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Polymorphism of the *COMT*, *MAO*, *DAT*, *NET* and *5-HTT* Genes, and Biogenic Amines in Parkinson's Disease

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Abstract: Epinephrine (E) and sympathetic nerve stimulation were described by Thomas Renton Elliott in 1905 for the first time. Dopamine (DA), norepinephrine (NE), E, and serotonin (5-HT) belong to the classic biogenic amines (or monoamines). Parkinson's disease (PD) is among the diseases in which it has been established that catecholamines may account for the neurodegeneration of central and peripheral catecholamine neural systems. PD is a chronic and progressive neurological disorder characterized by resting tremor, rigidity, and bradykinesia, affecting 2% of individuals above the age of 65 years. This disorder is a result of degeneration of DA-producing neurons of the *substantia nigra* and a significant loss of noradrenergic neurons in the *locus coeruleus*. In PD and other related neurodegerative diseases, catecholamines play the role of endogenous neurotoxins. Catechol-O-methyltransferase (COMT) and/or monoamine oxidase (MAO) catalyze the metabolism of monoamines. However, the monoamine transporters for DA, NE, and 5-HT namely DAT, NET, and SERT, respectively regulate the monoamine concentration. The metabolism of catecholamines and 5-HT involves common factors. Monoamine transporters represent targets for many pharmacological agents that affect brain function, including psychostimulators and antidepressants. In PD, polymorphisms of the *COMT*, *MAO*, *DAT, NET,* and *5-HTT* genes may change the levels of biogenic amines and their metabolic products. The currently available therapies for PD improve the symptoms but do not halt the progression of the disease. The most effective treatment for PD patients is therapy with L-dopa. Combined therapy for PD involves a DA agonist and decarboxylase, MAOs and COMT inhibitors, and is the current optimal form of PD treatment maintaining monoamine balance.

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INTRODUCTION

Parkinson's disease (PD) is a chronic and progressive neurological disorder characterized by resting tremor, rigidity, and bradykinesia, affecting 2% of individuals above the age of 65 years. PD is a result of degeneration of the dopamine-producing neurons of the *substantia nigra* (SN) [1] and a significant loss of noradrenergic neurons in the *locus coeruleus* (LC) [2]. To classical biogenic amines (or monoamines) may be included: dopamine (DA), norepinephrine (NE), and epinenephrine (E), as well as serotonin (5-HT) [3]. Experimental findings using animal models of PD suggest that NE may protect DA neurons from damage.

The catecholamines, DA, NE, and E belong to a class of chemical neurotransmitters and hormones, and regulate physiological processes as well as leading to the development of neurological, psychiatric, and cardiovascular diseases [4]. In the disease processes in which catecholamines have established roles, the neurodegeneration of central and peripheral catecholamine neural systems is involved. In PD and other related neurodegerative diseases, the catecholamines play the role of endogenous neurotoxins. Mechanisms of catecholamine-induced neurotoxicity involve nonenzymatic auto-oxidation of catecholamines [5] and formation of highly reactive deaminated catecholaldehyde metabolites that may induce the progression of neurodegenerative disease [4].

Catechol-O-methyltransferase (COMT) and/or MAO (monoamine oxidase) further catalyze the metabolism of monoamines. Sympathetic nerves contain only MAO, but adrenal chromaffin cells contain both MAO and COMT. The COMT enzyme is distributed in all organs. Monoamine transporters also play a role in maintaining the proper levels of catecholamines. However, the monoamine transporters play an important role in the concentration of monoamines in storage vesicles before their release and also act as a safeguard of neurons against high toxic levels of catecholamines. Monoamine transporters for DA, NE, and 5-HT - DAT, NET, and SERT, respectively, represent targets for many pharmacological agents that affect brain function, including psychostimulants and antidepressants [4, 6, 7].

In PD, polymorphisms of the *COMT*, *MAO*, *DAT, NET,* and *5-HTT* genes may change the levels of biogenic amines and their metabolic products [8-12].

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Available therapies in PD improve the symptoms but do not halt progression of the disease. The most effective treatment for PD patients is therapy with L-3,4-dihydroxyphenylalanine (L-dopa) [13]. COMT activity is an important factor determining the response to L-dopa treatment [9, 14-16]. The most effective treatment of patients with PD seems to be combination of L-dopa with inhibitors of aromatic L-aminoacid decarboxylase (AADC), MAOs and COMT, which would effectively correct levels of the drug (L-dopa) and the duration of its action, as well as monoamine concentration.

SYNTHESIS AND METABOLISM OF BIOGENIC AMINES IN PARKINSON'S DISEASE

Naturally occurring monoamines in the central nervous system (CNS) may be divided into two distinct groups depending on their amino-acidic substrate. The amino acid tyrosine (Tyr) gives origin to catecholamines [17], whereas tryptophan (Trp) is a substrate for 5-HT biosynthesis [18].

The most significant catecholamines in the human brain are DA, NE and E. Sympathetic nerve stimulation and E were first described by Thomas Renton Elliott in a 68-page treatise published in 1905 [19]. However, almost half a century ago, Ulf von Euler, Julius Axelrod, and Sir Bernard Katz described humoral transmitters in the nerve terminals and the mechanism for their storage, release, and catecholamine inactivation [17]. DA is synthetized by dopaminergic neurons, mostly located in the SN and other areas of the brain comprising the dopaminergic system [1, 2, 20].

NE, and to small extent E, occur in various brain areas and are responsible for alertness [21], decision-making [22] and other higher brain functions [23, 24].

The metabolism of CNS monoamines takes place in several compartments. The biosynthesis of biogenic amines takes place in the cytoplasm of neurons. The synthesized monoamines are then absorbed and stored inside specialized vesicles. The vesicles packed with monoamines are transported toward a synaptic knob, awaiting a stimulus. The proper action potential, reaching the trigger level, induces Ca^{2+} dependent movement of monoamine vesicles toward the presynaptic membrane, which induces exocytosis [25]. This process is followed by a release of the neurotransmitter into the synaptic cleft, where a portion of the molecules attaches to the proper receptors and triggers an action potential on the postsynaptic membrane, propagating the stimulus along the next neuron. Subsequently, several neurotransmitter molecules dissociate from receptors, and sideways with unbound neurotransmitters present in the synaptic cleft to undergo reuptake or enzymatic breakdown [4].

The catecholamines are synthesized by a sequential reaction (Fig. **1**), where the first step is tyrosine hydroxylation by a cytosolic enzyme, tyrosine hydroxylase (TH). The TH enzyme utilizes tetrahydrobiopterin (THBT) as a cofactor and molecular oxygen as a substrate for hydroxyl group formation. The product of this stage is L-dopa. The second step of catecholamine biosynthesis is decarboxylation of L-dopa to DA. This reaction requires the presence of pyridoxal phosphate (active form of vitamin B6), and is catalyzed by the enzyme AADC (also described as L-dopa decarboxylase or 5-HTP decarboxylase) [4, 17]. DA is a substrate for the next reaction – β-hydroxylation (performed by an enzyme dopamine β-hydroxylase, DBH) – yielding NE. This reaction requires the presence of ascorbic acid – vitamin C. NE is then utilized as a substrate for the further step – the formation of E, which is catalyzed by the enzyme phenylmethanolamine N-methyltransferase. This reaction requires S-adenosylmethionine (SAM) as a co-substrate to provide methyl groups [26].

Like DA, the biosynthesis of 5-HT occurs with the participation of coupled reactions, but with Trp as the primary amino acid substrate. The first reaction is hydroxylation of Trp, yielding 5-hydroxytryptophane (5-HTP), with THBT as a cofactor. This reaction is catalyzed by the enzyme tryptophan hydroxylase (TPH). The next step is decarboxylation of 5-HTP to 5-hydroxytryptamine (5-HT), and is catalyzed by AADC.

