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Associations of IL-1β and IL-6 gene polymorphisms with Parkinson's disease

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Abstract. – OBJECTIVE: The aim of this study was to explore the associations of interleukin-1β (IL-1β) and IL-6 gene polymorphisms with the pathogenesis of Parkinson's disease.

PATIENTS AND METHODS: A total of 200 patients with Parkinson's disease in our hospital were collected as the disease group. Meanwhile, 200 healthy subjects were taken as the control group. Peripheral blood samples were drawn from all research subjects. The polymorphic regions of IL-1β and IL-6 were amplified *via* polymerase chain reaction (PCR). Moreover, the polymorphisms were detected and analyzed, followed by further analysis based on the changes in gene expressions and Hoehn-Yahr grade of patients.

RESULTS: The allele distributions at IL-1β rs571556428 (p=0.015) and IL-6 rs543214973 (p=0.012) were statistically different between control group and disease group. In disease group, the G allele frequency at IL-1β rs571556428 and T allele frequency at IL-6 rs543214973 were significantly higher (p<0.05). Genotype distributions at IL-1β rs572292175 (p=0.017) and rs571556428 (p=0.000), and IL-6 rs543214973 (p=0.002) in disease group were also different from those in control group. In addition, the frequencies of CT genotype at IL-1β rs572292175, AA genotype at IL-1β rs571556428 and AA genotype at IL-6 rs543214973 in disease group were significantly lower (p<0.05). After modeling and analysis, it was found that the distribution of recessive model at IL-1β rs571556428 (p=0.012) and IL-6 rs543214973 (p=0.014) in disease group exhibited significant differences from those in control group. The frequencies of TA haplotype at IL-1β rs572292175 and rs571556428 (p=0.038) and GA haplotype at IL-6 rs1474348 and rs543214973 (p=0.047) in disease group were lower than those in control group (p<0.05). The polymorphisms at IL-1β rs571556428 and IL-6 rs1474348 were significantly associated with gene expression (p<0.05). Moreover, the expressions of IL-1β and IL-6 rose significantly in patients with GG genotype at rs571556428 and CG genotype at rs1474348, respectively (p<0.05). Furthermore, the polymorphism at IL-1β rs571556428 was significantly correlated with the grade of Parkinson's disease (p=0.000). Parkinson's disease was in a higher grade (grade 4-5) in patients with AA genotype, whereas in a lower grade (grade 1-2) in patients with GG and AG genotypes.

CONCLUSIONS: IL-1β and IL-6 gene polymorphisms are significantly associated with the pathogenesis of Parkinson's disease.

Key Words:
IL-1β, IL-6, Gene polymorphism, Parkinson's disease.

Introduction

Parkinson's disease is a nervous system disease, whose morbidity rate increases gradually in contemporary society. Currently, it poses a great impact on patients' quality of life and social-economic development[1,2]. Parkinson's disease frequently occurs in elderly people aged above 60 years old. It is speculated that this disease may be closely related to the aging-induced decline in functional dopaminergic neurons[3,4]. Dopaminergic neuron death is not only caused by aging, but also correlated with a variety of factors, including various harmful substances in the environment and the activation of immuno-inflammatory system *in vivo*[5,6]. Therefore, it is of important significance to explore the pathogenesis of Parkinson's disease for its prevention.

In recent years, it has been increasingly confirmed that gene polymorphism is one of the important factors affecting the susceptibility to various diseases[7], such as chronic obstructive pulmonary disease[8] and lung cancer[9]. Gene polymorphisms of interleukin-1β (IL-1β) and IL-6, important pro-inflammatory molecules in the immune system, may play an important role in the pathogenesis of Parkinson's disease.

Therefore, the aim of this study was to explore the associations of IL-1β and IL-6 gene polymorphisms with the pathogenesis of Parkinson's dis-

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ease. The polymorphisms at IL-1β rs572292175 and rs571556428, and at IL-6 rs1474348 and rs543214973 were detected in healthy subjects and patients with Parkinson's disease in our hospital. Meanwhile, the haplotype analysis and model analysis were performed. Our findings might help to deeply discuss the susceptibility factors of Parkinson's disease with reference to the correlations of gene polymorphisms with gene expression and Hoehn-Yahr grade of patients.

