Miri NS, et al. Iran J Immunol. 2020; 17(4):313-323.
https://doi.org/10.22034/iji.2020.87454.1803.

ORIGINAL ARTICLE

Toll-Like Receptor (TLR)-9 rs352140 Polymorphism is an Immunopathology Protective Factor in Parkinson's Disease in the Northern Iranian Population

Niloufar Sadat Miri[1], Payam Saadat[2], Abbas Azadmehr[1*], Morteza Oladnabi[3], Abdolreza Daraei[1]

[1]Cellular and Molecular Biology Research Center, [2]Mobility Impairment Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, [3]Gorgan Congenital Malformations Research Center, Golestan University of Medical Sciences, Gorgan, Iran

ABSTRACT

Background: Neuroinflammation and immunopathology in Parkinson's disease (PD) is believed to be associated with genetic and environmental factors. **Objective:** We conducted the current study to evaluate the Toll-like receptors (TLR4 and TLR9) genes polymorphism in the patients suffering from Parkinson's disease in northern Iran. **Methods:** In this study, we extracted DNA from peripheral blood samples of unrelated 100 cases of Parkinson's disease and 100 healthy-matched controls with the mean age of 69.98 and 71.94 years, respectively. Subsequently, single-nucleotide polymorphisms (SNPs) of TLR4 and TLR9 were genotyped through restriction fragment length polymorphism-polymerase chain reaction (RFLP–PCR). The outcomes were confirmed employing Sanger sequencing. For the analysis of our data, we utilized SNPStats software and SPSS 22. **Results:** Our findings indicated that the allele distribution was meaningfully different in the PD group compared with the healthy control (p=0.02) with respect to rs352140 belonging to TLR9 gene. Moreover, rs352140T allele carriers were observed to be correlated with PD reduced risk (TT + TC vs. CC). The dominant rs352140 model was approved as the most acceptable inheritance model for fitting the data (OR 0.41, 95% CI 0.23-0.75, p=0.0031). Additionally, haplotype analysis revealed a meaningful correlation between TLR9 polymorphisms and Parkinson's disease. **Conclusion:** The obtained results in this study indicated that rs352140T of TLR9 gene was a protective factor in Parkinson's disease. Furthermore, this SNP could be an efficient prognosis and may be a prophylactic or therapeutic target. However, future investigations are of great necessity.

Received: 2020-07-20, Revised: 2020-12-13, Accepted: 2020-12-20.
Citation: Miri NS, Saadat P, Azadmehr A, Oladnabi M, Daraei A. Toll-Like Receptor (TLR9) rs352140 Polymorphism is an Immunopathology Protective Factor in Parkinson's Disease in the Northern Iranian Population. *Iran J Immunol.* 2020; 17(4):313-323.

Keywords: Parkinson's Disease, Single-Nucleotide Polymorphisms (SNPs), Toll-Like Receptors 4, Toll-Like Receptors 9

*Corresponding author: Dr. Abbas Azadmehr, Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran, e-mail: azadmehr2010@gmail.com

INTRODUCTION

Neuroinflammation acts as a pivotal factor in the pathogenesis of various infectious and non-infectious neurodegenerative disorders, Parkinson's diseases for instance (1). PD is considered as the second most prevalent chronic progressive neurodegenerative disease with a multifactorial nature in which both genetic and environmental factors interact to develop this disease in older ages (2-5). Parkinson's disease is a disorder with progressive movement. Its clinical manifestations are characterized by motor features, including resting tremor, bradykinesia, rigidity, and postural instability (6,7). It has been shown that dopaminergic neurons are destroyed in PD and alpha-synuclein is found as intracellular lewy bodies in the dissection of postmortem brains. Alpha-synuclein is a monomeric protein that plays physiologic roles in restricting the mobility of synaptic vesicles and neurotransmitter release. This protein may contribute to PD immunopathogenesis when this soluble oligomeric toxic conformation is formed, it can lead to dopaminergic neuronal cell death (8,9). However, the cause of PD remains elusive. It has been found that the overactivation of immune cells, specifically microglia in CNS through pattern recognition receptor (PRR) molecules such as toll like receptors (TLRs) that identify pathogen- associated molecular patterns (PAMPs) and danger/damage-associated molecular patterns (DAMPs). Afterwards, it produces inflammatory mediators associated with immunopathogenesis of PD (10-12). Toll like receptors, such as TLR4 and TLR9, are expressed in microglia cells and recognized in the pathogenic and non-photogenic agents in CNS. These receptors are capable of activating microglia through NF-κB signaling pathways in order to produce inflammatory cytokines, such as IL-6, IL-1B, and TNF-α. There is ample evidence indicating that α-syn can directly activate local microglia and initiate inflammatory condition by the overexpression of TLR4 and TLR9 (13-16). On the other hand, previous studies have indicated that the upregulation of TLR9 and TLR4 and even NF-κB inhibitors could block the neurodegeneration process in animal models (17,18). Certain studies have investigated the association of TLR4 and TLR9 polymorphism genes with the development of autoimmune diseases including multiple sclerosis (MS) and Alzheimer's disease (AD) (19-22). For note, Iran is a country with a high heterogeneous where population genetic studies is largely neglected (23). Moreover, previous studies have shown the associations between variants of TLR4 and TLR9 Loci in different populations. Meanwhile, no studies have been conducted in Iran on the correlation of these genes with the Parkinson's disease. The present research, for the first time, aimed to investigate the effect of polymorphisms on TLR9 (rs187084 and rs352140) and TLR4 (rs1927911, rs1927914, rs10116253) genes concerning the susceptibility of PD in the north of Iranian population.

