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Mutations in PRKN and SNCA Genes Important for the Progress of Parkinson's Disease

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Abstract: Although Parkinson's disease (PD) was first described almost 200 years ago, it remains an incurable disease with a cause that is not fully understood. Nowadays it is known that disturbances in the structure of pathological proteins in PD can be caused by more than environmental and genetic factors. Despite numerous debates and controversies in the literature about the role of mutations in the *SNCA* and *PRKN* genes in the pathogenesis of PD, it is evident that these genes play a key role in maintaining dopamine (DA) neuronal homeostasis and that the dysfunction of this homeostasis is relevant to both familial (FPD) and sporadic (SPD) PD with different onset. In recent years, the importance of alpha-synuclein (ASN) in the process of neurodegeneration and neuroprotective function of the Parkin is becoming better understood. Moreover, there have been an increasing number of recent reports indicating the importance of the interaction between these proteins and their encoding genes. Among others interactions, it is suggested that even heterozygous substitution in the *PRKN* gene in the presence of the variants +2/+2 or +2/+3 of NACP-Rep1 in the *SNCA* promoter, may increase the risk of PD manifestation, which is probably due to ineffective elimination of over-expressed ASN by the mutated Parkin protein. Finally, it seems that genetic testing may be an important part of diagnostics in patients with PD and may improve the prognostic process in the course of PD. However, only full knowledge of the mechanism of the interaction between the genes associated with the pathogenesis of PD is likely to help explain the currently unknown pathways of selective damage to dopaminergic neurons in the course of PD.

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INTRODUCTION

Developments in science and technology in the second half of the twentieth century have led to an increase in life expectancy, which has contributed to an increased number of diseases typical for old age, including Parkinson's disease (PD). Currently, PD is one of the most common degenerative diseases of the central nervous system (CNS), affecting nearly 2% of the population over 65 years old and 5% over 85 years old. Moreover, estimates show that, faced with an increasingly aging population, the number of patients with this neurodegenerative disease will maintain an upward trend [1, 2].

PD is a slowly progressive disease that is clinically characterized by a slowdown in mobility, muscle rigidity, and resting tremor, which are a consequence of the loss of substantia nigra cells of the midbrain and a dramatic reduction of striatal dopamine (DA) [3].

Although PD was first described almost 200 years ago, it remains an incurable disease with a not completely understood etiology. At present, it is known that in the course of PD there is a decay of dopaminergic neurons of the midbrain substantia nigra as a result of the ongoing degenerative process. It is believed that the cause of this loss of nerve cells is the accumulation of pathological forms of naturally occurring brain proteins, such as alpha-synuclein (ASN), Parkin and tau protein [4]. It has been shown that these proteins form deposits disrupting cellular metabolism and neurotransmission within the structures of the brain involved in the disease.

In 1960, Herbert Ehringer and Leopold Hornykiewicz described the role of DA in the pathogenesis of PD. As a result, L-dopa was introduced to the therapy of PD in 1968 [5]. Although six years earlier (in 1962), Watson and Crick had received the Nobel Prize for the detection of the structure of DNA, the age of discoveries in the field of genetics of PD was yet to come. The key protein of PD, ASN, was discovered in synaptic vesicles of Torpedo Californica only in 1988 [6]. The first mutation in the *SNCA* gene encoding the ASN protein, which is also the first mutation identified in PD, was described in 1990 [7]. However, in 1998, the first mutation in the gene for the Parkin protein (*PRKN*) was described [8]. In fact, the turn of the XXI century has become a landmark in the study of the genetic determinant of PD.

Nowadays it is known that disturbances in the structure of pathological proteins can be caused by not only environ-

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mental factors, but also by genetic factors. Although the diagnosis of PD is still mainly based on clinical criteria, extended by neuroimaging studies, it is currently believed that genetic testing may be an important part of intravital diagnostics in patients with PD. Moreover, it seems that the explanation of the mechanism of the interaction between the genes associated with pathogenesis of PD is likely to help indicate unknown pathways of selective damage to dopaminergic neurons in the course of PD. Described as the first genes associated with PD, the *SNCA* and *PRKN* genes are currently also the best-studied and the most frequently suggested in the analysis of the etiology of PD. It is believed that mutations in the *SNCA* and *PRKN* genes may affect not only the manifestation of PD but also the progress and course of the disease.

In this paper, we presente the current state of knowledge about the mutations of the *SNCA* and *PRKN* genes and their encoded proteins: ASN and Parkin.

ALPHA-SYNUCLEIN IN PARKINSON'S DISEASE

Alpha-synuclein is a protein composed of 140 amino acids and is part of a family of proteins that includes the β- and γ-synuclein [9]. The structure of ASN contains an N-terminal, an amphipathic region containing the six conserved repeat amino acids KTKEGV, a central hydrophobic region, which includes the non-amyloid betacomponent domain (NAC), and the C-terminal acidic region [10]. For many years, it was believed that the ,,not-folded" chain of amino acids was the native structure of ASN, taking the helical form only in conjunction with the lipids of cell membranes. It was thought that ASN is a monomer, but recent studies have shown that under physiological conditions ASN largely takes the form of tetramers, and may take the helical form without connection to the lipid membrane [11].

Immunohistochemical studies have shown that ASN in cells can be found bonded to both the nuclear membrane and within the synaptic vesicles [12]. To a lesser extent, ASN occurs in the free form in the cytoplasm.

The functions of ASN are not fully understood; however, due to the cellular location of this protein, it has been suggested that the functions of ASN may be related to synaptic transport. Moreover, it is believed that interaction of ASN with cytoskeletal proteins may indicate its participation in the axonal transport of synaptic vesicles [13]. It has been shown that ASN may affect the rate of the synaptic vesicle by the modulation of lipid metabolism and prevention of lipid hydrolysis to the base and phosphatidic acid, which is responsible for creating synaptic vesicles from cellular membranes. It has been also shown that ASN regulates the activity of synaptic vesicles by binding and transport of fatty acids [14, 15], and that ASN is involved in the metabolism of membrane phospholipids by inhibiting phospholipase D2 activity (PLD2) [14]. There are also reports indicating that ASN participates in the process of differentiation and survival of the dopaminergic neuron progenitor cells of the mouse and human [16, 17]. It is also believed that ASN may have anti-apoptotic activity [18, 19].

It is known that ASN can be degraded either with the involvement of the ubiquitin-proteasome system or by lysosomal autophagy. It may be also degraded by cytoplasmic proteases including capanin I [20]. Under conditions that are adverse for neurons, ASN may change its structure and take the form of a beta harmonica. It is also known that disturbances in the structure of ASN observed in the course of PD may lead to aggregation of ASN and formation of soluble oligomers, leading to insoluble filaments and deposits in the nerve cells [21]. As has been shown, ASN is one of the main components of Lewy's body (LB) pathology, which are round or polymorphonuclear cellular inclusions in the cytoplasm of nerve cells. Moreover, it has been suggested that the formation of insoluble deposits of ASN and this aggregation process may give rise to the formation of LB [22].