The monoamines that are synthesized in the cytoplasm are transported into the monoamine vesicles by specialized enzymes. The process is performed by vesicular monoamine transporters (VMATs). There are two isoforms of the VMAT transporter described in literature: VMAT1, which is mostly localized in the neuroendocrine cells, and VMAT2, which is primarily located in the CNS [27].

VMAT2 is localized within the vesicle's membrane and its main role is to actively transport catecholamines, 5-HT and histamine into the monoamine vesicles [27, 28]. The vesicles store monoamine neurotransmitters and release them into the synaptic cleft in response to action potential.

The released monoamines are metabolized by several enzymes. COMT breaks down DA, NE and E yielding 3-methoxytyramine, normetanephrine (NMETA), and mentanephrine (META), respectively (Fig. **1**). The A isoform of MAO metabolizes DA, NE [29], and 5-HT, giving 3,4-dihydroxyphenylacetaldehyde, 3,4-dihydroxymandelic acid, and 5-hydroxyindoleacetic acid, respectively. There are also literature data indicating that xanthine oxidase may participate in catecholamine metabolism [30].

POLYMORPHISMS OF THE *COMT* GENE AND METABOLISMS OF BIOGENIC AMINES IN PARKINSON'S DISEASE

It is known that metabolic O-methylation of endogenous catecholamines and other catechols are catalyzed by COMT. A single *COMT* gene is located on the long arm of chromosome 22q11 and contains six exons [31, 32]. Most studies of variations in the *COMT* gene refer to missense mutation of this gene in exon 4 (substitution CGTG to CATG).

Single nucleotide polymorphisms (SNPs) at nucleotide 1947 in the *COMT* gene encode both the acid-soluble (S-COMT), and membrane-bound (MB-COMT) forms of this enzyme [33]. Moreover, there are three major genopytes of the *COMT* gene formed by four SNPs: one located in the *S-COMT* promoter region (A>G; rs6269), in the boundary region of intron 3rd of the *MB-COMT* gene, and three in the *S-COMT* and *MB-COMT* coding region at codons: two synonymous changes His62His (C>T; rs4633), Leu136Leu (C>G; rs4818), and one nonsynonymous alteration Val158 Met (A>G; rs4680) [34].

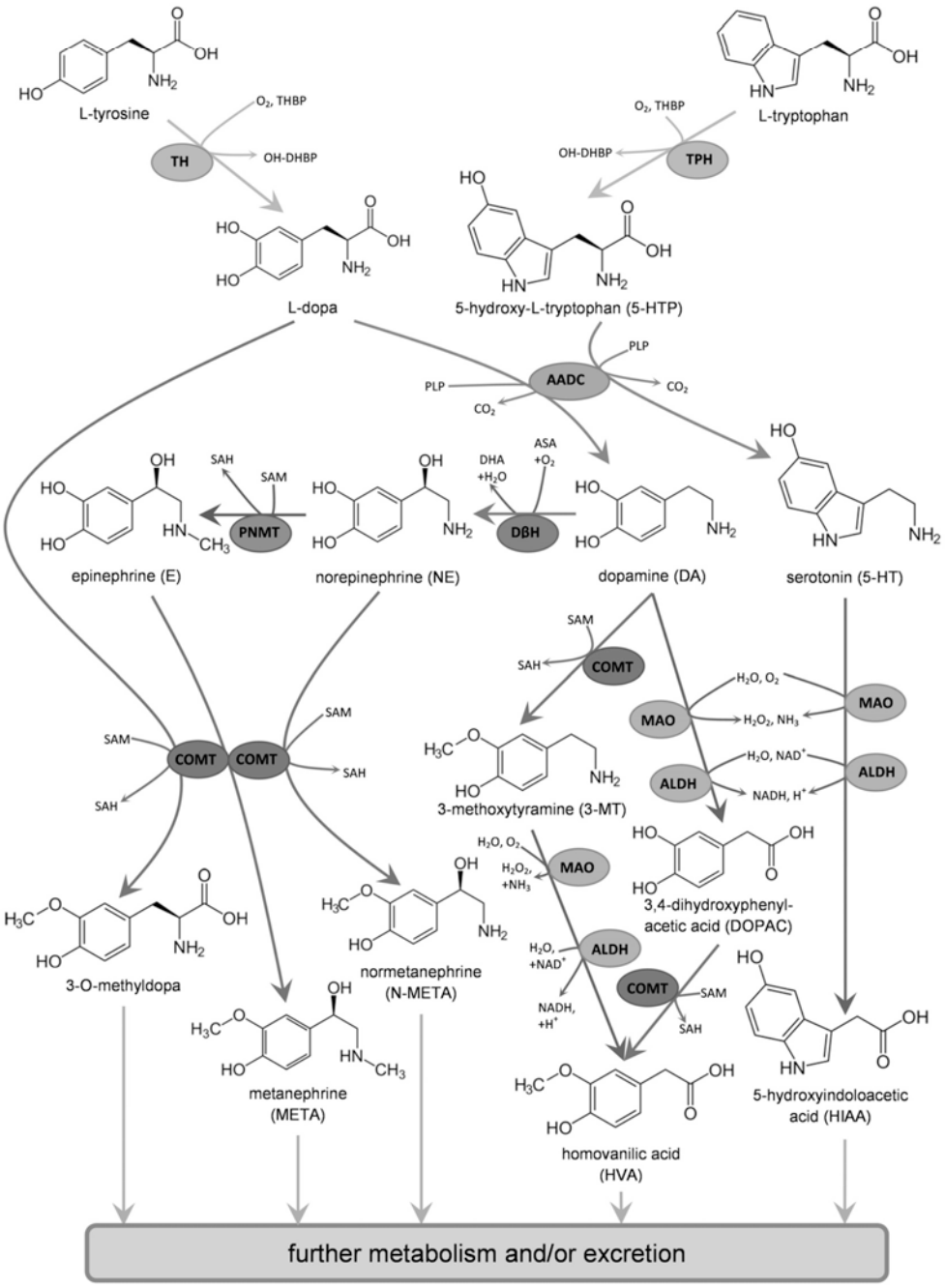

Fig. (1). The biosynthesis and metabolism of chosen biogenic amines. The amino acidic substrates are shown at the top of the figure, with the intermediates below them. The following row shows selected neurotransmitters and their corresponding metabolites below them. The enzymes are shown in ellipses. AADC –aromatic L-amino acid decarboxylase, ASA – ascorbic acid (vitamin C), ALDH – aldehyde dehydrogenase, COMT – catechol-O-methyltransferase, DβH – dopamine β-hydroxylase, DHB – dehydroascorbic acid, MAO – monoamine oxidase, PLP – pirydoxal phosphate (active form of vitamin B6), PNMT – phenylethanolamine N-methyltransferase, TH – tyrosine hydroxylase, TPH – tryptophan hydroxylase, SAM – S-adenosylmethionine, SAH – S-adenosylhomocysteine, THBP – tetrahydrobiopterin, OH-DHBP – hydroxydihydrobiopterin.

It has been shown that a common SNP in codon 158, a substitution of valine (Val) by methionine (Met), is associated with pain rating and μ-opioid system responses [35], cognition and common affective disorders [36-38]. Moreover, genotypes divergent from synonymous SNPs exhibited the largest difference in COMT enzymatic activity, owing to a reduced amount of translated protein. The major *COMT* genotype varies with respect to mRNA local stem-loop structures, determining the most stable structure and lower protein levels and enzymatic activity [34]. The COMT enzymatic activity in women is 20-30% lower than in men; it should be stated that in women, estrogens downregulate *COMT* gene transcription and may increase the risk of PD [39].

