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Mitochondrial Respiratory Chain Enzyme Activities, mtDNA Variants and Gene Expression Levels in Idiopathic Parkinson's Disease

[İdiyopatik Parkinson Hastalarında Mitokondriyel Solunum Zinciri Enzim Aktiviteleri, mtDNA Variantları ve Gen Ekspresyon Düzeyleri]

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ABSTRACT

Purpose: Mitochondrial dysfunction has been implicated in the development of idiopathic Parkinson's disease. In this study, it was aimed to investigate the relationship between mitochondrial respiratory chain enzyme deficiency and variants in ND2 and ND4 region of Complex I and expressions of these genes. Complex IV activity and glutathione level were also studied.

Methods: Activities of Complex I, Complex IV, citrate synthase and glutathione levels were measured from muscle biopsy samples of 19 idiopathic Parkinson's Disease patients. Entire ND2 and ND4 regions of Complex I were screened by sequencing and expression levels of these regions were also examined by using relative quantitative RT-PCR.

Results: A significant decrease in Complex I and IV activities was found in Parkinson's Disease patients (19.73 ± 8.24 U/mg protein, 11.51 ± 6.45 U/mg protein) compared with the control group (31.48 ± 8.28 U/mg protein, 30.02 ± 14.76 U/mg protein), respectively. Although several sequence variants were detected in ND2 and ND4 genes, they didn't account for the decrease in Complex I activity. ND2 (19 % and 21 %) and ND4 (14 % and 13 %) mRNA expressions were reduced in two of 19 patients.

Conclusion: Taken together, Complex I and IV dysfunctions and mitochondrial abnormalities might be the result of oxidative stress contributing to the pathogenesis of idiopathic Parkinson's Disease since we found that reduced glutathione levels were decreased 47 % in these patients.

Key Words: Idiopathic PD, Complex I, glutathione, ND2, ND4

ÖZET

Amaç: İdiyopatik Parkinson Hastalığı'nın ortaya çıkmasında mitokondriyel bozukluk oldukça önemlidir. Çalışmada mitokondriyel solunum zinciri enzim hasarı ile Kompleks I'in ND2 ve ND4 bölgelerindeki varyantlar ve bu genlerin ekspresyon düzeyleri arasındaki ilişkinin incelenmesi amaçlandı. Ayrıca Kompleks IV aktivitesi ile glutatyon düzeyleri değerlendirildi.

Yöntem: Kompleks I, Kompleks IV ve sitrat sentaz enzim aktivitelerini tayin etmek amacıyla 19 idiyopatik Parkinson hastasından elde edilen kas biyopsi örnekleri kullanıldı. Ayrıca Kompleks I'in ND2 ve ND4 bölgeleri sekanslama yapılarak tarandı ve bu bölgelerin ekspresyon düzeylerine göreceli kantitatif RT-PCR yöntemiyle bakıldı.

Bulgular: İdiyopatik Parkinson Hastaları'nda Kompleks I (19.73 ± 8.24 U/mg protein) ve Kompleks IV (11.51 ± 6.45 U/mg protein) aktivitelerinde kontrol grubuna (31.48 ± 8.28 U/mg protein, 30.02 ± 14.76 U/mg protein) kıyasla belirgin azalma gözlendi. Sekanslama sonucunda ND2 ve ND4 gen dizilerinde birçok sekans varyantı bulunmasına karşılık bunların Kompleks I aktivitesindeki düşüşten sorumlu olmadığı düşünüldü. Yine bu genlerin ekspresyon düzeylerine bakıldığında 19 hastadan sadece iki tanesinde Kompleks I'de % 19 ve % 21'lik; Kompleks IV'de ise % 14 ve % 13'lük azalma saptandı.

Sonuçlar: Bütün bu sonuçlar ve hasta grubun glutatyon düzeyindeki % 47'lik düşüş dikkate alındığında Kompleks I ve IV'ün hasarı ve mitokondriyel bozukluklar oksidatif stresin sonucu olabilmekte ve Parkinson Hastalığı'nın ortaya çıkmasına katkıda bulunabilmektedir.

