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ORIGINAL ARTICLE

Epigenetic analysis of heat shock activator complex in the peripheral blood of Parkinson's disease patients and its clinical significance

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ABSTRACT

Objectives: This study aimed to investigate the methylation changes of related genes in the peripheral blood and their clinical significance in Parkinson's disease (PD) and whether the methylation change of the gene encoding long noncoding RNA was different in the blood of patients and controls.

Patients and methods: This prospective cross-sectional, controlled study was conducted with 45 participants (22 males, 23 females; mean age: 60.7±5.9 years; range, 53 to 75 years) between June 2020 and June 2021. Drug-naive patients diagnosed with PD were included in this study. Those with PD and a Mini-Mental State Examination (MMSE) score >23 were defined as Group 1 (n=15), and those with PD and an MMSE score ≤23 were defined as Group 2 (n=15). Controls were included in Group 3 (n=15). The methylation changes of genes HSP70, HSP90, heat shock factor 1 (HSF1), heat shock RNA 1 (HSR1), and eukaryotic translation elongation factor 1 alpha (eEF1α) were investigated with methylation-specific real-time quantitative polymerase chain reaction analysis.

Results: The eEF1α was significantly more hypermethylated in Group 1. In Group 2, HSP70, HSP90 HSF1, HSR1, and eEF1α were significantly hypomethylated compared to Group 1 and Group 3 (for all genes $p<0.001$). The HSF1 hypomethylation was negatively correlated with MMSE and positively correlated with depression scores ($p=0.03$ and $p=0.013$, respectively). The correlation of eEF1α with MMSE and depression was the opposite of HSF1 ($p<0.001$ and $p=0.013$, respectively).

Conclusion: Cell line and autopsy studies indicate that eEF1α hypermethylation might be one of the main molecules triggering alpha-synuclein aggregation in the pathogenesis of PD. Therefore, eEF1α may be a molecule that can be used as a peripheral biomarker. The findings supported this idea as it was more hypermethylated in PD patients than in controls, whereas its negative regulator HSF1 was hypomethylated and correlated with the clinic. Furthermore, the worsening of cognitive functions and depression in PD patients may affect methylation levels of chaperone genes in the peripheral blood.

Keywords: Cognitive dysfunction, DNA methylation, eEF1α, heat shock activator complex, Parkinson's disease.

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide, often causing both motor and nonmotor symptoms in affected individuals, ultimately leading to morbidity. Therefore, PD is one of the most researched diseases for pathogenesis-oriented treatment rather than symptomatic treatments. Its pathogenesis is characterized by the accumulation of Lewy bodies consisting of alpha-synuclein in dopaminergic neurons in the substantia nigra pars compacta (SNpc) and by the resultant cellular degeneration.[1] A leading mechanism preventing the aggregation of

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alpha-synuclein is the signal pathways that activate in the presence of intracellular aggregation and provide cellular protection.[2] Endoplasmic reticulum stress (ERS) is the primary molecular mechanism preventing the intracellular accumulation of unfolded proteins (UFPs). Endoplasmic reticulum stress exerts its function by denaturing UFPs with heat shock proteins (HSPs) through the activation of PERK, IRE, and ATF6 signaling pathways and then refolding them properly or preventing aggregation of proteins involved in UFP degradation.[3] Unfolded proteins are refolded with HSPs, which are also known as chaperones. The HSP70 and HSP90 are the most significant HSPs that have been shown to be effective in PD. In cell line and animal studies examining the role of HSP70 in PD, it has been demonstrated that increased HSP70 reduces dopaminergic neuron loss and alpha-synuclein aggregation, while decreased HSP70 increases neurodegeneration.[4-6] Additionally, cell line studies investigating the role of HSP90 in the pathogenesis of PD have indicated that HSP90, in a similar way to HSP70, has a neuroprotective effect in the PD cell line model created with MPP+ (1-methyl-4-phenylpyridinium ion) toxicity.[7,8]

Heat shock factor 1 (HSF1) is the primary transcriptional factor of HSPs in PD. As long as there is no ERS in the cell, HSP70, HSP90, and HSF1 are bound to form a complex. When UFPs cause ERS, the intracellular temperature increases, and this heat-sensitive complex breaks up, releasing HSF1. Heat shock factor 1 is involved in proteostasis, synaptic fidelity, and memory consolidation.[9] Moreover, HSF1 expression has been shown to decrease alpha-synuclein aggregation.[9] On the other hand, induction of a cascade of calcium-signaling events at the neuromuscular junction by the cholinergic neurotransmission also leads to HSF1 activation. In addition, lack of HSF1 has been associated with neurodegeneration.[10]