Disorders in the structure and function of ASN are observed in a number of neurodegenerative diseases, such as PD, multisystem atrophy (MSA) or dementia with Lewy bodies (DLB), commonly called alpha-synucleinopathies.

It is known that the process of ASN aggregation is a negative phenomenon for neural cells, not only because of the high toxicity of the resulting aggregates, but also because of physiological function disorders caused by the reduction of bioavailability of this protein [23]. It has been shown that, in PD, the process of ASN aggregation may be modulated by a number of factors. These include, among others, oxidative stress and disruption of mitochondrial complex I. Improper functioning of the mitochondrial respiratory chain leads to excessive production of reactive oxygen species (ROS) and thereby enhances the aggregation process of ASN [24, 25].

It has also been suggested that there is an interaction between the ASN and tau proteins, leading to a synergistic induction of fibrillation of these proteins [26]. Further, it has been demonstrated that ASN can interact with β-amyloid protein and act as a nucleant aggregator for both itself and for β-amyloid. On the other hand, the presence of β-amyloid may give rise to nucleation of ASN [27]. However, it has been postulated that nonamyloidogenic proteins, such as β-synuclein, may protect against the ASN aggregation process [28].

It also appears that factors leading to the destabilization of the ASN tetramer structure may affect the aggregation process of ASN. A revolutionary discovery published in 2011 in Nature demonstrated that the ASN aggregation process must be preceded by ASN tetramer decomposition into easily aggregating monomers of this protein and tetramers of ASN can aggregate. The authors of that study also suggested that the aggregation process of ASN may involve new, as yet unknown, factors that can produce, among their effects, destabilization of the ASN tetramer structure [29].

Furthermore, it has been shown that a properly functioning ubiquitin-proteasome system (which is responsible for the degradation of proteins) and mutations in the gene encoding ASN have a substantial effect on the ASN fibrillation process.

MUTATIONS AND POLYMORPHISMS OF *SNCA* GENE IN PARKINSON'S DISEASE

The gene encoding the ASN protein is the *SNCA* gene and corresponds to the loci of PARK1 and PARK4. The *SNCA* gene is located on the long arm of chromosome 4

(4q21.3-22). The first described point mutation of *SNCA* determining the manifestation of PD was a G>A transversion at position 209 of 4th exon, which causes substitution of alanine (A) to threonine (T) at position 53 of the ASN protein [7].

Interestingly, an evolutionarily conserved *SNCA* gene found physiologically in mice, rats and canaries, has a T instead of an A at position 53 of the protein, while the substitution of these amino acids in humans leads to the manifestation of PD. The first reports describing the case of the Sicilian Contrusi family with familial Parkinson Disease (FPD) caused by A53T mutations in the *SNCA* gene have showed a faster progression and early onset of the disease. Most of the Contrusi family members with the A53T mutation of *SNCA* affected by PD showed a unilateral resting tremor, postural and gait disturbances, bradykinesia and rigidity. Some of these individuals exhibit dementia that varies considerably in severity. These patients respond well to L-dopa therapy, but they have a number of dyskinesias [7, 30].

Another point mutation in the *SNCA* gene was identified in a German family with a G>C transversion at position 88 in the 3th exon leading to the substitution of A to proline (P) in position 30 of the ASN protein. It has been also shown that, as a result of the A30P mutation, ASN is deprived of the ability to connect the N-terminal domain with the membrane of the synaptic vesicle transmitted fast axonal transport, leading to changes in ASN localization in the cell.

As it has been shown, PD caused by A30P mutations of *SNCA* was associated with a relatively early onset of disease and characterized a milder course than in patients with the A53T mutation in *SNCA* [31]. In the case of both mutations, a diversity of symptoms has been reported in the individual families bearing these mutations [7, 30, 31].

Moreover, it has been shown that the A53T and A30P mutations increase the likelihood of ASN oligomerization as opposed to fibrillation [32]. It is suggested that this property may indicate the crucial importance of oligomerization of ASN in the pathogenesis of PD. Other studies have shown that the A53T mutation enhances the aggregation of ASN and formation of filaments, in contrast to the A30P mutation [33].

A third point mutation in the *SNCA* gene causing a substitution of glutamic acid (E) to lysine (K) at position 46 of the protein has also been described [34]. As has been shown, the E46K mutation of *SNCA* changes the polarity of ASN and affects the occurrence of significant physico-chemical and molecular changes in this protein. It has also been suggested, that the E46K mutation may affect the release of neurotransmitters and lead to a more effective aggregation of ASN compared to the A53T and A30P mutations by altering the binding of ASN with the phospholipids of cell membranes [35].

Clinically, patients with the E46K mutation in the *SNCA* gene have demonstrated not only movement disorders and dementia, but also visual hallucinations [34]. Moreover, recent reports suggest that patients with the E46K mutation of *SNCA* exhibit neuropsychological disturbances at an early stage of the disease and that these may be a distinct feature of cognitive impairment [36].

POINT MUTATION IN *SNCA* GENE AND INTERACTION OF ALPHA-SYNUCLEIN WITH DOPAMINE

It is known that catecholamines, especially DA, can modulate the oligomerization of ASN in PC12 cell lines with overexpression of ASN [37]. It has also been shown that oxidation products of DA affect the aggregation of ASN *in vitro*, and that this process depends on the pH of the environment [38]. However, in the study by Da Costa *et al.* conducted on the nerve cell line TSM1, 6-hydroxydopamine caused severe aggregation of ASN that may reduce the bioavailability of this protein and inhibit its anti-apoptotic effects [39]. However, the studies carried out in PC12 cell lines with overexpression of ASN have indicated that DA and its oxidized derivatives cause inhibition of ASN aggregation at the level of oligomers (protofibrils) and prevent its further fibrillation [37].

On the other hand, it has been shown that physiological ASN can modulate catecholamine biosynthesis by reduction of tryptophan hydroxylase (HT) expression at the protein level and decrease the expression of the transcription factor Nurr1that initiates transcription of the HT gene, as well as reduction in the expression of other genes involved in the biosynthesis of DA, such as the cyclohydrolase GTP and DAA genes [40]. As it has been shown by studies conducted *in vitro* and in brain cells of mice and rats that ASN may lead to a reduction in HT activity directly by binding to the unphosphorylated (inactive) form of HT and increase its stability, as well as indirectly by modulating the activity of phosphatases and kinases responsible for the phosphorylation of HT; such as ERK, calcium-dependent kinases, calmodulins, and protein kinase C (PKC). However, a reduction in the activity of HT may affect the inhibition of DA biosynthesis [41-43].

As it has been shown, mutated ASN (A53T, A30P) also inhibits the activity of HT [39, 44]. However, due to the aggregation of mutant forms of ASN the concentration of its soluble pool decreases, which may result in inefficient inhibition of HT and consequently the increase of DA in turn leads to a cause of oxidative stress on the action of quinones and free hydroxyl radicals produced through the metabolism of DA [12, 45].

