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Gene Expression Levels Related to Histone Acetylation are Altered in Parkinson Disease Patients

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ABSTRACT

Introduction: Parkinson's Disease (PD) is a neurodegenerative disorder distinguished from other neurodegenerative disorders by the loss of dopaminergic neurons in the substantia nigra region of the brain, and is the most common neurodegenerative disorder, along with Alzheimer's Disease. PD is characterized by the presence of Lewy bodies when evaluated pathologically. Recent studies showed that the incidence of PH development as a result of genetic mutations alone is very low among all PD cases, and that environmental effects contribute significantly to the disease progression. The molecular mechanisms of diseases are associated with the maintenance of gene and protein expressions as a result of epigenetic regulations. The role of these regulations in the development and pathogenesis of neurodegenerative diseases is still not clearly understood.

Methods: In our study, we examined the expression levels of H3C1, H3C12, HDAC4, HDAC5, ANKRD11, ANKRD12, ITM2B and GABBR1, which are genes involved in epigenetic processes in patients with idiopathic PD. Seventy five patients diagnosed with idiopathic PD and 50 healthy controls were included in the study. Peripheral Blood Mononuclear Cell (PBMC) was obtained from whole blood taken from the patient and control groups, and then total RNA was isolated from PBMC.

Results: According to the comparison of the patient and control groups, the expression of H3C1, H3C12, ITM2B was high, and the expression of ANKRD11, HDAC4, HDAC5 and GABBR1 was low ($p<0.05$).

Conclusion: As conclusion, we propose that histone regulation is one of the epigenetic mechanisms related to the presence of PD.

Keywords: Epigenetics, histone deacetylases, histone regulation, neurodegenerative, Parkinson's disease

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INTRODUCTION

Although Parkinson's Disease (PD) was defined as a syndrome evaluated according to motor and non-motor symptoms in the early 1800 s, it was later discovered that non-motor disorders were also common in patients. Although aging is an important risk factor, PD has a low incidence among the entire elderly population (1).

Motor symptoms in PD are associated with loss of dopaminergic neurons of substantia Nigra (SN) and Lewy bodies. Lewy bodies are fibrils formed when monomeric α-synucleins (SNCA) come together to form β-sheets. 10% of PD cases are autosomal dominant with familial inheritance and the most common OD inheritance is mutations in the LRRK2 gene. PARK2 gene is one of the longest autosomal recessive (OR) inherited genes with mutations associated with PD (2). In addition to genetic factors, many epigenetic-related processes involved in mitochondrial functions, protein folding, neuroinflammation and oxidative stress play an important role in the pathophysiology of PD (3). Therefore, the pathogenesis of idiopathic PD is not only dependent on genetic factors but also on epigenetic mechanisms.

Highlights:

- **HDCA5 and GABBR1 have suppressive properties in the PD physiopathology.**
- **Histone regulations are prominent in the pathogenesis of PD.**
- **A negative correlation was found between HDCA5, GABBR1 and UPDRS scores.**

In idiopathic PD, which mainly occurs independent of inherited conditions and mutations, defects in mitochondria, as well as increased reactive oxygen species and the resulting oxidative stress, initiate the death cascade in neuronal cells. Processes such as exposure to environmental iron and calcium ions, inflammation leading to excessive microglial activation, overstimulation of some receptors, initiate irreversible neuronal loss and

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lead to the emergence of the pathogenesis of PD (4,5). Recent studies have shown that mitochondrial DNA homeostasis is disrupted in PD, resulting in the death of dopamine-related neurons. At the same time, ubiquitin proteasome system (UPS) disorders, excessive microglial activation and oxidative stress have been detected in PD patients. The effects of epigenetic factors have been demonstrated by methods such as DNA/RNA sequencing and transcriptomic analysis, mass spectrometry with post-mortem brain tissue, molecular analysis of cerebrospinal fluid and blood obtained from PD patients, and experimental animal models of PD (6). Histone acetylation levels in dopaminergic neurons in brain sections of postmortem PD cases were found to be higher compared to healthy individuals (7). In addition, when PBMCs obtained from PD cases and healthy controls were compared by microarray method, increased and decreased gene expressions were detected in different signaling pathways, including those related with epigenetic mechanisms (8). Based on these studies, we aimed to investigate the expression level of genes related to histone acetylation, the leading epigenetic mechanism in neuronal loss, in PD patients.