In the *COMT* gene, a substitution of Val for Met (Table **1**) has been shown in two *loci* at codon 158 (Val158Met) and codon 108 (Val108Met) [40, 41].

Table 1. Polymorphism of *COMT*, *MAO*, *DAT, NET* and *5-HHT* Genes in PD

Gene	Polymorphism	Population	Number of Patients	References
COMT	G>A (Val108Met)	Chinese cohort	PD patients n=70	Xie *et al.*, 1997 [40]
COMT	675A>G (Val158Met) rs4680	Finnish cohort	PD patients n=158	Syvanen *et al.*, 1997 [32]
COMT	G>A (Val108Met)	Japanese cohort	PD patients n=176	Yoritaka *et al.*, 1997 [41]
COMT	G>A (Val108Met)	Japanese cohort	PD patients n=109	Kunugi *et al.*, 1997 [42]
COMT	675A>G (Val158Met) rs4680	Canadian cohort	PD patients n=24	Chong *et al.*, 2000 [52]
COMT	675A>G (Val158Met) rs4680	Korean cohort	PD patients n=73	Lee *et al.*, 2001 [9]
COMT	675A>G (Val158Met) rs4680	Chinese cohort	PD patients n=39	Tan *et al.*, 2005 [53]
COMT	675A>G (Val158Met) rs4680 promoter region A>G rs6269 C>T (His62His) rs4633 C>G (Leu136Leu) rs4818	Polish cohort	PD patients n=39 n=322 n=248	Białecka *et al.*, 2005 [43] Białecka *et al.*, 2008 [45] Białecka *et al.*, 2012 [54]
COMT	649G>A (Val158Met) rs4680	Polish cohort	PD patients n=30 n=49	Bugaj *et al.*, 2011 [12] our unpublished data
COMT	675A>G (Val158Met) rs4680	Japanese cohort	PD patients n=240	Kiyohara *et al.*, 2011 [46]
MAO-A	DNRP (intron 2) (exon 14)	Caucasian cohort	PD patients n=91	Hotamisligil *et al.*, 1994 [55]
MAO-A	DNRP (intron 2)	Japanese cohort	PD patients n=71	Nanko *et al.*, 1996 [56]
MAO-A	(intron 1)	Caucasian cohort	PD n=78	Plante-Bordeneuve *et al.*, 1997 [57]
MAO-A	*Eco*RV	Caucasian cohort	PD n=145	Costa-Mallen *et al.*, 2000 [58]

(Table 1) contd….

Gene	Polymorphism	Population	Number of Patients	References
MAO-A	1460C>T (exon 14) rs1137070	Polish cohort	PD patients n=30 n=49	Bugaj *et al.*, 2011 [12] our unpublished data
MAO-B	G>A (intron 13)	Caucasian cohort	PD patients n=46	Kurth *et al.*, 1993 [59]
MAO-B	DNRP (intron 2)	Caucasian cohort	PD patients n=91	Hotamisligil *et al.*, 1994 [55]
MAO-B	G>A (intron 13)	Asian cohort	PD patients n=54	Morimoto *et al.*, 1995 [60]
MAO-B	G>A (intron 13)	Caucasian cohort	PD patients SPD n=112 FPD n=12	Ho *et al.*, 1995 [61]
MAO-B	DNRP (intron 2)	Japanese cohort	PD patients n=71	Nanko *et al.*, 1996 [56]
MAO-B	G>A (intron 13)	Caucasian cohort	PD patients n=62	Costa *et al.*, 1997 [62]
MAO-B	G>A (intron 13)	Caucasian cohort	PD patients n=204	Mellick *et al.*, 1999 [63]
MAO-B	G>A (intron 13)	Caucasian cohort	PD patients n=214	Hernan *et al.*, 2002 [64]
MAO-B	G>A (intron 13)	Polish cohort	PD patients n=210	Bialecka *et al.*, 2005 [43]
MAO-B	G>A (intron 13)	Indian cohort	PD patients n=70	Singh *et al.*, 2008 [65]
DAT	1215A>G	Japanese cohort	PD patients n=172	Morino *et al.*, 2000 [66]
DAT	1215A>G	Japanese cohort	PD patients n=204	Kimura *et al.*, 2001[67]
DAT	1215A>G	Asian cohort	PD patients n=102	Lin *et al.*, 2002 [10]
DAT	1215A>G	Indian cohort	PD patients n=70	Singh *et al.*, 2008 [65]
NET	1287G>A (exon 9) rs5569	Polish cohort	PD patients n=30 n=49	Bugaj *et al.*, 2011 [12] our unpublished data
5-HTT	5-HTTLPR rs25531	Caucasian cohort	PD patients n=393	Albani *et al.*, 2009 [68]
5-HTT	5-HTTLPR rs25531	Asian cohort	PD patients n=306	Zhang *et al.*, 2009 [69]
5-HTT	5-HTTLPR	Asian cohort	PD patients n=503	Lee *et al.*, 2011 [70]
5-HTT	5-HTTLPR	Scandinavian cohort	PD patients n=16	Guzey *et al.*, 2012 [71]

n- number of Parkinson's disease patients, PD = Parkinson's disease.

The MB-COMT form is usually present in cerebral nerve cells, while the cells expressing the S-COMT form are found in the liver, blood and kidney. Moreover, when nucleotide 1947 in the *COMT* gene is guanine (G), it codes for Val and a high activity of thermostable $COMT^{H}$ is formed, whereas if this nucleotide is adenine (A), it codes for Met and a low activity of thermolabile $COMT^{L}$ is produced [33]. It is known that the homozygous variant Met (allele $COMT^{L}$) is 3-4 times less active and metabolizes DA significantly slower than homozygous variant Val ($COMT^{H}$ allele). The study by Kunugi *et al.* [42] showed that the homozygous $COMT^{L}$ variant is a genetic risk factor of PD in the Japanese population. Studies in Caucasian and Chinese populations did not confirm any association between the *COMT* gene polymorphism and PD [40, 41]. The study by Białecka *et al.* [43] has shown that there is a statistically lower frequency of the $COMT^{LL}$ genotype in PD in the Polish population. Moreover, the $COMT^{LL}$ genotype is common in Caucasians and Southwest Asians, with a frequency of 40-50%, which is greater than in Northeast Asians and Africans, in whom the frequency is 20-30% [41, 42, 44]. The study by Białecka *et al.* [43] has also shown that there is a higher frequency of the $COMT^{L}$ allele in the Polish population, as 56.7% of the controls carried this allele. Białecka *et al.* [45] indicated that high, not low, COMT activity is a risk factor for PD.

It is believed that the *COMT* Val158Met (A>G; rs4680) gene encodes genotypes GG, GA and AA. The distribution of *COMT* genotypes is known to differ between various ethnic populations. In a Caucasian population the distribution is 25, 50 and 25% for the GG, GA and AA genotypes, respectively [12, 32, 45]. In Japanese and Korean populations, a significantly lower level of subjects with AA genotype and A-allele frequency has been found [9, 42].

Moreover, the distribution of genotypes in Chinese, Finnish, Korean, and Polish studies were not significantly different in the frequencies of different *COMT* genotypes between PD patients and controls [12, 32, 40, 45, 46]. However, the Japanese and Korean studies reported that the AA genotype occurred more frequently in PD patients than in controls and may be a risk factor for PD [9, 41, 42].