Anahtar Kelimeler: İdiyopatik Parkinson Hastalığı, Kompleks I, glutatyon, ND2, ND4

Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative disorder, clinically characterized by bradykinesia, tremor, rigidity, balance problems and pathologically by progressive loss of dopaminergic neurons in *substantia nigra* (1). Many degenerating neurons contain large cytoplasmic inclusions known as Lewy bodies (2,3). In a small number of patients, PD is a familial disorder caused by some Mendelian inherited gene mutations (eg. α-synuclein, parkin, DJ1, PINK1 etc.) (Reviewed in 3,4). Idiopathic PD is the most common form of PD. Oxidative stress, environmental toxins and genetic predisposition have been implicated in the etiology of idiopathic PD (5). The discovery of selective inhibition of Complex I by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and production of Parkinson-like symptoms provided a clue about the importance of mitochondria and Complex I in idiopathic PD (6,7). Complex I is the first enzyme complex in the electron transport chain and is located in the inner mitochondrial membrane. It contains 43 subunits, 7 of which are encoded by the mitochondrial DNA (mtDNA) (8). These subunits (ND1, ND2, ND3, ND4, ND4L, ND5, ND6) are all hydrophobic and essential for full activity (9). Variants in mitochondrial DNA (mtDNA) were detected by several investigators, but no PD specific mutation was found (10).

Recently, decreased enzymatic activities in other electron transport chain members has also been shown by different groups (11,12). In platelets of PD patients reduced Complex IV activities have been reported (11).

The Complex I and IV defects are not restricted to brain tissue. Several studies have reported reduced Complex I and/or Complex IV activities in muscle (13), platelet (11) and leukocytes (14) in PD patients. Although these findings give some evidence that PD is a systemic disorder, some researchers propose that the enzymatic defect is limited to *substantia nigra* (15).

Since oxidative stress is very common in mitochondria, mitochondrial glutathione (GSH) is the most important defence mechanism against free radicals (16). Decreased GSH levels were shown in the brain of patients with PD (16). Glutathione depletion in PC12 cell line resulted in selective inhibition of mitochondrial Complex I activity (17).

Although, there is a few study investigating the electron transport chain enzyme complexes in skeletal muscle tissue of PD patients, there is inconsistency whether enzyme activities were decreased or not. In our knowledge there is no study evaluated the relationship between mtDNA variants, the expression levels of Complex I subunits and Complex I enzyme activity. In this study, activities of Complex I, Complex IV and GSH levels were examined in muscle mitochondria of 19 idiopathic PD patients. In addition, variants in ND2 and ND4 subunits of Complex I were screened to determine whether any mtDNA variant identified in PD patients may play a role in the pathogenesis of idiopathic PD. The effects of these variants on complex activities and the gene expression levels were also examined.

Materials and Methods

Patients and controls

Patients with idiopathic PD underwent a neurological examination including assessment of the Hoehn and Yahr stage (between I-IV) at the Department of Neurology of Ankara Education and Research Hospital. The clinical features of 19 idiopathic PD patients are reported in detail in Table 1. All patients were under the treatment of anti-parkinsonian drugs (L-Dopa, Selegiline and/or Pergolide). As controls, objects who underwent diagnostic muscle biopsy performed for different indications were selected. These objects were clinically examined and none of them had any signs of neurodegenerative and chronic disorder. Femoral quadriceps muscle biopsies were taken from 19 idiopathic PD patients and 4 control

Table 1. Clinical features of patient and control groups, respiratory chain enzyme activities and GSH levels

