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Different Gene Expression Patterns of IL-1 Family Members in Parkinson's Disease: Results from Bayesian Regression Model

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ABSTRACT

Parkinson's disease, the second most prevalent neurodegenerative disorder lacking a recognized etiology, is influenced by oxidative stress and alterations in inflammatory cytokine levels. This study aimed to investigate the expression levels of *Interleukin(IL)1 receptor accessory protein (IL-1RAcP), IL1β*, *IL1a*, *IL33*, and *IL36* genes in blood cells and serum IL-1β levels in Parkinson's disease patients compared to healthy controls (HCs).

In this case-control study, 44 Parkinson's disease patients and 44 age- and sex-matched HCs were included. Gene expression levels were assessed using Quantitative Real-time PCR, and serum IL-1β levels were measured via enzyme-linked immunosorbent assay. Advanced statistical analyses using the Bayesian regression model in R software were employed.

Parkinson's disease patients exhibited elevated expression levels of *IL-1RAcP* and *IL1β* genes but decreased levels of *IL1a*, *IL33*, and *IL36* compared to HCs. Age-based differences were not significant. Regarding gender, *IL33* transcript levels were significantly higher in males, and serum *IL*-1β levels were increased in patients. Subgroup analysis by gender indicated alterations in *IL1β* and *IL-1RAcP* expression in both genders, while *IL1a*, *IL33*, and *IL36* showed reduced expression only in males. Remarkably, only female patients displayed significantly higher serum IL-1β levels than female HCs.

These findings suggest that dysregulation of immune-related factors plays a crucial role in Parkinson's disease.

Keywords: Interleukin-1 receptor accessory protein; Interleukin-1; Interleukin-33; Interleukin-36; Parkinson disease

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INTRODUCTION

Parkinson's disease (PD) is a condition characterized by the loss of dopaminergic neurons located in the

substantia nigra of the midbrain, which results in dopamine deficiency. Dopamine is a neurotransmitter involved in movement, memory, motivation, and other functions. In terms of prevalence, PD ranks second after Alzheimer's disease (AD) among neurodegenerative disorders of the central nervous system (CNS), with several motor symptoms like resting tremor, bradykinesia, muscle stiffness, and impaired voluntary movement, and non-motor symptoms including depression, dementia, and sleep problems. Around 1% of people over the age of 80 suffer from PD, and men are somewhat more susceptible to being affected than women.[1] There is a long delay between the initial injury to dopaminergic cells and the appearance of clinical symptoms; underscoring the importance of finding reliable biomarkers for early diagnosis to provide a better therapeutic intervention at the disease onset and/or to monitor the progression of the disease.[2] While the brain was once thought to have an immune privilege status, it is now recognized as an immunologically specialized organ with its resident immune cells. Inflammatory cytokines in the brain, cerebrospinal fluid (CSF), and plasma of PD patients reflect immune-neural connections, which may in turn result in the release of inflammatory mediators from microglial cells thereby contributing to chronic inflammation.[3] Chronic neuroinflammation appears to be a contributing factor to disease progression rather than an initiating factor. Although extensive evidence supports the involvement of inflammation in PD, the exact trigger of PD development has remained to be identified.[4]

The main markers of inflammation that are examined in various diseases and are considered the main indicators of inflammation usually include common inflammatory cytokines such as IL-1β, TNF-α, and IL-6, and so far, the role of emerging cytokines such as IL-33 and IL-36 has been studied much less. Among the cytokine families, the IL-1 family has great importance in the process of inflammation, but studies on the role of these cytokines in PD are very limited. The interleukin (IL)-1 family comprises of IL-1α, IL-1β, IL-18, IL-33, and IL-36 with pro-inflammatory activities, IL-37 with anti-inflammatory activity, and IL-1 receptor antagonist (IL-1Ra), IL-36 receptor antagonist (IL36Ra), and IL-38 as receptor antagonists. This family transmits signals by forming a heterotrimeric complex comprising a ligand, primary receptor, and accessory receptor.[5] The most significant co-receptor, IL-1 receptor accessory protein (IL-1RAcP or IL-1RAP), functions as a co-receptor for inflammatory cytokines within the IL-1 family, except for IL-18. Previous research suggests that IL-1RAcP plays a critical role in the progression of chronic inflammation and autoimmune diseases including psoriasis, type 1 diabetes, and AD.[6] While the role of IL-1RAP in various diseases, especially inflammatory and autoimmune diseases, has been discussed, studies on the expression of this molecule and its related cytokines such as IL-1α, IL-33, and IL-36 in neuroinflammatory diseases are very rare (except for IL-1β). In this context, two studies have been conducted on the relationship between IL-1RAP and Alzheimer's disease, which is the closest disease to PD.[7,8] There are also limited studies on the role of emerging cytokines of this family such as IL-33[9,10] and IL-36[11] and their role in Alzheimer's disease. Inflammatory cytokines of the IL-1 family are key signaling molecules in the innate and adaptive immune system that cause inflammation in response to a wide range of stimuli. The main mechanism of signal initiation in this family is a step-by-step process in which cytokines first bind to their receptor, and this cytokine-receptor complex recruits an accessory receptor. Most IL-1 family cytokines, including IL-1α, IL-1β, IL-33, and IL-36, can be potential pro-inflammatory stimuli through this accessory receptor. Chronic inflammation following long-term signaling of IL-1α, IL-1β, IL-33, and IL-36 receptors is an important process in the pathogenesis of many inflammatory disorders, including autoimmune diseases, psoriasis, type 1 diabetes, and Alzheimer.[12] Recent studies have shown that abnormal IL-1RAP signaling plays a key role in the pathogenesis of these chronic inflammatory diseases. IL-33 is an "alarmin" cytokine that stimulates inflammatory responses. This cytokine is mainly expressed through epithelial, endothelial, and myofibroblast cells.[13] It seems that the effects of IL-33 can be either pro-inflammatory or anti-inflammatory depending on the situation. For example, it has been reported that IL-33 plays a role in neuroinflammatory diseases such as Alzheimer and multiple sclerosis through mechanisms such as inducing macrophage differentiation towards M2 phenotype or expanding and activating Treg cells to create a microenvironment that is anti-inflammatory against these diseases.[14] On the other hand, it is expressed in various organs (such as the intestine, lung, and skin) and plays an important role in tissue injury and inflammation. Since in the future, the goal is to target the microbiota-gut-brain axis as a new therapeutic strategy for neurological diseases such as PD, studies on the role

of IL-33 in the microbiota-gut-brain axis are increasing.[15] IL-36 is expressed in several tissues and by various subsets of immune cells such as monocytes/macrophages, dendritic cells, T cells, and also non-immune cells such as paraneoplastic cells, keratinocytes, Langhans cells, and mucosal epithelium. Human primary macrophages inherently express this cytokine, but its expression is significantly induced by TLR stimulation.[16] In addition to being expressed by various cell types, IL-36 can stimulate a diverse set of cellular subsets to elicit pro-inflammatory responses. Like IL-1α, IL-1β, and IL-33, IL-36 exerts its pro-inflammatory effects by binding to the receptor and recruiting IL-1RAP, which leads to the activation of downstream MAPK pathways via JNK and ERK1/2 and also NF-κB-dependent transcription.[17] The role of IL-36 in various inflammatory diseases such as inflammatory bowel disease, psoriasis, rheumatoid arthritis, Sjogren's syndrome, and even kidney and lung inflammation has been investigated and confirmed.[18] However, studies on the relationship of this cytokine with neuroinflammatory diseases are very rare and there is no report on PD. According to available reports, the expression of IL36 changes in myasthenia gravis and optic neuritis.[16]