Heat shock factor 1 trimerizes through heat shock RNA 1 (HSR1), which is a long noncoding RNA (lncRNA), and also through the eukaryotic translation elongation factor 1 alpha (eEF1α) protein, to which HSR1 binds. Moreover, HSF1 also binds to DNA and controls the transcription of HSPs. In other words, HSF1-mediated HSPs are the main proteins considered to protect the cells from protein-related damaging factors.[11] On the other hand, it is considered that the increased UFPs and the increased aggregation of alpha-synuclein by the lesser transcription of HSF1 and the proteins that regulate its transcription in PD may be molecular mechanisms affecting the pathogenesis. Additionally, a leading mechanism in the regulation of transcription in these genes is DNA methylation.[12]

The primary aim of this study was to examine whether there were epigenetic changes of HSP70, HSP90, HSF1, HSR1, and eEF1α, which are thought to play a role in the pathogenesis of PD, and to investigate how the presence of cognitive dysfunction contributed to the methylation levels of these genes. The secondary aim of the study was to examine whether there was a correlation between methylation levels of related genes and clinical symptoms of PD.

PATIENTS AND METHODS

The prospective, cross-sectional, controlled study was conducted with patients who presented to the neurology department of İnonü University Faculty of Medicine and fulfilled the inclusion criteria between June 2020 and June 2021. The inclusion criteria were as follows: *(i)* fulfilling the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria; *(ii)* absence of other diseases that could affect chaperones, such as diabetes mellitus (DM), other neurodegenerative diseases, cancer, and coronary artery disease (CAD); *(iii)* age between 50 and 75 years; *(iv)* being drug naive. The exclusion criteria were as follows: *(i)* individuals using dopaminergic drugs and *(ii)* smokers. A total of 189 PD patients presented to the neurology outpatient clinic between June 2020 and June 2021. Of these, 159 patients were excluded from the study due to the presence of DM, CAD, cancer, and other neurodegenerative diseases (n=115), not meeting the age criterion (n=29), and refusal to participate in the study (n=15). As a result, the remaining 30 patients were included in the study (Figure 1). Additionally, a control group of 15 age- and sex-matched participants was included in the study, for a total of 45 participants (22 males, 23 females; mean age: 60.7±5.9 years; range, 53 to 75 years). Clinical tests were performed by the same neurologist.

The cutoff of Mini-Mental State Examination (MMSE) scores may vary according to age, education level, and demographic data.[13] The generally accepted cutoff value for the MMSE for educated individuals is 24 points. Values below 24 points are associated with cognitive impairment. In the validity and reliability study of MMSE for the Turkish population published by Keskinoğlu et al.[14] in 2009, MMSE scores <26 were