COMT plays a role in the inactivation of many biologically active or toxic catechols and metabolizes hormones and neurotransmitters, such as L-dopa, DA, NE and E, modulating susceptibility to PD [4, 43, 47-49]. It is known that the AA genotype of *COMT* gene encodes the COMT enzyme with low activity [9].

Similarly to the studies by Bugaj *et al.* [12], our unpublished data carried out on people of Caucasian origin, 49 PD patients (aged 35-82 years) and 48 healthy subjects (aged 35-82 years) were found to have different plasma levels of the monoamines NE and E and their urine metabolites (NMETA and META, respectively) in both PD patients and controls with *COMT* GG (15%, 30%), GA (59%, 51%) and AA (26%, 19%) genotypes (c.649G>A; rs4680). Blood from subjects was collected after 5 min. in the upright position (after a minimum 30 min. supine position). Urine was collected for 24 hours. Urine was stabilized by the addition of hydrochloric acid.

In these studies, the polymorphism of the *COMT* gene was determined with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and concentrations of plasma NE, E and urine NMETA and META were estimated using the high performance liquid chromatography system with electrochemical (HPLC/EC). Our study indicated that the highest plasma level of NE in both PD patients and controls was in subjects with the *COMT* GG genotype (273.9±122.8 pg/ml and 349.5±163.0 pg/ml, respectively). Additionally, in PD patients with the *COMT* AA genotype only, the level of NE was higher than in controls with the same genotype (231.3±196.5 pg/ml and 212.3±105.7 pg/ml, respectively). In contrast to NE, the plasma concentration of E was higher in PD patients with all analyzed genotypes of the *COMT* gene, but in PD patients with the AA genotype only, the increase was statistically significant as compared to controls (Kruskal-Wallis test, $p<0.01$; 67.9±28.6 pg/ml and 37.2±22.0 pg/ml, respectively). Our study has also indicated that the highest urine concentration of the NE metabolite NMETA was in control subjects with the *COMT* GG genotype and the lowest level in the control subjects with the *COMT* AA genotype (915.9±766.6 μg/24 hours and 349.6±267.4 μg/24 hours, respectively). In PD patients with all analyzed *COMT* genotypes, the level of NMETA was much lower than in controls but the difference was not significant. However, in PD patients with *COMT* GG, GA and AA genotypes, the level of the E metabolite META was much higher than in controls, and the highest in patients with the *COMT* AA genotype (Kruskal-Wallis test, $p<0.001$; 1380.2±2031.7 μg/24 hours in PD patients and 12.0±21.1 μg/24 hours in controls).

It seems that the reduced activity of the *COMT* AA genotype in PD patients has a stronger impact on the final step of catecholamine biosynthesis (NE to E) and E metabolite levels (META).

The study by Farrell *et al.* [50] showed that the *COMT* Val158Met genotype in PD may affect the cognitive, behavioral, and perhaps effective responses to drug therapy and might also influence their toxicity [51]. The basic strategy and most effective treatment for PD patients is therapy with L-dopa [13]. The COMT enzyme is involved in peripheral L-dopa metabolism to 3-O-methyldopa (3-OMD) and of DA to 3-methoxytyramine.

The studies by Reilly *et al.* [14] and Rivera-Calimlim *et al.* [15] suggested that COMT activity is an important factor determining the response to L-dopa treatment. The study by Reilly *et al.* [14], seems to be less objective as they measured the subjective improvement reported by patients after L-dopa therapy. Lee *et al.* [9] analyzed motor response to L-dopa therapy using the Unified Parkinson's Disease Rating Scale (UPDRS) and showed that COMT had a significant role in determining the response to L-dopa. In this study, PD patients with the *COMT* AA genotype coding for the low activity of COMT show a larger magnitude of response to L-dopa than those with other genotypes. Moreover, there is better clinical response to lower L-dopa doses used by PD patients with low and medium activity *COMT* genotypes. These results are most likely related to a slower catabolism of L-dopa, more stable serum and CNS drug concentrations, as well as a lower level of 3-OMD. However, the *COMT* genotype seems to be a minor factor in judging the beneficial effect of COMT inhibitor administration [16].

POLYMORPHISMS OF *MAO* GENES AND METABOLISM OF BIOGENIC AMINES IN PARKINSON'S DISEASE

The functional role of MAO is to control the intracellular redox state in neurons and other cells. Under physiological conditions, the redox potential is kept by antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. However, disturbances in monoamine metabolism and increased concentrations of ammonia and other metabolites, and may lead to a decrease in the activity of MAOs and increase the production of reactive oxygen species (ROS) [72]. ROS may damage neurons and glia in the CNS. Thus, it seems that disturbances of MAO activity may lead to the progression of neurodegenerative diseases, such as PD and dementias [73].

MAOs are divided into two isoenzymes, MAO-A and MAO-B. The two isoforms differ in substantial structural overlap, substrate preference, inhibitor selectivity, anatomical distribution, and their role in behavioral regulation [74]. It has been shown that MAO-A has a very high affinity for 5-HT (120-fold higher than MAO-B) and a lower affinity for NE. In contrast to MAO-A, MAO-B preferred e.g. 2-phenylethylamine (PEA) and it substrates. However, the degradation of DA, tryptamine and tyramine is mediated by both MAOs, but there is a difference in terms of the kind of animal species and tissue localization, with MAO-B preferentially acting in DA metabolism in humans [75].

In 1988, Bach *et al.* [76] demonstrated that MAO-A and MAO-B are encoded by the *MAO-A* and *MAO-B* genes, respectively. Both these genes have been described as containing fifteen exons and fourteen introns [77], and are located on the short arm of the human X chromosome (Xp11.23). Moreover, it has been shown that MAO-A and MAO-B consist of 527 and 520 amino acids, respectively, with molecular weights of 59.7 and 58.8 kDa, respectively [78]. The two genes share about 70% sequence identity and an identical intron-exon organization. However, exon 12 of the *MAO* genes encodes the covalent FAD-binding-site and is the most conserved exon. The peptide sequence of MAO-A and MAO-B shares 93.9% amino acid identity [77]. It has been shown that human MAO-A and MAO-B are both dimeric [79].

Both isoenzymes are expressed in most tissues (MAO-A e.g. in fibroblasts, MAO-B e.g. in platelets and lymphocytes) and in most brain regions, but certain areas display only one of the two enzymes. In the brain, MAO-A is found mainly in dopaminergic and norepinergic neurons. Conversely to MAO-A, MAO-B is present only in the cell bodies of serotonergic neurons. Localization of MAO-B in serotonergic neurons remains unclear because 5-HT is mainly metabolized by MAO-A [74, 80]. It seems that this localization of MAO-B in serotonergic neurons may serve a protective role for 5-HT [74].

Moreover, the antidepressant properties of MAO inhibitors are known to be mainly due to the inactivation of MAO-A and increased 5-HT concentration [81]. It has been shown that selective MAO-A deficiency is associated with behavioral syndrome and a point mutation in exon 8 of the *MAO-A* gene, resulting in the substitution of glutamine codon (CAG) with a stop codon (TAG) at position 296 of the amino acid sequence, which has been associated with regulation of impulsive aggression [82, 83]. Four polymorphisms have been described in the *MAO* genes in psychiatric disorders: 1) *MAO-A (CA)*n, a dinucleotide repeat polymorphisms in intron 2 [84], 2) a 23 bp variable-number tandem repeat (VNTR) near exon 1 [85], 3) *Fnu*4HI and *Eco*RV, two PCR-RFLPs [86], and 4) *MAO-A-u*VNTR (variable number of tandem repeats), a 30 bp VNTR polymorphism located 1.2 kb upstream of the MAO-A transcription initiation site [87]. It has been shown that variations of the first three described polymorphisms in the *MAO-A* gene are associated with high susceptibility to several mental conditions, including bipolar disorder [88]. However, the *MAO-A-u*VNTR promoter polymorphism might be associated with a high prevalence of panic disorder and major depression (in females) [89].