According to the information aforementioned, the purpose of the present study was to determine the expression levels of *IL1RAcP*, *IL1β*, *IL1α*, *IL33*, and *IL36* genes and serum levels of IL-1β (due to its high level in the serum) in PD patients compared with healthy controls (HCs).

MATERIALS AND METHODS

Patients and Healthy Controls

In this study, 44 PD patients and 44 age- and sex-matched HCs were included between June 2022 to January 2023. All of the patients, who were in stage two of PD based on the Hoehn and Yahr scale, showed symptoms including voluntary movement disorder, bradykinesia, resting tremor, and muscle rigidity. Levodopa (L-dopa + Carbidopa) was administered as an anti-Parkinson's drug and general treatment. The patients were selected under the direction of neurologists and based on the "Adams and Victor's Principles of Neurology" guideline. Patients with neurological or autoimmune diseases other than PD and metabolic disorders were excluded from the study. Also, if a subject displayed any symptoms of a neurological or immunological disorder, she/he was excluded from the HCs group. The patients had been admitted to "Sina" and "Besat" hospitals affiliated with Hamadan University of Medical Sciences. All samples were taken by trained individuals and also blindly. In the control group, the presence of any disease was considered as an exclusion criterion. The study procedure was validated by the Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1400.803). Moreover, informed consent forms were filled out and signed by all individuals included in the study.

RNA Extraction and cDNA Synthesis

The whole blood samples were taken from all the patients and HCs and collected in 5 ml EDTA tubes. Total RNA was extracted using RNX-Plus™ (SINACLONE, Tehran, Iran) according to the manufacturer's instructions. In the following, the Easy cDNA Synthesis Kit (PARSTOUS, Mashhad, Iran) was used to synthesize cDNA from 2 to 6 µg of total RNA.

Quantitative Real-time PCR (qRT-PCR)

To evaluate gene expression of *IL1RAcP*, *IL1β*, *IL1α*, *IL33*, and *IL36*, qRT-PCR method was carried out using Ampliqon™ 2X Real-Time PCR Master Mix Green without ROX (AMPLIQON, Odense, Denmark). The features of the primers (Sinaclone, Tehran, Iran) utilized in this study are detailed in Supplementary Table. Briefly, to perform qRT-PCR, a reaction mixture (20 µL) containing approximately 1 Ml of cDNA template was prepared. The temperature conditions for each reaction started with an initial hold at 9°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 58.5°C for 30 seconds, and 72°C for 35 seconds. All reactions were performed by the Rotor-gene Q thermal cycler (Qiagen, Germany) in duplicate. The housekeeping gene, GAPDH, was used as an internal control.

Enzyme-linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) kit for human IL-1β (FineTest, Wuhan Fine Biotech, China) was used to determine serum levels of IL-1β in PD patients and HCs according to the manufacturer's protocols. Briefly, 100 µL of diluted samples were added to the plate wells and incubated at 37 °C for 90 minutes. Then, 100 µL of biotin-labeled primary antibody solution was added, followed by incubation at room temperature for 60 minutes. In the next step, 100 µL of HRP-streptavidin conjugate solution was

added and the plate was incubated for a further 30 minutes at 37°C. The plate was washed after each incubation using the washing buffer provided by the kit. In the next step, 90 µL Tetramethylbenzidine (TMB) was added followed by incubation at 37°C in the dark for 10–20 minutes. Finally, a stop solution was added and OD values were read immediately at 450 nm.

Statistical Analysis

Relative quantification was performed using $2^{-\Delta\Delta Ct}$ method.[19] The boxplots were created to present differential gene expression in PD patients compared with HCs, based upon gender. The use of the Bayesian regression model provided more accurate estimations of the impacts of group, age, and sex, as well as the interconnections between them, on gene expression and subgroup analyses based on gender. It can also alleviate certain sample size-related problems. Furthermore, the area under the Receiver Operating Characteristic (ROC) curve (AUC), sensitivity (Se), and specificity (Sp) were calculated using the ROC regression model. Additionally, the correlation matrix was used to find possible correlations between each pair of parameters in both studied groups (total) and the findings are presented as correlation coefficients (ranging from -1 to +1) and *p*-values. All analyses were conducted using R (version 4.1.0) statistical software and P-values less than 0.05 were considered statistically significant

RESULTS

Demographic Data

In total, 44 PD patients (68.77±11.53 years) participated in this study. Also, the control group comprised 44 healthy adults who were sex- and age-matched (66.17±8.50 years.) with the Patients. Table 1 summarizes the demographic information of the study groups.

Gene Expression Analysis and ELISA Results

We found that transcription levels of *IL1RAcP* and *IL1β* were significantly upregulated in patients compared with HCs ($p<0.001$), and these upregulations were seen in both genders. On the contrary, *IL1α*, *IL33*, and *IL36* gene expression levels were significantly lower in patients compared to HCs ($p<0.001$ for *IL33* and *IL36*, $p=0.023$ for *IL1α*). Subgroup analysis within gender revealed that these downregulations were only seen in males ($p<0.05$ for all). We did not find any significant age-dependent differences in the gene expression levels. However, when considering gender, *IL33* showed a significant upregulation in males in comparison to females ($p=0.04$). In other words, *IL33* expression levels were significantly increased in male compared with female subjects. Additionally, serum levels of IL-1β were significantly higher in female ($p=0.001$) patients compared with female HCs ($p=0.012$) and did not differ significantly by either gender or age (Table 2 and Figure 1).

