

CLINICAL STUDY

Delta deletion 4977 in mitochondrial DNA in patients with idiopathic Parkinson's disease

Taravari A¹, Panov S², Petrov I¹, Petrova V¹, Medziti F¹, Haliti G¹*University Clinic of Neurology, Skopje, Macedonia.*

Abstract: *Aim:* To determine the presence of delta deletion 4977 in mitochondrial DNA in patients with Idiopathic Parkinson's disease (IPD).

Material and methods: This has been a prospective, clinically genetic study, lasting for whole two years. The clinical part of this study was made at the University Clinic of Neurology in Skopje, Unit for extrapyramidal diseases. The laboratory-genetic part of the study was elaborated at the Laboratory for molecular biology at the Institute for Biology, Faculty of Sciences, University "Sts. Cyril and Methodius". This study comprised a total of 32 subjects with a clinically verified diagnosis for idiopathic Parkinson's disease; 18 men and 14 women (with mean age of 52.7 years). Control group consisted of 31 randomly selected, literally healthy persons, at similar age, with similar gender distribution, and no clinical and anamnestic data for parkinsonism or similar clinical entities.

Results: Objective neurologic results of all 32 investigated subjects (100%) showed presence of rigor, tremor and bradykinesia. The difference tested between the investigated and control group, concerning the present or absent deletion and heteroplasma, has been highly statistically significant ($p=0.001$).

Conclusion: It could be concluded with a great statistical significance that deletion 4977 in mitochondrial genome has been registered more frequently in the group of patients with IPD (Tab. 10, Fig. 6, Ref. 36). Text in PDF www.elis.sk.

Key words: delta deletion, mitochondrial DNA, Parkinson's disease.

The term parkinsonism has been used to describe the motor characteristics in Parkinson's disease (PD), which serve for differentiation from idiopathic Parkinson's disease (IPD), and which manifest due to different clinical and pathophysiological mechanisms. In order to come to a definite diagnosis in our study, we used the Brain Bank Criteria of the United Kingdom Parkinson's Disease Society (UKPDS) (17).

IPD has a favourable clinical result when treated with dopamine agonists or Levo-dopa drugs. It appears most frequently unilaterally and has a characteristic clinical picture with generalized slowness and typical tremor (4–6 Hz) resembling that of money counting (22).

Essential change in IPD is the decreased dopaminergic neurotransmission in basal ganglia. Idiopathic Parkinson's disease is the most frequent type of parkinsonism, and is presented in almost 70 % of patients with parkinsonism (22).

Genetic factors have an essential role in clinical manifestation of the disease; the real role of these genes will be discovered in the future with ambitious investigations aimed especially at explain-

ing the role of molecular biology in inheritance not obeying the Mendel in IPD, as well as at our capability to use this precious knowledge to the benefit of our patients (8, 32).

In summary, two autosomal dominant genes (α -synuclein and LRRK2) and three autosomal-recessive genes (parkin, DJ-1 and PINK1) are definitely associated with the inherited and early-onset form of IPD. In addition to them, there are other objective mutations such as UCHL-1, synphilin-1 and NR4A2, which perhaps are not biologically significant (2, 6, 9, 11, 32).

These discoveries in the field of genetics of IPD enabled the development of two significant hypotheses for the possible pathophysiological mechanism for occurrence of parkinsonism. In short, these, possibly joinable hypotheses include:

1. Aberration in ubiquitin-proteasomal pathway (α -synuclein/parkin/UCHL-1), and
2. Oxidative stress/mitochondrial dysfunction (DJ-1/PINK1) (12).

Delta deletion 4977 in IPD

Mitochondria are closed membranous organelles found in all eukaryotic cells. They are very important cell components, playing a significant role in many cell functions such as metabolism (oxidative phosphorylation), apoptosis, and aging (15). A typical eukaryote cell contains about 2,000 mitochondria which comprise approximately one-fifth (1/5th) of the total cell volume (34). They are known to be semi-autonomous organelles which have their own

¹University Clinic of Neurology, Skopje, Macedonia, and ²Faculty of Natural Sciences, Chair for Genetics and Molecular Biology, Skopje, Macedonia.