	Idiopathic PD	Control
n	19	4
Age	67.21 ± 12.59	38.00 ± 18.02
Age of Onset	62.05 ± 14.22	-
Duration of the Disease	5.16 ± 3.67	-
Hoehn&Yahr Scale	1.89 ± 0.94	-
Complex I Activity * (Unit/ mg protein)	19.73 ± 8.24**	31.48 ± 8.28
Complex IV Activity* (Unit/ mg protein)	11.51 ± 6.45**	30.02 ± 14.76
GSH (nmol/mg protein)*	2.76 ± 2.08**	5.66 ± 1.93

*Each enzyme activity assay was carried out at least three times on each sample
**$p<0.05$

subjects under local anesthesia (2 % lidocaine), after obtaining informed consent. This study was approved by the Ethics Committe of Ankara Education and Research Hospital.

Isolation of muscle mitochondria

The mitochondria were isolated from femoral quadriceps muscles of idiopathic PD patients and controls as described previously (18), with some modifications. Muscle samples (25 mg) were transferred into the homogenization buffer H (5 mM Hepes pH 7.2, 210 mM mannitol, 70 mM sucrose, 1 mM EGTA, 0.5 % BSA) and sliced by using Thomas tissue slicer. The slices were homogenized in a glass-teflon homogenizer with 5 volumes of Buffer H. The homogenate was centrifuged at 510 x g for 5 min and the supernatant was centrifuged at 20 800 x g for 40 min. The resulting pellet was resuspended in 50 mM Tris-HCl pH 7.4, 150 mM KCl, 1 mM EDTA buffer and divided into two. The first half of the mitochondrial suspension was sonicated five times (10-second periods). The mitochondrial membrane fragments were diluted 1:1 in 0.25 M sucrose, 30 mM Tris-HCl pH 7.7, 1 mM EDTA and centrifuged at 20 800 x g for 10 min. The supernatant was then ultracentrifuged at 100 000 x g for 70 min and the pellet was resuspended in the same buffer. The final suspension was assayed for Complex I and Complex IV enzyme activities.

The second half of the mitochondrial pellet was used to determine mitochondrial GSH levels.

Mitochondrial Complex I and Complex IV enzyme assays

Complex I activity was measured by monitoring the oxidation of NADH at 340 nm for 3 min in 50 mM Tris-HCl pH 8.0, containing 250 mM sucrose, 0.2 mM EDTA, 2.5 mg/ml BSA, 5 mM $MgCl_2$, 2 mM KCN, 40 μM Coenzyme-Q, 2 μg/ml Antimycin A, and 0.13 mM NADH, in the absence and presence of 2 μg/ml rotenone (19). The level of rotenone-sensitive oxidation activity was determined by subtracting the activity observed in the presence of rotenone from the total activity observed in the absence of rotenone. Complex IV activity was measured by following the oxidation of reduced cytochrome c in 10 mM potassium phosphate pH 7.4, containing 20 μM reduced cytochrome at 550 nm (20). All spectrophotometric assays were carried out at 37 °C on a Milton Roy Spectronic 3000 diode-array spectrophotometer (Milton Roy, Ivyland, PA). Results were analyzed statistically by Mann-Whitney U and correlation tests.

Determination of mitochondrial glutathione levels

The pellet saved to determine mitochondrial GSH level was measured according to the method of Kosower et al. by using the fluorescent dye, monobromobimane (21).

Determination of protein concentration

The total amount of protein in the samples was determined according to the method of Lowry et al. (22).