It is known that the level of DA in the brain is controlled by the metabolism within the synaptic space. However, DA can also go back into the cell through the dopamine transporter (DAT), where DA is "enclosed" in synaptic vesicles [46, 47]. It has been suggested that ASN inhibits DAT, influencing the rate of DA uptake rather than the affinity DAT for DA [48]. It has also been shown that the mutant A30P ASN retains the ability to inhibit the activity of DAT, in contrast to the variant A53T [32, 33].

SNCA MUTATIONS AFFECTING THE EXPRESSION OF ALPHA-SYNUCLEIN

It is believed that the pathogenesis of PD may include not only point mutations of the *SNCA* gene, but also variants related to the regulation of ASN expression [33, 43, 49, 65]. It has been shown that aggregation of ASN may be caused by duplication or triplication of this gene. Furthermore, it has been shown that triplication of the *SNCA* gene leads to a

two-fold increase of the ASN level, while duplication of the *SNCA* gene increases the level of this protein one-and-a-half-fold [33]. Currently, it is known that triplication of the *SNCA* gene is associated with the occurrence of early onset PD (EOPD) with rapid progression of the disease, often with dementia and disorders of the autonomic nervous system [50]. However, PD caused by duplication of the *SNCA* gene revealed a little later that the disease progresses slowly, without evidence of dementia, and that the course of the disease in this case is similar to SPD [51].

Currently, it is also believed that orthostatic hypotension occurring in PD patients with triplication of the *SNCA* gene, and which has not been reported in cases of duplication of this gene, is likely to be associated with disorders in the formation of synaptic vesicles induced by dysfunction of ASN, and occurring in their biosynthesis of noradrenaline and adrenaline [50, 51]. This hypothesis seems to explain the results of many pathological studies that have shown that there is not only loss of dopaminergic neurons, but also loss of noradrenergic terminals, also in the sympathetic nervous system of the heart, in PD [52, 53].

The literature reports have indicated that the appearance of each additional copy of the *SNCA* gene may affect the time of onset of PD and result in worsening clinical symptoms. There have also been reports indicating a faster PD progression in patients with *SNCA* gene duplication in an Italian family, in which earlier onset of the disease (40 years) and rapid progression with early fluctuations and dyskinesias and developing dementia have been observed [54].

In 2008, the case of a patient with PD with duplication of the *SNCA* gene was reported, in whom the disease did not respond to treatment with L-dopa and the disease progressed very quickly (to 5 stages in a Hoehn and Yahr scale over several years) [55]. On the other hand, asymptomatic mutation carriers who did not show any preclinical symptoms, also in PET image or olfactory disorders, have been reported in some families with *SNCA* duplication. These findings have indicated that there is variable penetration of *SNCA* duplication for which the ratio is about 30-40% [56]. It is believed that the likely variable penetration of the duplication of *SNCA* may be associated with other genetic or environmental factors [36].

Moreover, elevated expression levels of *SNCA*-mRNA have been found in the affected regions of the PD brain and support the hypothesis that increases in ANS expression is associated, among other factors, with the development of SPD [57]. It is also known that the overexpression of ASN in neurons facilitates aggregation of this protein even when ASN is found in its correct structure.

Therefore, it has been suspected that not only mutations in the *SNCA* gene but perhaps also other factors affecting the expression of ASN may contribute to the manifestation of PD, including also SPD. The study conducted by Chiba-Falek *et al.* showed that, in the NACP-Rep1 region of the *SNCA* gene promoter, there is a polymorphic area differing in the count of dinucleotide repeats and affecting the level of ASN expression and the risk of PD [57]. The NACP-Rep1 region contains dinucleotide repeats (TC)x(T)2(TC)y(TA)2 (CA)z, and the region may vary in the number of repeats and include substitutions of nucleotides. However, it has been proven that a change in the number of repeats, more than nucleotide substitutions, affects the expression of ASN [45, 58, 59].

Five alleles of NACP-Rep1 of the *SNCA* gene promoter have been described as the most common in humans: -1, 0, +1, +2, +3. Generally, in theEuropean population, the most frequent was allele +1 of NACP-Rep1 [45, 58, 59]. It has been also shown that allele 0 of the NACP-Rep1 region in the *SNCA* promoter is two pairs shorter than allele +1, while allele -1 is shorter by 4 bp however alleles 2 and 3 are longer by 2 and 4 bp, respectively.

Functional analyses on the two most common NACP-Rep1 alleles +1 and +2 suggest that the +2 allele is associated with an up-regulation of *SNCA* expression, whereas the +1 variant shows reduced gene expression [60, 61].

In addition, allele +1 of the NACP-Rep1 region of the *SNCA* promoter, having 259 bp, significantly reduces the risk of PD in the populations of Europe, America and Australia [58, 62, 63]. A protective function of genotype +1/+1 has been also indicated in the Polish population in a study by the authors [49]. Moreover, our study uses logistic regression analysis to confirm the reduction in PD risk in the presence of allele +1 in a dose dependent manner, while another study failed to replicate the finding in the populations of Japan, Singapore and Italy [45, 64].

Further, the study by Fuchs indicated that the +1/+1 genotype of the PD-associated promoter repeat NACP-Rep1 is associated with lower protein levels in blood than the other genotypes, which may explain its protective role [58]. In our study in the Polish population we also observed that if PD occurred in a person with the +1/+1 genotype, compared to the other genotypes, the progression of the disease was slower and response to pharmacotherapy was better with low dose L-dopa treatment [49].

Increased levels of ASN may induce its aggregation, accumulation, and consequently neurotoxic effects; under physiological conditions, among the many functions of ASN is the maintenance of dopamine homeostasis in the central nervous system. There is therefore a possibility that patients with genotype +1/+1, the pathogenesis of PD was due to factors other than ASN, while a reduced level of ASN, conditioned by the +1/+1 genotype, may have an effect on milder course of disease [43, 65, 66].

Current literature indicates a different genetic distribution of NACP-Rep1 genotypes depending on geographical region and the analyzed population. In addition, the results are often opposite. Although the possible protective effect of the +1 allele is not currently a subject of discussion, the suggestion has been that the 0, +2 and +3 alleles may have no impact, of may increase the risk of onset of PD, and sometimes even that they may have a protective effect [62-64, 67].

The studies by Tan [62] and Myhre [68] observed a higher frequency of the +3 allele in PD cases compared with healthy controls, while in the study by both Tan *et al.* [45] and Spadafora *et al.* [64] no significant differences of the various genotypes between PD and controls were found in populations of Singapore and Italy. However, a 2012 study from Italy showed evidence of an association between the +2

allele on NACP-Rep1 and PD [67]. In 2006, a meta-analysis of 11 study populations provided strong evidence that the +3 allele was more frequent in PD cases, increasing risk of this disease, while the +2 allele did not differ between PD cases and unaffected controls; however, the authors suggested that the lack of association of the +2 allele in the meta-analysis could be due to the large fluctuation in its frequencies observed in the analyzed populations [63]. In our previous study in a Polish population, we demonstrated that the +2 and +3 alleles had a higher frequency in PD patients, increasing PD risk. Moreover, an increase in +2 allele was found to increase the risk of PD manifestation in a dose dependent manner [49].

