Nutrition Research Reviews (2019), **32**, 218–230
doi:10.1017/S0954422419000106

The role of one-carbon metabolism and homocysteine in Parkinson's disease onset, pathology and mechanisms

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disorder. It is characterised by the progressive degeneration of dopaminergic (DA) neurons. The cause of degeneration is not well understood; however, both genetics and environmental factors, such as nutrition, have been implicated in the disease process. Deficiencies in one-carbon metabolism in particular have been associated with increased risk for PD onset and progression, though the precise relationship is unclear. The aim of the present review is to determine the role of one-carbon metabolism and elevated levels of homocysteine in PD onset and pathology and to identify potential mechanisms involved. A search of PubMed, Google Scholar and Web of Science was undertaken to identify relevant human and animal studies. Case–control, prospective cohort studies, meta-analyses and non-randomised trials were included in the present review. The results from human studies indicate that polymorphisms in one-carbon metabolism may increase risk for PD development. There is an unclear role for dietary B-vitamin intake on PD onset and progression. However, dietary supplementation with B-vitamins may be beneficial for PD-affected individuals, particularly those on L-DOPA (levodopa or L-3,4-dihydroxyphenylalanine) treatment. Additionally, one-carbon metabolism generates methyl groups, and methylation capacity in PD-affected individuals is reduced. This reduced capacity has an impact on expression of disease-specific genes that may be involved in PD progression. During B-vitamin deficiency, animal studies report increased vulnerability of DA cells through increased oxidative stress and altered methylation. Nutrition, especially folates and related B-vitamins, may contribute to the onset and progression of PD by making the brain more vulnerable to damage; however, further investigation is required.

Key words: **Parkinson's disease: Dopamine: One-carbon metabolism: Homocysteine: B-vitamins**

Introduction

Parkinson's disease (PD) is the second most common of the neurodegenerative disorders[1,2]. It affects approximately 1 % of individuals over the age of 60 years worldwide[3,4] and continues to be a critical area of research focus. Symptoms and diagnoses typically appear later in life, at an average age in the range of 64–66 years[5]. Due to an increasingly ageing population[6], the number of those affected by PD is expected to grow substantially in coming years[7]. Some estimates suggest an increase of 65 % for those over age 40 years by 2031[3], and others for the amount of affected individuals to double by 2050[7].

PD is characterised by the progressive degeneration of dopaminergic (DA) neurons within the substantia nigra pars compacta (SNc) region of the midbrain. This degeneration appears to be specific, as mesolimbic DA neurons of the ventral tegmental area remain largely unaffected. The death of the SNc DA neuronal population results in a depletion of striatal dopamine, which in turn leads to a range of motor impairments. At the onset of symptoms, approximately 60 % of SNc DA neurons are generally believed to have already been lost[8]. As a result, individuals experience rigidity of movement, tremors at rest, and a slowing or absence of voluntary movement[8]. These impairments affect daily tasks such as walking, writing, dressing and eating, all of which contribute to an overall negative impact on quality of life. Using a measure incorporating eight functional categories (cognition, emotion, speech, vision, mobility, dexterity, pain and discomfort), individuals living with PD were found to have a significantly reduced quality of life, which translated to moderate to severe disability in 82 % of individuals[3]. Because PD is chronic and progressive, symptoms also deteriorate further over time, leading to greater detriments to quality of life for patients.

In addition to these effects, those with PD have shown significantly greater risk for developing dementia compared with controls[9,10]. In PD dementia, diagnosis of dementia occurs after at least 1 year has passed since PD diagnosis. This form of dementia can be characterised by dysfunction in executive and visuospatial abilities, attention and memory, with the latter typically less affected than seen in Alzheimer's disease[11,12].

Abbreviations: 5-methylTHF, 5-methyltetrahydrofolate; CBS, cystathionine-β-synthase; COMT, catechol-*O*-methyltransferase; DA, dopaminergic; L-DOPA, levodopa or L-3,4-dihydroxyphenylalanine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTHFR, methylenetetrahydrofolate reductase; MTRR, methionine synthase reductase; P5P, pyridoxal-5'-phosphate; PD, Parkinson's disease; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SNc, substantia nigra pars compacta; *SNCA*, α-synuclein gene.