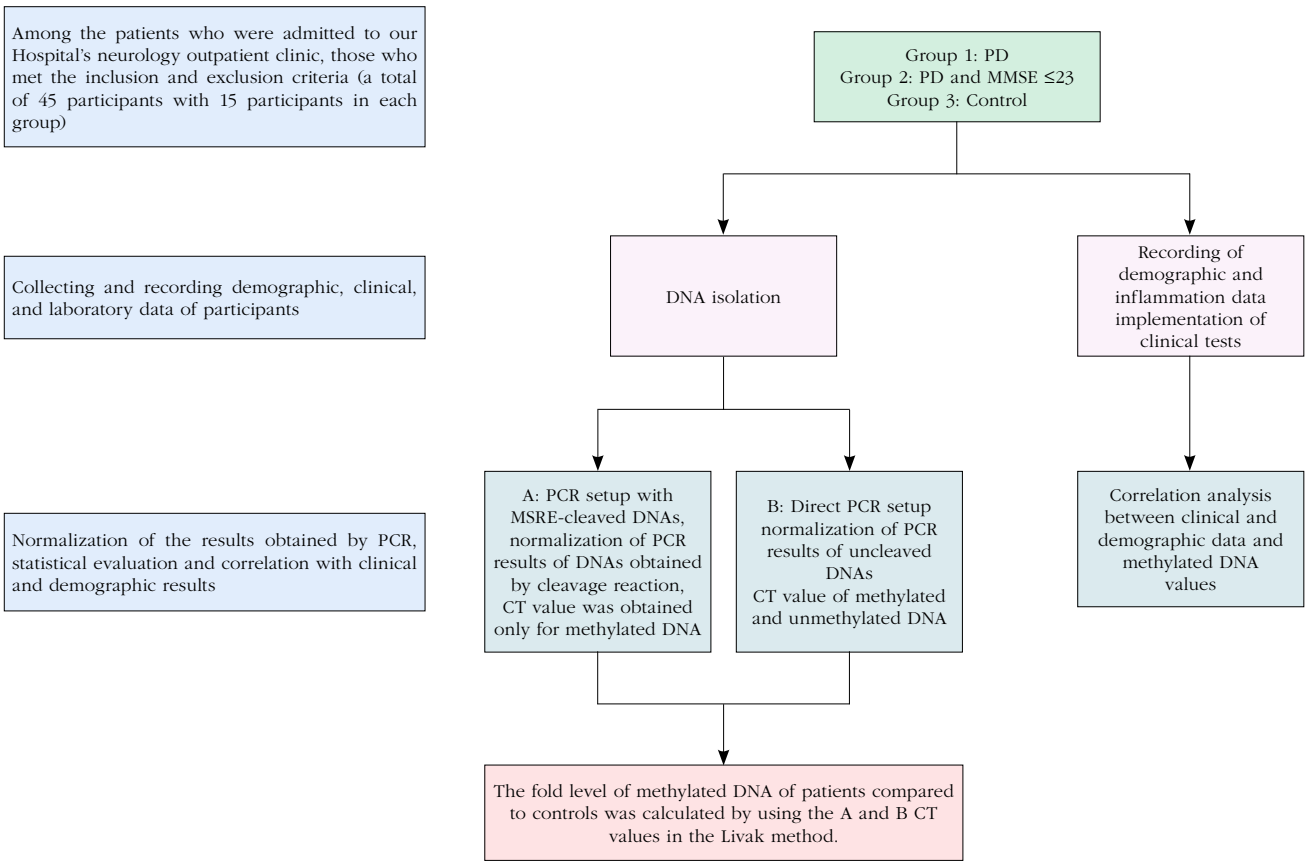

Figure 1. Flowchart of the study.

PD: Parkinson's disease; MMSE: Mini-Mental State Examination; MSRE: Methylation-sensitive restriction enzyme; DNA: Deoxyribonucleic acid; PCR: Polymerase chain reaction; CT: Cycle threshold.

considered the limit value for minimal cognitive impairment, while scores <24 were considered the limit value for mild cognitive impairment, and scores <22 were considered the limit value for cognitive impairment. Therefore, we considered values ≤23 as cognitive impairment. Patients with a diagnosis of PD with an MMSE score ≤23 were classified as PD patients with cognitive dysfunction. Participants were divided into three groups, with 15 in each group: PD patients with MMSE scores >23 (Group 1), PD patients with MMSE scores ≤23 (Group 2), and the control group (Group 3). The patients included in the study were evaluated, and their demographic characteristics were recorded by the same researcher. Extrapyramidal examination findings were assessed using the Unified Parkinson's Disease Rating Score (UPDRS), cognitive functions were assessed using MMSE, and depression scores were assessed using the Geriatric Depression Scale (GDS). Laboratory and inflammation markers were entered into the database. Patients' daily life activities were recorded using the Schwab and England Activities of Daily Living Scale (SEADL), and disease stage was assessed using the Hoehn and Yahr (H&Y) scale.

