic DNA was extracted from 3 mL of venous peripheral blood of PD individuals using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin) and stored in a -20°C freezer. This procedure has been performed in the "Laboratório de Genética Bioquímica e Sequenciamento de DNA Profa. Tânia Falcão (GENOMA)"-Universidade Federal Rural de Pernambuco (UFRPE).

NFKB1 (rs28362491) and TNF-α (rs1800629) variants were genotyped using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. A fragment of 289 pb from NFKB1

rs28362491 SNP was amplified using the following set of primers: sense 5'-TTTAATCTGTGAAGAGATGTGAATG-3' and antisense 5' CTCTGGCTTC-CTAGCAGGG and digested by the restriction enzyme Van91I, which yields three DNA fragments of 206, 48 and 35 bp for NFKB W allele, two DNA fragments of 254, 35 bp for NFKB D allele [19]. For TNF-α an amplicon of 107 bp was amplified using the following primers: sense 5'-AGGCAATAGGTTTTGAGGGCCAT-3' and antisense 5'- TCCTCCCTGCTGC-GATTCCG-3', that was then digested by restriction enzyme NcoI (Fermentas). The GG genotype resulted in two fragments of 87 and 20 bp, while the AA genotype produced one fragment of 107 bp. The GA genotype showed 3 fragments: 107, 87 and 20pb [20]. The digestion products were separated on a 3% agarose gel using the red gel dye and subsequently analyzed on the transluminator under UV light.

## 3 STATISTICAL ANALYSES

The genotype distribution and allele frequencies of the polymorphisms were obtained by direct counting. The Hardy-Weinberg equilibrium (HWE) test was applied to datasets using the Bioestat 5.0 program. Univariate and multivariate statistical analyses were performed using the R software, version 3.0.2 (http://www.R-project.org/). The association for categorical variables was verified with the chi-squared test. The Student's t-test was performed for continuous data with normal distribution and the Wilcoxon–Mann–Whitney test was performed for data without a normal distribution. The Kolmogorov–Smirnov test was used to evaluate normality. Genotype frequencies were analyzed by the $\chi 2$ test and the odds ratio (OR) with 95% confidence interval (CI) was calculated, using the Bioestat 5.0 program. Data are shown as mean ± standard deviation (SD) and as median with 25–75th percentiles, as appropriate.

Multiple Poisson regression analyses with robust standard errors were used to assess the effect of polymorphisms on the occurrence of visual hallucinations, since the outcome was a frequent event in our sample [21], while potential confounders to be considered in models were defined based on conceptual analyses of the literature and/or by means of a statistical definition. A formal Bonferroni correction for the number of analyzed SNPs would require a significance threshold of P = 0.025.

## 4 RESULTS

The clinical and demographic characteristics of the patients according to the presence/absence of fatigue and sleep disorder are described in Table 1. In this study, we verified that the prevalence of fatigue and sleep disorder were present in 26.2% and 15% of the patients, respectively. Regarding sleep disorder, there was a significant association with the use of the pramipexole (p = 0.020).

Our results of univariate analyses between TNF-α and NFKB polymorphisms (rs1800629 and rs28362491, respectively) and the occurrence of fatigue and sleep disorder are shown in Table 2. No statistical association was found between the genetic variants investigated and the occurrence these side effects (p>0.05).

In contrast, the multivariate analysis revealed an increased risk for the occurrence of fatigue in individuals carrying as the NFKB D/W genotype (Prevalence Ratio: PR=2.091; 95%CI, 1.155-3.784; p=0.015) and D/D genotype (PR= 2.237; 95%CI, 1.078-4.643; p=0.031) (Table 3). In addition, there was an increased risk to the occurrence of fatigue in PD patients with sleep disorder (PR= 5.226; 95%CI, 2.934-9.307; p= 0.000). Regarding the TNF-α gene, no significant association was observed between the occurrence of fatigue and/or sleep disorder (p > 0.05) (Table 3).