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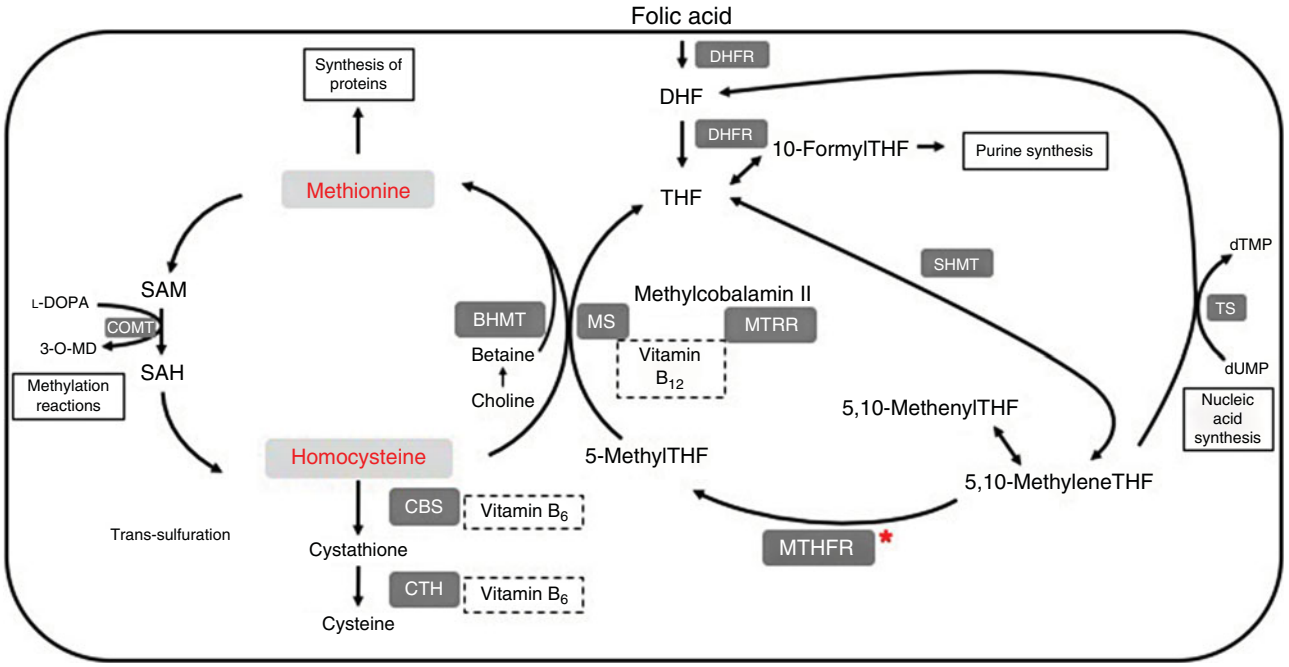

Fig. 1. Summary of one-carbon metabolism and levodopa (L-DOPA or L-3,4-dihydroxyphenylalanine) interaction. 3-*O*-MD, 3-*O*-methyldopa; BHMT, betaine homocysteine methyltransferase; CBS, cystathionine-β-synthase; COMT, catechol-*O*-methyltransferase; CTH, cystathionine γ-lyase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; MTRR, methionine synthase reductase; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; TS, thymidylate synthase.

A link is also suspected between another type of dementia, known as Lewy body dementia, and PD. In this form, symptoms of dementia occur before or in conjunction with motor impairment[13]. Cytoplasmic inclusions called Lewy bodies, consisting primarily of α-synuclein protein, can be found within brain tissue in PD resulting in Lewy body dementia, and may contribute to cognitive symptoms[14,15]. Though Lewy bodies are observed in other neurological conditions and are therefore not specific to PD, they are commonly found in patients with PD and are considered a pathological feature of the disease[15].

Research dedicated to PD continues to be of critical importance as the precise cause of the neurodegeneration affecting DA neurons remains unknown. As a result, current methods of treatment fail to target a cause, and instead treat only the identifiable symptoms. For instance, the widely used drug levodopa (L-DOPA (L-3,4-dihydroxyphenylalanine)) helps replenish lost striatal dopamine but fails to address the underlying cell death occurring within the SNc. Despite its ubiquitous use in the treatment of PD, L-DOPA can also have limited effectiveness, wear off over time, and elicit undesirable side effects[16]. As such, the need for further research to develop more effective and targeted treatments for those with PD is imperative.

