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ORIGINAL ARTICLE

Interleukin-18 promoter polymorphisms and idiopathic Parkinson disease: an Egyptian study

Ebtesam Fahmy[1] · Amany Rabah[1] · Sahar Sharaf[2] · Hanan Helmy[1] · Ahmed Kamal[3]

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Abstract

Etiology of sporadic Parkinson's disease (PD) is largely unknown. The contribution of genetic factors to the pathogenesis of PD is supported by the demonstration of high concordance in twins, increased risk among relatives of PD patients and existence of familial cases. This study aimed to examine the relation between interleukin 18 (IL-18) gene promoter polymorphisms and idiopathic PD, and its impact on clinical presentation and disease severity. 30 idiopathic PD patients and 15 age- and sex-matched healthy subjects were included. Disease severity was assessed using Unified Parkinson's Disease Rating Scale (UPDRS). Genetic testing for IL-18 gene promoter −607C/A single nucleotide polymorphisms (SNP) was done using real-time polymerase chain reaction (PCR) technique. A raised risk of PD development was found in patients with A/C and C/C genotypes of the site −607C/A (odds ratios = 1.83 and 1.98, respectively). The distribution of the genotypes showed no significant relation to gender or predominant clinical presentation. The age at onset of disease was significantly lower in C/C and A/A genotypes compared to A/C genotype ($p = 0.001$ and 0.04, respectively). Patients with A/A genotype showed significantly higher mentation score of UPDRS compared to patients with A/C and C/C genotypes ($p = 0.003$ and $p = 0.002$, respectively). Polymorphisms of IL-18 gene promoter increase the risk of developing idiopathic PD. The polymorphisms may affect phenotypic expression rather than being a direct cause of idiopathic PD.

Keywords Interleukin-18 · Promoter polymorphisms · Idiopathic Parkinson's disease

Introduction

Parkinson disease (PD) is a common neurodegenerative disorder characterized by progressive degeneration of dopaminergic neurons in the substantia nigra. Growing evidence revealed that immune responses and neuro-inflammatory processes might play an important role in the pathogenesis of PD [1].

Interleukin 18 (IL-18) is a pleiotropic pro-inflammatory cytokine that functions in the inflammatory cascade believed to be the key player in neuro-inflammation and neuro-degeneration. Reports revealed that IL-18 is involved in microglial activation which contributes to dopaminergic neuro-degeneration. Whether there is an association between genetic polymorphisms of IL-18 gene promoter and sporadic PD is largely unknown [2].

IL-18 gene promoter contains multiple transcription initiation sites. Polymorphisms of IL-18 gene promoter have been shown to influence a lot of chronic inflammatory diseases including diabetes, obesity, atherosclerosis and rheumatoid arthritis pathogenesis [3, 4]. Moreover, IL-18 gene

✉ Hanan Helmy
drhananelgendy76@gmail.com

Ebtesam Fahmy
em_fahmy@kasaralainy.edu.eg.com

Amany Rabah
amanyrabah@hotmail.com

Sahar Sharaf
sahar.abdelatty@kasaralainy.edu.eg.com

Ahmed Kamal
ahm_shams@hotmail.com

[1] Department of Neurology, Cairo University, Giza, Egypt

[2] Department of Clinical Pathology, Cairo University, Giza, Egypt

[3] Department of Neurology, Sahel Teaching Hospital, Cairo, Egypt

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promoter polymorphisms have been associated with Alzheimer's disease, which might indicate its contribution to the progress of neurodegenerative disorders [5].

A study by Xu et al. [6] has addressed the relation between IL-18 gene promoter polymorphisms and PD. However, there is still a need for more studies to increase awareness about genetics of neuro-inflammation, outline the relation between inflammatory interleukins and PD incidence and symptomatology, and attempt reproduction of the results in different ethnicities. This can aid to discover protective mechanisms to halt or decelerate the progression of the disease and direct appropriate genetic testing in susceptible individuals [7].

The present study was designed to examine the relation between polymorphisms of IL-18 gene promoter and idiopathic PD, and its impact on clinical presentation and disease severity.