Address for correspondence: A. Taravari, University Clinic of Neurology, Skopje, Macedonia.

genome and own mechanisms for replication, transcription and protein synthesis (28).

Mitochondria, as cell organelles, play an important role in metabolism of eukaryote cells. One of the most important mitochondrial functions is ATP synthesis (through carbohydrate catabolism and fat oxidation), which is a process that includes a series of enzymatic complexes associated with the inner membrane, and especially with the chain of electron transportation (27). Unfavourable side product of the respiratory chain and oxidative phosphorylation is the ROC production (reactive oxidative products: oxygen anions, hydrogen peroxide and hydroxyl radicals). ROC production occurs in conditions when electrons of respiratory chain of the Krebs' cycle engage directly in oxygen reaction. It is supposed that ROC production could be increased in conditions of excessive production of electrons which could appear in case of increased energy production or disturbed activity of the respiratory chain.

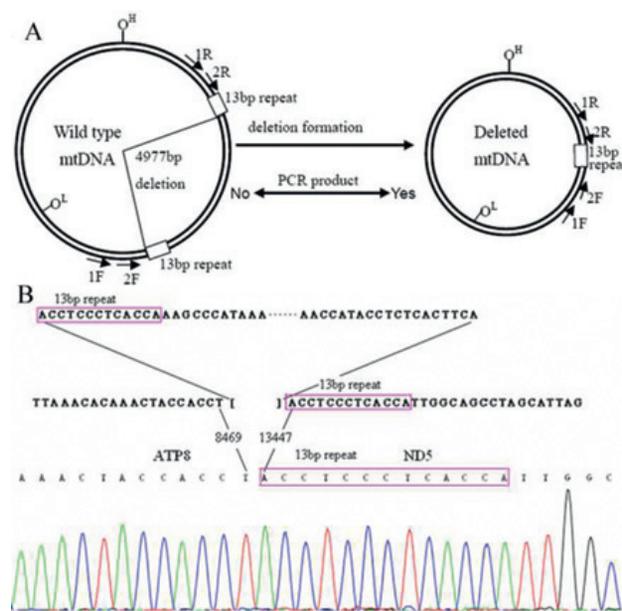
Inherited mitochondrial material differs in several features from the basic genetic material in humans. There are many factors that make the difference separating the mitochondrial genetics from that of Mendel's. Some of such most outstanding factors include the presence of several copies of genetic material in one cell, differences in the basic mechanisms significant for replication and control of transcription, and inheritance of mutation diseases from one parent. Differences between both genetic systems in human cells could be an evolution rest which enables to make distinction between the functional consequences occurred from mtDNA mutations (21).

Andersson et al (1981) published the sequence and structural organization of the human mitochondrial genome. It was the first sequenced mitochondrial genome built of 16,569 base pairs (1). According to the standard model of mitochondrial inheritance, mitochondrial mtDNA is maternal inheritance (13). It is a circular molecule which encodes 13–14 of the total of 87 proteins included in the chain of electron transportation, 2 ribosomal (12C and 16C) RNA molecules (rRNA) and 22 transportation RNA molecules (tRNA) indispensable for protein synthesis.

The polyploid nature of mitochondrial genome which has many thousands of mitochondrial copies in the cell (from 1,000 to 100,000, depending on cell type) and it increases the importance of the mitochondrial genetics, i.e. it helps in explaining the terms of homoplasmy and heteroplasmy. Homoplasmy is a term used to explain the development of identical copies of mitochondrial genome in the cell, while heteroplasmy denotes the presence of a mixture of two or more mitochondrial genotypes. These terms are especially important when mtDNA mutation is to be explained and understood as a leader of some diseases. A low level of heteroplasmic mutations in the cell has been known as microheteroplasmy and it is difficult to detect.

It is considered that a great number of diseases such as diabetes, cardiovascular diseases, lactic acidosis, specific forms of myopathies, osteoporosis, Alzheimer's and Parkinson's diseases, strokes and many others are partly caused through mitochondrial dysfunction (33).