Genetic and environmental factors have been the topic of numerous investigations, and have provided some insight into potential pathological mechanisms, such as α-synuclein aggregation and increased oxidative stress. Recently, emerging evidence has indicated a potential role for dietary factors[17], particularly B-vitamins, in the development of PD. Folate, a B-vitamin, is found naturally in foods such as leafy green vegetables and liver. A synthetic form of folate, folic acid, is found within vitamin supplements and has proven ability as a preventative measure against neural tube defects[18]. The protective effects of folates in neural tube defects led to mandatory fortification with folic acid in a number of countries worldwide[19]. In addition to the protective effects on neural tube development, folates are involved in nucleotide, protein and neurotransmitter synthesis, DNA repair, methylation, second messenger systems, ion channels, as well as the metabolism of homocysteine in the brain[20].

One-carbon metabolism is outlined in Fig. 1. Within cells, folate is converted to its main circulating form, 5-methyltetrahydrofolate (5-methylTHF), by the enzyme methylenetetrahydrofolate reductase (MTHFR). 5-MethylTHF is used by methionine synthase and vitamin B_{12} to remethylate homocysteine into methionine. The enzyme methionine synthase reductase (MTRR) maintains adequate levels of cobalamin for methionine synthase. Methionine can be converted to the methyl donor *S*-adenosylmethionine (SAM). After losing its methyl group, SAM becomes *S*-adenosylhomocysteine (SAH), which can then be metabolised further into homocysteine. Folate is therefore a cofactor in the metabolism of homocysteine, through its conversion to 5-methylTHF, which allows for remethylation of homocysteine into methionine.

Elevations in homocysteine can occur through several processes. Levels of folate and homocysteine generally show an inverse relationship, with low amounts of folate associated with elevations in homocysteine and vice versa. As a cofactor in the metabolism of homocysteine, low levels of folate result in a reduction in the amount of 5-methylTHF. Lower levels of 5-methylTHF mean a decline in the amount of homocysteine

https://doi.org/10.1017/S0954422419000106 Published online by Cambridge University Press

undergoing reconversion to methionine, allowing homocysteine to accumulate as a result. Therefore, a failure to obtain adequate levels of folates within the diet can be associated with high concentrations of homocysteine. Similarly, low dietary intake of vitamins B_6 and B_{12} also leads to increased levels of homocysteine.

Trans-sulfuration is an alternative pathway to reduce levels of homocysteine. In this reaction, cystathionine-β-synthase (CBS) converts homocysteine to cystathionine. The CBS knockout mouse model, the *CBS*$^{-/-}$ mouse, has significantly elevated plasma homocysteine levels (about 200 μM) and a low survival rate[21,22]. The mice that do survive do not develop neuropathologies[21,23]. This may be because *CBS*$^{-/-}$ mice do not have DNA methylation inhibition in brain tissue[22].

Alternatively, homocysteine levels can become elevated due to reduced levels of enzymes within one-carbon metabolism. For example, this can occur as a result of mutations in genes encoding for enzymes such as MTHFR or MTRR[24,25]. A polymorphism in *MTHFR* 677C→T leads to increased levels of homocysteine[26]. MTHFR-deficient mice have increased levels of homocysteine when compared with wild types[27]. Reduced levels of MTRR increase homocysteine in humans[28]. A gene trap mouse model of MTRR has mildly increased levels of homocysteine[29,30].

Increased levels of homocysteine have been associated with the development of several pathological conditions, including PD[31]. Interestingly, patients with PD have consistently been shown to have elevated levels of homocysteine compared with controls[31–36]. Furthermore, levels of homocysteine are increased in PD patients administered L-DOPA[37,38], as L-DOPA can be methylated using methyl groups from SAM, resulting in increases in SAH and homocysteine. Previous work has demonstrated that elevated levels of homocysteine increase risk for vascular disease[39]. PD patients with elevated levels of homocysteine may develop vascular disease[40,41].

At present, it is unknown how precisely nutritional factors might play a role in PD. Given the close relationship between B-vitamins and homocysteine, the latter of which has shown an association with PD, it seems likely that these dietary factors have at least some level of involvement. The importance of understanding all factors, both genetic and environmental, at play in the degenerative process is clear in order to develop more effective and targeted treatments for those with PD. Such knowledge could also lead to significant improvements in quality of life for those affected by PD. With this in mind, and with emerging evidence that has indicated a possible role for dietary factors in PD development[17], investigations into nutritional factors appear particularly timely since therapeutic development for PD has been slow. The aim of the present review is to determine whether one-carbon metabolism and homocysteine are involved in PD onset and related pathology and identify potential mechanisms related to this.