ROC Curves

The optimal prediction approach would produce a spot in the upper left corner of the ROC curve, reflecting 100% sensitivity which means zero false negatives, and 100% specificity which means zero false positives. After analyzing AUC (95% CI) values of transcripts, the following values were obtained: *IL1RAcP* with AUC (95% CI) of 88.93 [82.28%-95.58% CI], *IL1β* with AUC (95% CI) of 73.04 [62.29%-83.79% CI], *IL1α* with AUC (95% CI) of 65.91 [54.1%-77.72% CI], *IL33* with AUC (95% CI) of 76.52 [66.53%-86.51% CI], *IL36* with AUC (95% CI) of 72.29 [61.67%-82.91% CI], and IL-1β with AUC (95% CI) of 65.13% [53.02%-77.24% CI] (Figure 2). Comparisons of the Se, Sp and AUC values showed *IL1RAcP* could be considered as an effective tool in diagnostic panels of PD.

Correlation Analysis

The results of correlation analyses are depicted in Figure 3. According to the findings of our study, *IL1RAcP* and *IL1β* expression levels showed a significant positive correlation. Such an association was also found between the expression levels of *IL1α* with *IL33*, *IL1α* with *IL36*, and *IL33* with *IL36* ((r= 0.72, 0.67 and 0.54 respectively, $p<0.001$ for all). Since we found no correlation between age and gene expression levels, it could be assumed that the age factor does not influence the results of the present study.

Table 1. Demographic data of the groups as number or mean ± SD

Variables	PD patients	Healthy controls	*p*
Female/male (no. %)	11 (25%)/33 (75%)	14 (31.8%)/30 (68.2%)	0.478[a]
Age (Mean ± SD, year)	68.77 ± 11.53	66.17 ± 8.50	0.232[b]
Median of Age (Min-Max, year)	69 (38-89)	66.5 (41-81.5)	-
Disease duration (Mean ± SD, year)	6.94 ± 5.61	-	-
Age at onset (Mean ± SD, year)	61.86 ± 12.17	-	-

[a] Chi-square Test, [b] Independent t-test

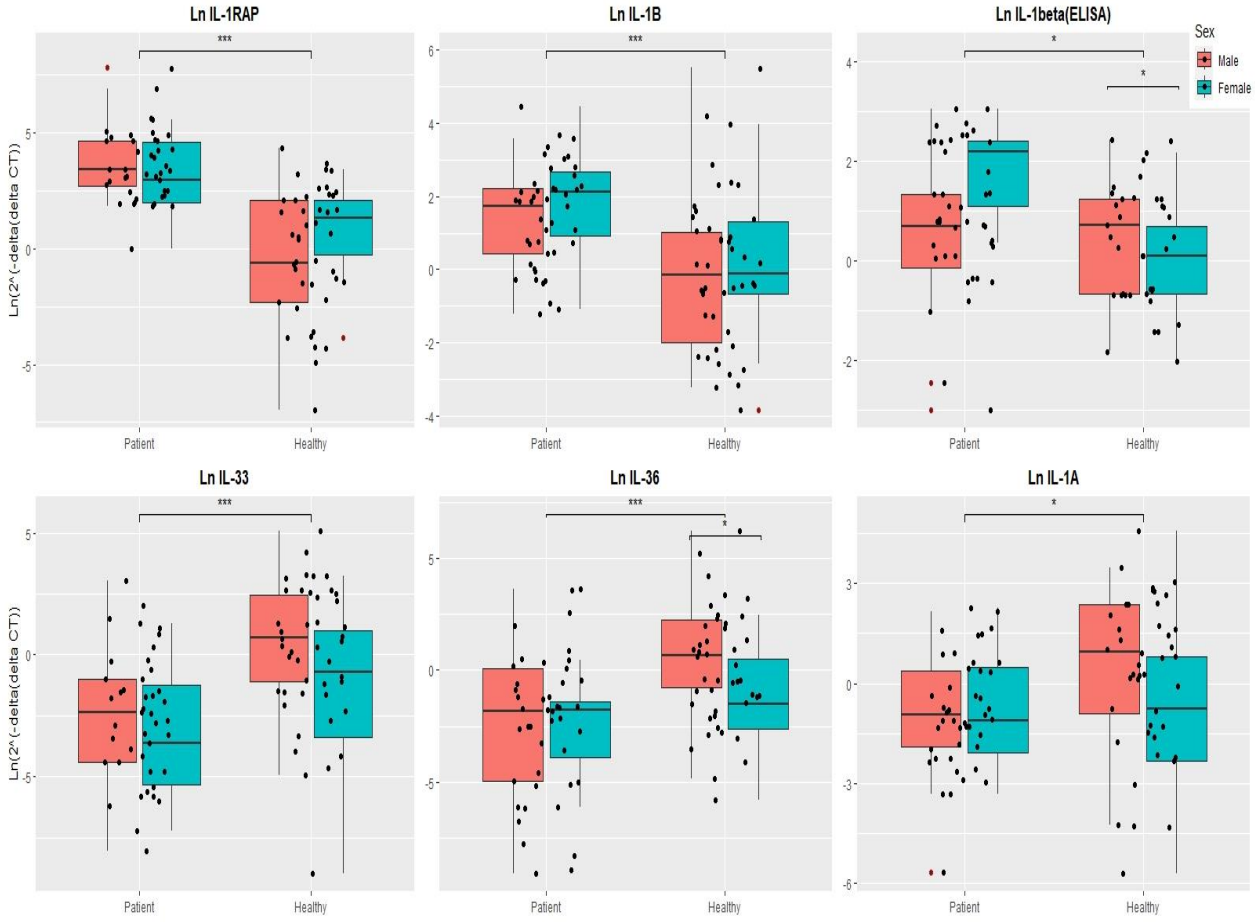

Figure 1. Comparison of relative expression of *Interleukin(IL)-1 receptor accessory protein (IL1RAP), Interleukin1β (IL1B), Interleukin1α (IL1A) Interleukin33 (IL33),* and *Interleukin36 (IL36)* genes as well as serum levels of Interleukin-1β (IL-1*B*) in Parkinson's disease patients and healthy controls based on gender using independent t-test.(p* 0.01-0.05, ***p* 0.01-0.001, ****p*<0.001)**

Table 2. Relative expression levels of *Interleukin-1 receptor accessory protein (IL1RAP), Interleukin1β (IL1B), Interleukin1α (IL1A) Interleukin33 (IL33),* and *Interleukin36 (IL36)* genes as well as serum levels of Interleukin-1β (IL-1β) in Parkinson's disease patients and healthy controls based on the results of the Bayesian regression model