One of the most frequent mutations appearing in mitochondrial genomes, and being connected with the process of aging and



Scheme 1. Schematic distribution of deletion Δ mtDNA⁴⁹⁷⁷ in human mitochondrial genome.

development of some diseases has been that of deletion 4977 bp of mitochondrial genome (Δ mtDNA⁴⁹⁷⁷). It is a deletion occurring between two 13 bp direct repetitions from normal mtDNA on nucleotide positions of 8470/8482 bp and 13447/13459 bp (16, 31) (Scheme 1). This deletion, as so many others, occurs laterally on some direct or indirect repeating sequences, and because mtDNA contains many such repetitions, it can have hundreds of possible deletions (29). It is supposed that the development of mitochondrial deletions is a consequence of the sliding of erroneous chains which could have appeared during the mitochondrial replication. In that case, the deleted region will be replicated better than the normal genome, and within time, it can cause its genome to become gradually larger (7, 35). Symptoms of these diseases can range from light to severe, depending on the level of the accumulation of deleted molecules (30).

Materials and methods

This study comprised a total of 32 examinees with clinically verified diagnosis of idiopathic Parkinson's disease, namely 18 men and 14 women with average age of 52.7 years. The youngest examinee was 30 years old, while the oldest one was 78. The control group consisted of 31 randomly chosen literally healthy persons at similar age, with similar gender distribution, and no clinical and anamnestic data for parkinsonism or other similar clinical entities.

This is a prospective, clinical and genetic study lasting for two years. The clinical part of this study was made at the University Clinic of Neurology, Medical School in Skopje. The laboratory-genetic part of the study was made in the Laboratory for Molecular Biology at the Institute for Biology, Faculty of Natural Sciences in Skopje.

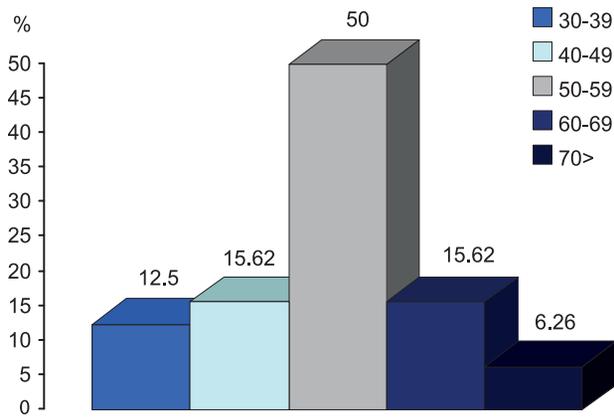


Fig. 1. Distribution of the examinees according to the age.

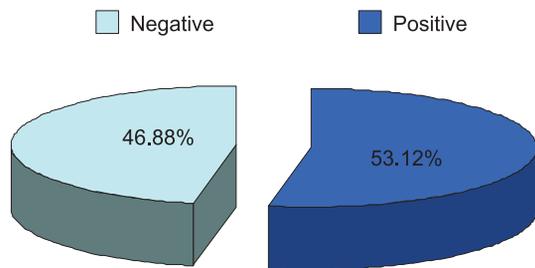


Fig. 2. Examinees' genetic anamnesis

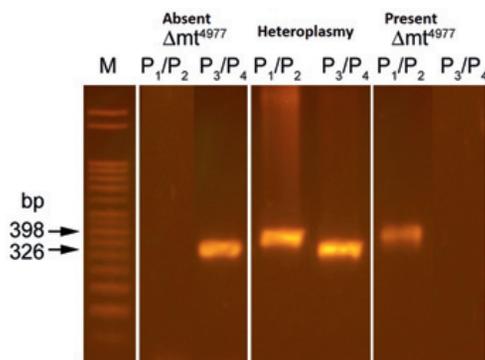


Fig. 3. Molecular detection of mt delta deletion. A composite electrophoregram of agarose gel is presented, photographed under UV-light. On M position DNA marker was applied with fragments of known length (in base pairs – bp). DNA samples are amplified with primer pairs P1/P2 and P3/P4. When deletion was absent, there was no deletion of the pair P1/P2, and in amplification of the pair P3/P4 electrophoregram band appeared, having a length of 326 bp. Conversely, when deletion was present, amplification of the pair P1/P2 resulted with a band being long 398 bp, and amplification product was absent with the pair P3/P4. In heteroplasmy, amplification products are present with both primers.