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## 5 DISCUSSION

Although studies had showed association of inflammation with Parkinson's disease, the mechanisms where by it contributes to the progression of the disease are still unclear [22]. Experimental and postmortem studies suggest that chronic inflammation associated with microglial activation could act as a stressor event promoting the recruitment of pro-inflammatory molecules such as tumor necrosis factor (TNFα) and nuclear factor kappa B (NFKB1) contributing to the progressive degeneration of dopaminergic neurons [23] and the appearance of non-motor symptoms such as fatigue and sleep disorders, once this symptoms may be due alterations in inflammatory molecules.

Fatigue n neurological diseases has been described as a significant decrease in energy level ou an increased persecution of efforts disproportionate to attempted activities [24). Friedman et al. (2018) reported that on-third of their patients rated fatigue as onde of the most disabling symptoms of Parkinson´s disease and more than 50% of the patients assessed had this symptom [25]. Others studies in different populations, however, reported prevalence ranging from 33# to 58% of the patients studied [2, 23, 24-31]. In our study the prevalence of fatigue was 26.2% suggesting that this difference can be explained once fatigue is assessed as categorical variable (presence or absence).

In general, fatigue is defined as a feeling of exhaustion for a certain period of time and is not explained by the effects of drugs, medical or psychiatric disorders [24]. In Parkinson's disease, fatigue is considered one of the most common and incapacitating non-motor symptoms, and may manifest itself even in the pre-motor stages of the disease and, once present, may persist and/or worsen over time [26,27].

Previous reports showed that the prevalence of sleep disorder ranged 50% to 81% [29,32–34]. In the present study, only 15% of the patients evaluated presented sleep disturbance. Again, we can suggest that this difference can be explained once sleep disorder is assessed as categorical variable (presence or absence).

Sleep disorder was described by James Parkinson as severe exhaustion and extreme drowsiness, and although the onset of motor symptoms in the PD often preceding non-motor symptoms, some patients may begin to exhibit non-motor symptoms in the course of the disease [2].

According to Titova and colleagues (2017) in a study evaluating non-motor characteristics of Parkinson's disease, the main treatments for non-motor disease symptoms are from non-pharmacological origin [35] . However, motor symptoms usually precede the non-motor symptoms of PD and have as main

treatment the use of drugs that, in the long time, may lead to the onset of adverse events, as well as triggering new symptoms of the disease [2,36–38]. In this line of reasoning, our results showed association between the use of prampexole and the development of sleep disturbance (p = 0.020).

The transcription factor NFKB1 was first discovered on T lymphocytes [39], but is present in all mammalian cells. The most well-known and studied role of this transcription factor is in the immune response, in which it regulates expression of essential genes in the inflammatory process and defense against parasites. In addition, it also acts on cell survival and proliferation, apoptosis and CNS function [40]. The TNFα protein initially known for its cytotoxicity, was originally identified in mouse serum after injection with Mycobacterium bovis Calmette-Guerin (BCG) bacillus and endotoxin. It is a proinflammatory cytokine, encoded by the TNF-α gene, that actively regulates a broad spectrum of biological processes, notably neuroinflammation [41,42].

NFKB1 is a transcription factor that modulates the balance of pro and anti-inflammatory cytokines. Wang et al. showed that the polymorphism -94 ins / del ATTG (rs28362491) involving the deletion of four nucleotides in the promoter region of the NFKB1 gene consists of three genotypes: homozygous or wild type (WW), homozygous or variant (DD); heterozygous ins / del (DW) and is within the promoter region of the NFKB1 gene, potentially affecting gene transcription and NFKB protein function, leading sequentially to loss of nuclear protein binding capacity and reduced activity.

Individuals carrying the mutant genotype may have the activity of this gene reduced and thus not be able to respond adequately to certain stimuli in order to aid in the transcription of genes whose products would act in an attempt to control the inflammatory process, and may end up making it chronic and aiding in the onset of various physiological complications [43]. These information can explain our data from multivariate analysis that revealed that individuals carrying DW or DD genotypes for NFKB gene had 2-fold increased risk for the occurrence of fatigue [31].

Recently, Herlofson et al. [31] showed that the development of fatigue would be associated with changes in metabolism, endocrine system and inflammatory markers and it is not related to the central nervous system. These information can explain our data from multivariate analysis that revealed that individuals carrying DW or DD genotypes for NFKB gene had 2-fold increased risk for the occurrence of fatigue.