Five milliliters of peripheral blood samples were obtained from each participant and stored at –20°C until laboratory analysis. Participants' DNA was isolated from the peripheral blood using the appropriate protocol with the QIAamp DNA Blood Maxi Kit (Qiagen, Maryland, USA). The quality and concentrations of the obtained DNAs were measured spectrometrically using a nanodrop spectrophotometer (DeNovix Inc., Wilmington, DE, USA). To evaluate methylation, an appropriate amount of DNA was cleaved with methylation-sensitive restriction enzymes (MSREs), HpaII and Hin6I, following the kit protocol. The presence of methylation prevents DNA digestion by the restriction enzymes, thus keeping the DNA intact and amplifying the methylated DNA region by polymerase chain reaction (PCR). However,

TABLE 1
Primers of associated genes and numbers of sites cleaved by MSREs

Genes	Forward primer	Backward primer	Hin6I	HpaII
HSP70	GGTGAGGGTGCGATCTTAGA	AGAAACTTCCGGTAGTGGGG	33	12
HSP90	GCCCCGATTCCCAAGCTAAG	CCAAAGAATCCAGCCGCAAG	33	22
HSF1	TGATCCCCACAACCAAGACA	CTCTCCAGCCGTTCCATTTG	30	23
HSR1	TCCCATCCTCCTCATCTCTCCA	GATGTTCTCCGCAACCTTCC	5	8
eEF1α	CAGAGGAAGACGCTCTAGGG	ATTACTCTCAGCTCCGGTCG	5	12

MSREs: Methylation-sensitive restriction enzymes; HSP70: Heat shock protein 70; HSP90: Heat shock protein 90; HSF1: Heat shock factor 1; HSR1: Heat shock RNA 1; eEF1α: Eukaryotic translation elongation factor 1 alpha.

unmethylated DNA molecules are cut by the enzymes and cannot serve as a template for the PCR.

The Hin6I recognizes and cleaves DNA at the 5′-GCGC-3′ and 3′-CGCG-5′ sites, and HpaII recognizes and cleaves DNA at the 5′-CCGG-3′ and 3′-GGCC-5′ sites. The DNA cleavage efficiencies were analyzed by 1% agarose gel electrophoresis. All of the DNA samples were cut under the same conditions (500 ng DNA, 10 IU enzymes, 37°C, and 8 to 12 h).

To investigate the importance of chaperones and related molecules in the pathogenesis of PD, the methylation changes of PD-related HSPs, HSP70 and HSP90, as well as the methylation changes of HSF1, which controls the transcription of these HSPs, lncRNA, which activates HSF1, and the eEF1α gene were investigated. Table 1 presents the primers used in the amplification of the promoter regions of these genes and the number of sites cleaved by MSREs.

A total of six PCR reactions were established for each participant, using three cleaved DNAs and three uncleaved DNAs. In total, 270 reactions were established for each gene in all 45 participants. Table 2 presents the reaction content, grading, and times.

The normalized data of the cycle threshold values obtained by the reactions were processed using the Livak method.[15] Melting curve analyses were also performed to ensure the fidelity of each amplification and amplified DNAs were also checked by 1% agarose gel electrophoresis. The flowchart of the study is presented in Figure 1.

Statistical analysis

Data were analyzed using IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). Comparisons of qualitative data were performed by Pearson's chi-square test. Normal distribution of the quantitative data was assessed using the Shapiro-Wilk test. Descriptives were expressed as median, minimum, and maximum values. Two independent groups were compared by the Mann-Whitney U test, and the Kruskal-Wallis test was used for more than two independent groups.

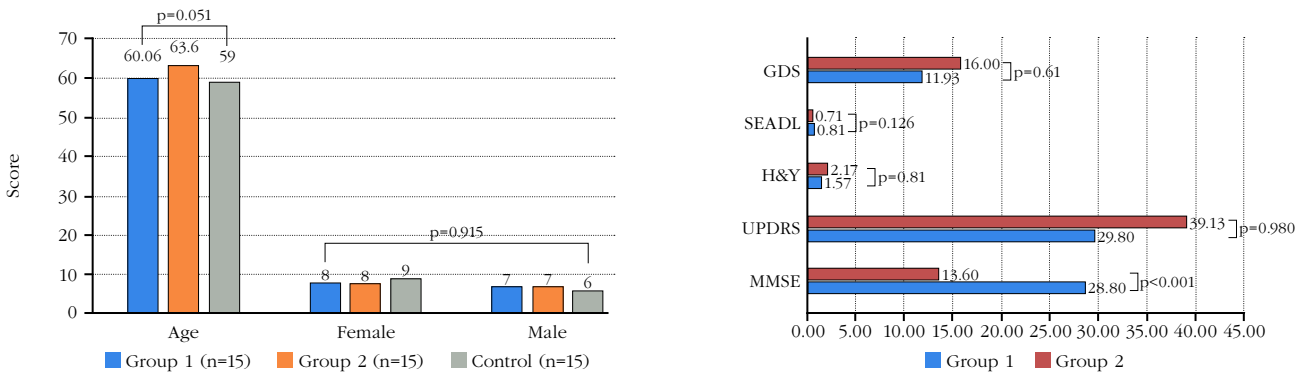

Figure 2. The demographic and clinical characteristics of the participants. There was no significant statistical difference for age between groups (p=0.51). There was no significant statistical difference between the groups in terms of sex.