All 32 volunteers have verified IPD. At the same time, samples of venous blood were analyzed, including 2 volunteers from the families of these patients. Genetic investigations by means of molecular and genetic analyses of the patients and their family members were made, namely the detection of delta deletion mtDNA 4977.

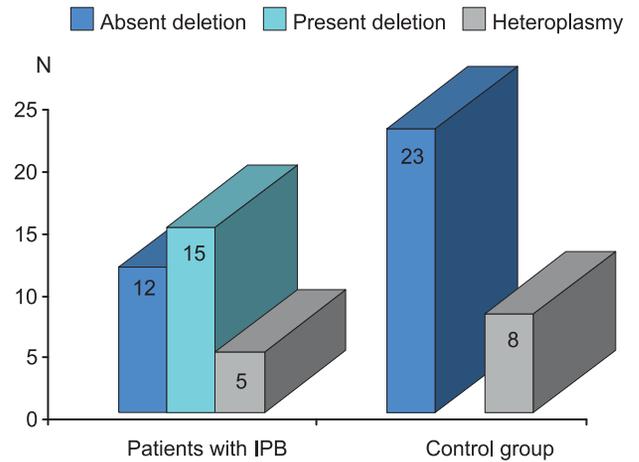


Fig. 4. Deletion (Δ mtDNA 4977) in mitochondrial genome.

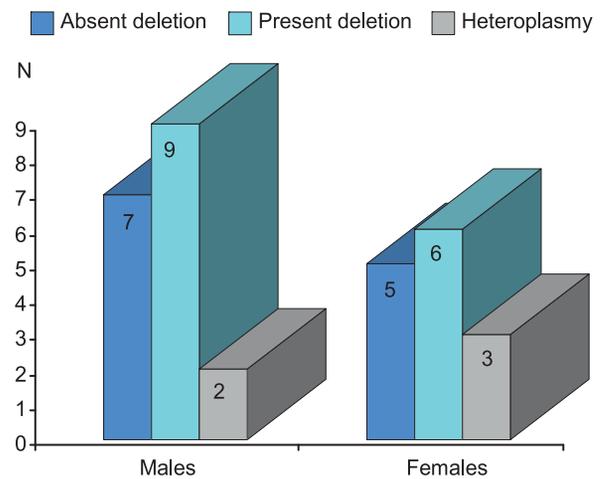


Fig. 5. Deletion (Δ mtDNA 4977) in mitochondrial genome concerning the examinees' gender.

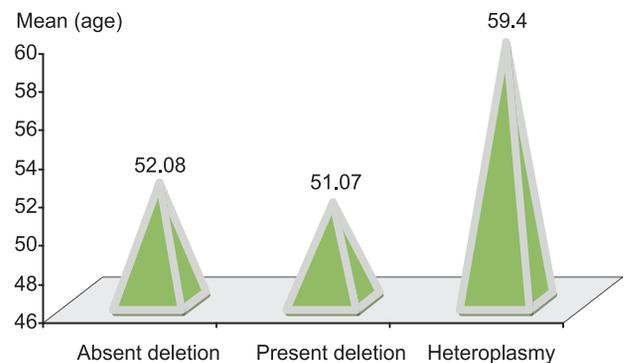


Fig. 6. Deletion (Δ mtDNA 4977) in mitochondrial genome concerning the examinees age with IPD.

Realization of the study was approved by the Ethical Committee of the Medical School, University "Ss. Cyril and Methodius".