MATERIALS AND METHODS

Study Subjects. The case group comprised 100 Parkinson's disease patients (48 females and 52 males) with a mean age of 69.98 years. They were diagnosed as sporadic PD under the supervision of a neurologist through determining the severity of the disease and the priority of the four symptoms including tremor, bradykinesia, rigidity, and postural instability. The severity of Parkinson's disease was based on the Unified Parkinson's disease Rating Scales (UPDRS) presented by Movement Disorder

Society (MDS) (24). We used Hoehn and Yahr's scale for examining the disease clinical staging (25). The patients with family history of Parkinsonism,secondary forms of parkinson's, neurological and psychiatric conditions were excluded. In addition, 100 healthy individuals (46 females and 54 males) with the mean age of 71.94 years, without any clear neurodegenerative history of disorder, were selected randomly and enrolled as the control group. The exclusion criterion was having a diagnosis of any immune-associated or other diseases. Two groups of case and control were matched according to their age and gender. For note, the entire population participating in the current study were Iranian unrelated families. The current study was confirmed by the Babol University of Medical Sciences Ethical Committee (IR.MUBABOL.HRI.REC.1397.130). Consent form was filled out by all our participants.

Genomic DNA Extraction and Screening of TLR4 and TLR9 SNPs. A total volume of 2 ml whole blood was taken from all the participants. Genomic DNA was extracted from peripheral blood DNA extraction kit (Favorgen, Taiwan and kowsar Biotech Company, Iran) according to the manufacturer's protocols. We stored the extracted DNA at -20ºC until polymerase chain reaction (PCR) amplifications were conducted. Polymorphisms of TLR9, namely rs187084 and rs352140, together with polymorphisms of TLR4, namely rs1927914, rs1927911 and rs10116253, were genotyped in all the subjects with polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) method with primers designed by online primerQuest tool–IDT (26). Table 1 represents all the primers and restriction enzymes used in this study.

Table 1. Characteristics and conditions of PCR-RFLP technique for genotyping of selected SNPs

Gene	SNP	Primer sequence	Product size (bp)	Restriction enzyme	Restriction fragments of genotype (bp)
TLR4	rs1927914	F:GAGAGCTATGATGAGGATTG R: TTGATGGAGTCTACAAGAG	308	SphI	308= TT 308,185,123= TC 185,123=CC
	rs10116253	F:GGGTGTAAAAGCCAGGTAGAGGAGT R:TGGAAAGTAGCAAGTGCAATGTAAT	601	BsmAI	601 = CC 601, 470, 131 = TC 470, 131 = TT
	rs1927911	F:GTCAAGATGTCCAGACCTTC R:GGGAGCGTTAGAGAATTAG	238	BsaJI	111,127= TT 59,68,111= CC 59,68,111,127= TC
TLR9	rs352140	F: CTTGGCTGTGGATGTTGTTG R: AGTCAATGGCTCCCAGTTC	239	Bst UI	136,103=CC 239,136,103=TC 239=TT
	rs187084	F: GCCTTCACTCAGAAATACCC R: CTAGCACACCGGATCATTG	214	Afl II	77,137=TT 214,77,137=TC 214=CC