In contrast to the results of Kay *et al.* [69], there was no association found in the Polish population between the 0 allele and the risk of PD, but the presence of this genetic variant was correlated with a decrease in the stage of disease in patients suffering for PD over 10 years compared to patients with the other variants of NACP-Rep1 [49].

Additionally, it was demonstrated that genotypes +2/+2 and +2/+3 were associated with faster progression of the disease in a Polish population, but had no influence on the response to therapy [49]. This hypothesis seems to correspond with the results of the study by Ritz [70], which showed that the risk of faster decline of motor function was increased four-fold in carriers of the +3 allele of the NACP-Rep1 promoter variant. Moreover, the study by Kay *et al.* [69] indicated a trend of decreasing age of onset with increasing allele size; whereas the study by Ritz demonstrated that age at onset of carriers of at least one +2 allele was earlier compared to noncarriers [71]. The present study has not shown any association between the NACP-Rep1 alleles and dementia, similarly to the study of de Marco [72].

It is currently believed that the aggregation of ASN in patients with PD can also be affected by factors such as alternative splicing, phosphorylation, or factors modifying the expression of the *SNCA* gene. However, the importance of these factors has not yet been fully confirmed. It seems that examination of genotypes of the NACP-Rep1 region of the *SNCA* promoter may not only help to explain the pathogenesis of PD, but may also facilitate in early PD diagnosis and help determine the degree of individual risk for this neurodegenerative disease.

Furthermore, it is currently believed that the efficiency of the ubiquitin-proteasome system can have a significant impact on the process of ASN fibrillation. As it has been shown, both in experimental neuronal cell cultures and *in vivo* experimental animal model of PD in rats, abnormal activity of the proteasome may lead to the aggregation of ASN due to its ineffective elimination. A critical protein for the proper functioning of the ubiquitin-proteasome pathway is Parkin protein.

ROLE OF PARKIN PROTEIN IN THE AGGREGATION OF ALPHA-SYNUCLEIN

Parkin is a cytoplasmic protein composed of 465 amino acids [73] and an evolutionary conserved gene product, with orthologs in *Caenorhabditis elegans*, *Drosophila melanogaster*, mouse, rat, and other species [74-78].

Expression of the Parkin protein has been shown in both neuronal and non-neuronal tissues. In the human brain, Parkin is expressed in neuronal cell bodies and glial cells in the gray matter, whereas in the white matter it is located mainly in cells with an astrocyte-like morphology [78]. It has also been shown that, although Parkin is localized in the cytosol, it is associated with the endoplasmic reticulum (ER) and the outer membrane of mitochondria [79, 80].

There are also some reports indicating localizations of Parkin in the Golgi apparatus, synaptic vesicle, and nucleus [81, 82]. It has been suggested, that Parkin may also be localized in mitochondria of proliferating cells but moves to the extra-mitochondrial cytoplasm when cells are under differentiated or quiescent conditions [83].

It has been shown that Parkin is composed of an N-terminal ubiquitin-like domain and the C-terminal domain with two RING finger motifs, whose presence can indicate a potential regulatory function of the Parkin [84]. Currently, it is believed that Parkin plays a vital role in the proper function of mitochondria. It has been shown that Parkin can regulate the transcription and replication of mitochondrial DNA in proliferating cells [83].

It is known that some of the Parkin molecules occur within the mitochondria [85], wherein they are associated with mtDNA and mitochondrial transcription factor A (TFAM), so that Parkin controls the operation of mitochondria in terms of such functions as the transcription and replication of mtDNA [83]. It is believed that Parkin rather protects against damage to mtDNA in conditions of oxidative stress, and may even induce repair mechanisms [85, 86].

It is also suggested that Parkin may affect the activity of the mitochondrial complex and may have an indirect effect on the level of oxidative stress [83]. Several mechanisms have been proposed to explain the regulation of cellular redox balance by Parkin. It has been shown that overexpression of wild-type Parkin leads an enhancement of the mitochondrial membrane potential and reduction of mitochondrial reactive oxygen species (ROS) production in neuronal cells [83, 87].

It has also been suggested that Parkin limits oxidative damage by induction of antioxidant enzymes. Several reports have indicated a connection between mitochondrial dysfunction and oxidative damage in Parkin deficient mice [88-91]. In transgenic mice and flies with knockout of Parkin, deficient mitochondria have been observed [92, 93]. Further studies have shown that Parkin deficient mice demonstrated a decrease in serum antioxidant capacity, reduced levels of peroxiredoxin-1, 2, and 6 in the midbrain, and increased lipid peroxidation in the CNS [94].

However, Parkin overexpression was not able to rescue human dopaminergic neuroblastoma cells from hydrogen peroxide- and rotenone-induced apoptosis [95].

It has been found that Parkin may downregulate the zinc finger-containing protein called PARIS, which is a major transcriptional repressor of PGC-1 expression [96]. It is known that PGC-1 is a number of the peroxisome proliferator-activated receptor gamma coactivator (PGC) family and has a central role in the regulation of transcriptional control in mitochondrial biogenesis. It is therefore probable that

Parkin may indirectly modulate mitochondrial metabolism [96].

It is also believed that Parkin is involved in the process of mitochondrial fusion and fission, a vital mechanism for mitochondrial metabolism, communication, and quality control.

Furthermore, it is suggested that Parkin can participate in managing irregular mitochondria to degradation in the phagosomes. Some reports have also indicated that Parkin might translocate specifically to mitochondria and induce mitochondrial autophagy [97].

Recently, there has been support of a model in which the translocation of Parkin to damaged mitochondria induces the degradation of mitochondrial fusion factor, mitofusin, leading to impaired mitochondrial fusion. The authors of that study have suggested that this process may serve to selectively isolate damaged mitochondria for their removal by autophagy [98].

However, Parkin functions mainly as an E3 ligase ubiquitin stimulating protein binding (directed to degradation in the proteasome) with ubiquitin, consequently preventing cell apoptosis [84, 99]. Ubiquitination is a vital cellular quality control mechanism that prevents accumulation of misfolded and damaged proteins in the cell. It is thought that substrates of Parkin include, among others, synphilin-1, CDC-rel1, cyclin E, p38 tRNA synthase, Pael-R and synaptotagmin XI.

As is apparent from the study by Zhang *et al.*, Parkin is also responsible for its own ubiquitination and degradation in the proteasome [84]. In 2001, Shimura *et al.* first described the presence of Parkin in the human brain complex containing Parkin with the glycosylated form of ASN (alpha-SP22), thus indicating the involvement of Parkin in ASN degradation via the ubiquitin-proteasome system [100, 101]. It has been shown that dysfunction of the Parkin protein can lead to ineffective elimination of ASN and the aggregation of this protein [102].