Table 2. Sequences of the primers used for the sequencing of ND2 and ND4 genes

Position	Base Sequence	Primer Sequence*	Cycle conditions
ND2	4434-4777	TACCCCGAAAATGTTGGTTA GCTATAGCTATTATGATTATT	94 °C for 5 min, 32 cycles at 94 °C for 30 s, 55°C for 30 s and 72 °C for 30 s, 72 °C for 10 min
	4750-5103	CATCATTAATAATCATAATAG TTAGGATAATATAAATAGTTA	94 °C for 5 min, 32 cycles at 94 °C for 30 s, 45°C for 30 s and 72 °C for 30 s, 72 °C for 10 min
	5061-5542	CCTAACATAACCATTCTTAAT GGCTCTTGGTCTGTATTTAAC	94 °C for 5 min, 32 cycles at 94 °C for 30 s, 50°C for 30 s and 72 °C for 30 s, 72 °C for 10 min
ND4	10728-11107	CTAGACTACGTACATAACCTA GATTAGTTCTGTGGCTGTGGA	94 °C for 5 min, 32 cycles at 94 °C for 30 s, 55°C for 30 s and 72 °C for 30 s, 72 °C for 10 min
	11078-11472	ATTATAACATTCACAGCCACA AGTTTTAAGAGTACTGCGGCAA	94 °C for 5 min, 32 cycles at 94 °C for 30 s, 55°C for 30 s and 72 °C for 30 s, 72 °C for 10 min
	11432-11808	ATCGCTGGGTCAATAGTACTT GTTTGAAGTCCTTGAGAGAGG	94 °C for 5 min, 32 cycles at 94 °C for 30 s, 60°C for 30 s and 72 °C for 30 s, 72 °C for 10 min
	11778-12170	GCATCATAATCCTCTCTCAA CACAATCTGATGTTTTGGTTA	94 °C for 5 min, 32 cycles at 94 °C for 30 s, 55°C for 30 s and 72 °C for 30 s, 72 °C for 10 min

*All the primers listed are 5`→3` direction

Isolation of mtDNA and sequencing of ND2 and ND4 genes

The mtDNA was isolated from the femoral quadriceps muscle tissues (20 mg) of idiopathic PD patients and controls by using the DNA Qiamp DNA mini kit (Qiagen, Germany). The entire mitochondrial ND2 and ND4 genes were amplified with PCR and nucleotide sequences between 4434-5542 and 10728-12170, respectively were sequenced. PCR and sequencing primers for ND2 and ND4 regions were shown in Table 2. PCR amplifications were performed in 50 μl reaction volumes containing 2 mM $MgCl_2$, 200 μM dNTP, 90 ng DNA, 2.5 U Taq DNA polymerase (Perkin-Elmer), 1X PCR buffer and 300 nmol of each primers. The cycling conditions were indicated in Table 2. PCR products were verified by electrophoresis on a 1 % agarose gel stained with ethidium bromide.

For sequencing, PCR products were purified using the QIAquick PCR Purification kit (Qiagen, Germany) and analyzed using an Applied Biosystems automatic sequencer.

RNA isolation and analysis of ND2 and ND4 gene expressions

RNA was isolated from 25-50 mg of femoral quadriceps muscle tissues of idiopathic PD patients and controls by using the RNAqueous-4PCR kit (Ambion, Texas, USA) according to the manufacturer's protocol. RNA used for cDNA synthesis was treated with DNAase I (Ambion) for 1 h at 37 °C. The DNAase I was inactivated by adding 0.1 M EDTA and 1 mg/ml glycogen followed by phenol/chloroform/isoamyl alcohol extraction. The purity of the isolated total RNA was verified by 1.2 % denaturing agarose gel electrophoresis. cDNA synthesis was performed by incubating 3 μg of total RNA, 1.25 μg oligo$(dT)_{12\text{-}18}$ primer (Life Technologies, USA) and 25 U of reverse transcriptase (Ambion, Texas, USA) at 42 °C for 1 h. Samples were processed according to Retroscript kit (Ambion, Texas, USA) manual. To quantitate the relative levels of ND2 and ND4 gene expressions, relative quantitative RT-PCR (QuantumRNA module; Ambion, Texas, USA) was performed. Initial experiments were performed to optimize the amplification efficiency of ND2 or ND4 transcripts by adjusting the ratio of 18S RNA primer/competimer. For ND2 amplification, the primers 5`-TAGCCCCCTTTCACTTCTGA-3` and 5`-GTGGTAAGGGCGATGAGTGT-3`; for ND4 amplification, the primers 5`-TGACTCCCTAAAGCCCATGT-3` and 5`-TGAGGGTGTTTTTCTCGTGTG-3` were used. For PCR amplification of cDNA products, PCR mixtures contained 0.15 μM ND2 or ND4 primers, 0.2 μM 18S RNA primer/competimer (3:7), 2.5 U SuperTaq polymerase (Ambion), 0.22 μM dNTP in a total volume of 50 μl. PCR conditions were as follows: Hot start 5 min at 80 °C, 5 min at 49 °C, 39 cycles of 30 s at 94 °C, 45 s at 66 °C, 10 s at 72 °C, final extension for 7 min at 72 °C. The RT-PCR products were electrophoresed on 1 % agarose gel stained with SyberGold fluorescence dye. The products were visualized with a Kodak DC290 camera and quantitated by ROI analysis.