GDS: Geriatric Depression Scale; SEADL: Schwab and England Activities of Daily Living Scale; H&Y: Hoehn and Yahr; UPDRS: Unified Parkinson's Disease Rating Score; MMSE: Mini-mental state examination.

TABLE 2
Reaction content and reaction grades and times of PCR

Reaction content	150 ng DNA
	10 microliter mastermix
	0.5 microliter primer mix, 10 microM (forward and backward)
	DNAase free water (Total reaction content 20 microliter)
Reaction grades and times	10 minute 95°C denaturing stage 1 cycle
	45 second 95-61-72°C annealing stage 30 cycle (for eEF1α primer 95-65-72°C annealing stage 30 cycle)
	10 minute 72°C extending stage 1 cycle

PCR: Polymerase chain reaction; DNA: Deoxyribonucleic acid; eEF1α: Eukaryotic translation elongation factor 1 alpha.

TABLE 3
Methylation differences of HSPs and regulatory factors between groups

	Control (n=15)		Group 1 (n=15)		Group 2 (n=15)		
	Median	Min-Max	Median	Min-Max	Median	Min-Max	*p*
HSR1	1458.23	45.25-198668[a]	16384	181.02-334118.42[a]	0.02	0-9703003.75[b]	<0.001
HSP90	512	2.41-9674.69[a]	3590.58	16-24491.61[a]	0.03	0-307451.64[b]	<0.001
HSP70	4124.49	184.82-15076.35[a]	5792.62	230.72-370727.6[a]	0	0-4107986.47[b]	<0.001
HSF1	107.63	0.35-15286.81[a]	128	5.66-58251.19[a]	0.18	0-27939.14[b]	<0.001
eEF1α	2.25	0.6-34.04[a]	44.02	9.13-256[b]	0.03	0-62.68[c]	<0.001

HSR1: Heat shock RNA 1; HSP90: Heat shock protein 90; HSP70: Heat shock protein 70; HSF1: Heat shock factor 1; eEF1α: Eukaryotic translation elongation factor 1 alpha; * The difference between groups with different superscripts was statistically significant.

Following the Kruskal-Wallis test, Conover's method was used for pairwise comparisons. The effect of gene methylation on depression, UPDRS, and total MMSE was determined with nonstandardized and standardized regression weights using the AMOS program (IBM Corp., Armonk, NY, USA). A *p*-value <0.05 was considered statistically significant.

RESULTS

Figures 2 present the demographic and clinical characteristics of the participants. There was no significant statistical difference for age between groups (p=0.51, Figure 2). There was no significant statistical difference between the groups in terms of sex (p=0.915, Figure 2).

The only gene that was different between Group 1 (MMSE >23) and Group 3 was eEF1α. This gene was significantly more hypermethylated in Group 1 than in Group 3. However, in Group 2, HSP70, HSP90 HSF1, HSR1, and eEF1α were significantly hypomethylated compared to Groups 1 and 3 (Table 3). While there was a statistically significant difference in MMSE scores between the patient groups (p<0.001), there was no statistically significant difference in UPDRS (p=0.980), H&Y (p=0.81), SEADL (p=0.126), and GDS scores (p=0.61, Figure 2).

Demographic characteristics of the participants did not affect the methylation changes in the groups. Correlations between clinical characteristics of the patient groups and methylation levels were evaluated with the AMOS path diagram (Figure 3). According to the analysis of moment structures, eEF1α hypomethylation was 0.877 times positively correlated with MMSE (p<0.001), and −0.703 negatively correlated with depression (p=0.013). Hypomethylation of HSF1 was −0.731 negatively correlated with MMSE (p<0.03) and 0.657 times positively correlated with depression (p=0.013). Path analysis results between other gene methylation levels and clinical findings are shown in Figure 3.