The examinees were diagnosed to have idiopathic Parkinson's disease (IPD). Their diagnosis was verified by means of detailed

Table 1. Distribution of the examinees according to the age

Age (years)	n	%
30–39	4	12.5
40–49	5	15.62
50–59	16	50.0
60–69	5	15.62
70 >	2	6.26
All	32	100
Mean=52.75±10.3	Median=50	Min=30
		Max=78

Table 2. Examinees genetic anamnesis

Genetic anamnesis	n	%
Positive	17	53.12
Negative	15	46.88
All	32	100

Table 3. Distribution of the symptoms: rigidity, tremor and dyskinesia

Symptoms	Rigidity		Tremor		Dyskinesia	
	n	%	n	%	n	%
Present	32	100	32	100	15	46.88
Absent	/	/	/	/	17	53.12
All	32	100	32	100	32	100

Table 4. Distribution of the symptoms: bradykinesia, reduced postural reflexes (RPR) and bradylalia

Symptoms	Bradykinesia		RPR		Bradylalia	
	n	%	n	%	n	%
Present	32	100	31	96.87	28	87.5
Absent	/	/	1	3.13	4	12.5
All	32	100	32	100	32	100

Table 5. Deletion (Δ mtDNA 4977) in mitochondrial genome

Δ mtDNA 4977	Patients with IPB	Control group
Absent deletion	12 (37.5%)	23 (74.19%)
Present deletion	15 (46.87%)	0
Heteroplasmy	5 (15.63%)	8 (25.81%)
All	32	31

anamnesis and detailed clinical neurologic examination while strictly obeying the Brain Bank Criteria, as well as by means of performed investigations, namely neurophysiologic investigations, brain nuclear imaging resonance, Doppler of the extracranial carotid arteries and neuropsychologic investigations.

Majority of the variants for PCR method enable their choice depending on the type of the analysis which is to be made. Beside the classical PCR reaction, for DNA deletion (Δ mtDNA⁴⁹⁷⁷), a nested PCR as well as PCR are very often used to amplify the long fragments (Long PCR).

In this study, for PCR amplification and detection of Δ mtDNA⁴⁹⁷⁷ deletion, two pairs of primers, namely mtDNA 4977-1/2

Table 6. Logistic Regression Analysis

Variable	B	Wald	df	Sig.	Exp(B)	95,0% CL for (B)	
						Lower	Upper
Age	0.009	0.109	1	0.741	1.009	0.957	1.063
Δ mt 4977	1.590	8.191	1	0.004	4.902	1.65	14.559
Constant	-1.128	0.572	1	0.45	0.324		

Table 7. Deletion (Δ mtDNA 4977) in mitochondrial genome concerning the examinees gender

Gender	Δ mtDNA 4977			All
	Absent deletion	Present deletion	Heteroplasmy	
Male	7 (21.88 %)	9 (28.13 %)	2 (6.25 %)	18 (56.25 %)
Female	5 (15.63 %)	6 (18.75 %)	3 (9.38 %)	14 (43.75 %)
All	12 (37.50 %)	15 (46.88 %)	5 (15.63 %)	32

Chi-square=0.64 df=2 p=0.73

(frw/rev) and mtDNA 4977-3/4 (frw/rev) (Sigma-Genosys) were used for corresponding separate detection of the mtDNA region when Δ mtDNA⁴⁹⁷⁷ is absent, i.e. deletion is present.

Results

As to gender structure, the examinees were composed of 18 (56.25 %) male and 18 (43.75 %) female examinees. The average age of examinees was 52.7±10.3 years. The youngest examinee was 30 years old, while the oldest one was 78 years old. The mean values of 50 years showed that 16 (50 %) of the examinees belonged to the age group of 50–59, even 23 (71.87 %) of the patients were older than 50 years (Tab. 1, Fig. 1).

Concerning the familial and genetic anamnesis, more than a half of examinees, i.e. 17 (53.1 %) proved that they had a patient with Parkinson's disease in their family (Tab. 2, Fig. 2).

Table 3 shows the distribution of neurologic symptoms of extrapyramidal origin, characteristic and typical for patients with IPD.

