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Homocysteine Level and Mechanisms of Injury in Parkinson's Disease as Related to *MTHFR*, *MTR*, and *MTHFD1* Genes Polymorphisms and L-Dopa Treatment

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Abstract: An elevated concentration of total homocysteine (tHcy) in plasma and cerebrospinal fluid is considered to be a risk factor for Alzheimer's disease (AD) and Parkinson's disease (PD). Homocysteine (Hcy) levels are influenced by folate concentrations and numerous genetic factors through the folate cycle, however, their role in the pathogenesis of PD remains controversial. Hcy exerts a neurotoxic action and may participate in the mechanisms of neurodegeneration, such as excitotoxicity, oxidative stress, calcium accumulation, and apoptosis. Elevated Hcy levels can lead to prooxidative activity, most probably through direct interaction with N-methyl-D-aspartate (NMDA) receptors and sensitization of dopaminergic neurons to age-related dysfunction and death. Several studies have shown that higher concentration of Hcy in PD is related to long-term administration of levodopa (L-dopa). An elevation of plasma tHcy levels can also reflect deficiencies of cofactors in remethylation of Hcy to methionine (Met) (folates and vitamin B12) and in its transsulfuration to cysteine (Cys) (vitamin B6). It is believed that the increase in the concentration of Hcy in PD can affect genetic polymorphisms of the folate metabolic pathway genes, such as *MTHFR* (C677T, A1298C and G1793A), *MTR* (A2756G), and *MTHFD1* (G1958A), whose frequencies tend to increase in PD patients, as well as the reduced concentration of B vitamins. In PD, increased levels of Hcy may lead to dementia, depression and progression of the disease.

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1. INTRODUCTION

Homocysteine (Hcy) is a sulfur-containing amino acid formed by demethylation of methionine (Met) via the methionine cycle and a metabolic cycle of folate (Fig. **1**). Elevated plasma Hcy concentration is an independent risk factor for cardiovascular diseases (stroke, heart disease), and its involvement in endothelial cell dysfunction is well established [1]. The last decade has also seen many studies of hyperhomocysteinamia (HHcy) in some neurological, psychiatric, and movement disorders, such as epilepsy, cognitive impairment in later life, Alzheimer's disease (AD), depression, anxiety, as well as primary dystonia, Huntington's disease, and idiopathic Parkinson's disease (PD) [2-8]. Thus, an elevated (greater than 14 μmol/L) concentration of plasma Hcy has been reported to correlate with a doubled risk of AD [5], and has even been shown to result in lowered cognitive functions in 25% of elderly persons without dementia [9]. An increase of total Hcy concentration in plasma and cerebrospinal fluid has also been reported to be the cause of cognitive decline and depression in PD patients [10-12]. The potential mechanism of action of Hcy in cognitive dysfunction may be the induction of vascular changes in the brain. Hcy exerts a neurotoxic action through different mechanisms, such as amino acid-mediated damage, mitochondrial dysfunction, free radical and cytosolic calcium accumulation, and apoptotic pathway activation [6, 13-17]. Elevated levels of Hcy may have neurotoxic effects, such as DNA damage, basal ganglia disorders, and a neurotransmitter imbalance in motor circuits [18-22], which can increase the risk of neural-cell movement dysfunction. High concentrations of Hcy in PD may increase the risk of this disease by direct toxic effects on dopaminergic neurons. Increasingly compelling data suggest that Hcy may play a role in sensitizing dopaminergic neurons to age-related dysfunction and death [18, 19, 23]. Recently, it has been reported that elevated Hcy levels are detected in the plasma of PD patients receiving levodopa (L-3,4 – dihydroxyphenylalanine; L-dopa) [24, 25]. For the treatment of PD, the administration of L-dopa remains the gold standard therapy, and increased plasma levels of Hcy have been found mainly in those patients with PD receiving L-dopa in long-term therapy [24, 26-28]. Patients receiving L-dopa for the first 5 years appear to be the most exposed to neurotoxic effects of Hcy, while continued

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administration of L-dopa has resulted in stably elevated Hcy levels [29]. L-dopa is methylated by catechol O-methyltransferase (COMT), which drives the production of S-adenosyl-Hcy and increases the production of Hcy (Fig. **1**). As indicated in the literature, L-dopa therapy leads to motor fluctuations and disabling involuntary movements called L-dopa-induced dyskinesia [30, 31].

