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TNF-α − 308 G/A and − 238 G/A promoter polymorphisms and sporadic Parkinson's disease in an Italian cohort

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ABSTRACT

The etiology of sporadic Parkinson's disease is (PD) still not understood but it is believed that a complex interplay between environmental and genetic factors could trigger the pathology. Pro-inflammatory TNF-α is released by activated microglia and is up-regulated in the brain and cerebrospinal fluid of PD patients; TNF-α modulates neuroinflammation and can activate the molecular mechanisms that lead to neurotoxicity and neuronal death. We analyzed two functional SNPs within the TNF-α gene promoter (rs361525 and rs1800629) in 354 Italian PD patients and 443 healthy controls (HC). In our cohort of patients, no significant associations could be observed between rs361525 and rs1800629 SNPs and either PD onset risk or PD-associated clinical parameters including age at onset of fluctuations, UPDRS-ME (Unified Parkinson Disease Rating Scale-Motor Examination), Schwab & England, Hohen & Yahr stage scale, and MMSE (Mini-Mental State Examination) score. Conflicting results on the role played by TNF-α rs1800629 SNP on PD onset risk are present in the literature. We could not find any association between TNF-α rs361525 and rs1800629 and PD.

1. Introduction

Parkinson's disease (PD), the second most common neurodegenerative disorder in humans, is caused by the progressive loss of dopaminergic neurons in the substantia nigra (SN) pars compacta (pc) of the midbrain, and is characterized by rigidity, resting tremor and bradykinesia. The etiopathogenesis of PD is still unclear but a genetic contribution to the etiology is well established. Thus, mutations in 18 specific chromosomal regions with a causative effect in familiar PD have been described [1]. The majority of PD cases are nevertheless sporadic, and in these latter cases it is believed that a complex interplay between environmental and genetic factors triggers the pathology (double-hit hypothesis).

Chronic inflammation and microglia activation are associated with neuronal death in neurodegenerative disorders, including PD [2]. Microglia activation can be caused by a exogenous, infective insults, by metabolites released by dying neurons and also as a reaction to α-synuclein aggregates caused by protein misfolding [3], a feature of PD. Notably, whereas mild microglia activation is associated with beneficial effects, chronic activation, as is the case in PD, results in death of otherwise viable cells [4]. Proinflammatory cytokines released by activated microglia including tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and IL-1β are upregulated in the brain and cerebrospinal fluid of PD patients [5]. 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; blockade of TNF-α receptors reduces dopaminergic neurons death in PD animal models [6]. Several single nucleotide polymorphisms (SNPs) in the TNF-α gene promoter are described; in particular − 308 G/A (rs1800629) − 238 G/A (rs361525) SNPs are functional polymorphisms: the presence of GG genotypes for both of them result in a stronger *in vitro* and *in vivo* transcriptional activity [7–9].

Previous studies regarding rs1800629 SNP gave conflicting results that we summarized in Table 1.With this work we wanted to shed light

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Table 1
Summary table of the existing literature about TNFα promoter polymorphisms and risk of PD onset.

First author, year. [Ref. No]	Country	Ethnicity	TNFα SNP	Genotyping method	HC	PD	Association observed
Ross OA [15]	Ireland	Irish	rs1800629	PCR-SSOP	93	90	No risk
Wu YR [16]	Taiwan	Asian	rs1799964, rs800630, rs1799724, rs1800629	PCR-RFLP	326	369	No risk
Pascale E [17]	Italy	Caucasian	rs1800629	PCR-RFLP	156	146	No risk
Bialecka M [18]	Poland	Caucasian	rs1800629	PCR-RFLP	300	316	AA genotype increases risk, especially in EO-PD
Wahner AD [19]	California USA	Rural Californian	rs1800629	PCR and pyrosequencing	269	289	AA genotype increases risk
Krüger R [20]	Germany	German	rs1800629	PCR-RFLP	177	237	AG genotype increases risk

Table 2
TNF-α rs361525 and rs1800629 genotypes and alleles distribution in PD patients and healthy controls (HC).