All the PCR Amplifications were performed at a final volume of 20 µL, containing 12.5 µL of Taq DNA polymerase 2x master mix red (Ampliqon®, Denmark), genomic DNA (50 ng), 5 pmol/L of each primer, and 3.5 µl of sterile water. The conditions related to the rs1927911 polymorphism of TLR4 were as follows: initial denaturation for 5 min at 94°C along with 36 denaturation cycles for 30 s at 94°C, annealing for 30 s at 56°C, extension for 30 s at 72°C, and an ultimate extension for 5 min at 72°C. Moreover, the rs10116253 polymorphism of TLR4 amplification conditions was carried out with an initial denaturation step: for 5 min at 94°C, 35 denaturation cycles for 30s at 94°C, hybridization for 30 s at 63°C, extension for 1 min at 72°C, and an ultimate extension for 5 min at 72°C. For the amplification, the target region included rs1927914 polymorphism of TLR4 as follows: initial denaturation for 5 min at 94°C along with 36 denaturation cycles for 30 s at 94°C, annealing for 30 s at 54°C, extension for 30 s at 72°C, and an ultimate extension for 5 min at 72°C. Furthermore, we determined the PCR conditions of TLR9. The reaction conditions for rs352140 polymorphisms of TLR9 were as follows: denaturation for 5 min at 94°C along with 36 denaturation cycles for 30 s at 94°C and 58°C, extension for 30 s at 72°C, and an ultimate extension for 5 min at 72°C. Regarding the rs187084 polymorphism of TLR9, the PCR temperature cycling was as follows: initial denaturation for 5 min at 94°C along with 36 denaturation cycles for 30 s at 94°C, annealing for 30 s at 60°C, extension for 30 s at 72°C, and an ultimate extension for 5 min at 72°C. The PCR products were digested by restriction enzymes (Ferments Co, Lithuania) according to the Table 1. All the reactions were incubated at 37°C overnight with 65°C inactivation for 20 min. However, the rs1927911 system underwent incubation overnight at 55°C and for the inactivation of the enzyme, the samples were put in 80°C for 20 min. The digestion products were visualized under UV radiation following the electrophoresis on a 1.5 % agarose gel containing SYBR safe DNA stain (Sinaclon Co, Iran). Furthermore, to confirm the PCR-RFLP results, we performed Sanger sequencing on some samples.

Statistical Analysis. Direct counting was utilized to estimate allele and genotype frequencies. SPSS Statistics 22.0 and SNPStats software were used to run all the statistical analyses (27). We employed SNPStats software to measure the Hardy–Weinberg equilibrium (HWE) between the observed and the expected genotype distribution, relative risk conferred by a specific genotype and allele, haplotype analysis, odds ratio (OR) adjusted by gender, 95% confidence intervals (CI) and age in the logistic model of regression (28). Different genetic models, including over-dominant, dominant, co-dominant, recessive, and log-additive inheritance, were evaluated with SNPStats software. In addition, the distribution difference in allele and genotype between the patients and controls was assessed using the Chi-square test. The power of the test was calculated using G-power 3.1.9.2 software (Universitat Kiel, Germany). P-values below 0.05 were regarded as statistically significant.

RESULTS

In the current study, we presented the clinical characteristics and demographic data of the subjects as could be seen Table 2. Moreover, Table 3 depicts the genotype and allele distributions for the five studied TLR polymorphisms in both the PD and

control groups. Our findings indicated a meaningful difference in allele distribution in the rs352140 polymorphism of TLR9 with a comparison between the PD group and the control group. Additionally, no meaningful differences were observed between the PD and control groups in terms of the other examined SNPs. Genotype distributions of five SNPs examined in this study were not consistent with those in Hardy-Weinberg equilibrium (HWE) ($p>0.05$) except for rs352140 polymorphism of TLR9. The frequencies of rs352140 polymorphisms were found in the Hardy–Weinberg equilibrium among all the subjects, patients, and controls. The frequencies of rs10116253 polymorphism of TLR4 gene was found in the Hardy–Weinberg equilibrium among the patients and the controls ($p>0.05$), which was not in all the subjects ($p<0.05$). The association analysis was performed following inheritance modeling of the rs352140 polymorphism of TLR9 effects using logistic regression. The rs352140 polymorphism showed a significant difference in the co-dominant (OR 0.27, 95% CI 0.11– 0.66, p=0.0054), dominant (OR 0.41, 95% CI 0.23–0.75, p=0.0031), and recessive models (OR 0.42, 95% CI 0.19–0.94, p=0.03) (Table 4).