Patients and Methods

General Data

A total of 200 patients with Parkinson's disease admitted to our hospital were enrolled in the disease group. The selection of patients was based on the guideline proposed by the International Parkinson and Movement Disorder Society (MDS). Meanwhile, 200 healthy subjects receiving physical examination recently were taken as the control group. No abnormalities were found in 200 healthy subjects after blood routine examination, hepatic-renal function test, chest X-ray and abdominal B ultrasound. General data (name, gender, age, etc.) and clinical data (disease history, family history, smoking and drinking history, etc.) were collected in both groups. In disease group, there were 113 males and 87 females, with an average age of (62.43±4.51) years old. In control group, there were 108 males and 92 females, with an average age of (61.24±5.82) years old. There were no statistically significant differences in such general data as age and gender between the two groups (p>0.05).

Diagnostic criteria for Parkinson's disease in disease group were as follows: 1) elderly patients; 2) patients with clinical symptoms such as static tremor, myotonia and bradykinesia; 3) those without characteristic changes in head CT and MRI; 4) those who responded to dopaminergic drugs, and 5) those without other neuropsychiatric diseases.

This investigation was approved by the Ethics Committee of Hebei General Hospital. Signed written informed consents were obtained from all participants before the study.

Clinical Sample Collection and Treatment

About 5-7 mL of peripheral blood was first collected using the anticoagulant tube from the elbow vein in both groups. Within 30 min, these blood samples were centrifuged at 3000 rpm for 5 min. Mid-layer nucleated cells were then carefully collected into new centrifuge tubes for extraction of genomic deoxyribonucleic acid (DNA).

DNA Extraction

DNA was extracted in both groups strictly according to the instructions of genomic DNA extraction kit (TIANGEN, Beijing, Ching). In brief, according to the sample volume, proteinase K solution was added into the centrifuge tube, as well as peripheral blood samples and buffer. The mixture was evenly mixed using a shaker for 1 min, followed by incubation at 65°C for 10 min. Subsequently, a certain volume of absolute ethanol was added, mixed evenly and transferred into the adsorption column. The buffer was then added into the adsorption column, and the mixture was centrifuged. Next, the elution buffer was added into the absorption column, and the resulting solution was genomic DNA. The mass of DNA extracted was detected using a spectrophotometer, and the optical density $(OD)_{260}/OD_{280}$ should be 1.8-2.0.

Polymerase Chain Reaction (PCR) Amplification and Analysis of IL-1β and IL-6 Gene Polymorphisms

Polymorphic regions at IL-1β rs572292175 and rs571556428, and at IL-6 rs1474348 and rs543214973 were amplified *via* PCR. Total PCR system was 25 μL, including 1 μL of forward primers, 1 μL of reverse primers, 1 μL of DNA template, 12.5 μL of Taq enzymes, and 9.5 μL of ddH_2O. PCR conditions were as follows: 95°C for 5 min, (95°C for 35 s, 56°C for 40 s, 72°C for 30 s) × 35 cycles, 72°C for 5 min, and continued at 4°C. Primers of polymorphic loci were shown in Table I. All PCR products were sent to Jiangxi Biotechnology Co., Ltd. (Nanchang, China) for sequencing. IL-1β and IL-6 gene polymorphisms were finally analyzed in both groups.

Detection of IL-1β and IL-6 Gene Expressions

The expressions of IL-1β and IL-6 were detected *via* real-time fluorescence quantitative PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference. Gene primers were designed using Prime Premier 5.0 and synthesized by Sangon (Shanghai, China) (Table I).

Table I. Primer sequences of loci and genes.

