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Research article

Tumor necrosis factor alpha polymorphisms are associated with Parkinson's disease age at onset

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ABSTRACT

The role of neuroinflammation in Parkinson's disease (PD) has been demonstrated through several different approaches. It was suggested an inflammation-derived oxidative stress and cytokine-dependent toxicity role in the nigrostriatal pathway degeneration and hasten progression of disease. Tumor necrosis factor alpha (*TNFA*) gene promoter polymorphisms might alter the expression of this cytokine contributing to the pro- and anti-inflammatory polarization. An increased *TNFA* expression might lead to inflammatory profile predominance. The aim of study was to determine if *TNFA* haplotypes are associated with PD age at onset. Five polymorphisms in *TNFA* gene were investigated in 226 patients with idiopathic PD in relation to age at onset. Haplotype grouping was based on allele expression. Logistic binary regression analysis showed that the genetic background leading to higher TNF-α expression confers a higher risk to develop PD earlier. Gender and ancestry did not differ between groups. High *TNFA* expression may contribute for faster dopaminergic neuron degeneration. In this context, a higher genetic pro-inflammatory profile confers a higher risk to develop PD earlier.

1. Introduction

The role of neuroinflammation in Parkinson's disease (PD) has been demonstrated through several different approaches, such as epidemiological studies, postmortem brain examinations and human clinical imaging. These studies suggested an inflammation-derived oxidative stress and cytokine-dependent toxicity role in the nigrostriatal pathway degeneration and hasten progression of disease in humans with idiopathic PD [1].

The loss of dopaminergic (DA) neurons in the substantia nigra (SN) and the presence of Lewy bodies within some remaining nigral neurons are the most prominent pathological features of PD [2]. Experimental models of PD suggested that DA neurons are extremely sensitive to tumor necrosis factor alpha (TNF-α) [3]. This cytokine is released through activation of angiotensin type-1 receptors, NADPH oxidase, Rho-kinase and NF-κB and it has a central role in angiotensin-induced DA cell death [4]. In PD models, this DA degeneration can be related with a gradual polarization from anti-inflammatory to inflammatory phenotype in activated microglia, correlated with increasing *TNFA* levels during this polarization process [5].

Polymorphisms in *TNFA* gene regulatory region might alter the amount of cytokine production contributing to this pro- and anti-inflammatory polarization. The level of TNF production in healthy individuals shows wide and stable variation, with high and low producer phenotypes present in the population, indicating a substantial genetic contribution to TNF regulation. TNF gene expression is controlled at the transcriptional level in resting human monocytes, while both transcriptional and posttranscriptional events regulate the level of TNF transcripts in TPA-activated cells. A number of groups have published functional studies of promoter variants, with many concentrating on the −308 SNP. The methodology of these studies has been broadly similar, with a variety of *in vitro* techniques applied to address the question of whether proteins bind to DNA probes containing allelic variants of the site under investigation (EMSA); and whether DNA constructs, consisting of reporter genes whose expression is regulated by allelic variants of promoters, show differences in transcriptional activity (transient transfection of reporter constructs). A study by Wilson et al. [6] has received considerable attention since its publication and showed large difference in transcriptional activity between allelic constructs, with the −308A construct having over eight-fold higher basal activity in Raji B cells. Four polymorphisms: a G to A substitution at position −308 (rs1800629), a C to T substitution at position −857 (rs1799724), a C to A substitution at position −863 (rs1800630), and a T to C substitution at position −1031 (rs1799964) within the promoter region

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of *TNFA* appear to have an effect on the transcriptional level of *TNFA* evaluated by response to Concanavalin A activated peripheral blood mononuclear cells measured by a luciferase assay. The transcriptional promoter activity of the −1031C/−863A or −857T allele in response to Concanavalin A stimulation was also about two-fold higher [7].

An increased *TNFA* expression might lead to inflammatory profile predominance and may contribute to a faster DA degeneration. Therefore, the aim of this study was to determine if *TNFA* haplotypes are associated with PD age at onset.

2. Materials and methods

A total of 226 patients with idiopathic PD were included in this study. Diagnostic processes and clinical evaluation were described elsewhere [8]. In brief, patients were diagnosed and recruited at the Movement Disorders clinics at "Hospital de Clínicas de Porto Alegre" in Brazil from 2006 to 2014. Diagnosis was based on UK Parkinson Disease Society Brain Bank criteria [9]. All patients were seen by the same experienced neurologists at the same clinic. Patients with atypical manifestations, secondary Parkinsonism or with familial history of the disease were excluded. The hospital Ethics Committee approved the study and all participants gave written informed consent to participate. The study was performed in compliance with the Declaration of Helsinki.

Genomic DNA was extracted from blood samples and five polymorphisms (rs1799964, rs1800630, rs1799724, rs1800629, rs361525) in *TNFA* promoter region were genotyped by TaqMan® SNP Genotyping Assay methods (Applied Biosystems, Foster City, CA). The PHASE software was used to infer haplotypes for each individual since these markers were in linkage disequilibrium. To avoid categories with few individuals, an expression criterion was applied for haplotype grouping. The expression level associated with each *TNFA* allele was used to create these categories. Alleles associated with higher *TNFA* expression were C for rs1799964, A for rs1800630, T for rs1799724, A for rs1800629 and A for rs361525 according to experimental published data [6,7]. Consequently, lower *TNFA* expression alleles were T, C, C, G and G, respectively. Logistic binary regression analysis was performed to estimate *TNFA* haplotype effect on age at onset. Statistical significance was defined as a two-tailed p-value < 0.05. The effect of TNF-α haplotypes on Levodopa dose was tested by ANOVA, Statistical analyses were performed using SPSS for Windows v18.0 (SPSS Inc., Chicago, IL, USA).