Despite significant progress in the treatment of PD, an increased incidence of diseases typical in the elderly, such as PD, is accompanied by an increase in the mortality of the population suffering from this degenerative disorder. It is believed that the most likely cause of PD is the pathological deposition of proteins in specific structures of the central nervous system (CNS), leading to impaired cellular metabolism and neuronal damage of macromolecular compounds, such as nucleic acids (DNA, RNA). As indicated in the literature [32, 33], the interaction of reactive oxygen species (ROS) with DNA leads to the oxidation of guanine and formation of 8-oxo-2'-deoxyguanosine (8-oxo2dG). Augmented levels of 8-oxo2dG were demonstrated in the brain and lymphocytes of patients with PD [29, 32-35]. This indicates a gradual increase of nucleic acid damage during the development of this disease, and a high level of oxidized guanine in DNA is considered a risk factor for senescence and neurodegenerative diseases (e.g. PD). In PD, oxidative stress is accompanied by the release of ferrous ions (an important substrate for oxidative reactions and for production of ROS) [36], decreased glutathione (GSH) levels, and by the impairment of mitochondrial respiratory chain complex I [36, 37].

As reported in the literature [38] and supported by our studies [8], increased levels of 8-oxo2dG in the pharmacotherapy of PD also reflects long-term administration of L-dopa. After oral intake, L-dopa undergoes metabolism, including the oxidative metabolism of dopamine, and auto-oxidation, and is transported across the blood-brain barrier. It is believed that both excessive peripheral oxidation of dopamine and the incorporation of oxidized guanine to DNA in PD patients treated with L-dopa are probably responsible for the accumulation of the degradation products in the mesencephalon, leading to the lesion of *substantia nigra pars compacta* (SNpc) [38]. The contribution of L-dopa therapy to oxidative damage in peripheral blood lymphocytes (PBLs) in PD patients is not clear [29, 39].

Influence on Hcy concentrations has also been reported with regards to a number of genetic factors participating in the folate metabolic pathway [40, 41] (Fig. **1**). It has been

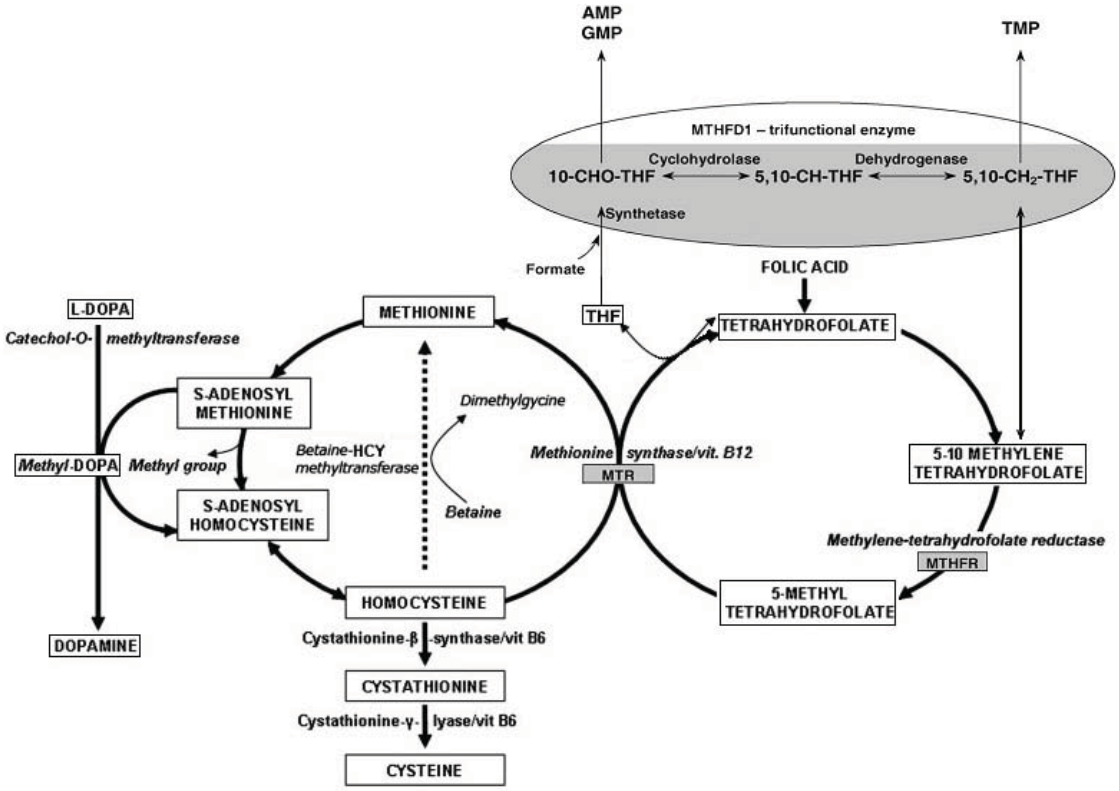

Fig. (1). Contribution of MTHFR, MTR and MTHFD1 in the folate metabolic pathway.