	Forward/reverse	Primer sequence
rs572292175	Forward	ATGATGGCTTATTACAGTGGCAA
	Reverse	GTCGGAGATTCGTAGCTGGA
rs571556428	Forward	AGCTACGAATCTCCGACCAC
	Reverse	CGTTATCCCATGTGTCGAAGAA
rs1474348	Forward	TTCGACACATGGGATAACGAGG
	Reverse	TTTTTGCTGTGAGTCCCGGAG
rs543214973	Forward	GAAATGCCACCTTTTGACAGTG
	Reverse	TGGATGCTCTCATCAGGACAG
IL-1β	Forward	TTCAGGCAGGCAGTATCACTC
	Reverse	GAAGGTCCACGGGAAAGACAC
IL-6	Forward	CTGTGACTCATGGGATGATGATG
	Reverse	CGGAGCCTGTAGTGCAGTTG
GAPDH	Forward	GCAACTGTTCCTGAACTCAACT
	Reverse	ATCTTTTGGGGTCCGTCAACT

Grading of Parkinson's Disease

Parkinson's disease was graded using the Hoehn-Yahr grading criteria as follows: grade 1: disease in the unilateral limb, grade 1.5: symptoms in the unilateral limb and trunk (axis), grade 2: symptoms in bilateral limbs with no balance disorder, grade 2.5: mild symptoms in bilateral limbs that are able to recover from the posterior drawer test, grade 3: mild-moderate symptoms in bilateral limbs that are unable to recover from the posterior drawer test, unstable posture, slow in turning, and limitation of many functions with self-care ability, grade 4: severe disability with ability to stand and walk without help, and grade 5: sitting in a wheelchair or lying in bed with complete dependence on others.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) was used for all statistical analysis. Measurement data were compared using *t*-test, and Hardy-Weinberg equilibrium test was adopted. Analysis of variance followed by Post-Hoc Test (Least Significant Difference) was performed for comparison of enumeration data among groups, and SHEsis website for haplotype analysis. $p<0.05$ was considered statistically significant.

Results

Allele Distributions at IL-1β rs572292175 and rs571556428 and at IL-6 rs1474348 and rs543214973 in Both Groups

As shown in Table II, the allele distributions at IL-1β rs571556428 ($p=0.015$) and IL-6 rs543214973 ($p=0.012$) were statistically different between control group and disease group. In disease group, G allele frequency at IL-1β rs571556428 and T allele frequency at IL-6 rs543214973 were significantly higher ($p<0.05$).

Genotype Distributions at IL-1β rs572292175 and rs571556428 and at IL-6 rs1474348 and rs543214973 in Both Groups

As shown in Table III, the genotype distributions at IL-1β rs572292175 ($p=0.017$) and rs571556428 ($p=0.000$), and IL-6 rs543214973

Table II. Allele distributions at IL-1β rs572292175 and rs571556428 and at IL-6 rs1474348 and rs543214973 in both groups.

Gene	Locus	Allele	Control group	Disease group	OR	95% CI	χ^2	p
IL-1β	rs572292175	C	206 (0.515)	216 (0.540)	1.12	0.83-1.45	0.51	0.478
		T	194 (0.485)	184 (0.460)				
	rs571556428	A	236 (0.590)	202 (0.505)	0.72	0.53-0.93	5.83	0.015
		G	164 (0.410)	198 (0.495)				
IL-6	rs1474348	C	182 (0.455)	190 (0.475)	1.08	0.82-1.43	0.32	0.571
		G	218 (0.545)	210 (0.525)				
	rs543214973	A	237 (0.593)	202 (0.505)	0.75	0.53-0.92	6.18	0.012
		T	163 (0.407)	198 (0.495)				

Table III. Genotype distributions at IL-1β rs572292175 and rs571556428 and at IL-6 rs1474348 and rs543214973 in both groups.