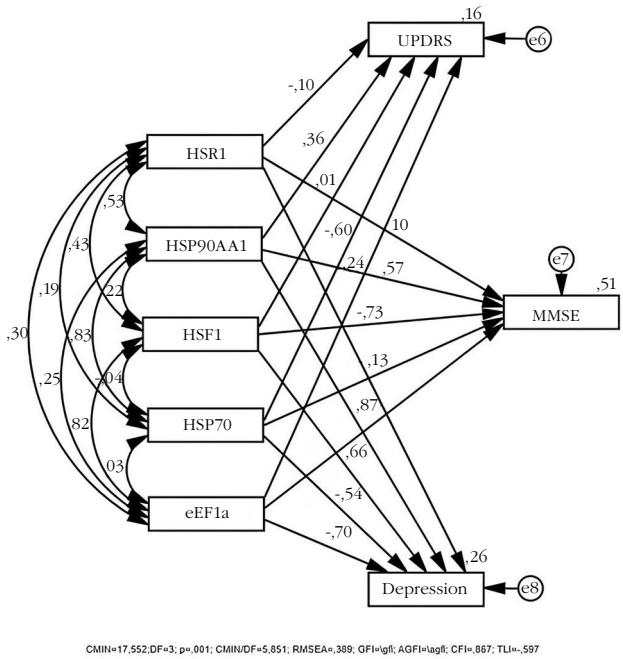

Figure 3. Path diagram of gene methylations and clinical findings. Results of full structural equation model analysis. According to the analysis of moment structures, eEF1α hypomethylation was 0.877 times positively correlated with MMSE ($p<0.001$).

UPDRS: Unified Parkinson's Disease Rating Score; HSR1: Heat shock RNA 1; HSF1: Heat shock factor 1; HSP70: Heat shock protein 70; eEF1α: Elongation factor 1 alpha; MMSE: Mini-mental state examination.

DISCUSSION

The main finding of this study was that eEF1α hypermethylation might be one of the main molecules triggering alpha-synuclein aggregation in the pathogenesis of PD. Another important finding was that the worsening of cognitive functions in PD patients may trigger hypomethylation of chaperone genes as a reaction to increased neurodegeneration.

The main trigger for the increased aggregation of alpha-synuclein to transform into Lewy bodies in PD is unknown. Therefore, disease-modifying treatments are the most critical but unmet need in PD. Although symptomatic treatments preserve patients' quality of life and functions for a while, disease progression cannot be prevented and continues to be a social and economic burden for the patient and the family. It is known that the pathogenetic processes in PD begin years before the clinical manifestation of PD symptoms.[1] Of note, hyposmia and gastrointestinal dysfunction are known to begin years before the onset of PD, and alpha-synuclein aggregation has been shown in the epithelium of these regions.[16] Although mutations or polymorphisms in alpha-synuclein cause Lewy body aggregation, this can be detected in a very small proportion of patients with PD.[17] However, this pathogenetic process remains largely unknown. Although the UFP response is likely to provide neuroprotection, various factors that trigger epigenetic changes and are known as facilitators, including inflammation, aging, energy depletion, and excitotoxicity, may be involved in the pathogenesis of idiopathic PD.[18] The transition of ERS to the terminal stage may cause this pathogenetic process. An example of this is the reduction of gene expression by hypermethylation of chaperones.

Heat shock proteins are expressed at low levels under normal conditions but show a dramatic increase in expression in response to cellular stress. Heat shock proteins serve as intracellular chaperones for other proteins. Molecular chaperones are ubiquitous, highly conserved proteins involved in newly synthesized protein folding. They promote protein folding, prevent protein aggregation, or target improperly folded proteins and transfer them to specific degradation pathways. Moreover, HSPs can regulate apoptosis by directly interacting with some components of the apoptotic pathway.[4,9,19] The main regulator of the heat shock response is HSF1.[19] It has been shown that the increased expression of HSP70 plays a neuroprotective role both directly and indirectly by promoting the HSF1 expression in animal models.[12,20] Additionally, increasing the expression of HSP70 by the activation of HSF1 is conducive to improving cognitive function.[21] In the aging brain, the levels of various chaperones, such as small HSPs and structural HSP70, change. This reduced inducibility in stress proteins, together with a decreased UPS activity, can increase neuronal fragility and cause neurodegeneration. The HSP70 and HSP90 appear to be involved in the pathogenesis of PD.[19,22]