	Variable	IL1RAP				IL1B				IL-1β (Serum)			
		Posterior Beta	SE	95% CrI	*p*	Posterior Beta	SE	95% CrI	*p*	Posterior Beta	SE	95%CrI	*p*
Total	Group (Control/Case)	-3.65	0.48	[-4.6, -2.72]	**<0.001**	-1.51	0.39	[-2.27,-0.74]	**<0.001**	-0.75	0.30	[-1.33,-0.16]	**0.012**
	Gender (Female/Male)	0.33	0.52	[-0.7, 1.36]	0.523	0.30	0.44	[-0.57, 1.16]	0.478	0.31	0.33	[-0.34, 0.95]	0.338
	Age	-0.02	0.02	[-0.07, 0.03]	0.415	2.60e-04	0.02	[-0.03, 0.04]	0.973	-0.03	0.01	[-0.06, 0.00]	0.061
Male	Group (Control/Case)	-4.14	0.60	[-5.33,-2.98]	**<0.001**	-1.38	0.45	[-2.27,-0.49]	**0.003**	-0.35	0.36	[-1.07, 0.34]	0.335
	Age	-0.02	0.03	[-0.08,0.04]	0.564	0.02	0.02	[-0.03, 0.07]	0.417	-0.03	0.02	[-0.07, 0.01]	0.091
Female	Group (Control/Case)	-2.40	0.82	[-4.04,-0.79]	**0.006**	-1.79	0.78	[-3.32,-0.21]	**0.030**	-1.86	0.49	[-2.83,-0.93]	**0.001**
	Age	-0.02	0.03	[-0.09,0.04]	0.491	-0.03	0.03	[-0.10, 0.04]	0.341	-0.01	0.02	[-0.06, 0.03]	0.539
		IL33				IL36				IL1A			
Total	Group (Control/Case)	2.70	0.59	[1.55, 3.87]	**<0.001**	2.52	0.64	[1.27, 3.79]	**<0.001**	1.01	0.44	[0.14,1.88]	**0.023**
	Gender (Female/Male)	-1.33	0.65	[-2.61,-0.06]	**0.040**	-1.05	0.70	[-2.41, 0.32]	0.130	-0.80	0.49	[-1.75, 0.18]	0.099
	Age	-0.02	0.03	[-0.08, 0.03]	0.431	-4.14e-03	0.03	[-0.07, 0.06]	0.892	-0.03	0.02	[-0.08, 0.01]	0.120
Male	Group (Control/Case)	3.03	0.65	[1.73, 4.30]	**<0.001**	3.11	0.79	[1.58, 4.68]	**<0.001**	1.48	0.47	[0.55,2.41]	**0.003**
	Age	4.32e-03	0.03	[-0.06, 0.07]	0.894	0.03	0.04	[-0.05, 0.11]	0.484	-0.02	0.03	[-0.07, 0.03]	0.443
Female	Group (Control/Case)	1.97	1.32	[-0.61, 4.63]	0.136	1.12	0.93	[-0.71, 2.96]	0.215	-0.20	1.05	[-2.25, 1.87]	0.829
	Age	-0.07	0.06	[-0.18, 0.05]	0.230	-0.06	0.04	[-0.14, 0.02]	0.132	-0.06	0.04	[-0.14, 0.03]	0.184

SE=Standard error, Crl=Credible intervals, Case=Parkinson's disease patients, Control=healthy controls

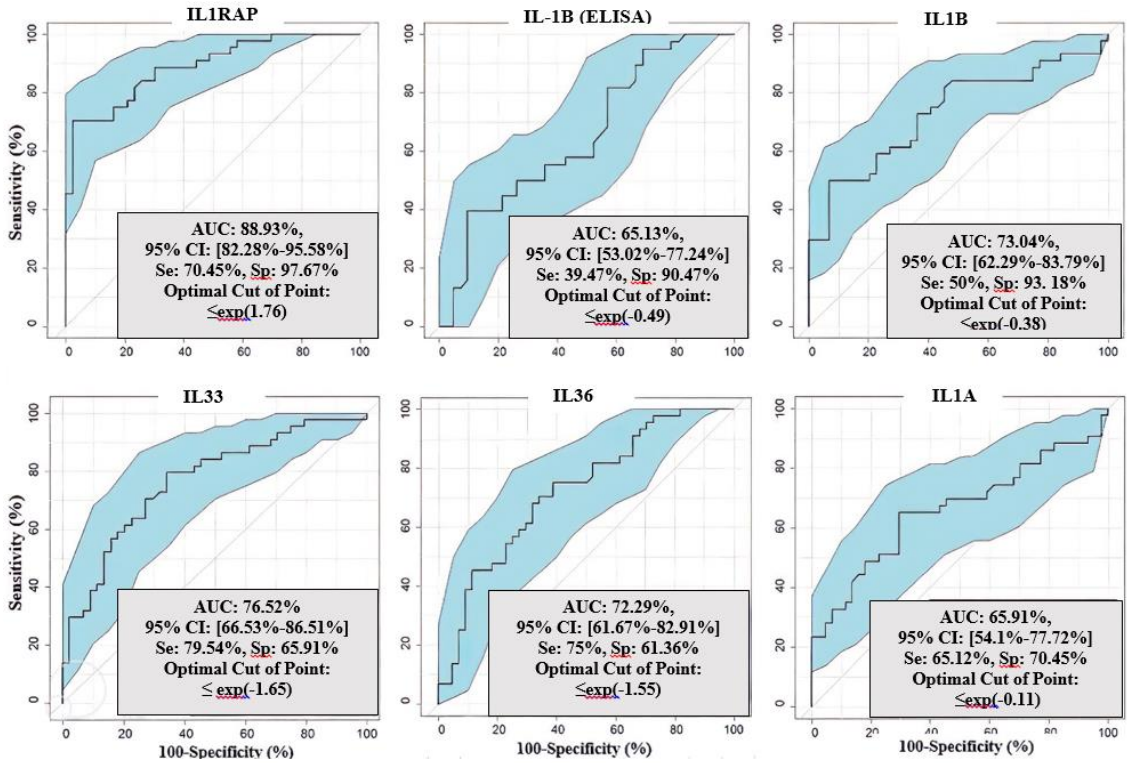

Figure 2. ROC curve analysis for Interleukin-1 receptor accessory protein (IL1RAP), Interleukin1β (IL1B), Interleukin1α (IL1A) Interleukin33 (IL33), and Interleukin36 (IL36) transcripts as well as serum levels of Interleukin-1β (IL-1β) based on the results of the Bayesian regression model

Z=Z-Score, Sp=Specificity, Se = Sensitivity, AUC=Area under curve, CI=Confidence interval