Results

The activities of Complex I and IV and GSH level were examined in muscle mitochondria of 19 idiopathic PD patients. For accurate evaluation of Complex I activity located in the inner mitochondrial membrane, samples with rotenone sensitivity ≥74 % were taken into consideration. A significant decrease (38 %) in Complex I activity was found in idiopathic PD patients (19.73 ± 8.24 nmol/mg protein) compared with the control group (31.48 ± 8.28 nmol/mg protein) ($p<0.05$) (Table 1 and Figure 1). In addition, Complex IV activities in idiopathic PD patients and the control group were found to be 11.51 ± 6.45 U/mg protein and 30.02 ± 14.76 U/mg protein, respectively ($p<0.05$) (Table 1 and Figure 1). No significant correlation between age, age of onset, the duration of the disease and Complex I and Complex IV enzyme activities were found in idiopathic PD patients (data not shown). GSH levels in idiopathic PD patients compared to control group indicated 47 % loss of GSH in idiopathic PD patients ($p<0.05$) (Table 1).

To identify the ND2 and ND4 variants that might predispose to idiopathic PD, mtDNA was isolated from muscles of 19 idiopathic PD patients. The ND2 and ND4 genes were sequenced by ABI PRISM 310 Genetic Analyzer. The base substitutions were detected along the genes encoding ND2 and ND4 subunits of Complex I compared to MITOMAP reference sequence and Human Mitochondrial Genome Database (www.mitomap.org, www.genpat.uu.se/mtDB/) (Table 3). Of these sequence variants shown in Table 3, only five of them cause an amino acid exchange: T4639C → I90T (ND2), A4833G → T122A (ND2), A4917G → N150D (ND2), G5046A → V193I, (ND2) and G5460A → A331T (ND2).

ND2 and ND4 gene expressions of patients from muscle mitochondria were determined relative to the 18S rRNA internal standard. The results of relative-quantitative RT-PCR showed that only two patients had a decrease in the expression of ND2 (19 and 21 %) (Figure 2A, lanes 7 and 8) and ND4 (14 and 13 %) (Figure 2B, lanes 7 and 8).

Discussion

Relationship between mitochondrial dysfunction and the pathogenesis of idiopathic PD have been documented in the literature (5, 9, 13, 23). Such studies suggest that particularly Complex I deficiency plays a pathogenic role in idiopathic PD. Complex I is one of the major site responsible for superoxide production in the cell. Inhibition of respiratory chain causes increased generation of free radicals. Increased oxidative stress results in protein, lipid and DNA damage in nigral dopaminergic neurons in PD (9).