Recently, a novel Parkin substrate - mitochondrial hexokinase I (HKI) - has been identified. It has been detected that Parkin ubiquitylation of HKI leads to its proteasomal degradation by a decrease in the membrane potential [103].

Apart from lysine48 (K48)-linked poly-ubiquitination, Parkin is also able to catalyze both mono-ubiquitination and K63-linked poly-ubiquitination, and influence a range of cellular processes such signal transduction, transcriptional regulation, and protein and membrane trafficking, without promoting substrate degradation [104-107].

Recent studies have shown that Parkin may play a role in cellular decision-making, choosing between two systems of degradation: proteasome activity (through its ability to K48-linked poly-ubiquitination associated with the proteasome) and macroautophagy (through K63 ubiquitination related to cell signaling and the formation of LB) [108-110]. In addition, according to the literature, Parkin may also interact with DA and indirectly influence the aggregation of ASN in nerve cells [111].

Studies in transgenic mice with a silenced Gpr37 gene have showed that Parkin may regulate the concentration of DA by modulating the activity of HT regulating the degradation process of DAT belonging to the substrate spectrum of Parkin, and by interaction with the GPR37 receptor [112].

Interestingly, studies with dopaminergic neuroblastoma cells with overexpression of Parkin have shown that Parkin may also protect neurons from apoptosis induced by the action of DA and its oxidative derivatives [112]. On the other hand, it has been shown that Parkin in cell cultures of rat neurons is subjected to oxidative derivatives of DA as well as a covalent modification by DA, leading to inhibition of Parkin activity [114, 115]. However, the main cause of Parkin dysfunction to be mutations in the gene encoding this protein (*PRKN*).

MUTATIONS OF *PRKN* GENE IN PARKINSON'S DISEASE

The *PRKN* gene, also known as PARK2, maps the long arm of chromosome 6 (6q25.2–q27) [116], contains 12 exons, and spans about 1.53 Mb, making it one of the largest genes in the human genome [77, 117].

It is known that the Parkin promoter functions as a bidirectional promoter, not only for Parkin but also regulating the transcription of a Parkin co-regulated gene (*PACRG*) that is antisense to *PRKN*, spans 0.6 Mb and contains five exons [118]. The role of the *PACRG* gene product is not currently known.

A *PRKN* mutation was first described in a Japanese family with an autosomal recessive juvenile PD (JPD) [119]. So far, more than 100 mutations of *PRKN* gene have been identified comprising both deletions and insertions of one or more exons, as well as point mutations that change the reading frame, cause premature termination of translation or amino acid substitutions, and almost half of the described mutations are missence / nonsence type [73, 77, 120].

It has been shown that the mutations in the *PRKN* gene are the most common genetic disorders in family cases of JPD, although their presence has been also demonstrated in LOPD, both in FPD and SPD [121-123]. Although *PRKN* gene mutations have been identified in all 12 exons of this gene, the most common seem to be mutations in exons 2, 4, 7, 8, 10 and 11. The vast majority of studied FPD cases that are conditioned by a *PRKN* mutation are inherited in an autosomal recessive manner, but heterozygous mutations related to PD have also been reported.

Furthermore, it has been shown that mutations in the *PRKN* gene occur at different frequencies both in Caucasians and in populations of African and Asian countries [32, 77].

However, the literature on the prevalence of mutations in *PRKN* and their involvement in the modulation of PD risk are very diverse and have a wide variation depending on the studied population and the age of subjects included in the study.

It has been suggested that mutations in *PRKN*, including homo- and heterozygous mutations, are detected in about 40-50% of early-onset FPD and in about 1.3-20% of SPD patients [124-128].

In the study by Abbas *et al.* [129], pointed mutations of *PRKN* in the European population were approximately twice as common as homozygous exonic deletions. In the European population, i *PRKN* mutations were reported in about 19% of SPD and 50% of early-onset FPD [32].

Furthermore, the study by Lucking *et al.* in SPD revealed that 77% with age of disease onset below 20 years had mutations in the *PRKN* gene, but in cases with age of disease onset between 31 and 45 years mutations were found only in 3% in the European population.

A larger case study has confirmed the reports of Lucking *et al*., and has shown *PRKN* mutations in 67% of cases with age of onset below 20 years and in 8% of cases with an age of onset between 30–45 years [130]. In another study involving 363 affected subjects from 307 families, *PRKN* mutations were identified in 2% of all late-onset families screened, thereby directly implicating the *PRKN* gene in LOPD [131]. *PRKN* mutations in EOPD were detected at a 5% frequency in a Korean population, and at an 11% frequency in a Japanese population [8]. In the Italian population, mutations of *PRKN* occurred with a frequency of 8-13%, 16% in French, 9% in German, and 4% in Americans, with 21% in North African and about 8% in Brazilian [32, 130, 132, 133].

The observation that mutations in the *PRKN* gene are common in juvenile- and early-onset PD and increasing evidence supporting a direct role for Parkin in late-onset disease make this gene a particularly compelling candidate for intensified investigation.

DELETIONS OF *PRKN*

In 1998, Kitada *et al.* [77] first described a homozygous deletion of exons 3–7 of the *PRKN* gene in autosomal recessive JPD.

Since then, the deletion of exons 1, 2, 3, 4, 5, 7, 8 and duplication of exons 2, 3, 4, 7, as well as deletions spanning multiple adjacent exons like exons , 2-4, 3-9, 3-5, 4-7, and 7-9 have been detected (Table **1**) [8, 118, 134-140]. Lucking *et al.* [32, 139] detected triplication of exon 2 of the *PRKN* gene.

It is suggested that single or multiple exon deletions and duplications occur with a frequency of 15.8% and account for about 50% of all mutations of the *PRKN* gene [141]. This high rearrangement rate of the *PRKN* gene can be explained by the fact that *PRKN* is located within the large common fragile site (CFS) *FRA6E* [142]. It has been observed that extremely large genes located in an unstable region are highly evolutionary conserved. Therefore, it is also suggested that those regions and the genes within them may be involved in proper cell function [142].

It has been shown that the most unstable region in the CFS *FRA6E* is located between exons 2 and 8 of *PRKN*, suggesting hot-spots for forming gaps and breaks in the large intronic regions [143]. Moreover, according to literature reports, exon deletions and duplications of the *PRKN* gene most frequently include the region between exons 2 and 9 [144].

Although many reports indicate an important role of *PRKN* exon 2 and 4 deletions in the pathogenesis of idiopatic PD [126, 145-147], in the study in a Polish population there was no detectable deletion of exon 2 and 4, as opposed to the German and Japan populations and the results obtained in the multipopulation study [3, 36, 49, 123, 148, 149]. However, the Polish population results were consistent with the studies by Kruger [150] (also showing no deletion of exon 4 in the German population), Sinha *et al.* [150], and Barsottini *et al.* [152], who also did not detect any deletion of exons 2 and 4 of *PRKN* in PD patients. However, deletion of other, not tested exons has not been ruled out in the Polish population. In turn, in the study by Oliveri *et al.* [153], no homozygous exonic deletions were detected in 118 LOPD patients in the American population, suggesting that deletion mutations of *PRKN* were not as common as in EOPD. Therefore, it is generally suggested that copy number variations (CNVs) of *PRKN* are most probably related with EOPD [154].