In all 32 examinees the objective neurologic finding showed the presence of rigor and hypertonia of extrapyramidal origin, respectively. Tremor was also present as one of the cardiologic symptoms of IPD in all 32 examinees. Dyskinesia as a symptom occurring due to long-term usage of substitution therapy (Levodopa) was registered in 15 (46.9 %) and absent in 17 (53.1 %) patients of our group with IPD.

Bradykinesia, the slowness of walking, as the main symptom among cardinal symptoms according to Brain Bank Criteria of United Kingdom Parkinson's Society for the Diagnosis of Parkinson's Disease, was present in all our examinees.

Reduced postural reflexes were positive in a dominant number of examinees, 31 (96.9 %).

Bradylalia, the speech changes (slow and monotonous speech) was recorded in 28 (87.5 %) examinees, while in 4 (12.5 %) of our patients, quite normal speech was recorded (Tab. 4).

Detection of specific mitochondrial DNA 4977 delta deletion

Molecular detection of mt delta deletion. A composite electrophoregram of agarose gel is presented, photographed under UV-

Table 8. Deletion (Δ mtDNA 4977) in mitochondrial genome concerning the ethnic nationality of the examinees

Nationality	Δ mtDNA 4977			All
	Absent deletion	Present deletion	Heteroplasmy	
Macedonian	4 (12.50 %)	8 (25.00 %)	0	12 (37.50 %)
Albanian	6 (18.75 %)	4 (12.50 %)	4 (12.50 %)	14 (43.75 %)
Turkish	2 (6.25 %)	3 (9.38 %)	1 (3.13 %)	6 (18.75 %)
All	12 (37.50 %)	15 (46.88 %)	5 (15.63 %)	32

Chi-square=5.59 df=4 p=0.23

Table 9. Deletion (Δ mtDNA 4977) in mitochondrial genome concerning the examinees' age with IPD

Δ mtDNA 4977	n	Age	SD
Absent deletion	12	52.083	11.62
Present deletion	15	51.067	7.56
Heteroplasmy	5	59.40	13.72

Analysis of Variance F=1.28 p=0.29

light. On M position, DNA marker was applied with fragments of known length (in base pairs – bp). DNA samples are amplified with primer pairs P1/P2 and P3/P4. When deletion was absent, there was no deletion of the pair P1/P2, and in amplification of the pair P3/P4, an electrophoregram band appeared in length of 326 bp. Conversely, when deletion was present, amplification of the pair P1/P2 resulted with a band in length of 398 bp, and amplification product was absent with the pair P3/P4. In heteroplasmy, amplification products are present with both primers (Fig. 3).

In this of part of the examination, the results relating to the detection of specific mitochondrial DNA 4977 delta deletion (Δ mtDNA 4977 deletion) are present. For carrying out this objective, 32 examinees were analyzed and diagnosed with IPD while 31 (46.9 %) conditionally healthy examinees constituted our control group.

In the study group of patients, the deletion of mitochondrial genome has been registered in 15 (46.9 %) of the examinees constituting our control group.

This deletion was detected only in 8 (25.8 %) examinees of the control group. The differences between the examinees and control group tested in relation to presence or absence of deletion or heteroplasmy was highly statistically significant ($p=0.001$). Deletion in mitochondrial genome has been registered highly significantly more often in the group of patients with IPD (Tab. 5, Fig. 4).

In order to quantify, or in fact to measure the relationship between the deletion in the mitochondrial genome and idiopathic Parkinson's disease, we performed Logistic Regression Analysis (Binary Logistic Regression). Results from this analysis are shown in Table 6.

As opposed to recent investigations showing that with age, the appearance of deletion Δ mtDNA 4977 in mitochondrial genome increases, our investigation showed that the examinees' age did

not prove to be a significant factor in relation to the latter deletion (ExpB=1.01 95% CL 0.957–1.063).

As to the deletion 4799 of the mitochondrial genome, it could be proved to represent an independently significant factor, i.e. the examinees with a verified deletion of mitochondrial genome had a 4.9-fold greater chance of developing idiopathic Parkinson's disease compared to the examinees without deletion (ExpB=4.9 95% CL 1.65–14.56).