Materials and methods

Publications using medical subject headings (MeSH) keywords, one-carbon metabolism, folate, folic acid, MTHFR, Parkinson's disease, L-DOPA and homocysteine were retrieved. Studies involving human subjects were categorised into three areas: polymorphisms in one-carbon metabolism; dietary intake of B-vitamins; and methylation. For each study we collected the following data: the country in which the study was conducted; whether government fortification was present; study design; sample size; and the main findings of the study. All of these findings are summarised in Tables 1 to 4. Case–control, prospective cohort, meta-analysis and non-randomised trials were included in the review. To understand the mechanistic interactions of one-carbon metabolism on PD pathology, we analysed *in vitro* and *in vivo* studies. For each study, we collected the model system information, design of the study, and the major findings. These results are summarised in Table 5.

Results

Polymorphisms in one-carbon metabolism may increase risk for Parkinson's disease development

Genetic factors may contribute to homocysteine accumulation through polymorphisms in enzymes involved in one-carbon metabolism. The data from our literature search are summarised in Table 1. One of the most common of these is a polymorphism in *MTHFR* 677C→T, which has been observed at greater frequencies in PD patients than the general population in some studies[24,42], while no differences have been seen in others[43]. An Italian case–control study found that the 677TT polymorphism was significantly more frequent in PD patients than controls, after controlling for age, sex, folate and vitamin B_{12} status[24]. In a prospective cohort study in the Netherlands, the TT variant was associated with increased risk for PD in individuals who smoke[42]. A study conducted in Poland found no differences in the distribution of MTHFR alleles in PD compared with control groups[43]. Regardless of their association with PD, polymorphisms in *MTHFR* such as 677C→T have been linked to elevated homocysteine concentrations in individuals, including those with PD[42–45]. It was also demonstrated that the T allele, along with disease status, influenced homocysteine levels in participants, and that significant interaction between these two factors existed[43]. Results from meta-analysis have also supported a relationship, finding an increased susceptibility associated with the T allele[45,46].

Other enzymes involved in one-carbon metabolism have also been shown to be affected, for example, MTRR, which maintains adequate levels of cobalamin during the conversion of homocysteine into methionine[47]. *MTRR* 1049GG was significantly associated with PD in a Chinese population in a case–control study[47]. These results suggest that there is an increasing risk for PD with the number of polymorphisms that interfere with normal one-carbon metabolism. It therefore appears that such polymorphisms in enzymes involved in one-carbon metabolism may contribute in some way to PD development.

Role of B-vitamins in Parkinson's disease onset and progression

Several studies have investigated the impact of dietary B-vitamins on PD development; the results are summarised in Table 2. A study in Mexico examined the nutritional status of patients with PD[48]. Patients reported both losing weight (73 %) and decreased food intake (67 %), which may have been the result of PD itself or

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Table 1. Impact of polymorphisms of enzymes in folate metabolism on Parkinson's disease (PD) onset and pathology

Reference	Country	Government fortification	Design	Sample size	Major finding
Gorgone *et al.* (2012)[24]	Italy	No	Case–control	PD: 60; control: 82	MTHFR 677TT polymorphism was significantly more frequent in PD patients than controls
de Lau *et al.* (2005)[42]	Netherlands	No	Prospective cohort	Cohort: 5920; incident PD cases: 65	TT variant was associated with increased risk for PD in smokers
Białecka *et al.* (2012)[43]	Poland	No	Case–control	PD: 320, controls: 254	No differences in the distribution of MTHFR alleles in PD compared with control groups were seen
Wu *et al.* (2013)[46]	–	–	Meta-analysis	Fifteen studies included	An increased susceptibility associated with the T allele in European as well as Asian populations was found
Zhu *et al.* (2015)[45]	–	–	Meta-analysis	Fifteen studies included	A significant association between 677TT and PD in Europeans but not Asians was observed
Fong *et al.* (2011)[47]	Taiwan	No	Case–control	PD: 211; controls: 218	MTRR 1049GG was significantly associated with PD. Risk was greater for those with several polymorphisms, including MTHFR 677TT

MTHFR, methylenetetrahydrofolate reductase; MTRR, methionine synthase reductase.