It has been reported that HSP70 is involved in different cellular processes, such as protein translation, translocation between membranes, assembly and disassembly of macromolecular complexes or aggregates, and apoptosis.[19] In addition, the inhibitory role of HSF70 in the aggregation of synuclein fibrils, which is the main component of Lewy bodies, has been defined in different studies.[4,6,8,23,24] Thus, it has been observed that HSP70 effectively inhibits synuclein fibril formation *in vitro*. Inhibition of fibrillation is known to result from the interaction of HSP70 with various

synuclein intermediates. The HSP70 can inhibit the formation of prefibrillar synuclein, a pathogenic strain in PD, and also delay fibril elongation.[19,25]

Another prominent chaperone known to play a role in PD is HSP 90, which does not function in de novo folding of proteins as in HSP70 but may affect the rate of stress-induced denatured protein refolding.[19] It has been shown that HSP90 is the predominant chaperone involved in synuclein-related pathologies. Similar to HSP70, HSP90 dissolves *in vivo* to colocalize with synuclein, suggesting a possible role in modulating folding and suppressing misfolded synuclein.[26] Falsone et al.[27] found that HSP90 blocked synuclein aggregation in an *in vitro* animal model.[28] In times of stress, HSP90 keeps unnatural client proteins in a structure capable of folding and prevents their irreversible denaturation. When nucleotide binds, HSP90 can direct client proteins to the proteasome, promoting ubiquitination and degradation. Therefore, HSP90 may play a role in the balance between stabilization and degradation of client proteins. In animal models of Parkinson's disease induced by MPP+ and rotenone, overexpression of HSF70 has been observed to not only protect cells from cytotoxicity but also reduce soluble protein aggregations in cells.[23,24,29] Similar to HSP70, expression of HSP90 was increased in rotenone-induced Parkinson's models.[19] In addition, some studies have found that inhibiting HSP90 leads to a decrease in oligomeric synuclein, attenuation of cytotoxicity of mutant A53T synuclein, and an increase in dopamine in PD.[28]

In our study, no significant difference was found between PD patients and controls in HSP70 methylation, while significant hypomethylation was detected in patients with PD who developed cognitive dysfunction. Additionally, hypermethylation of eEF1α was detected in PD patients, which could be explained by the fact that eEF1α inhibits HSF1 from binding to DNA and there was no elevation in the expression of chaperones such as HSP70 and HSP90. These findings suggest that unfolded alpha-synuclein cannot be corrected by the mediation of chaperones due to hypermethylated eEF1α and contributes to the formation and progression of PD pathogenesis. To the best of our knowledge, there is no data in the literature about eEF1α methylation levels in PD. In correlation with the literature, our data also support that increasing the expression of HSF1 and HSP70 by reducing hypermethylation of eEF1α levels could slow down alpha-synuclein accumulation in the early period. In our PD group with cognitive dysfunction, it was observed that all the chaperones evaluated in our study were hypomethylated, which could increase the gene expression. These findings also implicate that as the disease spreads in patients, chaperones reactively increase to provide secondary neuroprotection associated not only with alpha-synuclein aggregation but also with additional factors, such as the increase in intracellular calcium level and neurotransmission change.

Eukaryotic translation elongation factor 1 alpha is a protein member of the G protein family that is responsible for the enzymatic delivery of aminoacyl transfer RNAs to the A site of the ribosome during the protein translation process. There are two isoforms of eEF1α, including eEF1α1 and eEF1α2, which are expressed in different tissues. eEF1α1 is found in almost all types of tissues, while eEF1α2 is expressed only in tissues composed of nonproliferating cells, such as the brain, heart, and skeletal muscles. In neurons, eEF1α1 is expressed during embryonic development and during the first two weeks of postnatal development. Subsequently, expression of eEF1α2 increases and becomes dominant.[30] Recent studies have indicated that the RNA class known as noncoding RNA plays a regulatory role in protein expression.[11] Similarly, our findings indicated that HSF1, which increases the expression of HSPs, may cause dysfunction of chaperones of lncRNA, which enables specific binding of HSF1 to DNA and of lncRNA to eEF1α. The opposite MMSE and depression correlations of HSF1 and eEF1α2 in AMOS analysis also support the findings. On the other hand, HSR1 and eEF1α were hypomethylated in the PD group with cognitive dysfunction, while eEF1α was hypermethylated in Group 1 compared to Groups 2 and 3. A study also reported that the expression of eEF1α in the MPP+-induced cell line increased secondarily.[31] Based on the literature, we consider that the hypomethylation of eEF1α in Group 2 could be a reactive result aimed at providing neuroprotection, as in the study of Khwanraj et al.[31] Garcia-Esparcia et al.[32] showed that the expression of eEF1α in SNpc in the brain of patients with PD decreased, and it caused alpha-synuclein aggregation. Prommahom and Dharmasaroja[30] showed for the first time in the MPP+ PD model that eEF1α2 plays an important role in the regulation of autophagy in dopaminergic neuron-like cells and suggested that eEF1α2 may provide a mechanism for survival of dopaminergic neurons in conditions of neurotoxicity in terms of neurodegeneration pathogenesis.