Gene	Locus	Genotype	Control group	Disease group	χ^2	p
IL-1β	rs572292175	CC	43 (0.215)	62 (0.310)	8.11	0.017
		CT	120 (0.600)	92 (0.460)		
		TT	37 (0.185)	46 (0.230)		
	rs571556428	AA	79 (0.395)	46 (0.230)	14.17	0.000
		AG	78 (0.390)	110 (0.550)		
		GG	43 (0.215)	44 (0.220)		
IL-6	rs1474348	CC	40 (0.200)	43 (0.215)	0.35	0.838
		CG	102 (0.510)	104 (0.520)		
		GG	58 (0.290)	53 (0.265)		
	rs543214973	AA	76 (0.380)	45 (0.225)	11.83	0.002
		AT	85 (0.425)	112 (0.560)		
		TT	39 (0.195)	43 (0.215)		

(p=0.002) in disease group were also different from those in control group. The frequencies of CT genotype at IL-1β rs572292175, AA genotype at IL-1β rs571556428 and AA genotype at IL-6 rs543214973 in disease group were remarkably lower (p<0.05).

Analysis of Polymorphisms at IL-1β rs572292175 and rs571556428 and at IL-6 rs1474348 and rs543214973 in Both Groups

After modeling and analysis, it was found that the distributions of recessive model at IL-1β rs571556428 (p=0.012) and IL-6 rs543214973 (p=0.014) in disease group exhibited significant differences from those in control group (Table IV).

Haplotype Analysis of IL-1β rs572292175 and rs571556428 and IL-6 rs1474348 and rs543214973 in Both Groups

Haplotype analysis indicated that the frequencies of TA haplotype at IL-1β rs572292175 and rs571556428 (p=0.038) and GA haplotype at IL-6 rs1474348 and rs543214973 (p=0.047) in disease group were significantly lower than those in control group (Table V).

Associations of Polymorphisms at IL-1β rs572292175 and rs571556428 and IL-6 rs1474348 and rs543214973 With Gene Expression in Both Groups

The polymorphisms at IL-1β rs571556428 and IL-6 rs1474348 were significantly associated with gene expression (p<0.05). In addition, the expressions of IL-1β and IL-6 rose remarkably in patients with GG genotype at rs571556428 and CG genotype at rs1474348, respectively (Figures 1-4).

Associations of Polymorphisms at IL-1β rs572292175 and rs571556428 and IL-6 rs1474348 and rs543214973 With Grade of Parkinson's Disease in Both Groups

The polymorphism at IL-1β rs571556428 was significantly correlated with the grade of Parkinson's disease (p=0.000). Parkinson's disease was in a higher grade (grade 4-5) in patients with AA genotype, whereas in a lower grade (grade 1-2) in patients with GG and AG genotypes (Table VI).

Discussion

Parkinson's disease and Alzheimer's disease are the most prevalent and incurable neurodegenerative diseases in elderly people. The number of these patients rises with the social aging[10,11]. The pathogenesis of Parkinson's disease is mainly related to the degeneration and death of dopaminergic neurons. Therefore, the symptoms of patients can be partially alleviated by dopamine preparations[12]. Currently, studies have found that the onset of Parkinson's disease is associated with mitochondrial dysfunction caused by a variety of factors, as well as the uptake of neurotoxic substances[13]. Genetic factors, including mutation in related pathogenic genes, increase in copy number of chromosome and partial gene deletion, may also be important causes of the disease[14]. Due to random changes in some bases in the genetic process, gene polymorphisms are considered correlated with the onset of Parkinson's disease, such as those at DBH rs1611115[15] and SNCA rs2736990[16]. Therefore, the exploration of the association between gene polymorphisms and the onset of Parkin-

Table IV. Analysis of polymorphisms at IL-1β rs572292175 and rs571556428 and at IL-6 rs1474348 and rs543214973 in both groups.