Table 2. The demographic and clinical characteristics of all study subjects

Variable	PD group (n = 100)	Control group (n = 100)
Gender (male/female)	52/48	54/46
Age range(years)	49-97	51-92
Mean age ± SD (years)	69.98 ± 9.65	71.94 ± 9.56
Duration of PD (years)	6.1 ± 4.7	-
UPDRS score	50.16 ± 25.6	-
Hoehn and Yahr stage	3 (1–5)	-

Among them, the dominant model was selected as the most acceptable inheritance model for fitting the data since it had a lower value in terms of Akaike information criterion (AIC) (272.5). Similar results were demonstrated after adjusting gender and age (Table 4). Our results revealed the lack of association between TLR4 examined polymorphisms and the risk of PD ($p>0.05$). Even though T/C genotype in the subjects rs187084 of TLR9 showed the risk of PD, no significant associations were observed ($p>0.05$). It is noteworthy that the power of rs352140 SNP among genotype and allele were respectively 0.8270 and 0.8863.

Table 3. Allele and genotype frequencies of selected SNPs in TLR4 and TLR9 genes in patients and controls

Gene		Genotype					Allele		
TLR9	rs352140	N	CC (%)	TC (%)	TT (%)	P	C (%)	T (%)	P-value
	PD	100	26 (26)	53 (53)	21 (21)	0.06	105 (52.5)	95 (47.5)	0.02
	Control	100	46 (46)	44 (44)	10 (10)		136 (68)	64 (32)	
	rs187084	N	CC (%)	TC (%)	TT (%)	P	C (%)	T (%)	P-value
	PD	100	11 (11)	89 (89)	0 (0)	0.205	111 (55.5)	89 (44.5)	0.616
	Control	100	6 (6)	94 (89)	0 (0)		106 (53)	94 (47)	
TLR4	rs1927914	N	CC (%)	TC (%)	TT (%)	P	C (%)	T (%)	P-value
	PD	100	0 (0)	57 (57)	43 (43)	0.395	57 (28.5)	143 (71.5)	0.461
	Control	100	0 (0)	51 (51)	49 (43)		51 (25.5)	149 (74.5)	
	rs1927911	N	CC (%)	TC (%)	TT (%)	P	C (%)	T (%)	P-value
	PD	100	28 (28)	72 (72)	0 (0)	1.00	128 (64)	72 (36)	1.00
	Control	100	28 (28)	72 (72)	0 (0)		128 (64)	72 (36)	
	rs10116253	N	CC (%)	TC (%)	TT (%)	P	C (%)	T (%)	P-value
	PD	100	3 (3)	44 (44)	53 (53)	0.959	50 (25)	150 (75)	0.683
	Control	100	3 (3)	42 (42)	55 (55)		48 (24)	152 (76)	

Haplotype Analysis. Ultimately, the haplotypes of TLR4 and TLR9 genes were studied in the above-mentioned population (Table 5,6). Based on our results, Global haplotypes of TLR9 gene were associated with PD.

Table 4. Association of rs352140 polymorphism in TLR9 gene with PD under different genetic models

Model	Genotype	Patient (%)	Control (%)	OR (95% CI)	P-value	AIC	OR (95% CI)	P-value	AIC
	Not adjusted						**Adjusted by covariates***		
Codominant	C/C	26 (26%)	46 (46%)	1.00			1.00		
	T/C	53 (53%)	44 (44%)	0.47 (0.25-0.88)	0.0054	272.8	0.45 (0.24-0.86)	0.004	273.6
	T/T	21 (21%)	10 (10%)	0.27 (0.11-0.66)			0.26 (0.10-0.63)		
Dominant	C/C	26 (26%)	46 (46%)	1.00			1.00		
	T/C-T/T	74 (74%)	54 (54%)	0.41 (0.23-0.75)	0.0031	272.5	0.40 (0.22-0.73)	0.0023	273.4
Recessive	C/C-T/C	79 (79%)	90 (90%)	1.00			1.00		
	T/T	21 (21%)	10 (10%)	0.42 (0.19-0.94)	0.03	276.6	0.41 (0.18-0.92)	0.026	277.7
Over dominant	C/C-T/T	47 (47%)	56 (56%)	1.00			1.00		
	T/C	53 (53%)	44 (44%)	0.70 (0.40-1.22)	0.2	279.6	0.69 (0.39-1.21)	0.19	281