3. Results

For comparison purposes, patients were divided in two groups as performed in previous studies [10,11]: the early onset (EOPD, onset at or before 50 years; n = 63) and the late onset group (LOPD, onset at or after 51 years; n = 163). The EOPD and LOPD groups did not differ in relation to gender, ancestry, cognitive impairment, Hoehn & Yahr or Schwab & England scales. Levodopa daily dose differed between groups. Mean dose was higher in the EOPD group (Table 1). Levodopa daily dose was not associated with *TNFA* haplotypes (Table 2).

Table 1
Clinical and demographic data of PD patients stratified by age at onset.

	Early onset	Late onset	p-value
Gender (male)	35 (54.7)	82 (50.3)	0.559[a]
European ancestry	55 (87.5)	141 (86.5)	0.999[a]
Cognitive Impairment (without)	31 (55.4)	65 (45.5)	0.269[a]
Levodopa daily dose (mg)	756.0 (313.6)	686.3 (292.3)	0.043[b]
Hoehn & Yahr	2.5 (0.8)	2.5 (1.1)	0.353[b]
Schwab & England	71.1 (24.2)	71.4 (24.2)	0.857[b]

Data presented as n (%) or mean (± standard deviation). Tests performed in the table were: [a]chi-square and [b]Mann–Whitney *U* test.

Table 2
Levodopa daily dose according to the number of *TNFA* high expression alleles.

TNFA haplotypes[a]	Levodopa daily dose	p-value
No HE alleles	708.5 (270.7)	0.363
1–2 HE alleles	686.0 (291.2)	
3–4 HE alleles	762.2 (357.4)	

Data presented as mean (± standard deviation). Test performed in the table was one-way ANOVA.
[a] HE means high expression.

Table 3
Characterization of *TNFA* haplotypes according with the number of alleles associated with high expression.

TFNA haplotypes	n	Expression	Total high expression alleles
CACGG/CACGG	8	++−−−/++−−−	4
CACGG/CCCGA	6	++−−−/+−−−+	4
CACGG/CCCGG	1	++−−−/+−−−−	3
CACGG/TATGG	9	++−−−/−++−−	4
CACGG/TCCAG	13	++−−−/−−−+−	3
CACGG/TCCGG	42	++−−−/−−−−−	2
CCCGA/CCCGA	1	+−−−+/+−−−+	4
CCCGA/TCCAG	1	+−−−+/−−−+−	3
CCCGA/TCCGG	13	+−−−+/−−−−−	2
CCCGA/TCTGG	5	+−−−+/−−+−−	3
CCCGG/TCCGG	1	+−−−−/−−−−−	1
TACGG/TCCGG	1	−+−−−/−−−−−	1
TATGG/TCCGG	2	−++−−/−−−−−	2
TCCAG/TCCAG	4	−−−+−/−−−+−	2
TCCAG/TCCGG	25	−−−+−/−−−−−	1
TCCAG/TCTGG	11	−−−+−/−−+−−	2
TCCGG/TCCGG	56	−−−−−/−−−−−	0
TCCGG/TCTGG	24	−−−−−/−−+−−	1
TCTGG/TCTGG	3	−−+−−/−−+−−	2

Haplotypes derived in the following order: rs1799964/rs1800630/rs1799724/rs1800629/rs361525. The higher expression alleles in these variants were C, A, T, A, A, respectively.

Allele frequencies were directly obtained by gene counting. All markers were in Hardy-Weinberg equilibrium (data not shown). Haplotypes derived from *TNFA* alleles associated with lower expression were pooled as "low expression allele group", while haplotypes derived from alleles associated with higher expression of *TNFA* were grouped as "1 or 2 high expression alleles" and "3 or 4 high expression alleles" according to the number of high expression alleles included in the haplotype (a complete characterization of all haplotypes observed in this sample is shown in Table 3).

Haplotypes containing 3 or 4 high expression alleles showed a higher odds ratio (*OR*) for EOPD (OR = 2.629, CI 95% [1.062–6.775]; p = 0.039) in relation to haplotypes without high expression alleles (Table 4). The effect size Cohen's interpretation scale showed that this result has a medium effect size ($d = 0.53$). None of the clinical or demographical variables remained significant in the multivariate analysis.

Table 4
Age at onset according to the number of *TNFA* high expression alleles.

TFNA-haplotypes[a]	Early onset	Late onset	OR (95% CI)	p-value	ES[b]
No HE alleles	10 (15.9)	46 (28.2)	–		
1–2 HE alleles	37 (58.7)	89 (54.6)	1.912 (0.889–4.374)	0.105	
3–4 HE alleles	16 (25.4)	28 (17.2)	2.629 (1.062–6.775)	0.039	0.534

Data presented as n (%). Gender, ancestry, cognitive impairment, Hoehn & Yahr, Schwab & England scales, and levodopa daily dose were not significant in this analysis.
[a] HE means high expression.
[b] ES means Cohen's d effect size.