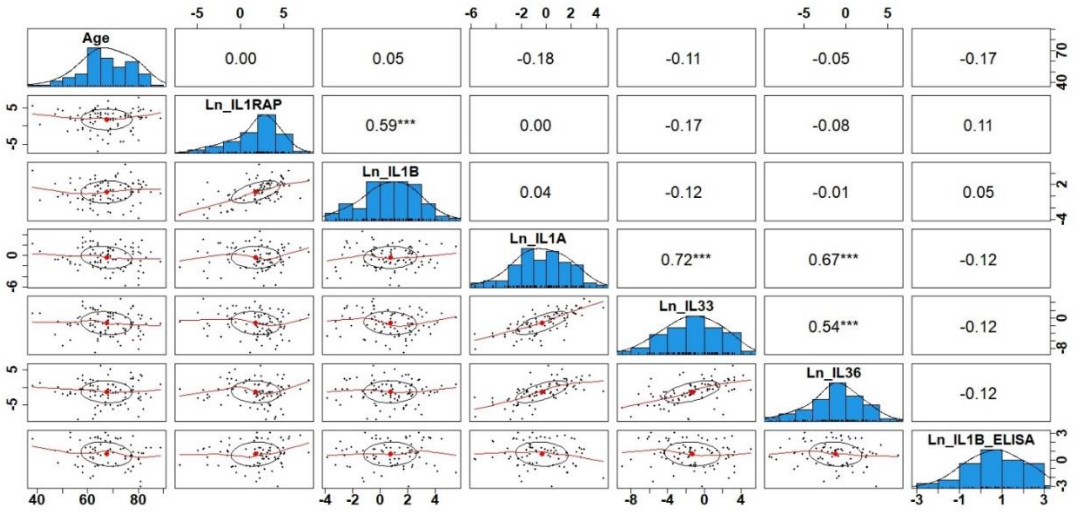

Figure 3. Correlations between the expression levels of Interleukin-1 receptor accessory protein (IL1RAP), Interleukin1β (IL1B), Interleukin1α (IL1A) Interleukin33 (IL33), and Interleukin36 (IL36) , serum levels of Interleukin-1β (IL-1β) , and age with each other based on the results of the Bayesian regression model. The correlation coefficients between the indices range from -1 to 0, signifying a negative correlation, and from 0 to +1, signifying a positive correlation (****p*<0.001)**

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DISCUSSION

The present study showed that *IL1β* and *IL1RAcP* gene expression levels were significantly higher in PD patients compared with HCs. Expectedly, serum levels of IL-1β were considerably increased in PD patients in comparison with HCs. However, transcript levels of *IL1α*, *IL33*, and *IL36* were found to be significantly lower in PD patients compared to HCs.

Based on the emerging data, dopaminergic neuron loss can be attributed to the prolonged inflammatory reactions and glial cell activation in both human PD and animal models of PD. Elevated proinflammatory cytokines in post-mortem PD brains strongly support this theory, as well.[20] McGeer and colleagues provided the first evidence for the involvement of inflammation in PD in 1988.[21] Indeed, neuroinflammation is described as a "double-edged sword" as it shows both neuroprotective and neurodegenerative properties.[22]

Despite having low blood levels, the majority of IL-1 family members seem to be endogenous activators of inflammation. According to previous reports, the IL-1 family members can cause cell death and neurodegeneration via a variety of mechanisms, such as inducing the expression of cell adhesion molecules and infiltration of immune cells into the CNS.[23,24] The evidence under discussion indicates that IL-1 family members have a significant role in mediating neurodegeneration after stroke, brain damage, and chronic CNS disorders such as multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), AD, and PD.[25,26] In the majority of neurodegenerative disorders, microglial cells exert their neuroinflammatory effects via "A1" astrocytes which produce proinflammatory mediators including IL-1α, IL-1β, and TNF-α.[27] According to several studies, PD patients have higher levels of IL-6, TNF-α, IL-2, IL-1β, IL-10, interferon-gamma (IFN-γ), and transforming growth factor-beta 1 (TGF-β1) in serum, CSF, and striatum, indicating that clinical features of PD are associated with inflammatory responses.[28,29] Furthermore, a significant increase in the expression levels of inflammatory cytokines has also been documented in the ascending and descending colon of PD patients.[30] Aside from the rise in pro-inflammatory cytokines including IL-1β, Karpenko and colleagues in 2018 reported reduced serum IL-1Ra in PD patients compared to HCs.[28] Expectedly, Menza et al, in 2010 showed that there is a strong relationship between proinflammatory cytokines and the development of key non-motor symptoms associated with PD such as depression, cognition, and sleep problems.[31] Contrarily, Kıçık and co-workers in 2020 showed that PD patients with mild cognitive impairment show much lower serum levels of NF-κB, IL-1β, and IL-18 in comparison to PD patients with normal cognition and HCs.[32]. Furthermore, Koziorowski and colleagues in 2012 reported an insignificant alteration of serum IL-1β in PD patients compared to HCs.[33] Based on a study in 2015 reporting higher serum levels of IL-1β but similar levels of IL-1α in PD patients compared with HCs and AD patients, it seems that the former cytokine plays a more important role in the inflammatory reactions observed in PD than the latter one.[34]

It has recently been proposed that dysfunctional IL-1RAcP signaling is a key player in several chronic inflammatory conditions caused by persistent cytokine receptor activation.12 Furthermore, IL-1RAcP has been found to influence synaptic formation and function as trans-synaptic cell adhesion molecules 1.[35] Therefore, the observed higher expression levels of *IL1RAcP* in PD patients in our study is not surprising as it serves a crucial role in the signal transduction of proinflammatory cytokines of the IL-1 family, particularly IL-1β.[36] IL-1RAcPb is unable to recruit IRAK4 and MYD88 and therefore possesses an immunomodulatory activity on the CNS neurons where it is exclusively produced. Thus, it has been suggested that IL-1RAcPb can regulate IL-1β mediated inflammation in the CNS.[37] It is worth noting that the upregulation of *IL1RAcP* gene in PD patients does not necessarily act as an enhancer of inflammation. Rather, it is likely that the increase in the level of IL-1RAcP is a result of inflammation and is used as a compensatory agent to control the inflammatory responses. Even though there are some reports on IL-1RAcP in AD, as the most similar neurodegenerative disease to PD, our study is the first to assess *IL1RAcP* gene expression in PD patients.[38]

Furthermore, In spite of the limited studies on IL-33 as an emerging cytokine of IL-1 family, in neurodegenerative diseases (particularly in PD), previous studies have shown that this cytokine is highly expressed in the glial cells around amyloid plaques of AD patients,[39] as well as in the serum, CSF,[40] peripheral leukocytes and CNS[41] of MS patients. Moreover, Kempuraj et al, in 2018 elucidated that activated mast cells during neuroinflammatory reactions, produce a variety of inflammatory mediators including IL-1β,