Table 2. Role of dietary intake of B-vitamins on Parkinson's disease (PD) onset and progression

Reference	Country	Government fortification	Design	Sample size	Major finding
dos Santos *et al.* (2009)[33]	Brazil	Yes	Case–control	PD: 52; controls: 69	PD patients with folic acid levels <13 ng/ml had elevated homocysteine while those with concentrations >13 ng/ml showed no significant difference with respect to controls
Murakami *et al.* (2010)[49]	Japan	No	Case–control	PD: 249; controls: 368	PD patients had lower folate intake than controls. Intake of folate, vitamin B_{12} and riboflavin intake were not associated with PD risk
Chen *et al.* (2004)[52]	USA	Yes	Prospective cohort	Cohort: 136 057; PD: 415	Higher dietary intake of folate, vitamins B_6 and B_{12} were not associated with lower risk of PD
de Lau *et al.* (2006)[53]	Netherlands	No	Prospective cohort	Cohort: 5920; incident PD cases: 65	No observed effects of dietary folate or vitamin B_{12} on PD risk. Low vitamin B_6 intake was associated with increased PD risk in smokers
Shen (2015)[51]	–	–	Meta-analysis	Ten studies included	Observed lower levels of vitamin B_{12} in both L-DOPA-treated and untreated PD patients compared with controls. Higher dietary vitamin B_6 intake was associated with decreased risk of PD
Ibrahimagic *et al.* (2016)[60]	Bosnia and Herzegovina	No	Non-randomised trial	PD: 30	Periodic supplementation with 5 mg/d folic acid for 1–2 months every 6 months reduced homocysteine levels in all patients studied
Christine *et al.* (2018)[50]	USA	Yes	Prospective cohort	PD: 679	In PD patients, lower levels of vitamin B_{12} at baseline resulted in reduced mobility. Higher levels of homocysteine caused more cognitive decline

L-DOPA, levodopa or L-3,4-dihydroxyphenylalanine.

Table 3. Impact of methyl groups generated by folate metabolism on Parkinson's disease (PD) patients

Reference	Country	Government fortification	Design	Sample size	Major finding
Obeid *et al.* (2009)[35]	Germany	No	Case–control	PD: 87; COMTi usage data: N/A	Low levels of SAM were associated with increased α-synuclein. Improved cognitive ability was also associated with higher SAM:SAH ratios
Jowaed *et al.* (2010)[62]	Germany	No	Case–control	PD: 6; controls: 6	Methylation of *SNCA* was reduced in PD patients in SNc, putamen and cortex samples compared with controls

COMTi, catechol-*O*-methyltransferase inhibitor; N/A, not available; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; *SNCA*, α-synuclein gene; SNc, substantia nigra pars compacta.

of L-DOPA use. PD patients in Japan also had lower folate intake than controls[49]. After adjusting for potential confounding factors, folate, vitamin B_{12} and riboflavin intakes were not associated with PD risk[49]. This is worth noting, as it has been observed in other studies that the increased homocysteine levels of PD patients may be due in significant part to low folate levels[33]. In one such study, PD patients with folate levels below 13 ng/ml had elevated homocysteine compared with controls, while those with folate concentrations above 13 ng/ml showed no significant difference with respect to controls[33]. Such lower levels of B-vitamins may contribute to increased homocysteine levels in PD patients, either on their own or through a loss of potential protective effects against homocysteine increases precipitated by other factors.

In advanced PD, patients have lower levels of serum vitamin B_{12}, which can lead to further neuropathy and cognitive impairment[50,51]. Lower B_{12} levels during early PD have also been reported to result in more mobility issues in patients, whereas higher levels of homocysteine resulted cognitive decline in patients[50]. Further studies have investigated whether dietary intake of B-vitamins may reduce the risk of PD. One such study in the USA examined intake of dietary folate in addition to vitamins B_6 and B_{12} and their relationship to the risk of PD[52]. They found that higher dietary intake of B-vitamins was not associated with lower risk of PD. A study in the Netherlands also found no effects of dietary folate or vitamin B_{12} on PD risk[53]. However, an association for vitamin B_6 was found, but was significant only in smokers. These findings are also supported by a meta-analysis, which found an overall decreased risk of PD with high vitamin B_6 intake (OR 0·65; 95 % CI 0·30, 1·01)[51].

It is important to consider that such studies are often based on diet questionnaires, which may not provide completely accurate data regarding dietary and supplement consumption and can be subject to recall error and bias[54–57]. Some countries also have mandated folic acid fortification of grains, which would increase baseline levels for their citizens[58,59]. Additionally, consumption is not necessarily equivalent to circulating levels due to differences in metabolic processes between individuals. As a result, future studies may wish to combine such questionnaires regarding intake with physiological measurements such as plasma samples to provide a more accurate picture of dietary factors. Studies including sufficient numbers of participants are also critical to parse potential interactive effects of L-DOPA on homocysteine levels.