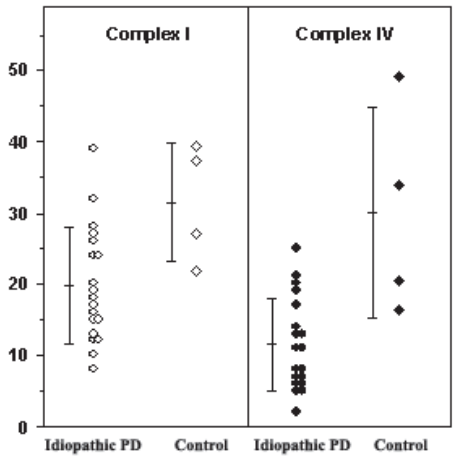

Fig. 1. Complex I and Complex IV activities in idiopathic PD patients and control groups. Dots represents individual subjects, bars standard deviations and lines are mean values. Complex I: ◇, Control; ❍, Idiopathic PD patients. Complex IV: ◆, Control; ●, Idiopathic PD patients.

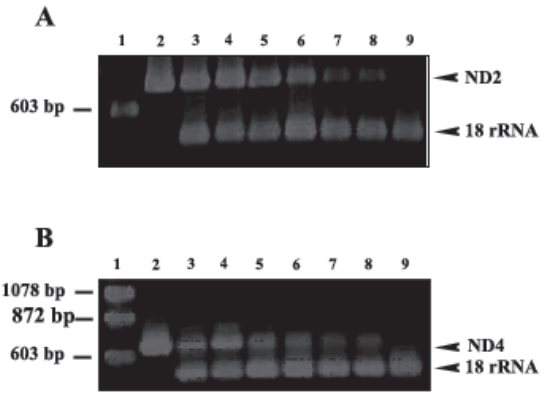

Fig. 2. Relative quantitative RT-PCR of ND2 and ND4 transcripts. A, ND2 transcript. Lane 1,ΦX174 marker; lane 2, ND2 using ND2 primers; lane 3, ND2 primers plus 18S RNA primers; lane 4-8, patients ND2 transcripts; lane 9, 18S RNA primers. Upper band is ND2 gene and lower band is 18S RNA primer. B, ND4 transcript. Lane 1, ΦX174 marker; lane 2, ND4 using ND4 primers; lane 3, ND4 primers plus 18S RNA primers; lane 4, Patients ND4 transcripts 4-8; lane 9, 18S RNA primers. Upper band is ND4 gene and lower band is 18S RNA primer.

Table 3. Variants in mitochondrial DNA encoding ND2 and ND4 subunits

Location	Base substitution	Amino acid change	#PD
ND2	T4639C	I90T	1/19
	T4646C	syn	
	A4715G	syn	
	A4958G	syn	
	T5004C	syn	
	G5046A	V193I	
	A5390G	syn	
	G5460A	A331T	
	A4833G	T122A	2/19
	G5417A	syn	
	A4917G	N150D	3/19
ND4	T10873C	syn	1/19
	A10876G	syn	
	T11299C	syn	
	C11326T*	syn	
	C11332T	syn	
	G11518A	syn	
	C11674T	syn	
	G11914A	syn	
	A11251G	syn	3/19
	A11467G	syn	4/19
	G11719A	syn	14/19

*Not reported in mitomap and mtDB - Human Mitochondrial Genome Database before

In addition to Complex I deficiency, depletion of GSH level was determined in *substantia nigra* (24, 25). Although the cause of neuronal degeneration induced by oxidative stress in PD is not known, mitochondrial dysfunction may contribute this process by generating free radicals or decreasing the ATP production.

Different results have been reported related to the decrease in electron transport chain enzyme activities in various tissues including brain, muscle, and platelets of idiopathic PD patients. Decrease in the muscle Complex I activity of idiopathic PD patients was found by the majority of researchers (13, 26-31). In contrast, some groups detected no decrease in Complex I and/or Complex IV activities in muscle tissues of idiopathic PD patients (28, 32, 33). In our study, we found significant decrease in both Complex I and Complex IV activities of the muscle tissues of idiopathic PD patients. Recently, it has been reported that the drug treatment in PD (such as L-dopa, Selegiline, Carbidopa) (34,35) and aging (35) does not affect mitochondrial Complex I and IV activities. Thus, decreased enzymes activities observed in our study is not due to the treatment of idiopathic PD patients. No significant correlation between age, age of onset, and duration of the disease with Complex I and Complex IV were determined in idiopathic PD patients.