IL-33, IL-6, TNF-α, IL-8, IL-17, and IL-18.[42] In another study by Xu and colleagues in 2022, it was found that IL-1β and IL-33 levels were higher in serum, striatum, and midbrain of PD patients in comparison with HCs.[43] In this context, it has been figured out that in several neurodegenerative disorders (e.g., AD, MS, and PD), elevated Glia maturation factor (GMF) in the CNS enhances the secretion of IL-33 from mouse astrocytes which in turn augments GMF-mediated release of IL-1β, TNF-α, and CCL2 from these cells.[44] On the contrary, *IL33* gene expression and protein levels have been reported to be significantly lower in brains of AD patients compared to healthy controls.[45] Consistently, Fu A. K.Y and his colleagues in 2016 found that AD patients have considerably higher levels of serum sST2, as a decoy receptor of IL-33, compared to healthy controls. They also showed that IL-33 administration reduces amyloid plaque deposition in transgenic mouse models of AD and modulates the immune response toward an anti-inflammatory situation along with reducing gene expression of proinflammatory cytokines, such as *IL6* and *IL1B*.[46] This finding may hold promise for novel therapeutic approaches for PD in the future. Currently, cytokine therapy is routinely used, to treat or alleviate symptoms in some neurological diseases (such as IFN-β in M.S). It was reported that IL-33 levels were significantly higher in the early-stage PD patients compared with advanced PD patients.[47] However, whether IL-33 has stimulatory or inhibitory effects on PD development remains to be determined. Lopetuso L.S in 2013 found that inflammatory bowel disease (IBD) patients exhibit elevated levels of IL-1 family members such as IL-1β and IL-33[48] providing evidence that PD and IBD might be interconnected. In fact, IBD may mildly predispose to PD.[49]

Similar to other members of the IL-1 family, IL-36 plays a significant role in promoting both innate and adaptive immune responses by increasing the polarization and proliferation of $CD4^+$ T cells and preventing their differentiation into regulatory T cells. This cytokine can also upregulate the expression of adhesion molecules and induce dendritic cells (DCs) maturation, and the release of inflammatory mediators like IL-8, IL-2, IL-12, IFN-γ, CCL2, and CCL20 due to its ability to promote the activation of NF-κB and AP-1.[50,51] Relatively few studies exist on the role of IL-36 and how it is altered in the neurodegenerative disorders. According to the reports, patients with MS,[52] AD,[53] ALS,[54] and Neuromyelitis optica spectrum disorder (NOSD)[55] exhibit higher gene expression and serum levels of IL-36. Based on the previous research conducted by our team,[56] there is an increased expression of IL-17 in PD patients. Additionally, there is a relation between IL-17 and IL-36.[57,58] Therefore, it is suggested that the IL-36/IL-17 pathway may possibly be involved in immune dysregulation occurred in PD. It seems that taking levodopa by PD patients might have contributed to reduced *IL1α*, *IL33*, and *IL36* expression levels observed in our study. In this regard, Reale and co-workers in 2009, after an *ex vivo* study on the culture supernatants of peripheral blood mononuclear cells (PBMCs) obtained from levodopa-treated PD patients and HCs, found that both LPS-induced and basal levels of RANTES, MCP1, CXCL8, MIP-1-α, IL-1β, IFN-γ, and TNF-α were significantly higher in patients compared with HCs. Furthermore, the results of ELISA tests on sera, confirmed the mentioned *ex vivo* findings.[59] Thus, it seems that IL-1α is more susceptible to levodopa than IL1β. It should be noted that in diseases where the levels of some cytokines changes compared to the control group, it is difficult to judge whether these cytokines play a role in causing - or exacerbating - the disease, or the disease causes their production and it has a compensatory role. This situation is much more complicated for cytokines such as IL-33 and IL-36, which have dual and even contradictory functions. The issue needs to be examined in detail. Furthermore, it should be noted that despite the fact that the two groups were accurately matched for age and sex, there may be differences between the two groups in other factors that may affect cytokine production. Such factors include diet, genetic differences, race, and other factors that may affect gene expression levels. Therefore, using a larger number of samples and matching the two case and control groups in multiple aspects is recommended.

In another part of our study, we found no significant age-related difference in gene expression or serum levels of the studied markers [in the case of IL-1β, the *p*-value (0.061) was close to the borderline of statistical significance]. Although it seems that age does not have a considerable effect on the expression of these markers, study on a larger sample size and the use of CSF instead of blood cells, may provide different results. Based on the gender, transcript levels of *IL33* -regardless of being patient or healthy- were shown to be significantly upregulated in male compared to female subjects. Thus, it seems that *IL33* expression is significantly affected by the gender. Moreover, we found that the decrease in the

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expression of *IL1α*, *IL33* and *IL36* genes has only occurred in men when comparing the patients with controls. This issue obviously indicates the effect of gender on the expression of these cytokines.

The correlation analysis was indicative of significant positive correlations between *IL1RAcP* and *IL1β* genes expression and also between expression levels of *IL1α*, *IL33* and *IL36* genes. According to the results obtained by ROC curve analyses, *IL1RAcP* gene expression has the potential to be included in diagnostic panels of PD due to its high Se and Sp values.

Based on the results of the study, it appears that the immune response dysregulation observed in PD is partly due to changes in the expression of IL-1 family cytokines and IL1RAcP. Among these cytokines, emerging ones such as IL-33 and IL-36 may also play a role. Additionally, some cytokines in this family that are primarily considered pro-inflammatory mediators, might show anti-inflammatory properties in certain situations and could act as a "double-edged sword". Furthermore, some of these cytokines including IL-1α, IL-33, and IL-36 show differential gene expression in males and females. The study recommends further investigations on IL-1 family members and their effects on various stages of PD to highlight the potential of these cytokines in this complex central nervous system disorder.