METHODS

Case Selection

Seventy-five patients diagnosed with PD who were followed up by the Istanbul University Istanbul Faculty of Medicine Movement Disorders Neurology Polyclinic and 50 healthy controls were included in the study. The diagnosis was based on the clinical PD criteria established by the UK Parkinson's Disease Association Brain Bank (9). The study was ethically approved by the Clinical Research Ethics Committee of Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (meeting decision number 20 dated 06.12.2019). An "Informed Consent Form" was obtained from all patients participating in the study. Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn-Yahr (H and Y) rating scales were used to assess the clinical stage and severity of the disease (10,11). Each patient was subjected to a standardized interview and neurological examinations were performed. Patients with a family history of PD, psychiatric comorbidities including depression, severe cognitive impairment or dementia, cardiovascular diseases, diabetes, head trauma, stroke, brain tumor or other systemic and neurological diseases including epilepsy were excluded.

Gene Expression Study (Real-time qPCR)

Peripheral Blood Mononuclear Cells (PBMC) were isolated from freshly collected blood by standard Ficoll-Paque density gradient centrifugation and stored at -80°C until study. Total RNA was extracted using a commercial RNA isolation kit according to the manufacturer's recommended protocol (innuPREP RNA Mini Kit 2.0, Analytik Jena). Quantification was performed in a spectrophotometer and sample quality was determined according to A260/A280 and A260/230 ratios (Thermo Scientific Nanodrop 2000® Spectrophotometer). All Total RNA samples were equalized to 1 µg and complementary DNA (cDNA) was obtained with SensiFAST™ cDNA Synthesis Kit (Bioline). RT-qPCR was performed using 1.5 µl cDNA, 6.5 µL ddH2O, 10 µL SYBR Green PCR Master Mix (Euroclone) and 2 µl primer set (Table 1) in a total of 20 µL reaction. RT-qPCR and melting curve analyses were performed on qTower3 (Analytik Jena). GAPDH gene was used as a reference gene for normalization. Relative gene expressions were analyzed according to the 2-ΔΔCt method (12).

Statistical Analyses

Categorical variables between groups were compared using chi-square test. A t-test was performed to evaluate the statistical significance of the difference in gene expression levels between the PD and healthy control groups. Pearson correlation analysis was performed to examine the relationship between clinical findings, UPDRS scores and gene expression. Values were expressed as mean (mean) ± standard deviation (SD) and p-values less than 0.05 were considered statistically significant. Statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS) program software (IBM).

RESULTS

Demographic Data

The total number of idiopathic Parkinson's patients included in the study was 75. Forty-five of the patients were male and 30 were female. The total number of the healthy control group was 50. Of the controls, 30 were male and 20 were female. Demographic and clinical features of the study groups are provided in Table 2.

Gene Expression Results

RNA isolations were performed from PBMCs obtained from PD and healthy control samples. After translation of RNAs into cDNA, real-time PCR studies for gene expression were successfully completed. Each sample was run in 3 replicates. GAPDH was used as housekeeping gene for normalization of Ct values obtained as a result of real-time PCR. The differences in expression levels between the two groups are shown in the graph in Figure 1.

While H3C1, H3C12, ANKRD12 and ITM2B genes were overexpressed in PD, ANKRD11, HDAC4, HDAC5 and GABBR1 genes showed reduced expression levels in PD patients compared to healthy controls.