A non-randomised trial examined the impact of folic acid supplementation on patients with PD[60]. Periodic supplementation with 5 mg/d folic acid for 1–2 months every 6 months reduced homocysteine levels in all patients studied[60]. If patients discontinued use for 2 months, this effect remained intact for 90 % of patients. After 4 months, homocysteine would return to elevated levels. This study had a small number of participants and failed to consider other factors such as *MTHFR* polymorphism or dietary B-vitamin intake but provides a basis for further investigation into whether B-vitamin supplementation may aid in reducing homocysteine levels, which may then lessen its harmful effects.

Reduced methyl groups generated from folate metabolism and impact on gene expression of Parkinson's disease-specific genes

Effects on methylation in PD patients are also of interest in the study of the disease. The results of our findings regarding methylation effects are summarised in Table 3. As discussed in previous sections, folates and other B-vitamins are linked to methylation reactions through one-carbon metabolism, particularly through effects on levels of the methyl donor SAM. The potential impact of changes in methylation patterns in PD has been reviewed previously[61]. Briefly, DNA methylation can block the recognition of binding sites by transcription factors, impairing gene expression by interfering with transcription. Additionally, other proteins can recognise methylated sites and recruit repressor proteins that then lead to gene repression[61].

A case–control study investigated methylation patterns of the human gene for α-synuclein (*SNCA*), and found that methylation of *SNCA* intron 1 led to a decrease in gene expression, while inhibiting methylation activated expression[62]. Methylation potential in patients with PD was reduced at this site in SNc,

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Table 4. Impact of levodopa or L-3,4-dihydroxyphenylalanine (L-DOPA) on B-vitamin metabolism in Parkinson's disease (PD)-affected patients

Reference	Country	Government fortification	Design	Sample size	Major finding
Yasui *et al.* (2003)[67]	Japan	No	Case–control	PD: 20; controls: N/A	No significant difference in homocysteine levels for PD patients before L-DOPA and controls. After patients began L-DOPA treatment, significant increases in homocysteine occurred
Yasui *et al.* (2000)[68]	Japan	No	Case–control	PD: 90; controls: 50	PD patients with *MTHFR* 677TT polymorphism had significantly higher levels of plasma homocysteine. Homocysteine levels were negatively correlated with serum folate
Camicioli *et al.* (2009)[32]	Canada	Yes	Case–control	PD: 51; controls: 50	Homocysteine levels were elevated in PD patients compared with controls; this was affected by multiple factors including PD, MTHFR genotype, B-vitamin intake, and folate level
Isobe *et al.* (2010)[69]	Japan	No	Case–control	PD: 18; controls: 16	Homocysteine levels in cerebrospinal fluid were significantly elevated in PD patients before L-DOPA treatment compared with controls. Levels after treatment were higher than levels before treatment, and greater for those taking higher doses of L-DOPA
Religa *et al.* (2006)[70]	Poland	No	Case–control	PD (treated): 99; PD (untreated): 15; controls: 100	PD patients had elevated levels of homocysteine which did not depend on the dosage of L-DOPA. Disease duration also significantly had an impact on homocysteine levels
Kocer *et al.* (2016)[71]	Turkey	No	Case–control	L-DOPA + COMTi: 30; L-DOPA: 58	No significant differences in homocysteine levels were observed between groups
Obeid *et al.* (2009)[35]	Germany	No	Case–control	PD: 87; COMTi usage data: N/A	COMTi did not significantly rescue SAM or reduce total homocysteine
Lamberti *et al.* (2004)[72]	Italy	No	Case–control	L-DOPA + COMTi: 20, L-DOPA: 26; controls: 32	Homocysteine was significantly increased in both groups of PD patients compared with controls. Those treated with L-DOPA+COMTi had significantly lower levels of homocysteine compared with those that received L-DOPA only
Kuhn *et al.* (1998)[40]	Germany	No	Case–control	PD: 22; controls: 22	Total homocysteine levels were significantly elevated in PD patients. There was a significant relationship between homocysteine and L-DOPA dose
Müller *et al.* (1999)[41]	Germany	No	Case–control	PD: 45; controls: 15	Plasma homocysteine levels were high in PD patients treated with L-DOPA compared with untreated PD patients and controls
Miller *et al.* (2003)[66]	USA	Yes	Case–control	PD: 20; PD + L-DOPA: 20	Plasma homocysteine levels were high in PD patients treated with L-DOPA when compared with PD patients naïve to L-DOPA. Plasma homocysteine levels were correlated with plasma folate and vitamin B_{12} in treated PD group

N/A, not available; MTHFR, methylenetetrahydrofolate reductase; COMTi, catechol-*O*-methyltransferase inhibitor; SAM, *S*-adenosylmethionine.