OR, odds ratio; 95% CI, 95% confidence intervals; AIC, Akaik's information criterion
*Covariates are gender and age

DISCUSSION

Parkinson's disease is a complex and heterogeneous disease; however, its exact cause remains poorly understood (29, 30). Various studies have been done to determine the impact of immunogenic factors on the Parkinson's disease development. Inflammation is one of the major causes of the pathogenesis of Parkinson's disease.
The innate immunity in these patients is associated with the activation of microglia by TLRs receptors (31). In activated microglia, TLR4 and TLR9 initiate signal transduction cascades, including the nuclear NF-κB pathway (32). Moreover, it was illustrated that certain NF-κB inhibitors can reduce the progression of neurodegeneration murine PD models (18). Recent studies on the immunopathogenesis of Parkinson's disease have revealed that aggregated α-synuclein may activate local microglia, leading to the production of pro-inflammatory cytokines through TLR4 and TLR9 pathway (15,33,34). In addition, it has been shown that the expression level of TLR4 and TLR9 increased in Parkinson's disease patients and that these gene polymorphisms might be associated with PD (15,21).

Table 5. Haplotype association of TLR4 gene polymorphism with PD

Gene	Haplotype	Frequency	OR (95% CI)	P-value
TLR4	TTC	0.4884	1.00	---
	TTT	0.1939	0.79 (0.30 – 2.04)	0.63
	CCT	0.1061	0.65 (0.25 – 1.68)	0.37
	CCC	0.0912	0.74 (0.24 – 2.31)	0.61
	CTT	0.0508	1.42 (0.35 – 5.76)	0.63
	TCC	0.0385	1.04 (0.27 – 4.00)	0.96
	CTC	0.0219	0.17 (0.02 – 1.76)	0.14
	Rare	0.0092	94277485.06	<0.0001

Global haplotype association P-value: 0.47
Interaction P-value: 0.089

Therefore, the current study examined the association between PD and TLR4 and TLR9 polymorphisms in the affected population in northern Iran. No studies have been reported concerning the role of the TLR4 and TLR9 variants in the Iranian population. Our study also evaluated the potential correlation between the TLR4 (namely, rs1927911, rs1927914 and rs10116253) and TLR9 (namely, rs352140 and rs187084) gene polymorphisms with the PD risk for the first time. The rs1912911 SNP is located

in the first intron whereas the rs1927914, rs10116253 and rs187084 SNPs are located in 5′-UTR. Moreover, the rs352140 SNP is located in exon 2 (P[Pro] P[Pro]) (35). In the current work, we showed that only rs352140 polymorphism may affect the development of PD. A significant difference was detected in the rs352140 allele distribution between the PD patients and the healthy controls.

Table 6. Haplotype association of TLR9 gene polymorphism with PD

Gene	Haplotype	Frequency	OR (95% CI)	P-value
TLR9	CT	0.3869	1.00	---
	TC	0.3269	0.44 (0.15 – 1.30)	0.14
	CC	0.2156	0.81 (0.22 - 3.01)	0.75
	TT	0.0706	0.40 (0.11 – 1.38)	0.15