Deletion of mitochondrial genome was present in 9 (28.1 %) male and 6 (18.7 %) female examinees, while not detected in 7 (21.9 %) male and 5 (15.6 %) female examinees. Heteroplasmy was almost equally present in examinees of both genders (2 vs 3).

The differences tested between the groups with deletion, and those without deletion and with heteroplasmy in relation to their gender was statistically non-significant ($p>0.05$).

The development of deletion in the mitochondrial genome in idiopathic Parkinson's disease, did not depend significantly on gender (Tab. 7, Fig. 5).

Statistically significant differences in ethnic nationality of examinees with deletion of mitochondrial genome, and those without deletion and with heteroplasmy ($p>0.05$) were not registered. This statistical comment was due to the difference tested in the distribution presented in Table 8. It can be noted that deletion of mitochondrial genome was present in 8 (25 %) Macedonians, 4 (12.5 %) Albanians, and 3 (9.4) Turks, while heteroplasmy was found in 4 (12.5 %) examinees of Albanian nationality and 1 examinee of Turkish ethnic community.

The average age of examinees in group with deletion of mitochondrial genome was 51.1 ± 7.6 years, the age of examinees without deletion was in range of 52.1 ± 11.6 years, while the average age of examinees with heteroplasmy was the greatest, namely 59.4 ± 13.7 years. The difference in average age of the examinees with deletion and those without deletion and with heteroplasmy was statistically non-significant ($p>0.05$) (Tab. 9, Fig. 6).

Deletion of mitochondrial genome was present in 9 (28.1 %) examinees with positive familial anamnesis for Parkinson's disease and 6 (18.7 %) with negative genetic anamnesis, while the deletion

Table 10. Deletion (Δ mtDNA 4977) in mitochondrial genome concerning the positive genetic anamnesis for Parkinson's disease

Genetic anamnesis	Δ mtDNA 4977			All
	Absent deletion	Present deletion	Heteroplasmy	
Positive	5 (15.63 %)	9 (28.13 %)	3 (9.38 %)	17 (53.15 %)
Negative	7 (21.88 %)	6 (18.75 %)	2 (6.25 %)	15 (46.88 %)
All	12 (37.50 %)	15 (46.88 %)	5 (15.63 %)	32

Chi-square=1.01 df=2 p=0.6

was not present in 5 (15.6 %) examinees with positive and 7 (21.9 %) with negative familial anamneses. Statistically significant difference ($p > 0.05$) among the groups with deletion and with heteroplasmy in the mitochondrial genome in relation to presence or absence of Parkinson's disease in the family was not registered (Table 10).

Discussion

Idiopathic Parkinson's disease has been approximately equally present in both genders, with a discretely greater presentation in male gender. Our study showed that the gender distribution for 32 patients with clinically verified diagnosis for idiopathic Parkinson's disease (IPD) in accord with Brain Bank Criteria yielded 18 (56.25 %) men and 14 (43.75 %) women. In most studies, as in those of Haaxma et al (2007), Lyons et al (2009) and Linder et al. (2010) performed in various time periods and on various populations, the results were similar to those in our study (14, 20, 24).

In our study the mean age of examinees was 52.7 years. The youngest examinee was only 30 years, the oldest one was 78. The mean age with the values of 50 years showed that 16 (50 %) of examinees belonged to the age group of 50–59 years, and even 23 (71.87 %) of examinees were older than 50 years.

As to the mean onset of idiopathic Parkinson's disease, the analysed papers indicate domination of those with the mean onset in the beginning of the sixth decade of life (3, 23, 36).

In their epidemiologic study performed in Norway on 554 patients with IPD, Alves et al (2009), among others, stated that the mean age at the onset of IPD symptom manifestation was 54.3 years which was compatible with the average age of our 32 examinees (3).