5,10-Methylenetetrahydrofolate (5,10-CH_2-THF) is either oxidized by MTHFD1 dehydrogenase to 5,10-methenyltetrahydrofolate (5,10-CH-THF) or reduced by MTHFR to 5-methyltetrahydrofolate (5-CH_3-THF). Methionine synthase (MTR) uses 5-CH_3-THF as a methyl group donor to remethylate homocysteine (Hcy) to methionine (Met). Methionine adenosyltransferase converts methionine to S-adenosylmethionine (SAM), which donates a methyl group to various methyl transferases, including catechol-O-methyltransferase (COMT). Methylation of L-dopa is catalyzed by COMT, which drives the production of S- adenosylhomocysteine (SAH), which can be reversibly hydrolyzed to Hcy. The production of Hcy is regulated by the enzymes MTR, cystathionine β-synthase, and betaine-Hcy methyltransferase. B-vitamins including folate, vitamin B6, and vitamin B12 are cofactors for these enzymes, and deficiencies of these cofactors can lead to elevated Hcy.

demonstrated that a specific predisposition to HHcy is modulated by genetic polymorphisms of the folate-cycle key enzymes regulating Hcy metabolism. One of these enzymes is 5,10-methylene tetrahydrofolate reductase (MTHFR), a folate-dependent enzyme, which plays a key role in regulating Hcy metabolism (Fig. **1**). MTHFR supplies methyltetrahydrofolate for methionine synthase (methyltetrahydrofolate-homocysteine methyltransferase, MTR), which converts Hcy to Met. B-vitamins, including folic acid (FA), vitamin B6 and vitamin B12, are cofactors for these enzymes, and deficiencies of these cofactors may lead to elevated Hcy levels. Another enzyme linked to the transformation of Hcy to Met is the trifunctional enzyme methylenetetrahydrofolate dehydrogenase/ methenyltetrahydrofolate cyclohydrolase/ formyltetrahydrofolate synthetase (MTHFD1). However, the extent to which genetic variations of the folate cycle genes influence susceptibility to PD remains unclear. Elevated plasma Hcy levels were found in patients with PD, but mainly in patients receiving L-dopa therapy [24-28]. Therefore, it is unclear whether the Hcy increase seen due to PD preceded or was caused by the L-dopa. Indeed, several open trials have been carried out in order to reduce Hcy levels in patients with PD, using vitamin B12, folic acid supplements, or treatment with COMT inhibitors [42, 43].

2. METABOLISM OF BIOTHIOLS

Elevated Hcy level is a risk factor for vascular diseases, cognitive impairment and dementia, and neurodegenerative diseases (e.g. PD). Direct conversion of Hcy is largely influenced by genetic and environmental factors. Genetic and nutritional factors (including drugs) are important determinants of Hcy metabolism, and what's more, the possible interactions between these factors can determine the production of increased levels of Hcy [44-46]. The level of Hcy is controlled depending upon the concentration of its metabolites: Cys and Met. Met is supplied with food, and its transformation to Hcy involves several steps (Fig. **1**). At the first step, Met is transformed to SAM (S-adenosylmethionine), the main donor of methyl groups in many reactions, and is then demethylated so SAH (S-adenosylhomocysteine), and hydrolyzed to Hcy (Fig. **1**).

Intracellular Hcy has an integral role in methylation processes and participates in two main metabolic pathways: transsulfuration to form cystathionine and glutathione, and remethylation to form Met (Fig. **1**). Under physiological conditions, approximately 50% of Hcy is catabolized by transsulfuration and converted to cystathionine and then to cysteine (Cys). These reactions are converted by cystathionine β-synthase (CBS) and γ-cystathionase (CTH), which require a derivative of vitamin B6. Remethylation of Hcy, catalyzed by Met synthase, is activated by a low concentration of Met. MTR is a vitamin B12-dependent enzyme responsible for transfer of methyl groups from 5-methyltetrahydrofolate (5-CH_3-THF) to Hcy. 5-CH_3-THF is formed by the MTHFR enzyme during the NADPH-dependent reduction of 5,10-methylenetetrahydrofolate (5,10-CH_2-THF). 5,10-CH_2-THF is also a substrate for MTHFD1, an NADP-dependent trifunctional enzyme, and can also be used as a coenzyme in the biosynthesis of thymidine (Fig. **1**). These reactions (with cofactors folate, vitamins B12 and B6) are donors of methyl groups necessary for the synthesis of proteins, DNA, RNA, phospholipids, myelin, and catecholamines.