	Gene	Locus	Genotype	Control group	Disease group	χ^2	p
Dominant model	IL-1β	rs572292175	CC+CT	163 (0.815)	154 (0.730)	3.15	0.207
			TT	37 (0.185)	46 (0.230)		
		rs571556428	AA+AG	157 (0.785)	156 (0.780)	3.95	0.139
			GG	43 (0.215)	44 (0.220)		
	IL-6	rs1474348	CC+CG	142 (0.710)	147 (0.735)	3.79	0.150
			GG	58 (0.290)	53 (0.265)		
		rs543214973	AA+AT	161 (0.805)	157 (0.785)	2.59	0.274
			TT	39 (0.195)	43 (0.215)		
Recessive model	IL-1β	rs572292175	CC	43 (0.215)	62 (0.310)	5.87	0.053
			CT+TT	157 (0.785)	138 (0.690)		
		rs571556428	AA	79 (0.395)	46 (0.230)	8.82	0.012
			AG+GG	121 (0.605)	154 (0.770)		
	IL-6	rs1474348	CC	40 (0.200)	43 (0.215)	4.04	0.133
			CG+GG	160 (0.800)	157 (0.785)		
		rs543214973	AA	76 (0.380)	45 (0.225)	8.54	0.014
			AT+TT	124 (0.620)	155 (0.775)		
Heterozygous model	IL-1β	rs572292175	CC	43 (0.215)	62 (0.310)	4.31	0.116
			CT	120 (0.600)	92 (0.460)		
		rs571556428	AA	79 (0.395)	46 (0.230)	5.16	0.076
			AG	78 (0.390)	110 (0.550)		
	IL-6	rs1474348	CC	40 (0.200)	43 (0.215)	5.2	0.074
			CG	102 (0.510)	104 (0.520)		
		rs543214973	AA	76 (0.380)	45 (0.225)	2.13	0.345
			AT	85 (0.425)	112 (0.560)		
Homozygous model	IL-1β	rs572292175	CC	43 (0.215)	62 (0.310)	3.52	0.172
			TT	37 (0.185)	46 (0.230)		
		rs571556428	AA	79 (0.395)	46 (0.230)	4.05	0.132
			GG	43 (0.215)	44 (0.220)		
	IL-6	rs1474348	CC	40 (0.200)	43 (0.215)	2.79	0.248
			GG	58 (0.290)	53 (0.265)		
		rs543214973	AA	76 (0.380)	45 (0.225)	5.37	0.068
			TT	39 (0.195)	43 (0.215)		

son's disease can help to clarify the pathogenesis of the disease. Furthermore, this may have certain significance for early screening and prevention of the disease.

Cytokines are important mediators in regulating immune and inflammatory responses in the body. They mainly include ILs, tumor necrosis factors and chemokines[17]. In patients with Parkinson's disease, a neurodegenerative disease, immune system disorders may occur. Meanwhile, various cytokines and immune cells may cause certain damage to dopaminergic neurons[18,19]. As important members of the IL family, IL-1 and IL-6 possess a potent pro-inflammatory effect. They can synergistically stimulate the proliferation and differentiation of T cells, enhance immune response, and mediate the protection of immune cells on tissues and cells[20]. Moreover,

Table V. Haplotype analysis of IL-1β rs572292175 and rs571556428 and IL-6 rs1474348 and rs543214973 in both groups.

Gene	Haplotype	Control group	Disease group	OR	95% CI	χ^2	p
IL-1β	CA	118.25 (0.296)	107.95 (0.270)	0.881	0.647-1.198	0.653	0.419
	CG	87.75 (0.219)	108.05 (0.270)	1.317	0.953-1.820	2.785	0.095
	TA	117.75 (0.294)	94.05 (0.235)	0.737	0.537-1.010	3.608	0.038
	TG	76.25 (0.191)	89.95 (0.225)	1.232	0.875-1.735	1.427	0.232
IL-6	CA	94.77 (0.237)	85.74 (0.214)	0.879	0.630-1.224	0.584	0.445
	CT	87.23 (0.218)	104.26 (0.261)	1.264	0.913-1.751	1.993	0.158
	GA	142.23 (0.356)	116.26 (0.291)	0.743	0.552-1.000	3.853	0.047
	GT	75.77 (0.189)	93.74 (0.234)	1.31	0.932-1.841	2.415	0.120