Table 5. Mechanisms through which folate metabolism may be involved in Parkinson's disease onset and progression

Reference	Animal model	Design	Major finding
Duan *et al.* (2002)[76]	C57BL/6 mouse	First study: mice maintained on control or FADD for 3 months before MPTP administration. Second study: mice given MPTP then homocysteine administered to striatum or SNc	Reduced numbers of surviving DA neurons following MPTP in FADD compared with a control diet. Direct infusion of homocysteine into SNc produced similar effects following MPTP
Lee *et al.* (2005)[77]	C57BL/6 mouse	Mice received i.p. injections of homocysteine daily for 36 d	Elevated homocysteine decreased tyrosine hydroxylase immunoreactivity as well as locomotor activity
Bhattacharjee *et al.* (2016)[82]	Swiss albino mouse	Mice given MPTP or saline daily for 5 d, followed by L-DOPA for 28 d	L-DOPA treatment led to increased homocysteine in both MPTP- and saline-treated mice. Levels were higher in MPTP treated- *v.* saline-treated mice. L-DOPA did not increase SNc DA cell death compared with MPTP alone
Liu *et al.* (2000)[65]	Swiss albino mouse	Mice received single or multiple i.p. injections of L-DOPA	L-DOPA increased the amount of SAH, decreased SAM, and decreased SAM:SAH ratios
Srivastav *et al.* (2015)[86]	*Drosophila*	Recessive allele resulting in reduced mRNA and null Parkin protein was introduced. Supplemental folate was then added	*Drosophila* given folic acid supplementation showed recovery from detrimental effects of parkin mutation
Miller *et al.* (1997)[84]	Rat	Rats received Ro 41-0960 (COMT inhibitor) i.p. injection before L-DOPA i.p. injection. A control group of animals was not administered the inhibitor	Rats that received the inhibitor did not have higher levels of homocysteine or SAH, or reduced levels of SAM
Daly *et al.* (1997)[80]	Rat	Study 1, control and folic acid-deficient diet rats injected with L-DOPA i.p., homocysteine levels measured. Study 2, rats injected with L-DOPA i.p. for 0, 1 or 17 d. One injection per d	Rats maintained on folic acid-deficient diet had higher levels of homocysteine after L-DOPA treatment. One injection of L-DOPA resulted in higher levels of homocysteine compared with 17 d
Ordonez & Wurtman (1974)[83]	Rat	Rats were maintained on control, folic acid-deficient or -supplemented diet before L-DOPA administration	Rats deficient in folic acid have reduced levels of methionine in brain tissue and serum after L-DOPA administration
Taufek & Bone (1980)[79]	Rat	Rats injected i.p. with L-DOPA and monoamine oxidase inhibitor for 3 weeks. One group received methionine supplementation	L-DOPA decreases concentrations of SAM and methionine. These effects were reversed by an adequate intake of methionine

FADD, folic acid-deficient diet; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SNc, substantia nigra pars compacta; DA, dopaminergic; i.p., intraperitoneal; L-DOPA, levodopa or L-3,4-dihydroxyphenylalanine; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; COMT, catechol-*O*-methyltransferase.

putamen and cortex samples compared with controls. When an inhibitor of DNA methylation was applied, methylation of the *SNCA* region in question was specifically reduced, and increased amounts of both α-synuclein mRNA and protein were produced.

The potential clinical consequences of reduced methylation in PD patients were investigated in another case–control study[35]. Levels of SAM and α-synuclein were shown to be inversely related in this study, with low levels of SAM associated with increased α-synuclein. Improved cognitive ability was also associated with higher methylation potential, as assessed by higher SAM:SAH ratios. The researchers offered two possible explanations for their findings. They suggest that decreased SAM leads to hypomethylation of *SNCA*, which results in greater α-synuclein expression. This is consistent with the findings of Jowaed *et al.*[62]. Second, they propose that increased SAH may act to inhibit processes involved in the repair of damaged proteins such as α-synuclein. Damaged α-synuclein is prone to aggregation, thus this action would also lead to increased accumulation. Therefore, the potential effects of homocysteine in PD extend beyond contributions to neurodegeneration and oxidative stress and may also influence gene expression and pathological protein aggregation.