3. HCY IN PD: POSSIBLE MECHANISMS OF INJURY

Hcy or its oxidized metabolite, homocysteine acid (HA), is considered a risk factor for neurodegenerative diseases, including AD and PD [8, 10]. It has been shown that Hcy may pass the blood/brain barrier, and its plasma level corresponds to the concentration in the brain [47]. In AD, a decreased level of SAM was documented [48], paralleled by a decreased methylation of DNA and augmented levels of β-amyloid [49, 50]. A decreased content of SAM was also demonstrated in the course of PD [51]. These studies indicate that increased plasma levels of Hcy in AD and PD may also arise from changes in the processes of Hcy remethylation to Met and transsulfuration to Cys. According to Miner *et al.* [52], this increase may be due to lower levels of Hcy metabolism cofactors as a result of physiological aging. Both in people over 60 years of age and in these degenerative diseases, a decreased concentration of Met has been observed coupled with elevated levels of Cys and a decreased ratio of Met and Cys to Hcy. As demonstrated in our previous reports [8, 29], a decrease in the Met-to-Hcy ratio may be linked to transformation of Hcy to thiolactone in endothelial cells, and the derivative of sulfonic sulfur containing thiol compounds may be involved in the development of Hcy-induced arteriosclerotic lesions [53]. Additionally, the demonstrated [8, 29] increased plasma Cys level in AD, PD and in individuals older than 60 years of age may result from an increased release of this amino acid from proteins due to substitution by the circulating Hcy or due to diminished transformation of Cys into GSH, which is important for the maintenance of redox homeostasis in the body.

Hcy may be neurotoxic by the stimulation of N-methyl-D-aspartate (NMDA) receptors [5] (as a glutamate receptor ligand) and oxidative stress (as a result of autooxidation of Hcy) [54]. Neurotoxicity was confirmed in studies on AD, where the high level of Hcy was found to potentially modulate the synthesis of β-amyloid and possibly potentiate glutamate toxicity, enhance calcium influx into the neurons, or have a deleterious role in vascular changes [49, 50]. In PD, a high concentration of Hcy may increase the risk of the disease through its direct toxic effect on dopaminergic neurons. *In vitro* studies on human dopaminergic neurons have documented a significant increase in neurotoxicity accompanying high Hcy levels [18]. Hcy added to cultured human dopaminergic cells resulted in the inhibition of mitochondrial complex I and caused oxidative stress, which increased the vulnerability to cell death [18]. Another possible mechanism of the role of Hcy in dopaminergic transmission has been presented by Agnati *et al.* [55], and allosteric modulation of dopamine D2 receptors showed that Hcy acted as an allosteric D2 receptor antagonist in an animal model, selectively reducing the affinity of D2 receptors for agonists but not for antagonists. The molecular mechanism of this modulation showed that Hcy forms non-covalent complexes with two arginine (Arg)-rich epitopes of the third intracellular loop of the D2 receptor [55]. These implications may result in the complications seen in PD therapy, such as the effect of high levels of Hcy on the reactivity of patients to L-dopa and dopamine agonists commonly used in the treatment of PD. On

the other hand, the clinical observations of dyskinesia that becomes gradually higher in PD patients with elevated Hcy may suggest that Hcy acts in the opposite manner (hypersensitivity of receptors) [56].

Furthermore, elevated Hcy levels in PD have been shown to carry the potential for deterioration of cognitive and motor functions, for depression, and for an increased predisposition to develop vascular diseases [24]. Gorell *et al.* [57] indicated that patients with PD have shown an increased risk for cardiovascular disease and stroke, and the increased total plasma Hcy (tHcy) level is a potential risk factor for vascular disease. This may be due to its cytotoxic activity, especially for endothelial cells, cytokine induction, the activation of procoagulant factors, abnormal serum lipid metabolism, and a change in rheological properties of the blood [1, 3, 15, 54]. In PD, both Hcy and Cys, the product of Hcy metabolism, may promote pathological alterations such as atherosclerosis and thrombogenesis [8, 53, 58, 59]. Muller and Kohn [59] indicated that only PD patients with an elevated level of Hcy above 15 μM showed an increase of Cys plasma levels, and that elevated concentrations of both risk factors (Hcy, Cys) may intervene in the neurodegenerative process.