Subjects and methods

This is a case–control study conducted on 45 subjects (30 PD patients and 15 age- and sex-matched healthy subjects). Patients were selected from Al Sahel Teaching Hospital and Neurology outpatient clinic of Cairo University Hospitals in the period from March to November 2012. An informed consent was taken from the patients prior to participation to ensure complete satisfaction.

Inclusion criteria were:

1. Patients (of both sexes) fulfilling the diagnostic criteria of idiopathic PD proposed by Jankovic and Shannon [8] which include:

- Parkinsonism characterized by typical rest tremor, bradykinesia and rigidity.
- Asymmetrical manifestations at onset.
- Significant response to a satisfactory trial of L-dopa.
- Absence of atypical features suggesting alternative diagnosis.
- None of the following: early prominent dementia, symmetrical signs, bulbar dysfunction, early gait disorders, falls within the first year of onset, wheelchair dependence within 5 years, early autonomic failure, sleep apnea, inspiratory stridor, apraxia, alien limb or cortical sensory loss.

2. Age range from 40 to 75 years.

Exclusion criteria were: other causes of Parkinson disease, intake of antipsychotic drugs, substance abuse, alcohol intake and metabolic disturbances (as chronic liver disease or elevated serum copper).

Methods

Patients were submitted to:

Thorough clinical assessment Including detailed history taking and complete neurological examination.

Grading of disease severity Using Unified Parkinson's Disease Rating Scale (UPDRS) which consists of 6 sections: mentation (mental activity), behavior, and mood (4 questions), activities of daily living (ADL) (13 questions), motor examination (14 questions), complications of therapy (11 questions), Modified Hoehn and Yahr Staging Scale and Schwab and England Activities of Daily Living Scale [9].

Laboratory workup Included complete blood count, liver function tests, kidney function tests, fasting blood sugar, and electrolytes (including sodium, potassium, calcium and phosphorus levels). Serum copper level was estimated for patients, to exclude hepato-lenticular degeneration.

Brain imaging Non-contrast computed tomography (CT) and/or magnetic resonance imaging (MRI) of the brain were done for patients to rule out secondary causes of parkinsonism.

Genetic testing for interleukin 18 gene promoter polymorphisms Determination of the polymorphisms at the −607C/A position in the promoter of the IL-18 gene was done for both patients and controls using real-time polymerase chain reaction (PCR). This comprises two steps, DNA extraction and genotype detection. Deoxyribonucleic acid extraction was done using High-Pure PCR Template Preparation kit, by Roche. For the detection of −607C/A single nucleotide polymorphism in interleukin 18 gene promoter region real-time PCR was done on the LightCycler® 2.0 instruments, using LightCycler® FastStart DNA Master^{PLUS} SYBR Green I supplied by Roche. To prove that only the desired PCR product has been amplified, a melting curve analysis after PCR was performed. LightCycler® 2.0 PCR systems automatically calculated the negative derivative of the change in fluorescence and generated a melting curve for each sample. The melting peaks for different genotypes are as follows:

- Homozygote −607 A/A: 1 melting peak at 85 °C.
- Heterozygote −607 A/C: 2 melting peaks at 84 and 85 °C.
- Homozygote −607 C/C: 1 melting peak at 84 °C.

Statistical analysis

Data were analyzed using the Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative

data were expressed as frequency and percentage. Independent sample *t* test was used to compare between two variables. A one-way analysis of variance (ANOVA F) was used to compare between more than two variables. Post hoc was used to test possible combinations of groups to determine where the significant differences are located (typically only if the omnibus "*F*" is significant). The odds ratio (OR) was calculated to assess the presence of an increased risk of disease development in relation to any of the IL-18 gene promoter polymorphisms. The OR of each genotype was calculated independently, using the following formula [10]:

$$\mathrm{OR} = \frac{a/b}{c/d} = \frac{a \times b}{b \times c},$$

where a is the number of patients with a specific genotype, b is the number of controls with that genotype, c is the number of patients with the other genotypes, and d is the number of controls with the other genotypes. p value < 0.05 was considered significant, p value < 0.001 was considered highly significant, and p value > 0.05 was considered insignificant.