Table 1. Known *PRKN* Exonic Deletions and Multiplications

Mutation	Reference
Del ex 2	[134, 136]
Del ex 4	[134, 135]
Del ex 5-7	[135]
Del ex 5	[8, 136]
Del ex 3	[8, 134, 136]
Del ex 3-6	[134]
Del ex 5-6	[134]
Del ex 6-7	[135]
Del ex 8-9	[137]
Del ex 7	[134, 136]
Del ex 3-4	[8, 134]
Del ex 3-9	[155]
Del ex 2-3	[134]
Del ex 2-4	[134, 135]
Dup ex 2	[136]
Dup ex 3	[155]
Dup ex 4	[136]
Trip ex 2	[32]

It is currently known known that CNVs of *PRKN* exons may occur in heterozygous configuration as pseudodominant mutations (like deletions of exon 2 or 4 [134] or duplication of exon 4 [136]) or in combination with other *PRKN* mutations [134, 136, 155], as well as in a homozygous configuration as recessive mutations (like deletions of exons 3 and 5 [8], 7 [136], or triplication of exon 2 [77]).

The results of rearrangements in the *PRKN* gene depend on the size and localization of the mutation, especially the exact breakpoints of rearrangements. However, only a few studies have analyzed the localization of the exact breakpoints of large rearrangements found in the *PRKN* gene [124, 156-158].

Most of the deletions and duplications of the *PRKN* gene are frameshift mutations that lead to a premature stop codon several positions downstream. It has also been suggested that

such mutations as genomic deletion of exon 4 of *PRKN* may induce splicing of exons 3-4 in the brain, leading to an in-frame, albeit truncated transcript. It has been also shown that truncated proteins are not even expressed at detectable levels in the brains of patients with deletions of exons 3 or 4 [79, 101], which strengthen the concept of loss-of-function mutations as the predominant pathomechanism in PD. Moreover, the study by Shimura *et al.* demonstrated that one UbcH7 belonging to the E2 family binds to the RING-IBR-RING box of Parkin, and deletions or point mutations within this region abolished the interaction of Parkin and UbcH7 [116]. Finally, it is currently known that rearrangements of *PRKN* exons in effect may lead to a loss of function of Parkin [159].

POINT MUTATIONS OF *PRKN*

Point mutations have been found in all the domains of Parkin, although the majority of point mutations localize to the RING-IBR-RING domain in the C-terminal half of Parkin and, in particular, to the first RING domain.

It has been suggested that point mutations might cause a greater overall loss of function than whole exonic deletions, because the intact but inactive protein may compete for substrate effectively and deleteriously affect normal ubiquitination mediated pathways in the cell [118]. More than 100 point mutations are currently known within the *PRKN* gene. The most important of them are presented in (Table **2**).

Although point mutations in the *PRKN* gene are characteristic for JPD and EOPD, also it has been suggested that these mutations may be involved in the pathogenesis of LOPD. However, studies testing common mutations and polymorphisms for association with LOPD have produced mixed results [153, 160-162].

Furthermore, there is no question that Parkin-associated parkinsonism is recessive; that is, both alleles are mutant, but despite previous reports it remains debatable whether a heterozygous mutation can cause or increase the risk of PD [138, 139, 163, 164].

In 2002, Hedrich *et al.* [165] evaluated 50 cases (under 50 years) from a variety of ethnicities for Parkin mutations and identified seven cases with compound heterozygous mutations and six cases with a single heterozygous mutation, leading them to hypothesize that the loss of a single Parkin allele may be associated with EOPD.

However, some authors suggest that mutations of *PRKN* gene do not appear to be associated with typical idiopathic PD [146, 162] while others indicate an association of *PRKN* mutation with idiopathic PD in populations of Europe, America and in multipopulation studies [32, 131, 166].

In the Italian population, the presence of *PRKN* mutations in the homozygous or compound heterozygous configuration was shown in patients with EOPD in 8.2% of cases and in 2.7% heterozygous cases [127], while in Swedish patients with EOPD the heterozygous mutation of *PRKN* gene was found only in 1.5% of patients and the homozygous form was not shown in any of the analyzed persons [167]. In the German population the frequency of *PRKN* mutations was 9% [168], in the Brazilian population 8% [134], and in the American population they were less than 4% [133] while in the Japanese population they reached 66% [8]. A small contributin of *PRKN* mutations in the pathogenesis of EOPD have been shown in the Polish population [169], while the studies in LOPD have shown that *PRKN* mutation in the Polish population occurs at a frequency of 20.6% [49, 123], which is similar to SPD in European populations [36].

Table 2. Missense, Nonsense and Frameshift *PRKN* Mutations (Modified on the Basis of [141])

Exon	Mutations
1	c.1A>T, c. 13G>A
2	c.29G>A, c.34G>C, c.43G>A, c.52G>A, c.92C>A, c.95A>C, c.97C>T, c.98G>A, c.101delA, c.101_102delAG, c.101A>G, c.110C>T, c.118C>T, c.124C>T, c.125G>A, c.125G>C, c.136G>A, c.136G>C, c.155delA, c.156_157insT, c.160T>A, c.164C>T, c.167T>A
3	c.220_221insGT, c.235G>T, c.245C>A, c.256G>A, c.300G>C, c.310C>T, c.337_376del40, c.397_399delCCA, c.428A>G
4	c.434G>A, c.458V>G, c.483A>T, c.497G>A, c.500G>A, c.511C>T, c.518C>T
5	c.536delG, *c.574A>C,* c.574A>G, c.600C>G
6	c.632A>G, c.633A>T, c.634delT, c.634T>G, c.635G>A, c.645C>A, c.688G>A, c.701G>A, c.714G>A, c.719C>G, c.719C>T, c.727G>A, c.730G>A
7	c.758G>A, c.758G>T, c.759C>G, c.766C>T, c.772G>A, c.794A>G, c.799T>C, c.804T>A, c.813A>T, c.814C>A, c.818A>G, c.823C>T, c.838G>A, c.848T>C, c.850G>C, c.865T>G, c.871delG
8	c.892A>C, c.893T>G, c.930G>C, c.931C>T, c.933G>T
9	c.968_973delGTGTCC, c.971delT, c.983G>A, c.1000C>T, c.1001G>A, c.1015G>T, c.1041_1042delGA, c.1046_1047delAA, c.1051A>C, c.1076G>A
10	c.1096C>T, c.1097G>A, c.1138G>C
11	c.1175_1176delGA, c.1180G>A, c.1183G>T, c.1186A>G, c.1192G>A, c.1204C>T, c.1205G>A, c.1225G>T, c.1244C>A, c.1252T>C, c.1283_1284insA
12	c.1286G>A, c.1289G>A, c.1292G>T, c.1310C>T, c.1321T>C, c.1330G>C, c.1335G>A, c.1358G>A, c.1372A>C, c.1378_1379insG, c.1393G>A

On the other hand, it is probable that one heterozygous mutation in *PRKN* may be sufficient to increase the risk of PD and induce preclinical changes in the substantia nigra. Studies using positron emission tomography (PET) shed new light on the issue of an association between the heterozygous mutation of *PRKN* gene with an increased risk of PD. In this study, the authors found a significant reduction of F-18-dopa uptake in the caudate, putamen, ventral, and dorsal midbrain compared with control subjects, and demonstrated that Parkin heterozygotes, although asymptomatic, may exhibit nigrostriatal dysfunction that in some individuals may contribute to LOPD [170].