Genotype	PD patients		HC subjects		p value	OR (95% CI)
	N	(%)	N	(%)		
rs361525 (− 238 G/A)						
AA	1	(0.3)	1	(0.2)	1	1.25 (0.08–20.09)
GA	42	(11.9)	52	(11.7)	1	1.01 (0.66–1.56)
GG	311	(87.9)	390	(88.0)	1	0.98 (0.64–1.51)
Total	354		443		0.99	
Allele						
A	44	(6.2)	54	(6.1)		
G	664	(93.8)	832	(93.9)		
Total	708		886		1	1.02 (0.68–1.54)
rs1800629 (− 308 G/A)						
AA	5	(1.4)	5	(1.1)	0.76	1.26 (0.36–4.37)
GA	80	(22.6)	88	(19.9)	0.38	1.18 (0.84–1.66)
GG	269	(76.0)	350	(79.0)	0.35	0.35 (0.60–1.17)
Total	354		443		0.59	
Allele						
A	90	(12.7)	98	(11.1)		
G	618	(87.3)	788	(88.9)		
Total	708		886		0.35	1.17 (0.86–1.59)

N = absolute number; % = percentage.

on TNF-α contribution to PD risk and we analyzed rs1800629 and rs361525 polymorphisms within the TNF-α gene promoter in a cohort of sporadic Italian Parkinson's disease patients and healthy controls.

2. Materials and methods

2.1. Study population and clinical data

The study population included 354 consecutive patients with PD (160 females and 194 males), and 443 age-matched healthy controls (HC) (269 females and 174 males) recruited among relatives (mostly patients' spouses with no maternal relationship for at least four generations). A recent meta-analysis study underlined the validity of the measurement of hyperechogenicity of substantia nigra for differential diagnosis of Parkinson's disease [10]. Unfortunately our subjects couldn't be evaluated by transcranial sonography but all patients enrolled in the study attended regular follow-up appointments with neurologists trained in movement disorders that evaluated their conditions and symptoms over time to confirm PD diagnosis. A complete anamnesis was collected to make sure that all the patients included in the study suffered from sporadic and not familiar PD. All the healthy controls were examined as well by neurologists that excluded the presence of any movement disorder and in particular PD.

Subjects were recruited at three separate institutions, the Unit of Parkinson's disease and Movements disorders of the IRCCS “C. Mondino” of Pavia, the Ospedale di Circolo and Fondazione Macchi in Varese, Italy, and the Neurologic Rehabilitation Unit of the Don C. Gnocchi Foundation, IRCCS in Milano, Italy. The following demographic and clinical variables were recorded in all patients: age, gender, age at onset of symptoms, current disability measured by the UPDRS-ME (Unified Parkinson Disease Rating Scale-Motor Examination), Hohen & Yahr stage [11] during ON and OFF periods and Schwab & England scale [12]. Cognitive performances were measured by the MMSE (Mini-Mental State Examination) [13] evaluated in ON phase and was corrected for age and education. All PD patients were undergoing dopaminergic therapy. The MMSE was normal (> 24) in 82.9% of the PD patients. PD patients had a mean age at onset of

Table 3
Distribution of two SNP of TNFα promoter (rs361525 and rs1800629) genotypes in Italian PD patients. Kruskall-Wallis test for age at onset, age at onset of fluctuations, UPDRS-ME in ON phase, Hohen & Yahr stage in ON phase, Shwab England scale and MMSE in ON phase (not normally distributed) are presented.

	rs361525 (− 238 G/A)				rs1800629 (− 308 G/A)			
Genotype	AA	AG	GG	p value	AA	AG	GG	p value
Age at onset, m.r.[a]	85.00	170.21	166.26	0.68	186.90	172.47	164.42	0.73
Age at onset fluctuations, m.r.[b]	–	87.23	80.12	0.52	106.83	73.94	82.24	0.41
UPDRS-ME ON, m.r.[c]	51.50	130.02	140.58	0.43	148.00	140.38	138.40	0.95
HY ON, m.r.[d]	–	24	200	0.75	144.50	112.49	112.14	0.74
Shwab England scale, m.r.[e]	250.50	153.23	142.95	0.34	167.90	148.29	142.91	0.73
Corrected MMSE ON, m.r.[f]	238.50	138.09	138.14	0.44	160.00	144.70	136.19	0.63

N = number of patients; m.r. = mean rank; HY = Hohen & Yahr scale.
[a] Calculated for 332 PD patients.
[b] Calculated for 161 PD patients.
[c] Calculated for 277 PD patients.
[d] Calculated for 224 PD patients.
[e] Calculated for 288 PD patients.
[f] Calculated for 276 PD patients.