Ethical statement

The ethical committee of the faculty of medicine, Cairo University, has allowed this study.

Results

Demographic and clinical characteristics

Patient group included 30 PD patients [17 females (56.70%) and 13 males (43.30%)]. Their age ranged from 45 to 72 years, with a mean of 59.37 ± 7.43 years. The control group included 15 healthy subjects [8 females (53.33%) and 7 males (46.67%)]. Their age ranged from 42 to 63 years, with a mean of 52.87 ± 8.21 years.

The age at disease onset ranged from 42 to 64 years, with a mean of 52.33 ± 5.60 years. The disease duration ranged from 1 to 15 years, with a mean of 7.03 ± 3.65 years. Tremors were predominant in 24 patients (80%), whereas bradykinesia was predominant in 6 patients (20%).

Distribution of interleukin 18 gene promoter polymorphisms

The distribution of IL-18 gene promoter polymorphisms at position −607C/A in patients and controls is presented in Table 1. No significant difference was detected between PD patients and controls regarding the distribution of the polymorphisms (A/A, C/C or A/C) of the IL-18 gene promoter ($p > 0.05$).

Table 1 IL-18 gene promoter −607C/A polymorphism distribution in patients and controls

Gene promoter −607 C/A form	Patients		Controls		*p* value
	No.	%	No.	%	
A/A	11	36.70	9	60.00	0.240
C/C	7	23.30	2	13.30	0.690
A/C	12	40.00	4	26.70	0.584
A%	57.00		73.30		
C%	43.00		26.70		

Table 2 The relative risk of PD development in patient subgroups distributed by genotype

Patient subgroup	Odds ratio	*p* value
A/A patients	0.39	0.14
C/C patients	1.98	0.44
A/C patients	1.83	0.38

Although a raised risk of PD development was found in patients with C/C and A/C genotypes, the odds ratios being 1.98 and 1.83, respectively, statistical significance was not achieved for any of the subgroups ($p > 0.05$) (Table 2).

Comparison between male and female patients

No significant difference was detected between male and female patients as regards the distribution of IL-18 gene promoter polymorphisms ($p = 0.80$) (Fig. 1).

Comparison between patients distributed according to predominant clinical presentation

No significant difference was detected between patients presented with bradykinesia or tremors regarding age at disease onset, UPDRS scores or distribution of IL-18 gene promoter polymorphisms ($p > 0.05$) (Fig. 2).

Comparison between Parkinson's disease patients distributed according to interleukin 18 gene promoter polymorphisms

A significant difference was found between IL-18 gene promoter polymorphisms regarding the age at disease onset ($p = 0.005$). Post hoc test revealed that patients with C/C genotype showed a lower age at onset compared to patients with A/C genotype (p value $= 0.001$). Patients with A/A genotype showed a significantly lower age at onset compared to patients with A/C genotype ($p = 0.04$). However, no significant difference was found between patients with C/C and A/A genotypes ($p > 0.05$) (Table 3).

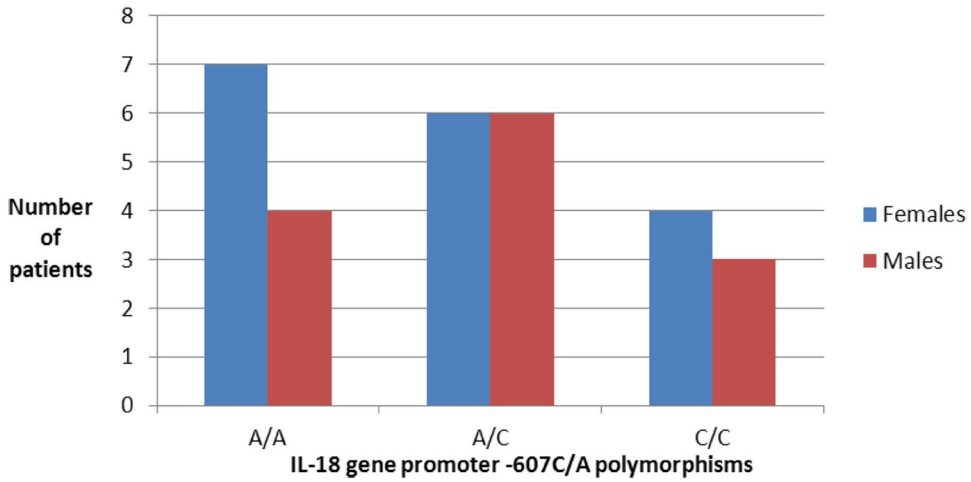

Fig. 1 Distribution of IL-18 gene promoter polymorphisms in male and female patients