Despite of Lindqvist et al. [44] had found significant and positive correlation between high level of TNFα and the presence of non-motor symptoms such as depression, anxiety and fatigue, no significant association between TNFa polymorphism and occurrence of fatigue was observed in the present study. Our data suggest that this polymorphism could not be is associated with the occurrence of fatigue. To the best of our knowledge this is the first study that correlated fatigue and sleep disorder in Parkinson's disease with polymorphisms in the promoter region of TNFα and NFKB1 genes (rs1800629 and rs28362491, respectively).

## 6 CONCLUSION

In conclusion, our study suggested that NFKB1 variant and use of the prampexole may have significant influence on increasing risk for the occurrence of fatigue and sleep disorder, respectively in a Brazilian PD population.


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### Conflict of interest

The authors declare that they have no competing interests.

### Consent to participate

The work was done according to all ethical principles and criteria of good scientific practice and data used from official databases that were processed statistically. The study does not contain personal data about patients nor does it include animal studies.

### Consent to publish

All authors agree with the publication of the research.

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Table 1. Clinical and demographic characteristics of Brazilian PD patients: all and stratified for fatigue and sleep disorder.

| | All | Fatigue | | P (value) | Sleep Disorder | | P (value) |
|---|---|---|---|---|---|---|---|
| | | No | Yes | | No | Yes | |
| Patients (%) | 180 | 133 (73.8) | 47 (26.2) | | 153 (85%) | 27 (15%) | |
| Men (%) | 107 (59.4) | 79 (59.4) | 28 (40.6) | 0.879 | 91 (59.5) | 16 (40.5) | 0.848 |
| Age at onset | 57.5 | 59 | 54 | 0.129 | 58 | 53 | 0.108 |
| Median (range) years | (48-64) | (48-66) | (49-60) | | (48-65) | (48-61) | |
| Disease Duration | 7.68± | 7 | 7 | 0.979 | 7 | 7 | 0.312 |
| Mean± s.d. | 4.06 | (5-10) | (5-9.5) | | (5-9) | (5.5-12) | |
| Levodopa Therapy | 6.72± | 6 | 6 | 0.880 | 6 | 6 | 0.269 |
| duration | 3.74 | (4-9) | (4-8) | | (4-9) | (4-9.5) | |
| Mean ± s.d. | | | | | | | |
| Levodopa dosage | 720.13± | 600 | 800 | 0.208 | 600 | 600 | 0.961 |
| Mean ±s.d. | 312.16 | (500-800) | (600-1000) | | (600-900) | (500-900) | |
| HY stage | | | | | | | |
| (%) | | | | | | | |
| 1(%) | 23(12.8) | 19(14.3) | 4(8.5) | 0.444 | 21(13.7) | 2(7.5) | 0.552 |
| 2(%) | 91(50.5) | 67(50.4) | 24(51.0) | 0.929 | 80(52.3) | 11(40.7) | 0.369 |
| 3(%) | 45(25) | 29(21.7) | 16(34.0) | 0.141 | 34(22.2) | 11(40.7) | 0.070 |
| 4(%) | 18(10) | 15(11.3) | 3(6.5) | 0.497 | 15(9.8) | 3(11.1) | 0.889 |
| 5(%) | 3(1.7) | 3(2.3) | 0(0.0) | 0.707 | 3(2.0) | 0 (0.0) | 0.935 |
| Pramipexole | 108(60.0) | 79(59.4) | 29(61.7) | 0.917 | 87(56.9) | 21(77.7) | **0.020** |
| Smoking | 20(11.1) | 14(10.5) | 6(12.7) | 0.880 | 18(11.8) | 2(7.4) | 0.739 |
| Alcoholism | 22(12.2) | 15(11.3) | 7(14.9) | 0.695 | 19(12.4) | 3(11.1) | 0.898 |
| Agrotoxic | 22(12.2) | 13(9.7) | 9(19.1) | 0.153 | 18(11.8) | 4(14.8) | 0.898 |

± SD, standard derivation. P (values) calculated by t test or by Wilcoxon–Mann–Whitney U-test (quantitative variables with or without normal distribution, respectively), and $X^2$ test (categorical variables). Significant p-values are shown in bold.