The role of the *MAO-A* gene polymorphism in the pathogenesis of PD is not clear. The results of association studies between the *MAO-A* gene and this disease are divergent (Table **1**). Hotamisligil *et al.* [90] have shown that the *MAO-A Eco*RV and *Msp*I polymorphisms were three times more frequent in PD than in controls. Costa-Mallen *et al.* [58] showed that the *MAO-A Eco*RV polymorphism was not significantly associated with PD. Moreover, the *MAO-A* gene polymorphism in intron 1 in both Japanese [56] and Caucasian [57] populations was also not significantly associated with PD. However, the study by Parsian *et al.* [11] demonstrated that *MAO-A* gene polymorphisms were strongly associated only with total, familial (FPD) and sporadic (SPD) PD. There were no significant differences between FPD and SPD.

The literature data indicate an association between mutations in exon 14 of *MAO-A* (c.1460C>T; rs1137070) and attention-deficit/hyperactivity disorder (ADHD) and mental diseases [91]. Similarly to Bugaj *et al.* [12], our unpublished data on 49 PD Polish patients (see above) have shown that the *MAO-A* CC and CT (c.1460C>T; rs1137070) genotypes occur with the same frequency (44%) with each other, and at double the frequency of the rare genotype TT (12%).

The *MAO-B* gene polymorphisms in PD are summarized in (Table **1**). Both polymorphisms in intron 13 of the *MAO-B* gene as well as in exon 14 have been reported to be associated with an increased risk of PD in Caucasians, while no correlation was found in Asian population [43, 59, 60, 62]. In the Indian population, a strong correlation between the *MAO-B* G variant (intron 13A/G) and PD was demonstrated [65].

Hotamisligil *et al.* [90] have shown that *MAO-A* polymorphism regulates gene expression and increases enzyme activity and ROS generation. MAOs are involved in the neurodegenerative process in PD through production of ROS and oxidative deamination of DA [92].

The oxidative deamination of catecholamines was described originally by Derek Richter in the mid-1930s. The study by Richter [29] described the first step of oxidation, involving the MAO enzyme, resulting in the formation of

deaminated aldehydes from DA, 3,4-dihydroxyphenylacetaldehyde (DOPAL), and NE, E, 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL).

MAOs, or oxygen oxidoreductases (deaminating), are mitochondrial-bound proteins catalyzing the oxidative deamination of key brain neurotransmitters, such as DA, NE, E, 5-HT, as well as a number of trace amines, such as tyramine and tryptamine [74]. MAOs exhibit their action with the participation of flavin adenine dinucleotide (FAD) as a cofactor, and lead to the formation of toxic aldehydes and ammonium from amines. It is known that aldehyde metabolites of DA, NE, and E deaminated by MAO undergo further metabolism by other enzymes e.g. COMT [4]. However, the main metabolic pathway of 5-HT consists of the conversion of this monoamine into 5-hydroxyindoloacetic acid (5-HIAA) by MAO and aldehyde dehydrogenase (ALDH) [18] (Fig. **1**).

Similarly to studies by Bugaj *et al.* [12], our unpublished data carried out on 49 PD patients and 48 healthy subjects (as described previously) have shown a different level of the monoamines NE, E, and 5-HT as well as NE and E metabolites (NMETA, META) in both PD patients and controls with the *MAO-A* CC, CT and TT genotypes (c.1460C>T; rs1137070). In this study, a polymorphism of the *MAO-A* gene was determined with the PCR-RFLP method and concentrations of plasma NE, E, 5-HT and urine NMETA, META were estimated using the HPLC/EC technique. Our study indicated that the highest plasma level of NE was observed in *MAO-A* CT heterozygote PD patients (229.9±149.7 pg/ml) and in control subjects with the common *MAO-A* CC genotype (289.8±172.0 pg/ml). Additionally, both PD patients and controls with the mutated *MAO-A* TT genotype have the lowest level of NE (141.6±67.6 pg/ml and 198.1±77.4 pg/ml, respectively). In contrast to NE, the plasma concentrations of E and 5-HT were higher in PD patients with all analyzed genotypes of the *MAO-A* gene as compared to controls (the lowest level in all subjects was found in PD and controls with the mutated *MAO-A* TT genotype) but were not statistically significant. Our study has also found that the highest urine concentration of the NE metabolite NMETA was in control subjects with the *MAO-A* CT genotype and the lowest level in the control subjects with the *MAO-A* TT genotype (637.4±405.7 μg/24 hours and 101.5±104.8 μg/24 hours, respectively). In PD patients with the *MAO-A* CC and CT genotypes, the level of NMETA was significantly lower than in controls (Kruskal-Wallis test, $p<0.05$; *MAO-A* CC, PD, 66.9±104.9 μg/24 hours, controls 501.2±565.1 μg/24 hours and Kruskal-Wallis test, $p<0.001$; *MAO-A* CT, PD, 147.8±308.6 μg/24 hours and controls, 637.4±405.7 μg/24 hours) and only with *MAO-A* TT was it higher than in controls (349.1±730.5 μg/24 hours), but not significantly. However, in PD patients with the *MAO-A* CC, CT and TT genotypes, the level of the E metabolite META was much higher than in controls and was statistically significant in patients with the *MAO-A* CC genotype (Kruskal-Wallis test, $p<0.001$; 1054.3±1085.3 μg/24 hours in PD patients and 10.5±24.5 μg/24 hours in controls) and in patients with the *MAO-A* CT genotype (Kruskal-Wallis test, $p<0.001$; 767.4±1503.1 μg/24 hours in PD patients and 7.8±16.2 μg/24 hours in controls).

It seems that in PD, the *MAO-A* CT genotype is associated with high plasma levels of monoamines (NE, E, 5-HT) and one of the highest levels of their metabolites (NMETA, META). It also seems that it would be reasonable to use MAO-A inactivation for its antidepressant effects (by increase in monoamine levels) as an effective strategy in the therapy of PD patients, especially for those with the mutated *MAO-A* TT genotype and with depressive symptoms.

MONOAMINE TRANSPORTER POLYMORPHISM AND BIOGENIC AMINES IN PARKINSON'S DISEASE

Monoamine transporters of neurotransmitters such as DA, NE and 5-HT, namely dopamine transporter (DAT), norepinephrine transporter (NET) and serotonin transporter (SERT), respectively, play key roles in controlling monoamine levels and modulating monoamine reuptake. These transporters also participate in monoamine synthesis in neurons, their transport, and the maintenance of monoamine homeostasis. Monoamine transporters also have an affinity for molecules other than monoamines, such as amphetamines and neurotoxins, e.g. DAT may transport 1-methyl-4-phenyl-1,2,3,6-terahydropyridine (MPTP) [93]. Moreover, monoamine transporters dysfunction may lead to neurotransmitter imbalance, which accompanies the progression of many disorders, such as depression, ADHD, schizophrenia and drug addiction [94, 95].

DAT plays a role in the reinforcing and behavioral stimulant effects in animals and humans, and regulates dopaminergic transmission involved in locomotion, cognition, emotion, and reward. Deficit in the dopaminergic system may lead to the development of neurological and psychiatric disorders, such as depression, ADHD and PD [95].