It is also known that vascular dementia and cognitive impairment worsen the prognosis of PD patients, and it is important to minimize the risk of their occurrence as much as possible. Increased levels of Hcy in PD patients may reflect the lack of cofactors in Hcy remethylation to Met (folates and vitamin B12) and in response to Cys transsulfuration (vitamin B6). Literature reports and our previous findings indicate that decreased levels in these vitamins are observed in both AD [60] and PD [42, 43], and that dietary supplements or the administration of pharmaceutical preparations of these vitamins can significantly prevent the intensification of vascular lesions and dementia in the patients. According to Lamberti *et al.* [43], administration of vitamin B12 and folates decreases the plasma level of Hcy, particularly in PD patients treated with L-dopa, and in this way prevents against the intensification of vascular lesions and dementia in these patients.

4. INFLUENCE OF L-DOPA TREATMENT ON THE LEVEL OF OXIDATIVE DAMAGE TO DNA IN PD

In many neurodegenerative diseases, including PD, oxidative stress and excitotoxicity seem to play a pivotal role in their pathogenesis. The accumulation of pathological forms of proteins, which arise as a result of posttranslational modifications or mutations of the encoding genes, results in pronounced neurotoxic effects on the CNS. It is believed that these proteins form inclusion bodies (Lewy bodies) in the cytoplasm of dopaminergic neurons of PD patients; these Lewy bodies contain deposits of dopamine level-controlled ubiquitin and α-synuclein [61, 62]. Augmented expression of α-synuclein in PD may intensify oxidative stress [62]. Bergman *et al.* [63] showed that dopaminergic neurons in the SNpc undergo oxidative damage in PD patients, and are accompanied by a decrease in dopamine levels in the *caudate nucleus*. It also has been shown that ferrous ions released from the damaged *substantia nigra* of patients with PD may provide an important substrate for oxidative reactions and the formation of ROS [64]. Production of ROS may lead to damage of macromolecular compounds such as nucleic acids (DNA, RNA), proteins, and lipids. DNA may undergo oxidation of guanine to 8-oxo2dG, which is considered a marker of oxidative damage to DNA in both normal aging and in neurodegenerative disorders. The studies by Migliore *et al.* [65] and Mecocci *et al.* [66] show that, in aging organisms, the level of 8-oxo2dG also significantly increases in PBLs. The study of Dorszewska *et al.* [8] indicates that the 8-oxo2dG levels are not significantly higher between 22 and 76 years of age, supporting the data from Alam *et al.* [34] that shows that the level of oxidatively modified guanine in DNA is insignificantly higher in the brain of individuals between 43 and 91 years of age. The increase in the level of 8-oxo2dG was also demonstrated in the PBLs of patients with PD [65, 67]. It is believed that the cause of the raised level of oxidatively modified nucleic acids in PD is both an overproduction of free radicals and a decrease in the level of enzymatic and non-enzymatic antioxidant repair systems [65].

As indicated in the literature [38, 64-66] and by our studies [8, 29], L-dopa therapy and the duration of its administration in PD patients can affect the increase in the level of 8-oxo2dG in DNA. However, there are also reports of a negative correlation between oxidative stress and L-dopa dosage in the PBLs of patients with PD [39]. Some studies (e.g. [68]) suggest a toxic effect of L-dopa on neuronal cell *in vitro,* while *in vivo* studies in animal models are contradictory. Since less than 5% of an oral dose of L-dopa is delivered to the brain, the remaining plasma levels of the drug undergo peripheral oxidative metabolism and may generate ROS. It is likely that the peripheral oxidation status in PD might be affected by L-dopa therapy [68]. The study of Spencer *et al.* [69] showed that the augmented oxidative stress in PD patients treated with L-dopa might influence the degree of reduction of antioxidants (GSH), disturbed mitochondrial transport, and excessive oxidation of dopamine. Our results [29] indicated that L-dopa can modify the level of 8-oxo2dG in the PBLs of PD patients. Patients seem to be particularly exposed to oxidative stress during the first five years of treatment with L-dopa, and following prolonged periods of time (over 10 years) of administration of this drug [29]. Interestingly, a significant increase in oxidative DNA damage has been observed in the IVth stage of PD development (according to the scale of Hoehn and Yahr), even though 8-oxo2dG levels were increased between stages I and III. It seems that the increasing levels of oxidatively altered nucleic acids in PD patients involve overproduction of free radicals, as well as decreased levels of enzymatic and non-enzymatic antioxidants, and less effective repair mechanisms. Samples from AD patients have been found to contain decreased activity of 8-oxoguanine glycosylase 1 (OGG1), and more oxidative DNA damage that may induce of apoptosis [33, 70].