The results of the study by Khan *et al.* have been reproduced in an independent study by subsequent transcranial sonography, revealing substantia nigra hyperechogenicity in 5 out of 7 asymptomatic carriers of *PRKN* mutations, and by functional MRI analysis of heterozygous *PRKN* mutation carriers have demonstrated reorganization of striatocortical motor loops, probably due to compensation of latent nigrostriatal dysfunction [171, 172].

This hypothesis may explain the presence of single heterozygous substitution in the *PRKN* gene in some persons from control groups and suggests that in those persons it cannot exclude preclinical changes or PD manifestation in later age.

The observation of patients with both normal and mutant alleles may reflect that haploinsufficiency is a risk factor for the disease or that certain mutations are dominant, conferring dominant-negative or toxic gain of function [173]. It is also known that Parkin is S-nitrosylated *in vitro* and *in vivo*, and S-nitrosylation inhibits Parkin's E3 ligase activity and its protective function [100]. Thus, it has been suggested that a heterozygous mutation of the PRKN gene coupled with nitrosative stress could lead to the manifestation of haploinsufficiency, accounting for the observation of disease-associated heterozygous mutations.

Association of a heterozygous mutation of the *PRKN* gene with SPD, mainly with LOPD, has also been shown in the study in a Polish population involving 90 SPD patients and 113 control subjects. In the analyzed population, 5 missense heterozygous substitutions (c.500 G>A, c.520 C>T, c.823 C>T, c.930 G>C, c.1180 G>A) in the *PRKN* gene were seen in 4 exons (4,7,8 and 11). In this study, the frequency of polymorphisms c.500 G>A, c.1180 G>A and c.930 G>C was significantly higher in PD cases and increased the risk of PD manifestation [123].

The c.500 G>A transition, located in 4 exon in the cysteine-rich unique Parkin domain (UPD), [174] has thus far been reported to not be associated with PD [162, 175] and to be associated with increased risk of PD in sporadic PD patients [151, 161]. In the Polish population there are data in EOPD indicating a similar frequency of this substitution in both the EOPD patients and in the control group [176]. It seems that the high frequency of the c.500 G>A polymorphism in the control group in this study may be due to the low age of control subjects, who may subsequently demonstrate neurological disorders in a later age. Our study indicates that the presence of the c.500 G>A substitution in the *PRKN* gene may significantly increase risk of LOPD [123].

The c.1180 G>A transition in exon 11, which is located between the IBR and RING2 domains, has been detected with a different frequency inter alia in populations of Europe, America, and Mexico, and has not been detected in the study populations of Japan [129, 175, 177, 178]. However, a significant association of this polymorphism with risk of PD has not been detected so far. Importantly, most of these studies involved FPD or SPD but with early onset and thereby the control groups contain young individuals, which may explain the high frequency of polymorphism presence in controls.

The c.930 G>C transversion is situated between the RING1 and IBR domains of Parkin and was first described in the South African population, where this substitution has been observed only in one PD patient but not in controls [179].

Similar to the above results were shown in a population of North America in the report by Pankratz *et al.* [146]. However, in the study by Bardien *et al.* [179], the c.930 G>C transversion was also observed in the patient's 51-year-old brother, who did not exhibit any signs of PD. This observation and the results of Polish studies [49, 123] suggest that the analyzed substitution in exon 8 may have incomplete penetration or lead to preclinical changes and increase the risk of PD in conjunction with other genetic or environmental factors.

On the other hand, the c.823 C>T mutation located in exon 7 was detected in our study only in PD and not in controls [49, 123]. Current research shows that the frequency of the c.823 C>T mutation is low. Results of our study, as well as the study by Sinha [151] who detected the c.823 C>T substitution in 0.2% of the PD group but none in the control group, suggests that this substitution may be characteristic for PD and demonstrate high penetration. It is known that the c.823 C>T mutation is located within the RING1 domain. The carboxyterminal half of Parkin comprises a specific arrangement of three zinc-finger domains: two RING fingers flank a domain known as the in-between RING (IBR) domain. The RING–IBR–RING domain binds to specific coenzymes and substrates [116]. Previous studies have shown that missense mutations within RING domains retain ubiquitin ligase activity [180] and confer a toxic gain of function, leading to Parkin protein aggregation [181].

Moreover, in two patients (and no controls) in the analyzed Polish population, the novel mutation c.520 C>T was detected [49, 123]. To date, a silent mutation in codon 174, namely c.522 C>T, has been detected and appears to not be pathogenic in nature [178]. The c.520 C>T substitution results in the amino acid change L174F. Although localization of this substitution in the UPD of Parkin, not in the RING-IBR-RING region, suggests that this mutation may not significantly impair the activity of Parkin, the c.520 C>T transition may be one of the factors increasing PD risk; however, this hypothesis should be confirmed in future studies.

Thus our study seems to confirm the important role of the heterozygous mutation of the *PRKN* gene in the modulation of PD risk. Although the role of the heterozygous *PRKN* mutation is not fully understood at present, it is believed that *PRKN* gene polymorphisms may be involved in the pathogenesis of SPD [3, 47] in several ways, including by altering the solubility of Parkin and leading to its aggregation (for example due to mutation R275W) [64] or by the reduction of the enzymatic activity of Parkin, as is the case with the amino acid substitution induced by the C924T mutation of *PRKN* and the accumulation of inefficiently ubiquitinated proteins that form aggregates (but often not taking the form of typical LB). West *et al.* was demonstrated that the variant of *PRKN* promoter associated to a lower expression of Parkin was significantly more frequent in patients with PD in comparison with the control group, indicating an important role of this variant in reducing the expression of Parkin in the pathogenesis of PD [38]. It also appears that the reduced efficiency of Parkin that also occurs in the case of heterozy-

gous mutation of the *PRKN* gene may lead to an increased risk of PD manifestation. Moreover, it seems that mutations affecting the splicing process may also significantly alter the structure and function of the Parkin protein. It has been shown that some splice donor variants of the *PRKN* gene may affect splicing [182] to produce an in-frame truncated transcript, while others may lead to exon deletions and a frame shift resulting in an alteration in Parkin function. It is also known that Parkin and PINK1 together may be involved in the same pathway upstream of the mitochondrial fission/fusion machinery and mutations in both have been shown to result in an increase of mitochondrial fission in mammalian cells [183, 184]. It has been suggested that point mutations in *PRKN* might also impair this function and in the process of induction of mitophagy by Parkin, resulting in increased cellular toxicity [97, 185]. Alternatively, some mutations of Parkin compromise its function by destabilizing the protein and accelerating its degradation via the proteasome [186].