Depletion of total glutathione is the first biochemical change in the *substantia nigra* of PD patients. Recent studies have shown that depletion in both cytosolic and mitochondrial glutathione level causes increased oxidative stress and selective inhibition of Complex I (36). We measured the mitochondrial GSH level and found decrease in GSH level in muscle tissues of idiopathic PD patients. Together with depletion of antioxidant GSH, decrease in respiratory chain enzyme activities lead to the increased generation of free radicals and bioenergetic defect in idiopathic PD. Depletion of GSH may contribute to the inactivation of Complex I activity via protein thiol oxidation. Another explanation of decreased electron transport chain enzyme activities might be due to the increased levels of mtDNA mutations induced by oxidative damage.

The genetic alteration of mitochondrial enzymes may change the electron transfer by mimicking the effect of enzyme inhibitors. Some alterations may induce the over-production of reactive oxygen species (ROS) while some others may depress them. Although several mtDNA mutations associated with idiopathic PD were reported, none of them were found to be directly responsible for PD (10, 37-39). Previous studies reported an association between PD and mitochondrial Complex I ND2-ND4 region variants (40-42). In this study, genetic variants in ND2, ND4 region, mitochondrial Complex I activity and the relationship between genetic variants and enzyme activities were evaluated. Since it is difficult to provide muscle specimen from the objects, in the researches evaluating electron transport chain enzymes (12, 30), participants number are relatively low. This issue was the limitation of our study.

In *substantia nigra* of PD patients an increased frequency of heteroplasmic G5460A transition of the ND2 subunit of Complex I has been reported (40). In this study, we identified G5460A polymorphism in only one patient's muscle specimen. In our study, in three patients, we detected A4917G mutation, which is associated with Leber's hereditary optic neuropathy (LHON). In previous reports, A4833G transition was found in striatal muscle mtDNA of PD patients (10). In our study, 2 patients had this base substitution. The sequence analysis of ND4 gene revealed that G11719A polymorphism, which does not cause an amino acid change were identified in 14 of 19 patients. As a result, the sequence results from this and other studies demonstrate that idiopathic PD patients with Complex I and IV deficiencies carry several mitochondrial ND2 and ND4 polymorphisms. In a study measuring muscle mitochondrial complex activity and DNA polymorphisms it is reported that point mutations are almost equally distributed all over the muscle fibers and L-dopa treatment does not affect the amount of point-mutated mtDNA (31). These findings led us that the variants found in this study may due to PD and some polymorphisms may play a role in the pathogenesis of PD. However, these variants are probably not directly associated with the pathogenesis of idiopathic PD. There are numerous pathogenic variants that may show varying defects on specific enzyme activities whereas no significant effect of ND2 and ND4 variants on Complex I and IV activities were detected.

The polycistronic nature of mtDNA is a drawback in individual assessment of gene expression. However, mtDNA gene expression levels are still used in different studies with some limitations (43-45). Evaluating mitochondrial gene expressions gives a general idea if there is a decrease in mtDNA transcripts. Apart from that, difference in expression levels for genes which is transcribed on the same polycistronic RNA, may suggest the possible role of posttranslational mechanisms (45). In our study, decreased expression levels of ND2 and ND4 genes were found in only two patients compared to the control group. The observed decrase in the Complex I and IV enzyme activities are not related to their gene expression levels since the decrease in enzyme activities are observed in all patients.

It is possible to relate Complex I and IV dysfunctions and mitochondrial abnormalities as a result of oxidative stress which contributes to the pathogenesis of idiopathic PD. Single base substitutions detected in this study might not account for the respiratory chain dysfunction. However the accumulation of multiple mutations or polymorphisms might be important for the ethiology of PD. Further studies may help to imply whether there is a relationship between these polymorphisms and susceptibility for PD.

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