Table 2. Univariate analyses showing the relation between *TNFα* rs1800629, *NFKB1* rs28362491 and the occurrence of fatigue and sleepdisorder in Brazilian PD patients.

| | Total | Fatigue | | $X^2$ (p) | OR | P* | Sleep Disorder | | $X^2$ (p) | OR (IC 95%) | P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *TNFα* | 180 | No (133) | Yes (47) | | | | No (153) | Yes (27) | | | |
| G/G | 119(66.0) | 87 (65.4) | 32 (68.1) | 3.25 (0.20) | 1.00 | | 101(66.0) | 18 (67.0) | 0.83 (0.66) | 1.00 | |
| G/A | 47(26.0) | 38 (28.6) | 9 (19.1) | | 0.64(0.28-1.48) | 0.21 | 39 (25.0) | 8 (30.0) | | 1.15 (0.46-2.86) | 0.62 |
| A/A | 14(8.0) | 8 (6.0) | 6 (12.8) | | 2.04(0.66-6.33) | 0.35 | 13 (8.0) | 1 (4.0) | | 0.43 (0.05-3.51) | 0.69 |
| *NFKB1* | | | | | | | | | | | |
| W/W | 48(26.7) | 39 (29.3) | 9 (19.1) | 2.31 (0.32) | 1.00 | | 38 (25.0) | 10 (37.0) | 2.33 (0.31) | 1.00 | |
| W/D | 89(49.5) | 65 (48.9) | 24 (51.1) | | 1.60 (0.68-3.79) | 0.31 | 79 (52.0) | 10 (37.0) | | 0.48 (0.18-1.25) | 0.32 |
| D/D | 43(23.8) | 29 (21.8) | 14 (29.8) | | 2.09 (0.80-5.49) | 0.20 | 36 (24.0) | 7 (26.0) | | 0.74 (0.26-2.15) | 0.77 |

Abbreviations:.P*: P values; $X^2$: Chi- square.

Table 3. Multiple Poisson regression model adjusted for clinical variables and *TNFα* (rs1800629) and *NFKB1* (rs28362491) polymorphisms predicting fatigue in Parkinson's disease patients.

| | Prevalence Ratio | 95% CI | p - value |
|---|---|---|---|
| Gender (Male) | 0.790 | 0.479-1.304 | 0.357 |
| rs1800629 | | 0.90-1.46 | 0.241 |
| A/A | 2.083 | 0.920-4.716 | 0.078 |
| G/A | 0.646 | 0.358-1.166 | 0.147 |
| rs 28362491 | | | |
| D/D | 2.237 | 1.078-4.643 | **0.031** |
| D/W | 2.091 | 1.155-3.784 | **0.015** |
| Age | 0.991 | 0.966-1.016 | 0.474 |
| Disease duration (years) | 0.951 | 0.787-1.151 | 0.608 |
| Levodopa therapy duration | 1.026 | 0.857-1.229 | 0.778 |
| PDSS | 5.226 | 2.934-9.307 | **0.000** |
| HY | 0.974 | 0.651-1.456 | 0.897 |
| Levodopa dose | 1.001 | 1.0-1.001 | 0.131 |
| Dopamine Agonists | | | |
| Pramipexole use | 0.793 | 0.483-1.300 | 0.357 |
| Pain | 0.624 | 0.149-2.617 | 0.519 |
| Bradykinesia | 1.147 | 0.400-3.290 | 0.798 |
| Rigidity | 1.020 | 0.501-2.075 | 0.957 |
| Tremor | 1.335 | 0.563-3.167 | 0.512 |
| Smoking | 1.063 | 0.485-2.328 | 0.878 |
| Alcoholism | 1.319 | 0.660-2.640 | 0.433 |
| Agrotoxic | 1.581 | 0.979-2.554 | 0.061 |

Abbreviations:.PDSS: PD Sleep scale; HY: Hoehn and Yahr. Significant p-values are shown in bold.