In addition, Pearson Correlation Analysis revealed that there was a negative correlation between HDCA5 and UPDRS IV (p=0.045, r=

Table 1. Primers used in qPCR analysis

Gene	Forward primer	Reverse primer
H3C1	TCCGCCGTTATCAGAAGTCCAC	GCTCTGGAAACGCAGGTCTGTT
H3C12	TCCGCAAACTGCCATTTCAGCG	GGCGTGAATAGCACAGAGGTTG
ANKRD11	CCTAGATGACGACACGCCTTTG	GTCTCGCCTTTCCTGTTGCTCT
ANKRD12	GGCTGCTATTCGAGGAGATGTG	GCTTCATGCAGTGGTGTCCAAC
HDAC4	AGGTGAAGCAGGAGCCCATTGA	GGTAGTTCCTCAGCTGGTGGAT
HDAC5	CGCTGAGAATGGCTTTACTGGC	GTGTAGAGGCTGAACTGGTTGG
GABBR1	CCTGAACAAGACATCTGGAGGAG	GCTGGCATCAAACACCACATGG
ITM2B	AGAAGAGCCTGGTGTTGGTGCA	CCACAGTAGTACACGTCATCTGG
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

Table 2. Summary of demographic information of the groups

	PD (n=75)	Healthy control (n=50)	p value
Gender (female/male)	10/20	20/30	>0.05
Age (mean ± SD)	63.16±10.60	62.98±10.54	>0.05
Disease onset (mean ± SD)	53.41±10.68		
Disease duration, year (mean ± SD)	9.75±5.51		
UPDRS total (mean ± SD)	46.74±24.01		
UPDRS I	7.53±4.58		
UPDRS II	8.42±6.54		
UPDRS III	31.71±15.77		
UPDRS IV	3.01±3.67		
H&Y scale	1.93±0.44		

H&Y scale: Hoehn-Yahr Rating Scales; UPDRS: Unified Parkinson's Disease Rating Scale.

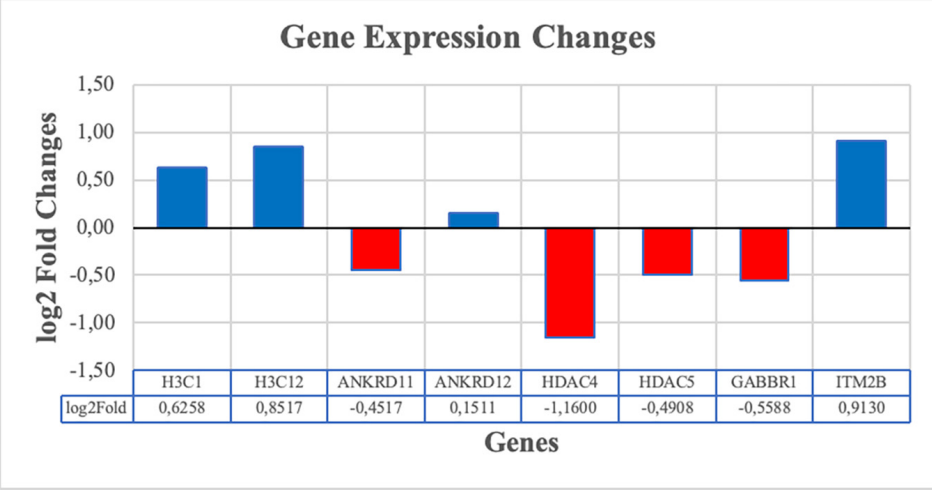

Figure 1. Log2 fold changes in expression level of PD group compared to healthy control group.

-0.233), HDCA5 and UPDRS Total (p=0.056, r=-0.222), GABBR1 and UPDRS IV (p=0.036, r=-0.243), GABBR1 and UPDRS Total (p=0.013, r=-0.286).

DISCUSSION

Idiopathic PD occurs in approximately 80% of all PD patients and is a neurodegenerative process that mainly affects neurons in the substantia nigra. The substantia nigra is a region containing 850000 cells and at least around 60% of these cells need to be lost before the emergence of PD symptoms (13). Thus, clinical symptoms appear a long time after disease onset due to the slow progression of cell loss and high system tolerance, and evidently, hereditary predisposition, exposure to environmental toxins and aging play an important role in the progression of PD.