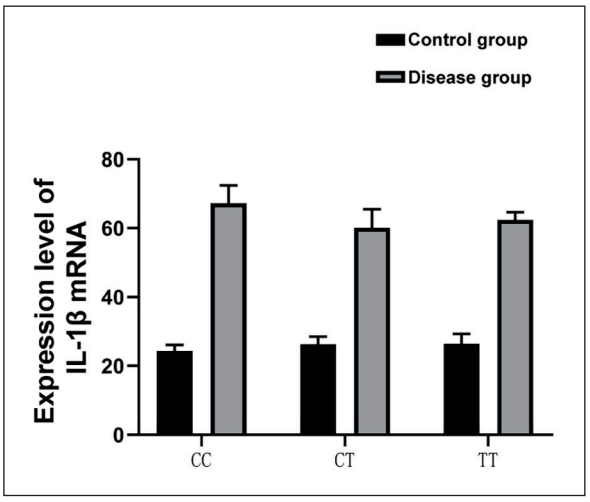

Figure 1. Association between polymorphism at IL-1β rs572292175 and gene expression.

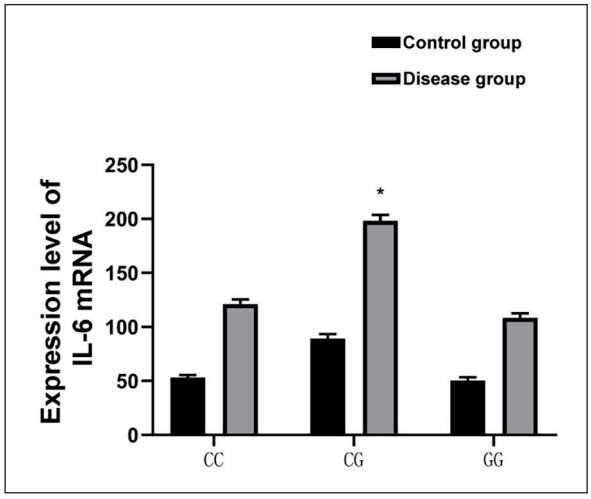

Figure 3. Association between polymorphism at IL-6 rs1474348 and gene expression (*p<0.05).

IL-1β and IL-6 gene polymorphisms play important roles in the occurrence and development of various diseases[21]. In this study, the differences in the polymorphisms at IL-1β rs572292175 and rs571556428 and at IL-6 rs1474348 and rs543214973 in peripheral blood nucleated cells were compared between control group and disease group. The results indicated that the allele distributions at IL-1β rs571556428 (p=0.015) and IL-6 rs543214973 (p=0.012) were significantly different between control group and disease group. In disease group, the G allele frequency at IL-1β rs571556428 and T allele frequency at IL-6 rs543214973 were higher (p<0.05). Besides, the genotype distributions at IL-1β rs572292175 (p=0.017) and rs571556428 (p=0.000), and IL-6 rs543214973 (p=0.002) in disease group were also different from those in control group. The frequencies of CT genotype at IL-1β rs572292175, AA genotype at IL-1β rs571556428 and AA genotype at IL-6 rs543214973 in disease group were remarkably lower (p<0.05). The above results demonstrated that IL-1β and IL-6 gene polymorphisms could cause changes in people's susceptibility to Parkinson's disease. All our findings might have important significance for the prevention and control of Parkinson's disease in high-risk group.

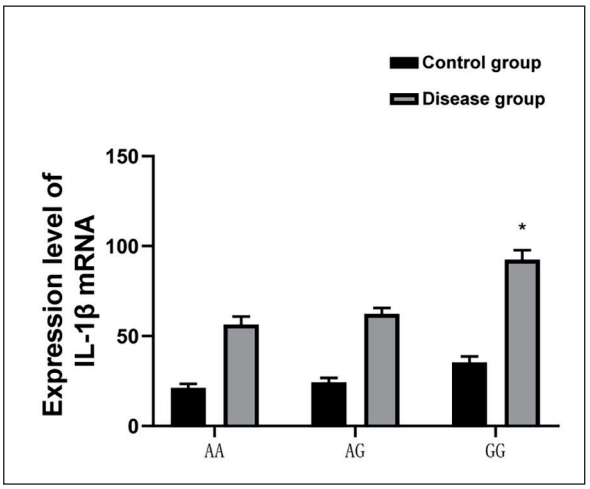

Figure 2. Association between polymorphism at IL-1β rs571556428 and gene expression (*p<0.05).