5. INFLUENCE OF L-DOPA TREATMENT ON THE PLASMA LEVEL OF BIOTHIOLS IN PD

Reports in the literature [24-28, 42] and our previous results [8, 29] have been shown that plasma Hcy levels in PD correspond to pharmacotherapy with L-dopa. The study of Miller *et al.* [71] indicates that L-dopa may induce elevated levels of Hcy during its methylation to 3-O-methyldopa (3-OMD) with involvement of COMT both in PBLs and in ni-

grostriatal neurons. This reaction requires SAM as a methyl group donor (Fig. **1**). The demethylated SAM is then immediately converted to SAH, which then forms Hcy via deadenosylation, whose production is regulated by MTR, CBS and betaine-Hcy methyltransferase (BHMT) enzymes. Thus, chronic L-dopa treatment may deplete SAM, while increasing SAH formation, which ultimately leads to the elevation of Hcy [40, 41]. The remaining plasma levels of L-dopa undergo peripheral oxidative metabolism to generate ROS, which occurs via decarboxylation and methylation, and may be inhibited outside of the nervous system to obtain clinically meaningful motor improvement.

High levels of Hcy are considered a risk factor for both vascular and degenerative diseases. In PD patients, this may involve a simultaneous increase in oxidation (high 8-oxo2dG level) during the period of treatment with L-dopa and the consequently hindered Hcy metabolism. Long-term administration of L-dopa is thought to promote benign vascular lesions in patients with PD and may result in cognitive disturbances or dementia, particular at late stages of L-dopa treatment [1]. Elevated levels of Hcy in the *substantia nigra* of PD patients have been demonstrated after just 3 months of L-dopa treatment [72]. Patients in the first 5 years of L-dopa treatment appear to be the most exposed to neurotoxic effects of Hcy, while the continued administration of L-dopa has resulted in stably elevated Hcy levels. It seems that Hcy elevates within six weeks to a few months after L-dopa initiation [73]. Further administration of L-dopa resulted in only a constantly high level of Hcy, and it seems that disturbed metabolism of Hcy to Met and Cys occurs throughout L-dopa use. The study of Jara-Prado *et al.* [74] indicates that the excitotoxicity in PD may be caused not only by pathological protein (α-synuclein) but also by excessive interaction of the sulfuric amino acids with NMDA receptors in the CNS. The study by Florczak *et al.* [29] has also indicated that the sulfuric amino acids levels are affected by the duration of L-dopa pharmacotherapy. Moreover, only PD patients with HHcy (Hcy above 15 μM) may have a disturbed metabolism of Hcy to Cys [59].

6. POLYMORPHISMS OF *MTHFR, MTR, MTHFD1* AND THE LEVEL OF BIOTHIOLS IN PD

6.1. Methylenetetrahydrofolate reductase (MTHFR)

MTHFR is a folate- and vitamin B12-dependent enzyme, which plays a key role in regulation of the folate and Hcy metabolic pathways (Fig. **1**). This regulatory enzyme catalyzes an irreversible conversion of 5,10-CH_2-THF to 5-CH_3-THF, which is the predominant form of circulating folate and serves as a substrate for the remethylation of Hcy to Met. Under normal dietary habits, half of the available Hcy receives the methyl group from 5-CH_3-THF, recycling Met, whereas the second half is transsulfured to Cys.