In our study, in a similar way to the findings of Garcia-Esparcia et al.,[32] hypermethylation that caused decreased eEF1α gene expression was observed in Group 1. Studies on neurodegenerative diseases have shown a correlation between the methylation levels in peripheral blood and those in brain tissue.[33,34] These findings suggest that the methylation levels assessed in the peripheral blood of our patients could be similar to those in the intracranial environment, and these levels could be used as a biomarker and a peripheral indicator of pathogenesis. In addition, the fact that the blood samples in Group 1 were collected at the time of the first diagnosis prior to the use of dopaminergic drugs and that the patients had no diseases such as DM, CAD, and additional neurodegenerative diseases, smoking, and cancer, which could affect the epigenetic regulation of chaperones due to ERS, further strengthen the relationship between PD and the data obtained in the study.

Chaperone-based therapies have recently emerged as important treatment modalities in the pathogenesis of neurodegenerative diseases. Of note, these therapies promote HSF1 through the inhibition of HSP90, indirectly increasing the expression of HSP70 and HSP90 and reducing alpha-synuclein aggregation. Additionally, there are treatments aimed at directly increasing the expression of HSP70 in addition to chemical chaperones and adeno-associated virus-mediated chaperone gene therapies.[35] Our findings suggest that treatments that will increase the expression of lncRNA-mediating proteins, such as eEF1α, particularly in the early diagnosis stage prior to the development of cognitive dysfunction, may play an important role in preventing the progression of neurodegeneration. In addition, the findings also implicated that eEF1α can be used as an important peripheral blood biomarker that can be used as an indicator of both PD and the development of cognitive dysfunction in PD.

There are some limitations to this study. First, the study had a small patient population. However, we considered that the risk of technical inaccuracies would increase in proportion to the increasing number of participants. Furthermore, it would be difficult to find more patients who met the exclusion criteria, and PCR analyses would require a larger number of reactions as the number of patients increased. Therefore, further studies investigating gene methylation panels in larger populations may substantiate our findings. Another limitation was that the parameters examined were studied from peripheral blood rather than brain tissue. However, it was not possible to use brain tissue for ethical reasons. Finally, MMSE is a tool that does not assess all aspects of cognitive impairment in PD patients.

In conclusion, while there was no additional factor that could affect ERS and thus chaperones in PD patients, our findings indicate that eEF1α, a protein to which lncRNAs that regulate chaperones bind, may have an important role in the pathogenesis of PD and serve as a peripheral biomarker. Accordingly, further treatment studies are recommended for the development of substrates for this molecule. The findings also demonstrate that one of the primary mechanisms regulating the expression of chaperones is methylation change and that there is a need for regulating the factors that may affect methylation in PD. Finally, given that the coexistence of cognitive dysfunction with PD implies that chaperone expression can be changed by adding additional clinical and cellular factors, the importance of early diagnosis of PD and early initiation of pathogenesis-oriented treatments is of paramount importance.

Ethics Committee Approval: The study protocol was approved by the Malatya Clinical Trials Ethics Committee (date: 05.02.2020, no: 2020/05). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Study Design: Y.I.G., A.K.; Participant selection: Y.I.G., I.T.; Lab work: Y.I.G., M.D., N.Ö.; Database and statistical analysis: Y.I.G., I.T., H.G.G.; Literature analysis and article writing: Y.I.G., I.T., M.D., N.Ö., H.G.G., A.K.

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