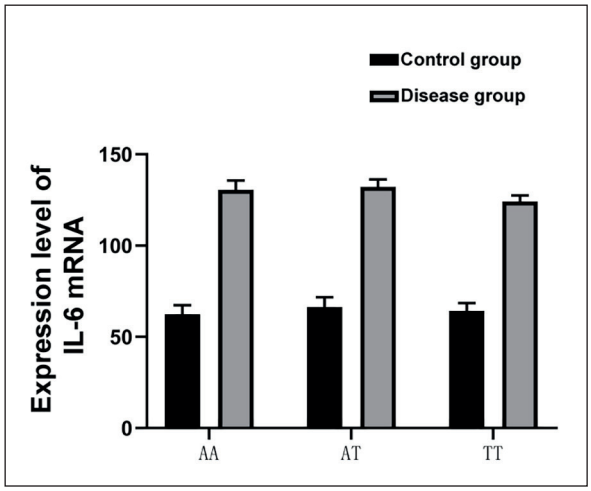

Figure 4. Association between polymorphism at IL-6 rs543214973 and gene expression.

Table VI. Associations of polymorphisms at IL-1β rs572292175 and rs571556428 and IL-6 rs1474348 and rs543214973 with grade of Parkinson's disease in both groups (%).

Gene	Locus	Genotype	Grade 1	Grade 1.5	Grade 2	Grade 2.5	Grade 3	Grade 4	Grade 5	*p*
IL-1β	rs572292175	CC	12	16	22	18	19	4	9	0.212
		CT	17	24	12	18	14	5	10	
		TT	23	21	11	16	18	9	2	
	rs571556428	AA	1	4	11	13	14	29	28	0.000
		AG	17	24	29	12	3	5	10	
		GG	34	19	8	12	9	10	8	
IL-6	rs1474348	CC	3	10	13	18	28	11	17	0.178
		CG	4	27	21	11	23	12	2	
		GG	2	16	27	12	14	20	9	
	rs543214973	AA	6	24	16	19	21	11	3	0.362
		AT	9	20	11	21	18	17	4	
		TT	13	18	26	19	11	7	6	

After further modeling and analysis, it was discovered that the distributions of recessive model at IL-1β rs571556428 (p=0.012) and IL-6 rs543214973 (p=0.014) in disease group exhibited significant differences from those in control group. The frequencies of TA haplotype at IL-1β rs572292175 and rs571556428 (p=0.038) and GA haplotype at IL-6 rs1474348 and rs543214973 (p=0.047) in disease group were remarkably lower in control group (p<0.05). It could be seen that the effect of gene polymorphisms on Parkinson's disease might not be caused only by a single locus or genotype, but by the combined effect of multiple loci and genotypes.

Polymorphisms at IL-1β rs571556428 and IL-6 rs1474348 were significantly associated with gene expression (p<0.05). Meanwhile, the expressions of IL-1β and IL-6 rose significantly in patients with GG genotype at rs571556428 and CG genotype at rs1474348, respectively (p<0.05). The above findings suggested that the immuno-inflammatory level was higher in patients with Parkinson's disease, and the immune system might play an important role in the disease and has a correlation with gene polymorphisms. In addition, experimental results showed that the polymorphism at IL-1β rs571556428 was significantly correlated with the grade of Parkinson's disease (p=0.000). Parkinson's disease was in a higher grade (grade 4-5) in patients with AA genotype, while in a lower grade (grade 1-2) in patients with GG and AG genotypes.

Conclusions

All these results indicate that the polymorphism at IL-1β rs571556428 is not only related to the pathogenesis, but also to the severity of Parkinson's disease. However, its specific mechanism remains to be further explored.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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