There are three common genetic polymorphisms in the coding region of the *MTHFR* gene, C677T, A1298C and G1793A, which, respectively, involve the Ala222Val, Ala429Glu, and Arg594Gln amino acid substitutions. Individually, these polymorphisms may contribute to the reduction of a specific enzyme's activity, impairing Hcy metabolism, which leads to the observed increase in plasma Hcy levels. As compared to the 677CC wild-type genotype, the 677TT homozygotes have decreased MTHFR activity by approximately 70%, and the 677CT heterozygotes by approximately 40% [75, 76]. It is also known that the *MTHFR* C677T polymorphism leads to increased heat lability and reduced enzymatic capability for methylation of Hcy [76-78]. This polymorphic variant, with an allelic frequency ranging from 12% to 35%, is one of the circumstances predisposing to an increased tHcy plasma level. About 10% to 13% of the Caucasian population are homozygous for this mutation, and thus *MTHFR* 677TT genotype carriers may have dramatically elevated tHcy as a consequence of the significant decrease in enzyme activity, especially if supplies of folate and vitamin B12 are limited [26, 44, 76, 79, 80]. The A1298C and G1793A polymorphisms are also associated with enzyme activity decline, although to a lesser extent than the C677T *MTHFR* polymorphism, and this effect is more evident in the homozygous than in the heterozygous state [81]. An association between higher plasma tHcy levels and the A1298C polymorphism is controversial, however, van der Put *et al.* [82] found that combined heterozygosity at the 677 and 1298 polymorphic sites was associated with reduced MTHFR activity, higher tHcy, and decreased plasma folate levels [82, 83].

The involvement of MTHFR in pathomechanisms of neurodegenerative diseases through its influence on Hcy homeostasis have been demonstrated [84-91], indicating that in the Polish population [8], as well as in populations of Northern Ireland [86], Italy [87] and Japan [88, 89], the TT (C677T), CC (A1298C) and AA (G1793A) genotypes of *MTHFR* are the least frequent and their incidence is, to some extent, increased in AD patients. However, these reports as well as literature review [26, 85, 90, 91], revealed that the most pronounced increases of tHcy were noted in AD and PD patients with the C677T *MTHFR* TT genotype, particularly for patients with low plasma folate levels. Moreover, in PD and AD patients bearing the *MTHFR* CT genotype, the process of Hcy to Cys transsulfuration was disturbed, resulting in higher Cys levels [8]. The most evident alterations in plasma Cys patterns were noted in AD patients carrying the 677CT genotype along with the 1298AA genotype of *MTHFR* [8]. This combination of *MTHFR* genotypes, potentially in the presence of additional polymorphisms, appears to be reflected in a higher incidence of severe dementia in the patients, measured using the MMSE scale. In the case of PD patients, the C677T and G1793A *MTHFR* polymorphisms have pointed in general to a prevalence of patients with common CC and GG genotypes (respectively) at each of the analyzed stages of PD (stages I to IV in the scale of Hoehn and Yahr) [8]. On the other hand, the heterozygous AC genotype of the A1298C polymorphism has been observed in PD patients more frequently at various stages of the disease, and the most pronounced disturbances in Hcy remethylation and transsulfuration have been manifested in *MTHFR* 1298AC genotype carriers [8]. Nevertheless, synergistic effects in the folate metabolic pathway of *MTHFR* and other gene polymorphisms in the pathogenesis of PD probably exist [90].

Since some studies have shown that higher levels of Hcy are related to L-dopa treatment in PD patients [8, 24-29, 42, 72], the *MTHFR* polymorphisms could enhance the effect of L-dopa on Hcy in PD patients, and cause an additional eleva-

tion in the concentration of Hcy. Several investigations have demonstrated that the *MTHFR* 677T allele causes an elevation in plasma Hcy levels in patients receiving L-dopa [26, 85, 91]. Yuan *et al.* [92] have recently reported that there is a relationship between the CT and TT genotypes of the *MTHFR* C677T polymorphism in PD patients and an elevation in Hcy levels after L-dopa administration. The study compared PD patients (mostly treated with L-dopa) with healthy controls, and suggested that the tHcy level was affected by the interaction of B-vitamin intake and the *MTHFR* 677TT genotype, but not by the *MTHFR* 1298CC genotype [92]. Previously, Nakaso *et al.* [80] revealed that the elevated Hcy plasma levels were associated with the *MTHFR* 677TT genotype and therapy with L-dopa. Accordingly, Yasui *et al.* [72] estimated that the mean Hcy levels in CC and TT homozygotes had increased from 10.9 to 14.6 μmol/L and 11.9 to 29.3 μmol/L with L-dopa therapy, respectively. These findings show that differences in Hcy levels among CC and TT genotypes are not only the result of MTHFR enzyme activity itself, but also due to differences in the capacity for L-dopa-associated metabolism in each genotype. However, the influence of connecting the C677T and A1298C *MTHFR* polymorphisms and L-dopa therapy on plasma Hcy levels in PD patients has not been clearly established [93].