However, the results demonstrated no significant correlations between TLR4 (namely rs1927911, rs1927914 and rs10116253) and TLR9 (namely, rs187084) polymorphisms and the PD risk. Our findings indicated that the above-mentioned polymorphisms may not contribute to PD susceptibility. However, further study is suggested. These five SNPs were previously studied in PD patients of another population. In agreement with our findings, no associations were found with rs10116253 and rs1912911 polymorphisms of TLR4 gene in Chinese population (36). In contrary to a study by Jing Zhao *et al.*, our results demonstrated that the rs1927914 of TLR4 gene was not associated with the Parkinson's disease. According to their findings, the rs1927914 polymorphism could reduce the risk of PD development. Moreover, they reported that the rs1927914C allele was as a protective agent against sporadic PD in a Han Chinese population. Despite the risk for developing PD in rs187084 of TLR9, T/C subjects, no significant associations were observed. Nevertheless, further study is suggested. In inheritance modeling analysis, the dominant, co-dominant, and recessive models indicated a meaningful correlation between rs352140 and PD. Based on the dominant model, T/T and T/C subjects showed about 59% reduction in the risk of PD compared to C/C subjects. Our findings indicated that rs352140 polymorphism of TLR9 might decrease the risk of PD development in the north of Iranian. Our results revealed that the T allele of rs352140 may have the potential to reduce the PD development and might also be regarded as a protective factor in Parkinson's disease. Similar results were reported by Konghua Zhu *et al.* (37). They realized that rs352140 T variant allele was less in female PD cases than that in the matched group. They also reported less susceptibility of rs352140 T allele carriers to PD. Moreover, previous studies have assessed discrepancies between the cases of PD and controls at onset and gender subgroup level (36,37). They revealed male and female PD pathogenesis to be potentially different and possibly associated with sex hormones (36,37). Thus, further investigations are recommended to determine the effects of these genetic changes, for

instance onset age, sex, and to generalize them to populations of various ethnicities in other countries for increasing the chance of developing PD.

ACKNOWLEDGEMENTS

We thank the deputy of research and technology and cellular and molecular biology research center, health research institute, and Babol University of Medical Sciences for financial support of this project (Grant No. 4730). This manuscript is extracted from Miss Niloufar Sadat Miri's thesis work for receiving the medical Master of Science degree in Medical Immunology. We are deeply grateful to those patients who took part in this research and profoundly appreciate their collaboration which made this study possible.

REFERENCES

1. Amor S, Puentes F, Baker D, van der Valk P. Inflammation in neurodegenerative diseases. Immunology. 2010; 129:154-69.
2. Reeve A, Simcox E, Turnbull D. Ageing and Parkinson's disease: why is advancing age the biggest risk factor? Ageing Res Rev. 2014; 14:19-30.
3. Warner TT, Schapira AH. Genetic and environmental factors in the cause of Parkinson's disease. Ann Neurol. 2003; 53 Suppl 3:S16-23; discussion S23-15.
4. Saadat P, Ahmadi Ahangar A, Samaei SE, Firozjaie A, Abbaspour F, Khafri S, et al. Serum Homocysteine Level in Parkinson's Disease and Its Association with Duration, Cardinal Manifestation, and Severity of Disease. Parkinsons Dis. 2018; 2018:5813084.
5. Wang X, Fan Y, Wang L, Chen B, Lu Y, Luo D. The association between the C-reactive protein gene +1444C/T polymorphism and Parkinson's disease susceptibility in a Chinese population. Gene. 2020; 753:144808.
6. Pahwa R, Factor SA, Lyons KE, Ondo WG, Gronseth G, Bronte-Stewart H, et al. Practice Parameter: treatment of Parkinson disease with motor fluctuations and dyskinesia (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology. 2006; 66:983-95.
7. Safari M, Jafari B, Zarbakhsh S, Sameni H, Vafaei AA, Mohammadi NK, et al. G-CSF for mobilizing transplanted bone marrow stem cells in rat model of Parkinson's disease. Iran J Basic Med Sci. 2016; 19:1318-24.
8. Ross CA, Poirier MA. Protein aggregation and neurodegenerative disease. Nat Med. 2004; 10 Suppl:S10-7.
9. Melki R. Alpha-synuclein and the prion hypothesis in Parkinson's disease. Rev Neurol (Paris). 2018; 174:644-52.
10. Ziakas PD, Prodromou ML, EI Khoury J, Zintzaras E, Mylonakis E. The role of TLR4 896 A>G and 1196 C>T in susceptibility to infections: a review and meta-analysis of genetic association studies. PLoS One. 2013; 8:e81047.
11. Creagh EM, O'Neill LA. TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. Trends Immunol. 2006; 27:352-7.
12. Dabagh-Gorjani F, Anvari F, Zolghadri J, KamaliSarvestani E, Gharesi-Fard B. Differences in the expression of TLRs and inflammatory cytokines in pre-eclamptic compared with healthy pregnant women. Iran J Immunol. 2014; 11:233-45.
13. Pandey S, Agrawal DK. Immunobiology of Toll-like receptors: emerging trends. Immunol Cell Biol. 2006; 84:333-41.
14. Harper SJ, Wilkie N. MAPKs: new targets for neurodegeneration. Expert Opin Ther Targets. 2003; 7:187-200.
15. Beraud D, Twomey M, Bloom B, Mittereder A, Ton V, Neitzke K, et al. alpha-Synuclein Alters Toll-Like Receptor Expression. Front Neurosci. 2011; 5:80.