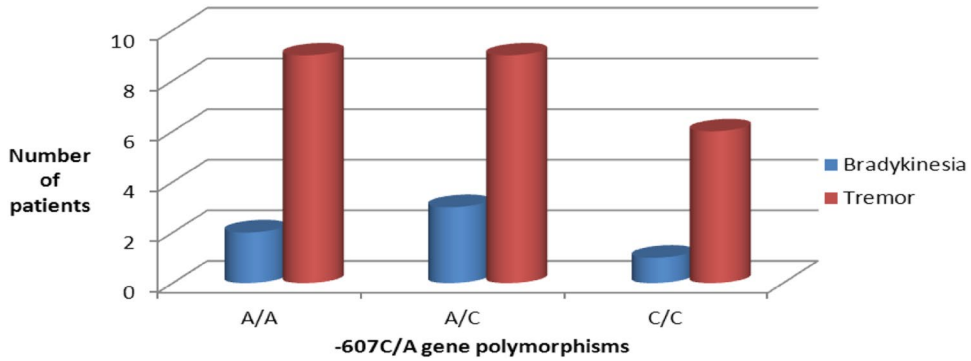

Fig. 2 IL-18 gene promoter polymorphisms in PD patients distributed according to main clinical presentation

Table 3 Comparison between different genotypes in the IL-18 gene promoter −607C/A site as regards age at onset of PD

Genotype −607C/A	No.	Age at onset (years)		ANOVA test	Post hoc test *p* values
		Range	Mean ± SD		
A/A	11	44–62	51.55 ± 5.48	$F = 6.53$ ($p = 0.005$)[a]	A/A vs. A/C = 0.04[a]
C/C	7	42–53	47.71 ± 3.99		C/C vs. A/C = 0.001[b]
A/C	12	50–64	55.75 ± 4.43		A/A vs. C/C = 0.11

[a]Statistically significant

[b]Highly significant

Comparing patient subgroups distributed according to genotype as regards the UPDRS scoring, a statistically significant difference was detected in the mean scores of mentation, mood and cognition parameter ($p = 0.002$). Post hoc test revealed that patients with A/A genotype had higher mean scores, denoting greater affection, than patients with A/C or C/C genotypes ($p = 0.003$, 0.002 respectively) (Table 4).

Discussion

Polymorphisms at −607C/A site of IL-18 gene promoter were found to influence the expression of IL-18 gene. These polymorphisms were previously studied in PD patients and linked to disease incidence, with C/C genotype showing a greater risk for disease development [6].

In the present study, C/C and A/A genotypes of IL-18 gene promoter presented at significantly earlier age compared to A/C genotype, which may suggest that these genotypes enhance early IL-18 gene transcription. Enhanced IL-18 gene transcription increases pro-inflammatory IL-18 production, and earlier damage of the dopaminergic neurons of substantia nigra in susceptible individuals. So, younger age at onset in patients with C/C and A/A genotypes could present a defective neuro-protection leading to dopaminergic neuronal damage. Nigral dopaminergic neurons appear to be the first to suffer from enhanced neuro-inflammation or impaired neuro-protection [2].