Today, pathophysiological studies have shown that neuronal loss due to gene mutations in sporadic PD patients is due to multiple causes, whether genetic or environmental. Nevertheless, studies have clearly shown that pathological processes due to cell death caused by defectively synthesized enzymes, proteins or gene substrates due to gene mutations are an important factor in PD. However, emergence of pathological conditions due to non-genetic factors may lead to PD susceptibility through the loss of function of different cellular structures (14,15).

In recent years, alterations in the mechanisms that maintain cellular homeostasis in neurodegenerative diseases that develop largely without direct genetic mutations but through epigenetic modifications, have begun to be investigated (16).

The acetylation of histones is dynamically regulated by histone acetylase (HAT) and histone deacetylase (HDAC) in a process called acetylation. HDACs are active regulators of axon growth, oxidative stress, synaptic plasticity and cognition. There are studies showing different neuropathological findings depending on the localization, expression changes and inhibition of histone deacetylases (17). In a study using a PD cell model, it was shown that exposure to paraquat, a neurotoxin, can induce histone H3 acetylation in N27 dopaminergic cells in a time-dependent manner. The marked increase in histone acetylation was partially associated with an increase in histone expression. However, both the activity and protein levels of histone deacetylases HDAC4 and HDAC7 were decreased. In addition, HDAC1, HDAC2, HDAC4, HDAC6 and SirT1 levels were found to be significantly lower in the midbrain tissues of PD

cases compared to matched controls (18). Other studies have also shown that neurotoxin-induced changes in HDAC activity or level occur through autophagy mechanisms, thus autophagy-mediated HDAC deficiency abnormally increases histone acetylation in dopaminergic neurons and ultimately causes neurodegeneration (19,20). The results suggest that environmental toxins abnormally upregulate histone expression and induce histone acetylation. Similarly, in our study, increased H3 histone expression and decreased HDAC4 and HDAC5 expression were observed in PD cases compared to healthy controls.

GABBR1 is a gene encoding a subunit of the GABAB receptor that binds to the potassium channel via the G protein. GABAB receptors consist of two subunits, GABBR1 and GABBR2, which act as inhibitors of calcium channels at the presynaptic membrane and activators of potassium channels at the postsynaptic membrane (21). In the PD model generated using MPTP and 6-HDA, the functioning of GABAB receptors was shown to be altered, resulting in the need for GABAergic inhibition upon dopamine deprivation (22). Furthermore, a recent meta-analysis of genome-wide microarray data from the study of various tissues of the human brain showed that GABBR1 gene expression is down-regulated in PD and Alzheimer's disease (23). Similarly, in our study, patients with PD also showed significantly reduced GABBR1 expression levels.

The Unified Parkinson's Disease Rating Scale (UPDRS) is a tool used to measure the severity as well as the progression of Parkinson's disease (PD). Developed by neurologists in 1987 to track the response to medications used to monitor and reduce the signs and symptoms of PD, the UPDRS was developed as the gold standard and is used for patients diagnosed with idiopathic PD at any stage according to the Hoehn and Yahr Staging Scale (H and Y). It addresses many aspects of Parkinson's, including motor and non-motor complications, and is suitable for use in a clinical setting.

There was a weak negative correlation between HDCA5 and GABBR1 versus total UPDRS and UPDRS IV scale scores, indicating that patients with higher HDCA5 and GABBR gene expression levels show trends towards displaying decreased UPDRS scores and thus lower clinical severity of PD. Therefore, it is tempting to speculate that these two genes have suppressive properties in the physiopathology of PD.

Histone acetylation, one of the epigenetic mechanisms, provides important information to understand the disease process because it is the mechanism by which transcriptional activity is regulated. Our study is valuable in terms of comparing histone acetylation-related gene expressions in idiopathic PD cases in the Turkish population for the first time. On the other hand, the small number of histone acetylation-related genes and the lack of analysis at the acetylation level are among the limitations of our study.

Ethics Committee Approval: The study was ethically approved by the Clinical Research Ethics Committee of Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (meeting decision number 20 dated 06.12.2019).

Informed Consent: An "Informed Consent Form" was obtained from all patients participating in the study.

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