STATEMENT OF ETHICS

The present study was performed according to the ethical standards of the institutional and/or national research committee (INRC) and the 1964 Helsinki declaration and its later amendments. The study protocol was approved by the Ethical Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1400.803). Informed consent forms were obtained from all patients and healthy controls. The protocols were done in accordance with the relevant guidelines and regulations.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Simon DK, Tanner CM, Brundin P. Parkinson Disease Epidemiology, Pathology, Genetics, and Pathophysiology. Clin Geriatr Med. 2020;36(1):1-12.
2. Zeng XS, Geng WS, Jia JJ, Chen L, Zhang PP. Cellular and Molecular Basis of Neurodegeneration in Parkinson Disease. Front Aging Neurosci. 2018;10:109.
3. Tansey MG, Wallings RL, Houser MC, Herrick MK, Keating CE, Joers V. Inflammation and immune dysfunction in Parkinson disease. Nat Rev Immunol. 2022;22(11):657-73.
4. Villumsen M, Aznar S, Pakkenberg B, Jess T, Brudek T. Inflammatory bowel disease increases the risk of Parkinson's disease: a Danish nationwide cohort study 1977-2014. Gut. 2019;68(1):18-24.
5. Sims JE, Smith DE. The IL-1 family: regulators of immunity. Nat Rev Immunol. 2010;10(2):89-102.
6. Fields JK, Günther S, Sundberg EJ. Structural Basis of IL-1 Family Cytokine Signaling. Front Immunol. 2019;10:1412.
7. Zettergren A, Höglund K, Kern S, Thorvaldsson V, Johan Skoog M, Hansson O, et al. Association of IL1RAP-related genetic variation with cerebrospinal fluid concentration of Alzheimer-associated tau protein. Sci Rep. 2019;9(1):2460.
8. Ramanan VK, Risacher SL, Nho K, Kim S, Shen L, McDonald BC, et al. GWAS of longitudinal amyloid accumulation on 18F-florbetapir PET in Alzheimer's disease implicates microglial activation gene IL1RAP. Brain. 2015;138(10):3076-88.
9. Jiang T, Zheng T, Li W, Liu N, Wang M. IL-33/ST2 signaling pathway and Alzheimer's Disease: A systematic review and meta-analysis. Clin Neurol Neurosurg. 2023:107773.
10. Liang C-S, Su K-P, Tsai C-L, Lee J-T, Chu C-S, Yeh T-C, et al. The role of interleukin-33 in patients with mild cognitive impairment and Alzheimer's disease. Alzheimers Res Ther. 2020;12(1):1-9.

11. Masoumi J, Vakilian A, Sayadi A, Shekari N, Khorramdelazad H. Assessing the gene expression of interleukin-36 in Alzheimer's patients. Gene Reports. 2020;21:100823.
12. Zarezadeh Mehrabadi A, Aghamohamadi N, Khoshmirsafa M, Aghamajidi A, Pilehforoshha M, Massoumi R, Falak R. The roles of interleukin-1 receptor accessory protein in certain inflammatory conditions. Immunology. 2022;166(1):38-46.
13. Gabryelska A, Kuna P, Antczak A, Białasiewicz P, Panek M. IL-33 mediated inflammation in chronic respiratory diseases—understanding the role of the member of IL-1 superfamily. Front Immunol. 2019;10:692.
14. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005;23(5):479-90.
15. Sun Y, Wen Y, Wang L, Wen L, You W, Wei S, et al. Therapeutic opportunities of interleukin-33 in the central nervous system. Front Immunol. 2021;12:654626.
16. Walsh PT, Fallon PG. The emergence of the IL-36 cytokine family as novel targets for inflammatory diseases. Ann N Y Acad Sci. 2018;1417(1):23-34.
17. Yi G, Ybe JA, Saha SS, Caviness G, Raymond E, Ganesan R, et al. Structural and functional attributes of the interleukin-36 receptor. Journal of Biological Chemistry. 2016;291(32):16597-609.
18. Yuan Z-C, Xu W-D, Liu X-Y, Liu X-Y, Huang A-F, Su L-C. Biology of IL-36 signaling and its role in systemic inflammatory diseases. Front Immunol. 2019;10:2532.
19. Rao X, Huang X, Zhou Z, Lin X. An improvement of the 2ˆ(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. Biostat Bioinforma Biomath. 2013;3(3):71-85.
20. Wang Q, Liu Y, Zhou J. Neuroinflammation in Parkinson's disease and its potential as therapeutic target. Transl Neurodegener. 2015;4:19.
21. McGeer PL, Itagaki S, Akiyama H, McGeer EG. Rate of cell death in parkinsonism indicates active neuropathological process. Ann Neurol. 1988;24(4):574-6.
22. Koprich JB, Reske-Nielsen C, Mithal P, Isacson O. Neuroinflammation mediated by IL-1beta increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease. J Neuroinflammation. 2008;5:8.
23. Choudhury SP, Bano S, Sen S, Suchal K, Kumar S, Nikolajeff F, et al. Altered neural cell junctions and ion-channels leading to disrupted neuron communication in Parkinson's disease. npj Parkinson Disease. 2022;8(1):66.
24. Lee Y, Lee S, Chang SC, Lee J. Significant roles of neuroinflammation in Parkinson's disease: therapeutic targets for PD prevention. Arch Pharm Res. 2019;42(5):416-25.
25. Berglöf E, Andre R, Renshaw BR, Allan SM, Lawrence CB, Rothwell NJ, Pinteaux E. IL-1Rrp2 expression and IL-1F9 (IL-1H1) actions in brain cells. J Neuroimmunol. 2003;139(1-2):36-43.
26. Lin CC, Edelson BT. New Insights into the Role of IL-1β in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis. J Immunol. 2017;198(12):4553-60.
27. Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW, 2nd, Mochly-Rosen D. Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. Nat Neurosci. 2019;22(10):1635-48.
28. Karpenko MN, Vasilishina AA, Gromova EA, Muruzheva ZM, Miliukhina IV, Bernadotte A. Interleukin-1β, interleukin-1 receptor antagonist, interleukin-6, interleukin-10, and tumor necrosis factor-α levels in CSF and serum in relation to the clinical diversity of Parkinson's disease. Cell Immunol. 2018;327:77-82.
29. Qin XY, Zhang SP, Cao C, Loh YP, Cheng Y. Aberrations in Peripheral Inflammatory Cytokine Levels in Parkinson Disease: A Systematic Review and Meta-analysis. JAMA Neurol. 2016;73(11):1316-24.
30. Rolli-Derkinderen M, Leclair-Visonneau L, Bourreille A, Coron E, Neunlist M, Derkinderen P. Is Parkinson's disease a chronic low-grade inflammatory bowel disease? J Neurol. 2020;267(8):2207-13.
31. Menza M, Dobkin RD, Marin H, Mark MH, Gara M, Bienfait K, et al. The role of inflammatory cytokines in cognition and other non-motor symptoms of Parkinson's disease. Psychosomatics. 2010;51(6):474-9.
32. Kıçık A, Tüzün E, Erdoğdu E, Bılgıç B, Tüfekçıoğlu Z, Öztürk-Işik E, et al. Neuroinflammation Mediators are Reduced in Sera of Parkinson's Disease Patients with Mild Cognitive Impairment. Noro Psikiyatr Ars. 2020;57(1):15-7.
33. Koziorowski D, Tomasiuk R, Szlufik S, Friedman A. Inflammatory cytokines and NT-proCNP in Parkinson's disease patients. Cytokine. 2012;60(3):762-6.
34. Dursun E, Gezen-Ak D, Hanağası H, Bilgiç B, Lohmann E, Ertan S, et al. The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum

levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. J Neuroimmunol. 2015;283:50-7.
35. Yoshida T, Shiroshima T, Lee SJ, Yasumura M, Uemura T, Chen X, et al. Interleukin-1 receptor accessory protein organizes neuronal synaptogenesis as a cell adhesion molecule. J Neurosci. 2012;32(8):2588-600.
36. Brikos C, Wait R, Begum S, O'Neill LA, Saklatvala J. Mass spectrometric analysis of the endogenous type I interleukin-1 (IL-1) receptor signaling complex formed after IL-1 binding identifies IL-1RAcP, MyD88, and IRAK-4 as the stable components. Mol Cell Proteomics. 2007;6(9):1551-9.
37. Smith DE, Lipsky BP, Russell C, Ketchem RR, Kirchner J, Hensley K, et al. A central nervous system-restricted isoform of the interleukin-1 receptor accessory protein modulates neuronal responses to interleukin-1. Immunity. 2009;30(6):817-31.
38. Ramanan VK, Risacher SL, Nho K, Kim S, Shen L, McDonald BC, et al. GWAS of longitudinal amyloid accumulation on 18F-florbetapir PET in Alzheimer's disease implicates microglial activation gene IL1RAP. Brain. 2015;138(Pt 10):3076-88.
39. Xiong Z, Thangavel R, Kempuraj D, Yang E, Zaheer S, Zaheer A. Alzheimer's disease: evidence for the expression of interleukin-33 and its receptor ST2 in the brain. J Alzheimers Dis. 2014;40(2):297-308.
40. Jafarzadeh A, Mahdavi R, Jamali M, Hajghani H, Nemati M, Ebrahimi HA. Increased Concentrations of Interleukin-33 in the Serum and Cerebrospinal Fluid of Patients with Multiple Sclerosis. Oman Med J. 2016;31(1):40-5.
41. Christophi GP, Gruber RC, Panos M, Christophi RL, Jubelt B, Massa PT. Interleukin-33 upregulation in peripheral leukocytes and CNS of multiple sclerosis patients. Clin Immunol. 2012;142(3):308-19.
42. Kempuraj D, Selvakumar GP, Zaheer S, Thangavel R, Ahmed ME, Raikwar S, et al. Cross-Talk between Glia, Neurons and Mast Cells in Neuroinflammation Associated with Parkinson's Disease. J Neuroimmune Pharmacol. 2018;13(1):100-12.
43. Xu J, He X, Xu Y, Chen X, Li M, Zhang L, et al. Characteristics of systemic inflammation and brain iron deposition in Parkinson's disease patients. Ann Clin Transl Neurol. 2022;9(3):276-85.
44. Kempuraj D, Khan MM, Thangavel R, Xiong Z, Yang E, Zaheer A. Glia maturation factor induces interleukin-33 release from astrocytes: implications for neurodegenerative diseases. J Neuroimmune Pharmacol. 2013;8(3):643-50.
45. Chapuis J, Hot D, Hansmannel F, Kerdraon O, Ferreira S, Hubans C, et al. Transcriptomic and genetic studies identify IL-33 as a candidate gene for Alzheimer's disease. Mol psychiatry. 2009;14(11):1004-16.
46. Fu AK, Hung KW, Yuen MY, Zhou X, Mak DS, Chan IC, et al. IL-33 ameliorates Alzheimer's disease-like pathology and cognitive decline. Proc Natl Acad Sci U S A. 2016;113(19):E2705-13.
47. Xu J, He X, Xu Y, Chen X, Li M, Zhang L, et al. Characteristics of systemic inflammation and brain iron deposition in Parkinson's disease patients. Ann Clin Transl Neurol. 2022;9(3):276-85.
48. Lopetuso L, Chowdhry S, Pizarro T. Opposing Functions of Classic and Novel IL-1 Family Members in Gut Health and Disease. Front Immunol. 2013;4.
49. Zhu Y, Yuan M, Liu Y, Yang F, Chen WZ, Xu ZZ, et al. Association between inflammatory bowel diseases and Parkinson's disease: systematic review and meta-analysis. Neural Regen Res. 2022;17(2):344-53.
50. Harusato A, Abo H, Ngo VL, Yi SW, Mitsutake K, Osuka S, et al. IL-36γ signaling controls the induced regulatory T cell-Th9 cell balance via NFκB activation and STAT transcription factors. Mucosal Immunol. 2017;10(6):1455-67.
51. Li Q, Liu S, Li L, Ji X, Wang M, Zhou J. Spinal IL-36γ/IL-36R participates in the maintenance of chronic inflammatory pain through astroglial JNK pathway. Glia. 2019;67(3):438-51.
52. Alsahebfosoul F, Jahanbani-Ardakani H, Ghavimi R, Sedaghat N, Etemadifar M. Serum level of interleukin 36 in patients with multiple sclerosis. J Immunoassay Immunochem. 2018;39(5):558-64.
53. Hill MA, Gammie SC. Alzheimer's disease large-scale gene expression portrait identifies exercise as the top theoretical treatment. Sci Reports. 2022;12(1):17189.
54. Liu G, Fiala M, Mizwicki MT, Sayre J, Magpantay L, Siani A, et al. Neuronal phagocytosis by inflammatory macrophages in ALS spinal cord: inhibition of inflammation by resolvin D1. Am J Neurodegener Dis. 2012;1(1):60-74.
55. Tang J, Zeng X, Yang J, Zhang L, Li H, Chen R, et al. Expression and Clinical Correlation Analysis Between Repulsive Guidance Molecule a and Neuromyelitis Optica Spectrum Disorders. Front Immunol. 2022;13.
56. Moradi S, Zamani A, Mazdeh M, Ramezani M, Komaki A, Talebi-Ghane E, Eftekharian MM. An inclusive study on cytokine gene expression in Parkinson's disease:

Advanced analysis using Bayesian regression model. Hum Immunol. 2023;84(2):123-9.
57. Fischer B, Kübelbeck TV, Kolb AL, Ringen J, Waisman A, Wittmann M, et al. IL-17A-driven psoriasis is critically dependent on IL-36 signaling. Front Immunol.14:1256133.
58. Mercurio L, Failla CM, Capriotti L, Scarponi C, Facchiano F, Morelli M, et al. Interleukin (IL)-17/IL-36 axis participates to the crosstalk between endothelial cells and keratinocytes during inflammatory skin responses. PLoS One. 2020;15(4):e0222969.
59. Reale M, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, Onofrj M. Peripheral cytokines profile in Parkinson's disease. Brain Behav Immun. 2009;23(1):55-63.