The gene for DAT, known as *DAT1*, is located on chromosome 5p15. The protein encoding region of the gene is over 64 kb long and comprises 15 exons [96]. The study by Singh *et al.* [65] on the polymorphism of the *DAT* (1215A>G) gene demonstrated that there is no significant association of the specified polymorphism of the *DAT* gene with PD, similarly to the reports by Kimura *et al.* [67] and Lin *et al.* [10] (Table **1**). However, Morino *et al.* [66] showed a significantly decreased frequency of G the allele of the *DAT* (1215A>G) polymorphism in PD patients and the contribution of DAT in the pathogenesis of PD. In addition, it has been shown that polymorphisms in gene coding enzymes such as DAT, involved in the detoxication mechanism, oxidative stress, and DA regulation, may modify the risk to development of PD [65].

It is known that reduced functional activity of monoamine transporters, e.g. DAT and NET, may lead to neuronal injury and the development of PD [4]. Clinical studies of PD patients have indicated that the norepinergic system may be affected before the dopaminergic system and can have an impact on non-motor preclinical symptoms of this disease, such as REM-sleep disorder and autonomic dysfunction, dementia, and depressive symptoms [97, 98]. Moreover, the norepinergic system may protect dopaminergic neurons from damage by neurotoxins, e.g. MPTP [99]. It is known that increased NE levels reduce the neurotoxic effect of such toxins, but do not completely

protect dopaminergic neurons [100]. Research carried out *post mortem* showed that, in the LC of PD patients, there is no compensation system and there is a decrease in norepinenergic function [101].

The *NET* gene, also called *SLC6A2*, is located on human chromosome 16 locus 16q12.2. This gene is encoded by 14 exons. Based on the nucleotide and amino acid sequence, NET consists of 617 amino acids [102]. There is evidence that SNPs in the *NET* gene may be underlying factors in some disorders. Park *et al.* [103] showed the possible role of the G1287A and A3081T genotypes of *SLC6A2* in the pathophysiology of ADHD. The NET enzyme also mediates norepinergic signaling involved in emotion, neuroplasticity, memory, depression and dementia [104], which may indicate the role of *NET* as a candidate gene associated with major depression. In the Korean population, it has been shown that the polymorphism *NET* (182T>C) is associated with major depression. Another polymorphism of the *NET* gene tied to depression is a substitution of G to A at position 1287 in exon 9 (*NET* 1287G>A) [98]. Depression is common in patients with PD, and it seems that polymorphisms of the *NET* gene may be involved in the pathogenesis of this disease [105].

Bugaj *et al.* [12] and our unpublished data on 49 PD Polish patients and 48 controls (as mentioned previously) have shown that the *NET* GG and GA (c.1287G>A; rs5569) genotypes occurred with similar frequency in both groups (PD, GG, 33% and 30%; controls GA, 59% and 55%, respectively) and the mutant AA genotype was almost half as common in PD than in controls (8% and 15%, respectively). NET is the main transporter for the removal of NE from the synapse and mainly restricted to the hippocampus and cortex. Damage to the norepinenergic system significantly impairs motor function [106]. Additionally, deficiency in the norepinenergic system and disturbances in NE levels play a role in the development of PD [100].

Similarly to studies by Bugaj *et al.* [12], our unpublished data carried out on 49 PD patients and 48 healthy subjects (see above) showed different levels of the monoamines NE, E, 5-HT, as well as NE and E metabolites (NMETA, META) in both PD patients and controls with the *NET* GG, GA and AA genotypes (c.1287G>A; rs5569). In this study, a polymorphism of *NET* was determined using PCR-RFLP, and concentrations of plasma NE, E, 5-HT and urine NMETA, META were estimated using HPLC/EC. Our study indicated that the plasma level of NE was lower in PD patients with all analyzed *NET* genotypes than in controls. The highest NE level was in PD heterozygotes *NET* GA (213.0±137.1 pg/ml), and the lowest in patients with the *NET* wild-type GG genotype (190.1±89.8 pg/ml). In contrast to NE, the plasma concentrations of E and 5-HT were higher in PD patients with all analyzed *NET* genotypes as compared to controls (except in PD, E levels with the mutant *NET* AA genotype) but the differences were statistically insignificant. Our study also indicated that the urine concentration of NMETA was higher in control subjects with all analyzed *NET* genotypes. However, in PD patients, a statistically significantly lower level of NE was seen only in patients with the *NET* GA genotype (Kruskal-Wallis test, $p<0.01$; PD, 136.0±350.8 μg/24 hours and controls, 513.4±472.8 μg/24 hours, respectively). In PD patients with *NET* GG, GA and AA genotypes, the level META was much higher than in controls and the difference was statistically significant in patients with the *NET* GG genotype (Kruskal-Wallis test, $p<0.001$; 1575.4±2021.6 μg/24 hours in PD patients and 11.2±28.5 μg/24 hours in controls) and GA genotype (Kruskal-Wallis test, $p<0.001$; 561.2±722.9 μg/24 hours in PD patients and 34.6±116.2 μg/24 hours in controls).

Our studies indicate the likely impact of genetic determinants of the *NET* gene on the level of biogenic amines (however, the differences are not statistically significant) and their metabolites in PD, especially in patients with the GA genotype, where the differences reach statistical significance.

The study by Jonsson *et al.* [107] has also shown that, in 66 healthy volunteers, DNA polymorphisms of the *NET* gene were associated with monoamine metabolites in cerebrospinal fluid (CSF) 3-methoxy-4-hydroxyphenylglycol (MHPG) levels but not homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations. These data have also indicated the possibility of interactions between the 5-HT and NE systems in the brain. Thus, it seems that *5-HTT* (coding SERT) and *NET* variants may participate differentially in the regulation of the NE turnover rate under presumed steady-state conditions in the CNS.

The gene encoding the SERT protein is called solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (*SLC6A4*). In humans the *5-HTT* gene is found on chromosome 17 at locus 17q11.1–q12 [8]. The following mutations have been described in the *5-HTT* gene:

- Length variation in the serotonin-transporter-gene-linked polymorphic region (5-HTTLPR)
- rs25531 — a SNP in the 5-HTTLPR
- rs25532 — another SNP in the 5-HTTLPR
- STin2 — a VNTR in the functional intron 2
- G56A on the second exon
- I425V on the ninth exon.

Moreover, the promoter region of the *5-HTT* (or *SLC6A4*) gene contains a polymorphism with *short* and *long* repeats in the 5-HTT-linked polymorphic region (5-HTTLPR or *SERTPR*). The short variation has 14 repeats of a sequence while the long variation has 16 repeats. The short variation leads to diminished transcription of *SLC6A4*, and it has been found that this diminished transcription can partly account for anxiety-related personality traits. The 5-HTTLPR polymorphism may be subdivided further, with 14 allelic variants (14-A, 14-B, 14-C, 14-D, 15, 16-A, 16-B, 16-C, 16-D, 16-E, 16-F, 19, 20 and 22) in groups of Japanese and Caucasian people [8, 108, 109].

It is known that the *5-HTT* gene may play an important role in the onset and development of mental diseases, pain perception, depressive symptoms and PD [68-71, 110-112]. At least 40% PD patients exhibit depressive symptoms and almost 50% may score within the depressive range with involvement of disturbances in the serotonergic system [105]. Our unpublished data carried out on 49 PD patients (men-

tioned previously) has shown that 43% of PD patients exhibited lower levels of 5-HT before on the use of drugs.

The influence of variations in genes encoding SERT on the expression of these structures in the brains of patients with PD is not clear. The study of the influence of the 5HTTLPR and 5HTR2A polymorphisms of the *5-HTT* gene encoding the 5-HT transporter conducted on 16 PD patients showed a significantly decreased expression of SERT in *gyrus cingulatus* and *nucleus caudatus*. In this study we did not observe any significant associations between genetic polymorphisms and the extent of radioligand SERT binding or between the polymorphisms and a diagnosis of PD [71].