CO-EXISTENCE OF MORE THAN ONE MUTATION IN THE *PRKN* GENE

It has been suggested that haploinsufficiency may be considered as a reduction of normal gene expression accompanied by a loss of normal protein activity. Moreover, many reports seem to point to the existence of a second, undetected mutation in these patients, perhaps in the promoter or intronic regions [173].

In our study in the Polish population, more than one mutation in the *PRKN* gene was detected in 5% PD patients: 4 patients had two substitutions and one had tree substitutions, while all control subjects who had substitution in *PRKN* had only one mutation [49, 123]. These results suggest that the presence of more than one heterozygous mutation in the *PRKN* gene may be required for PD manifestation. This hypothesis was first proposed by Abbas *et al.* [129], and subsequent reviews generally assume the existence of a second, undetected mutation [173]. Our study also indicates a probability that the patient who had one mutation in *PRKN* may have more genetic changes that are in a region of the gene that we did not test, so extension of studies to other regions of the *PRKN* gene is necessary to clarify this issue.

It should be noted that the concurrence of more than one *PRKN* mutation involved both point mutations and exon deletions/duplications. Some of the reported cases of concurrent mutations of *PRKN* are presented in (Table **3**).

CLINICAL FEATURES IN PD PATIENTS WITH *PRKN* MUTATIONS

In PD patients with a *PRKN* substitution, a slower progression of the disease has generally been observed when compared with PD patients without a mutation. It has also been observed that PD patients with *PRKN* mutations have a better response to L-dopa therapy than in PD patients without substitutions. This observation is generally consistent with the typical descriptions of *PRKN* patients which present with slow disease progression [32, 129] and a good response to L-dopa treatment; although it has been shown that patients with a *PRKN* mutation were more likely to develop treatment-induced motor complications earlier in the treatment of PD [32, 170]. Similar observations have been reported by Lohman *et al.*, who analyzed the phenotype of *PRKN* mutation carriers. The authors indicated that the course of the disease was similar to SPD but with earlier and more symmetric onset, dystonia and hyperreflexia as the initial sign, a relatively benign disease course with slower disease progression, sleep benefit, and better response to low doses of L-dopa but these were complicated with early motor fluctuations and development of dyskinesia [187].

Table 3. Examples of Co-existence of *PRKN* Mutations

Mutation 1	Mutation 2	Reference
Del ex 3	**Del ex 7**	[134]
Del ex 3	**Del ex 4**	[134]
Del ex 3	**Del ex 2-3**	[134]
Del ex 2	**Del ex 5**	[136]
Del ex 5	**Dup ex 3**	[155]
Del ex 3-4	**Trip ex 2**	[32]
Del ex 3	**K211N**	[134]
Del ex 3	**G430D**	[134]
Del ex 4	**C431R**	[138]
Del ex 7	**A82E**	[136]
R256C	**R275W**	[129]
D280N	**R334C**	[32]
K161N	**202-203delAG**	[134]
R275W	**438-477del**	[134]
255delA	**Del ex 3**	[134]
255delA	**Del ex 5**	[134]
1072delT	**Del ex 7**	[136]

However, it is believed that there are no specific clinical features that distinguish patients with *PRKN* mutations from other EOPD forms [188]. In the study by Lohmann *et al.*, the authors suggested that *PRKN* mutation carriers are clinically indistinguishable from other EOPD patients except for a lower L-dopaequivalent dose (LED) and later development of L-dopa-related motor complications [187].

Interestingly, rare atypical presentations have also been described, and a wide variability in onset age and phenotype might be observed even within the same family (variation of up to 20 years in the age of onset has been observed) [32, 188], indicating that there are strong modulating factors, either genetic or environmental.

Early cognitive impairment is rare in PD patients with *PRKN* mutations, and moderate cognitive deficits have only been reported for 2 patients with disease duration over 20 years [189].

Moreover, in our study the presence of a mutation in the *PRKN* gene was not associated with dementia or depression

in PD patients, which is consistent with previous reports [49, 123, 190]. Interestingly, there also appears to be no correlation of certain types of mutations with distinctive clinical features. This suggests that substitutions of amino acids resulting from missense mutations are as detrimental to Parkin function as are truncation and deletion mutations.

GENETIC FACTORS AND INTERACTIONS PARKIN-ALPHA-SYNUCLEIN

Investigation of clinical features in patients with digenic combinations of heterozygous mutations might provide us with more insight into the effects of these variants. It is known that ASN is one of the substrates for Parkin and interacts with the UBL of Parkin [101]. It has also been demonstrated, that mutated Parkin may lead to the formation of ASN deposits by undermining ASN degradation (no capacity of glycosylated ASN to bind Parkin) [102].

In our study we showed for the first time that the concurrence of *PRKN* heterozygous substitution and variants +2/+2 and +2/+3 of NACP-Rep1 region of the *SNCA* gene promoter occur in PD patients, while genotypes +2/+2 and +2/+3 have not been detected in controls with a mutation in *PRKN* [49, 123]. It is known that one of the mechanisms by which the recessive loss of Parkin could eliminate dopaminergic neurons would involve some neurotoxic substrate proteins, which would accumulate when there is insufficient Parkin for its ubiquitin-proteasome system-dependent degradation [66]. Therefore, the results of our study may suggest that even heterozygous substitution in the *PRKN* gene in the presence of the variants +2/+2 or +2/+3 of NACP-Rep1 in the *SNCA* promoter may increase the risk of PD manifestation, probably due to ineffective elimination of over-expressed ASN by mutated Parkin.

CONCLUSION

Despite numerous debates and controversies in the literature on the role of *SNCA* and *PRKN* gene mutations in the pathogenesis of PD, it is evident that these genes play a key role in maintaining DA neuronal homeostasis, and that the dysfunction of DA neuronal homeostasis is relevant both to FPD and SPD with different onset. During recent years, the importance of ASN in the process of neurodegeneration and the neuroprotective function of Parkin have become better understood. Recently, there have been an increasing number of reports indicating the importance of the interaction between these proteins and their encoding genes. It currently appears that the path described so far in the pathogenesis of PD and the identified *SNCA* and *PRKN* gene mutations increasingly allow for the improvement of the diagnostic and prognostic process in the course of PD. However, only a full knowledge of the mechanisms in which ASN and Parkin are involved and the dispelling of all doubt about the presence of specific variants of the *SNCA* and *PRKN* genes may allow us in the future to develop and implement a rapid and certain diagnosis and more effective pharmacotherapy.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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