16. Lehnardt S, Massillon L, Follett P, Jensen FE, Ratan R, Rosenberg PA, et al. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. Proc Natl Acad Sci USA. 2003; 100:8514-19.
17. Ghosh A, Roy A, Liu X, Kordower JH, Mufson EJ, Hartley DM, et al. Selective inhibition of NF-kappaB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. Proc Natl Acad Sci USA. 2007; 104:18754-9.
18. Peterson LJ, Flood PM. Oxidative stress and microglial cells in Parkinson's disease. Mediators Inflamm. 2012; 2012:401264.
19. Wang YL, Tan MS, Yu JT, Zhang W, Hu N, Wang HF, et al. Toll-like receptor 9 promoter polymorphism is associated with decreased risk of Alzheimer's disease in Han Chinese. J Neuroinflammation. 2013; 10:101.
20. Dominguez-Mozo MI, Garcia-Montojo M, Lopez-Cavanillas M, De Las Heras V, Garcia-Martinez A, Arias-Leal AM, et al. Toll-like receptor-9 in Spanish multiple sclerosis patients: an association with the gender. Eur J Neurol. 2014; 21:537-40.
21. Yu JT, Miao D, Cui WZ, Ou JR, Tian Y, Wu ZC, et al. Common variants in toll-like receptor 4 confer susceptibility to Alzheimer's disease in a Han Chinese population. Curr Alzheimer Res. 2012; 9:458-66.
22. Reindl M, Lutterotti A, Ingram J, Schanda K, Gassner C, Deisenhammer F, et al. Mutations in the gene for toll-like receptor 4 and multiple sclerosis. Tissue Antigens 2003; 61:85-8.
23. Mehrjoo Z, Fattahi Z, Beheshtian M, Mohseni M, Poustchi H, Ardalani F, et al. Distinct genetic variation and heterogeneity of the Iranian population. PLoS Genet. 2019; 15:e1008385.
24. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. Mov Disord. 2008; 23:2129-70.
25. Goetz CG, Poewe W, Rascol O, Sampaio C, Stebbins GT, Counsell C, et al. Movement Disorder Society Task Force report on the Hoehn and Yahr staging scale: status and recommendations. Mov Disord. 2004; 19:1020-8.
26. https://www.idtdna.com/primerquest/.
27. Sole X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 2006; 22:1928-9.
28. http://bioinfo.iconcologia.net/SNPStats.
29. Verstraeten A, Theuns J, Van Broeckhoven C. Progress in unraveling the genetic etiology of Parkinson disease in a genomic era. Trends Genet. 2015; 31:140-9.
30. Agliardi C, Guerini FR, Zanzottera M, Riboldazzi G, Zangaglia R, Bono G, et al. TNF-alpha -308 G/A and -238 G/A promoter polymorphisms and sporadic Parkinson's disease in an Italian cohort. J Neurol Sci. 2018; 385:45-8.
31. Kim YS, Joh TH. Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. Exp Mol Med. 2006; 38:333-47.
32. Guo LH, Schluesener HJ. The innate immunity of the central nervous system in chronic pain: the role of Toll-like receptors. Cell Mol Life Sci. 2007; 64:1128-36.
33. Noelker C, Morel L, Lescot T, Osterloh A, Alvarez-Fischer D, Breloer M, et al. Toll like receptor 4 mediates cell death in a mouse MPTP model of Parkinson disease. Sci Rep. 2013; 3:1393.
34. Trotta T, Porro C, Calvello R, Panaro MA. Biological role of Toll-like receptor-4 in the brain. J Neuroimmunol. 2014; 268:1-12.
35. https://www.ncbi.nlm.nih.gov/snp/.
36. Zhao J, Han X, Xue L, Zhu K, Liu H, Xie A. Association of TLR4 gene polymorphisms with sporadic Parkinson's disease in a Han Chinese population. Neurol Sci. 2015; 36:1659-65.
37. Zhu K, Teng J, Zhao J, Liu H, Xie A. Association of TLR9 polymorphisms with sporadic Parkinson's disease in Chinese Han population. Int J Neurosci. 2016; 126:612-6.