The study of the promoter SNP rs25531(A>G) of the *SLC6A4* gene conducted on 393 Caucasian PD patients has shown an association between the 5-HTTLPR polymorphism and the risk of PD. In this population, however, the rs25531 SNP and the 5-HTTLPR/rs25531 genotype were not associated with the risk of PD (Table **1**) [68].

It is known that mutations associated with the *5-HTT* gene may result in changes in 5-HT transporter function and 5-HT levels. The decrease of 5-HT in the synaptic cleft is commonly considered as a cause of depression. The reuptake of 5-HT released into the synaptic cleft is mediated by SERT. A significant reduction of 5-HT transporters has been shown in PD [71]. Many studies have focused on the relationship between the 5-HTT-linked polymorphic region (5-HTTLPR) and depression. The study by Zhang *et al.* [69] conducted on 306 PD patients has shown no evidence for an association between variants of the 5-HTTLPR and rs25531 alleles and depressive symptoms in Chinese PD patients.

However, the study by Lee *et al.* [70] has analyzed the association between the promoter region of SLC6A4 and peak-dose dyskinesias (PDSK) in 503 Korean PD patients treated with L-dopa for at least 5 years. This study showed no significant association of PDSK with any of the studied genetic variants. It seems that there might be a genetic susceptibility for the development of diphasic dyskinesia in PD patients on chronic L-dopa therapy, and the underlying pathophysiological mechanism of this predisposition might be distinct from that of PDSK.

The impact of polymorphisms of the *5-HTT* gene in the susceptibility to PD, dyskinesias and incidence of depression needs further investigation.

MONOAMINES AND PHYSIOLOGICAL FUNCTION AND L-DOPA THERAPY RESPONSE IN PARKINSON'S DISEASE

In PD, an augmented expression of α-synuclein (ASN) may intensify oxidative stress [113]. Oxidative stress and excitotoxicity play an important role in the pathogenesis of PD. Bergman *et al.* [114] have demonstrated that dopaminergic neurons in PD patients undergo oxidative damage of the compact portion of the SN, and DA levels decrease in the *putamen*, a region of *caudate nucleus*. The ASN protein appears to be important for maintaining the functional integrity of synaptic vesicles [115]. Mutations of the ASN protein may lead to destabilization and permeabilization of vesicular membranes and loss of vesicular monoamine contents, e.g. DA. It seems that monoamine distribution changes seen in PD could contribute to the pathogenesis of this disease, especially by increasing interneuronal deamination and production of neurotoxic catecholaldehydes, and SN degeneration (Figs. **1**, **2**) [4].

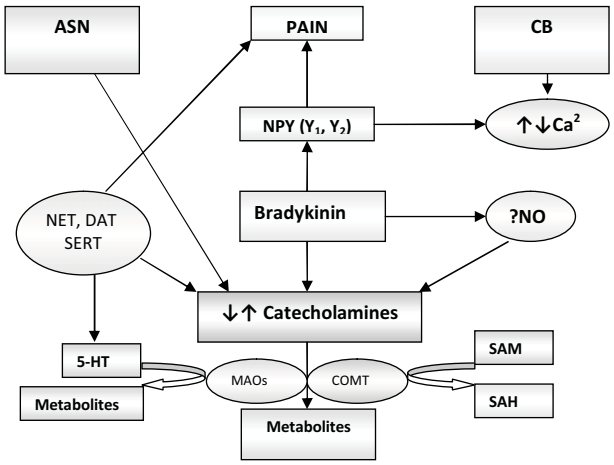

Fig. (2). Function and metabolism of monoamines in Parkinson's disease. ASN- α-synuclein; NET- norepinephrine transporter; DAT- dopamine transporter; SERT- serotonin transporter; 5-HT- serotonin; MAOs- monoamine oxidases; COMT- catechol-O-methyltransferase; SAM- S-adenosylmethionine; SAH- S-adenosylhomocysteine; NO- nitric oxide; NPY- neuropeptide Y, Y_1 and Y_2 receptors; CB- calbidin-D. (↑) increase and (↓) decrease of biochemical parameter levels.

Changes of catecholamine levels in PD may lead to disturbances in physiological functions. It is believed that changes in visual processing in PD, including deficient contrast sensitivity and abnormalities in visually evoked potentials, are the result of deficiencies in the retinal DA system [116], and digestive dysfunctions, such as constipation, may also be related to defects in the gastrointestinal DA system [117].

Monoamines are also associated with blood pressure regulation by a mechanism involving bradykinin. Bradykinin is a vasoactive nonapeptide in the CNS that promotes vasodilation via the bradykinin B_2 receptor (B2R), stimulating the endothelial production of nitric oxide (NO), prostaglandin I_2, and regulates neuronal function along with inducing adrenal catecholamine secretion [118]. It has been shown that NO acts as a neuromodulator and/or a neurotransmitter in the brain [119] and in the peripheral autonomic nervous system [120]. The role of NO in modulating catecholamine secretion is still not clear. The literature data indicate that NO may induce catecholamine secretion [121], however, there are contradictory reports implying that NO may inhibit adrenal catecholamine release [118, 122]. It has been shown that dysfunction of autonomic reflexes with inadequate increase of plasma catecholamine levels and an almost fixed heart rate lead to a decrease in blood pressure (BP). It is known that PD involves abnormalities of the catecholamine system reflected by sympathetic denervation [4] and a loss of noradrenergic nerves [4, 100]. However, sympathetic denervation in PD is most pronounced in orthostatic hypotension [123, 124].

Neurogenic orthostatic hypotension, which occurs commonly as a primary chronic autonomic disturbance,

usually results from deficient release of NE when the patient stands up. Orthostatic hypotension occurs in 50–80% of PD patients with disease duration of more than 15 years. Our unpublished data carried out on 49 PD patients (see above) has shown 14% PD patients with orthostatic hypotension. In PD patients, orthostatic hypotension is associated with neuroimaging evidence of cardiac sympathetic denervation and decreased synthesis, release, reuptake, and turnover of NE in the heart [123, 125]. Moreover, in PD these sympathetic disturbances may also occur in other organs, e.g. thyroid gland, renal cortex and skin [123].

The factors regulating monoamine levels in PD, such as COMT [34], MAO-A [89], the *5-HTT* gene encoding SERT [111], and bradykinin [126] are involved in pain perception. Moreover, bradykinin is a neuropeptide that activates B2R receptors on the terminals of specialized pain-sensing neurons known as nociceptors. Another neuropeptide, neuropeptide Y (NPY), also modulates nociceptors. NPY is expressed in the peripheral nervous system and in the CNS and modulates several physiological functions including satiety, anxiety and vascular tone [127]. NPY can be divided into subtypes of receptors, namely. Y_1 and Y_2, and both receptors are collocated with bradykinin [128]. Activation of Y_1 and Y_2 receptors may lead to an increase or decrease of Ca^{2+} levels. Both receptors are co-expressed with calcitonin gene-related peptide (CGRP), an important neurotransmitter in mediating nociception and neurogenic inflammation [129]. Another protein regulating Ca^{2+} levels is calbindin-D (CB). CB is localized within nerve cells and is often less vulnerable to degeneration in neurodogenerative diseases such as PD [130]. In PD, the presence of pain is a common symptom [131]. Older papers have indicated that, in patients with PD, the next common symptom of tremor is an effect due to decreased levels of E [132].