Table 4 Comparison between patients with different genotypes in IL-18 gene promoter −607C/A site as regards mean UPDRS scores

UPDRS sections	Genotype −607C/A						*p* value
	A/A		A/C		C/C		
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	
Mentation, behavior and mood	2–11	7.27 ± 3.10	0–8	3.50 ± 2.39	0–7	2.86 ± 2.61	ANOVA: 0.002[a] Post hoc: A/A vs. A/C 0.003[a] A/A vs. C/C 0.002[a] A/C vs. C/C 0.623
ADL	9–24	15.73 ± 4.31	3–31	15.58 ± 8.49	15–27	20.00 ± 3.74	0.29
Motor examination	11–27	17.64 ± 4.39	2–36	18.25 ± 8.66	12–25	19.43 ± 4.54	0.85
Complications of therapy	0–12	4.55 ± 4.18	0–13	4.08 ± 4.42	0–9	2.71 ± 3.35	0.65
Modified (H&Y) scale	1–3	2.23 ± 0.72	1–4	2.38 ± 1.00	1.5–3	2.21 ± 0.57	0.88
S&E scale	40–90	70.00 ± 13.42	30–90	65.83 ± 16.21	70–80	78.57 ± 3.78	0.15
Total score	25–58	40.64 ± 10.01	8–64	37.33 ± 15.84	27–52	42.29 ± 8.83	0.68

ADL activities of daily living, *H&Y* modified Hoehn and Yahr Scale, *S&E* Schwab and England Activities of Daily Living Scale, *UPDRS* Unified Parkinson's Disease Rating Scale

[a]Statistically significant

In a previous study done on an Ashkenazi Jew cohort, Hakansson et al. [11] found that G/G genotype at −174 G/C site of the IL-6 gene promoter was associated with earlier PD onset than G/C or C/C genotypes. However, Ross et al. [12] and Infante et al. [13] found no correlation between the IL-6 gene promoter polymorphisms and the age at PD onset in Irish and Spanish cohorts, respectively. This can be explained in view of differences in ethnic backgrounds which may influence the genetic map of individuals, as well as the subjective nature of reporting the symptom onset in different patients, which makes accurate determination of the age at disease onset a matter of difficulty. To the best of our knowledge, no previous study examined the correlation between IL-18 gene promoter polymorphisms and age at onset of PD.

Although C/C and A/C genotypes of IL-18 gene promoter showed an increased relative risk for PD development (OR = 1.98 and 1.83, respectively), this finding did not reach a statistical significance due to small sample size which was attributed to financial issues. In the study of Xu et al. [6], the relative risk of PD development in patients with C/C genotype was 1.80, which was statistically significant owing to a larger sample size. However, more research is needed to generalize this finding, as our study was confined to Egyptian ethnicity and that of Xu et al., was confined to Han Chinese ethnicity.

Patients with A/A genotype had higher scoring in the mentation, mood and behavior parameter of the UPDRS compared to A/C or C/C genotypes. The A/A genotype is more associated with intellectual impairment, thought disorder and depression. This implies that patients with A/A genotype had greater affection of the CNS structures responsible for the development of cognitive, behavioral and mood.

In this study, no significant difference was detected between patients presented with bradykinesia or tremors regarding distribution of IL-18 gene promoter polymorphisms which may be attributed to a small number of patients which was a limitation in this study. However, a relationship between genetic mutations and PD symptoms was detected in previous research. Paisan–Ruiz et al. [14] reported an association between G2019S mutation of the LRRK2 gene and the tremor-dominant PD in patients with familial disease. Haugarvoll et al. [15] and Healy et al. [16] also reported an association between LRRK2 mutation and the asymmetrical tremor-dominant PD phenotype. In an Egyptian cohort, Hashad et al. [17] found that G2019S mutation was associated with a higher degree of motor affection in the form of resting tremor and postural instability.

With the limitation of this study, it could be concluded that A/C and C/C genotypes at −607C/A SNP of IL-18 gene promoter had increased relative risk of developing PD. The C/C and A/A genotypes were related to earlier onset

presentation of PD. The A/A genotype was associated with greater cognitive, behavioral and mood changes. Future studies with inclusion of larger samples are warranted for further exploration of the association between interleukin 18 gene promoter polymorphisms and idiopathic PD.

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Author contributors All authors were involved in protocol development and data analysis. SS carried out the molecular genetic studies. EF wrote the first and final draft of the manuscript. AK was involved in patient recruitment. EF and HH reviewed and edited the manuscript and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest No potential conflict of interest relevant to this article is reported. No official funding.

Ethical approval The study was approved by the Ethical committee of the institution.

Infomed consent Patients were informed, and they consented to conduct the study.

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