6.2. Methionine Synthase (MTR)

In the event of Met deficit and low concentrations of SAM, most Hcy undergoes remethylation to Met by MTR (Fig. **1**). MTR with methylcobalamin, a derivative of cobalamin (vitamin B12), as a cofactor, catalyzes the transfer of a methyl group from 5-CH_3-THF to Hcy, leading to the formation of Met [94]. Mutations in the *MTR* gene as well as severe deficiency of vitamin B12 result in homocysteinuria, hyperhomocysteinemia and hypomethioninemia [95]. Some studies have shown that differences in the plasma levels of Hcy and folate are associated with variations in *MTR* genotypes [2, 8, 90, 95]. The frequently analyzed *MTR* A2756G polymorphism (Asp919Gly) reduces the enzyme activity for *de novo* synthesis of Met by the conversion of Hcy (Fig. **1**). The study of Matsuo *et al.* [96] also indicates that the low catalytic activity of MTR results in the hypomethylation of DNA (low levels of SAM). The heterozygous genotype of the *MTR* A2756G polymorphism is probably also associated with augmented levels of Hcy in AD and PD patients [8]. This increase in Hcy is likely due to low activity of MTR, caused by excessive oxidation of cobalamin [97] related to oxidative stress, which is more pronounced in aging and degenerative disorders. Thus, impairment in Hcy metabolism can be attributed to a reduction in the enzyme activity due to either the *MTHFR* C677T or the *MTR* A2756G polymorphisms, which result in the elevated Hcy levels that increase the risk for the development of vascular cell insults and neural cell degeneration.

6.3. Methylenetetrahydrofolate Dehydrogenase (MTHF D1)

The trifunctional enzyme, methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase represents another folate-dependent enzyme linked to the transformation of Hcy to Met. MTHFD1 catalyzes the transformation of tetrahydrofolate to 10-formyl, 5,10-methenyl and 5,10-methylene derivatives (Fig. **1**). 10-formyltetrahydrofolate and 5,10-methylenetetrahydrofolate serve as donors of methyl groups in DNA biosynthesis [98]. It seems that, similarly to *MTHFR*, the homozygotes of the *MTHFD1* G1958A polymorphism may be involved in the pathogenesis of cardiovascular diseases associated with elevated levels of Hcy, or in the development of folate level-related neural tube defects [99]. In the literature, however, less numerous data are available on the involvement of MTHFD1 in the pathogenesis of degenerative diseases, and its role in disturbances of thiol turnover in these disorders has not been fully elucidated. However, the study of Dorszewska *et al.* [8] indicated that heterozygotes (GA) and homozygotes (AA) of the *MTHFD1* G1958A polymorphism were responsible for increased levels of Hcy in AD and PD patients. According to these results, the GA genotype was also linked to an increase in the Cys level in PD patients, whereas in AD patients the AA genotype was related to increases of the Hcy and Cys levels. Thus, significant differences of Cys and Hcy levels in the GA genotype carriers were seen between AD and PD groups [8]. These results indicate that significant differences in the intensity of turnover of the circulating biothiols in PD and AD could be affected by the folate-dependent enzyme encoded by the *MTHFD1* gene.

7. CONCLUSION

Long-term therapy with L-dopa in PD patients plays an important role in the elevation of plasma Hcy levels. L-dopa administration intensifies oxidative stress in dopaminergic neurons and in the peripheral blood lymphocytes of PD patients, and also induces changes in the concentrations of Hcy and Cys, which are risk factors of vascular diseases. An elevation of plasma Hcy levels can also reflect deficiencies of cofactors in Hcy remethylation to Met (folates and vitamin B12) and in the transsulfuration reaction to Cys (vitamin B6). The *MTHFR*, *MTR* and *MTHFD1* polymorphisms promote elevated levels of biothiols in PD. The effect of this elevation of Hcy levels is remarkable with regard to the genotypes of the *MTHFR* TT (C677T), CC (A1298C) and AA (G1793A), MTR AG (A2756G), and MTHFD1 GA (G1958A) and AA (G1958A) polymorphisms, the frequencies of which tend to increase in PD patients. Moreover, only polymorphisms of the folate-dependent enzyme MTHFD1 have pointed to significant differences in the intensity of turnover of circulating biothiols between the two neurodegenerative diseases AD and PD, which differ in the localization of neurotoxic lesions in the CNS. Current literature and our results indicate that, both in AD and in PD, decreased levels of vitamins B6, B12 and folates are observed, and their supplementation using diet or pharmacological preparations may markedly counteract the progression of these diseases.

CONFLICT OF INTEREST

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

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