The basic strategy in the treatment of PD is the supplementation of missing DA in the form of L-dopa. L-dopa therapy results in improved activity in daily living, enhanced quality of life, and improved survival. However, the long-term use of L-dopa is associated with the development of motor fluctuations and dyskinesia. In addition, L-dopa therapy possesses further limitations. It has little or no effect on certain motor features (e.g. gait and balance dysfunction) and a non-motor symptom complex (autonomic dysfunction, pain syndromes, sleep disorders, mood disturbances and dementia) [13]. During long-term treatment with L-dopa, improvement of motor function is obtained by increasing the central DA level of PD subjects. The DβH enzyme is responsible for the conversion of DA to NE and L-dopa induced increase of DA levels, and may lead to an increase of NE expression in norepinergic neurons in PD. When the level of dopaminergic neurons is decreased, noradrenergic terminals in the stratum appear to be responsible for removing DA from the synapse [133]. DβH activity decreases in PD patients that are not treated with L-dopa. However, administration of L-dopa does not relieve non-motor symptoms in PD patients who have low levels of NE [134]. L-dopa is a precursor not only of DA but also other catecholamines.

There are potentially three pathways for peripheral metabolism of administrated L-dopa. These are decarboxylation to DA, O-methylation to 3-methoxytyrosine, and transamination to 3,4-dihydroxyphenyl pyruvic acid [135]. L-dopa may be converted by peripheral tissues to other catecholamines: NE, E or their metabolites (NMETA, META) [4, 124]. Thus, inhibitors of peripheral decarboxylation (e.g. carbidopa) and of O-methylation (e.g. entacapone) lead to increased availability of catecholamines and decreased required dose of L-dopa.

Our unpublished data carried out on 49 PD patients, of which 42 PD patients (aged 35-82 years) were treated with standard L-dopa therapy, with average daily dosage of 480 mg / 24 h. and 7 PD patients untreated with L-dopa (aged 41-81 years), and 48 healthy subjects (see above) have shown different level of the monoamines NE, E, 5-HT and the metabolites NMETA, META in both PD patients treated and untreated with L-dopa. In this study, concentrations of plasma NE, E, 5-HT and urine NMETA, META were estimated using HPLC/EC technique. Our study indicated that the plasma level of NE was insignificantly lower in PD patients treated with L-dopa as compared to PD patients untreated with L-dopa and controls (213.1±116.9 pg/ml, 261.0±196.3 pg/ml and 253.6±142.8 pg/ml, respectively). In contrast to NE, the plasma concentration of E was similar in both PD patients treated with L-dopa and PD patients untreated with L-dopa, and also higher than controls (Kruskal-Wallis test, $p<0.05$; 65.6±40.9 pg/ml, 70.8±43.7 pg/ml and 43.4±36.2 pg/ml, respectively).

The study by Davidson *et al.* [135] carried out in 59 PD patients similarly demonstrated that the urine NE level was changed insignificantly after L-dopa administration and was the lowest in PD patients receiving L-dopa. However, correspondingly to our study, the urine E level was also the highest in PD not receiving L-dopa and the difference was statistically significant as compared to controls ($p=0.018$).

Our study has also indicated that the urine concentration NMETA was lower in PD patients treated with L-dopa than in PD patients untreated with L-dopa and controls (Kruskal-Wallis test, $p<0.001$; PD treated with L-dopa, 149.2±343.4 μg/24 hours and controls, 513.4±472.8 μg/24 hours, respectively). However, the level of META was much higher in PD patients treated with L-dopa than in PD patients untreated with L-dopa and controls (Kruskal-Wallis test; PD patients treated with L-dopa, $p<0.05$, 1153.6±1655.9 μg/24 hours, and PD patients untreated with L-dopa, 54.±65.2 μg/24 hours, and controls, $p<0.001$, 9.6±20.4 μg/24 hours).

Davidson *et al.* [135] showed that, unlike our research, PD patients receiving L-dopa had significantly higher urine concentrations of both NMETA and META than in PD patients not receiving L-dopa and controls. Reported by Davidson *et al.* [135], significant differences in the levels of both NMETA and META could be the result of variable doses and time of administration of L-dopa, and the use of AADC and COMT inhibitors.

In our study, the level of 5-HT was similar in PD patients treated with L-dopa and in PD patients untreated with L-dopa and also higher than controls (controls 0.100±0.100 μg/ml; PD not receiving L-dopa 0.137±0.230 μg/ml; PD receiving L-dopa 0.148±0.172 μg/ml) however the difference did not reach statistical significance.

Hinz *et al.* [136] demonstrated that L-dopa administration leads to a decrease in 5-HT concentration, entailing disease symptoms associated with inadequate 5-HT levels (e.g. depression), side effects, adverse reactions, and tachyphylaxis of L-dopa. However, our study has shown a high level of 5-HT after L-dopa administration in PD. Our results may support the hypothesis of Ng *et al.* [137], that exogenously administered L-dopa may enter central 5-HT terminals and undergo decarboxylation to the amine with resultant displacement of the endogenous indoleamine from vesicular stores.

It appears that treatment of PD patients with L-dopa resulting in an abnormal level of biogenic amines and their metabolites is likely to be dependent on dosage and timing of administration. It seems that, in PD, combination therapy involving a DA agonist and AADC, MAOs, COMT inhibitors is the optimal form of PD therapy and helps maintain monoamine homeostasis.

CONCLUSION

As indicated by our unpublished data, in PD the polymorphisms in genes related with the metabolism of catecholamines (NE, E), e.g. *COMT* (c.649G>A), and other monoamines (5-HT), e.g. *MAO-A* (c.1460C>T), as well as in the transport and release of monoamines (NE), e.g. *NET* (c.1287G>A), can significantly affect the level of biogenic amines and/or their metabolites (NMETA, META).

It seems that, in PD patients, the reduced activity of COMT resulting from the (c.649G>A) AA genotype has a stronger impact on the final step of catecholamine biosynthesis (E level) and META level. Moreover, in PD, the *MAO-A* (c.1460C>T) CT genotype is associated with high plasma levels of monoamines (NE, E, 5-HT) and one of the highest levels of their metabolites (NMETA, META). It also seems reasonable to use MAO-A inactivation due to its antidepressant effects (by increased monoamine levels) as an effective strategy in the treatment of PD patients especially with the mutant *MAO-A* (c.1460C>T) TT genotype and with depressive symptoms. Our studies indicate the likely impact of genetic determinants of the *NET* (c.1287G>A) gene on the level of biogenic amines (however, not significant) as well as a significant influence on their metabolites in PD, especially in patients with the GA genotype.

It is worth noting that the metabolism of catecholamines and 5-HT is conjugated by common factors. Decarboxylation of both 5-HTP and L-dopa is performed by the same enzyme (AADC) in the neuronal cytoplasm, thus oversupply of either substrate would competitively inhibit formation of DA and 5-HT, respectively. Such a phenomenon may be observed in PD patients treated with L-dopa, in which a large supply of L-dopa may inhibit the decarboxylation of 5-HTP, leading to decreased levels of 5-HT in the human brain. Furthermore, an increased level of L-dopa might inflict overexpression of AADC, as discussed earlier, resulting in further DA and 5-HT imbalance in PD patients treated with L-dopa. The break down of 5-HT and catecholamines is performed to some extent by both MAOs, thus increased levels of monoamines in PD patients taking L-dopa would competitively inhibit the metabolism of 5-HT, followed by its increased levels in the patients' brains.

We still need therapies to provide a robust antiparkinsonian benefit through the day, to eliminate dyskinesias, and to slow down or stop the progress of the disease. Perhaps controlling the level of monoamines and their metabolism would help to improve the outcomes of treatment of patients with PD.

CONFLICT OF INTEREST

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

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