In their 10-year analysis, Lopez IC et al (2010) have practically started to examine 64 cases of *de novo* IPD being treated with Levodopa, followed-up and examined every six months within a period of 10 years. The analysis of all symptoms and signs was made with UPDRS, starting with motor fluctuations, freezing phenomenon, postural reflexes and dyskinesia. By this paper, progression of all IPD symptoms was followed up within a period of 10 years, in their regular follow-up and regular correction of therapy depending on clinical picture. There was a statistically significant difference in progression of symptoms between patients who manifested the disease at younger age and those who developed the first symptoms at a more advanced age (18).

Ivy N. Miller and Golomb AC (2010) made an interesting, clinico-epidemiological study, in which it was stated that the disease was manifested approximately equally in both genders, however definitively some symptoms appeared more frequently in female patients while the others were more frequent in male patients. Motor symptoms occurring as a result of decreased function of the dopaminergic system, i.e. those which we investigated dominantly, dominated among men, whereas women dominantly develop only rigidity out of motor symptoms and non-motor signs. It is supposed that some still unknown hormonal mechanisms had most probably some influence on such gender differences in manifestation (19).

In the study group of patients, deletion of the mitochondrial genome was registered in 15 (46.9 %) examinees, while 5 of them had heteroplasmy.

Parihar MS et al (2008) made a very interesting paper with 274 patients who were diagnosed with IPD after strict criteria. Similarly to our study, genetic investigations for many genes were made, including alpha synuclein, while more investigations were performed for mitochondrial DNA. In two patients, mutations of LPPK 2 gene were found, mutation of alpha-sy; nuclein gene was found in none, while in 70 % of the patients, delta deletion 4977 of mtDNA was found to be present. These results are quite identical with the findings in our study (25).

Charles Arthur et al (2009) investigated the premise that IPD, in fact its sporadic form, was due to disturbance in the function of enzyme systems in mitochondria. Among their examinees, they therefore investigated several potential mutations of the mitochondrial genome, including the deletion 4977. The authors came to a conclusion that there was a disturbance in the enzyme complex 1–4 in mitochondria, while in the sporadic form of IPD they did not succeed in finding a particular mitochondrial mutation which was specific only for IPD. They also concluded that the presence of delta deletion of mtDNA was very frequent in this disease, and it was statistically significantly more present in the older group of patients with sporadic form of IPD (5).

Reeve et al (2008) made a very important and interesting study dealing with delta deletion of 4977 of mtDNA. Several types of mitochondrial deletion were investigated in separate cells of substantia nigra in three groups of examinees, namely in control group, patients with IPD, and patients with other forms of parkinsonism. The authors found out 89 types of different deletions of mitochondrial DNA, among which was also delta deletion 4977. They did not succeed in finding a statistically significant presence of a definite type of deletion in some of the special types of PD (26).

Conclusion

It could be stated, with great statistical significance, that deletion in mitochondrial genome was more frequently registered in the group suffering from IPD. It was present in 62.5 % of our examinees with IPD, while in the control group this delta deletion 4977 of mtDNA was present only in 25.8 % of examinees. The difference tested between the examined and control group as to the presence of deletion, and its absence and heteroplasmy was highly statistically significant ($p = 0.001$).

As opposed to recent investigations showing that with age, the appearance of deletion Δ mtDNA 4977 in mitochondrial genome increases, our investigation showed that the examinees' age did not prove to be a significant factor in relation to the latter deletion ($\text{ExpB} = 1.01$ 95% CL 0.957–1.063).

The deletion 4977 of mitochondrial genome has been proved to be an independently significant factor, i.e. the examinees with verified deletion of mitochondrial genome had a 4.9-fold greater chance of developing idiopathic Parkinson's disease when compared to examinees without the deletion ($\text{ExpB} = 4.9$ 95% CL 1.65–14.56).

The examinees' mean age in group with deletion in mitochondrial genome was 51.1 ± 7.6 years, the examinees without deletion had the mean age of 52.1 ± 11.6 years, while the mean age of examinees with heteroplasmy was the greatest, namely in range of

59.4±13.7 years. The difference in mean age of examinees with deletion, without deletion, and those with heteroplasmy was statistically non-significant ($p>0.05$).

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