Treatment with L-DOPA (levodopa (L-3,4-dihydroxyphenylalanine)) makes an impact on one-carbon metabolism

L-DOPA is the most widely used treatment for PD and has been reported to affect folate metabolism[37,38]. Data are summarised in Table 4. Numerous studies have demonstrated that patients with PD consistently show elevated levels of homocysteine compared with controls[31–35,40,41,63]. However, it remains uncertain if this is due to PD, as additional research has indicated that L-DOPA can also contribute to elevated homocysteine. L-DOPA increases the amount of homocysteine in circulation by receiving a methyl group provided by SAM, converting SAM to SAH which is then readily hydrolysed into homocysteine[64,65]. This reaction involves the enzyme catechol-*O*-methyltransferase (COMT). Because L-DOPA is the most widely used treatment for PD, this no doubt has an impact on measurements

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taken from PD patient populations, as the majority are taking this drug. Indeed, several studies have found that homocysteine levels were significantly higher in PD patients treated with L-DOPA compared with non-treated individuals[25,44,66]. A case–control study examined PD patients before receiving L-DOPA compared with controls, and observed no significant difference in homocysteine levels[67]. After patients began receiving L-DOPA, all but one individual experienced a significant increase in plasma homocysteine. Researchers also observed that the increases were most notable for those with the 677C→T *MTHFR* polymorphism. Relative increases of 35·0 (SD 21·3) % for the CC genotype, 44·8 (SD 36·7) % for CT, and 156·2 (SD 108·5) % for the TT genotype were observed[67]. The high standard deviations are probably due to the small numbers of participants in this study, which had only twenty participants in total, and six, eight and six patients with the CC, CT and TT genotypes, respectively. Nevertheless, this study indicates a potential influence of polymorphisms in folate metabolism on the effects of L-DOPA on homocysteine levels. Another study investigating the impact of L-DOPA on PD patients found that homocysteine levels were elevated in TT genotype participants and that homocysteine levels were negatively correlated with serum folate levels[68].

Such an impact as a result of additional factors has also been suggested in other studies. For example, another case–control study reported that elevated levels of homocysteine in PD patients compared with controls were affected by multiple factors, including PD, *MTHFR* 677C→T polymorphism, B-vitamin intake and folate level[32]. When total homocysteine levels were examined in cerebrospinal fluid of PD patients both before and after L-DOPA treatment and in healthy controls, it was reported that PD patients had significantly higher levels of homocysteine than controls before treatment, and levels after treatment were significantly higher than before treatment in those with PD[69]. The difference in the total homocysteine concentration before and after treatment was also greater for those who took higher doses of L-DOPA (450 *v.* 300 mg/d), again indicating that L-DOPA is affecting homocysteine levels. Conversely, another study examined patients on different dosages of L-DOPA in comparison with controls, including non-treated patients, and found that while PD patients had elevated levels of homocysteine, levels did not depend on the dosage of L-DOPA that patients were receiving[70]. Also observed was a trend toward higher homocysteine levels in non-treated PD patients compared with controls[70], though there were only a small number of participants in this group (fifteen compared with ninety-nine L-DOPA-treated patients). They also found that disease duration significantly influenced homocysteine levels[70]. These results tend to indicate the potential for interaction between L-DOPA use, polymorphisms in folate metabolism, B-vitamin intake, and disease duration in the development and progression of PD. Based on these findings, PD patients who have been administered L-DOPA for a long period of time may benefit from supplementation of folic acid or other B-vitamins[41,66].

Another approach that researchers have employed to disentangle the potential effects that L-DOPA has on homocysteine is examining the effects of COMT inhibitors in conjunction with L-DOPA treatment. As mentioned earlier, COMT is involved in the *O*-methylation of L-DOPA that converts SAM into SAH, which is further hydrolysed into homocysteine (Fig. 1). If elevated homocysteine levels are due to increased amounts produced from SAH through this mechanism, inhibiting COMT should result in a reduction in homocysteine levels compared with individuals taking only L-DOPA. Results from such investigations have been conflicting. In one study, the COMT inhibitor, Entacapone, was administered to one group of PD patients receiving L-DOPA, while another received L-DOPA alone[71]. No significant differences in homocysteine levels were observed between the groups. Similar results were reported in another study that found that COMT inhibitors did not significantly rescue SAM or reduce total homocysteine[35]. Such findings suggest that if homocysteine levels are in fact elevated by L-DOPA use, this may occur through means other than via COMT-mediated methylation of L-DOPA. However, several key significant differences were observed between the treatment groups, including age, disease duration and age at disease onset, which may have had confounding effects on homocysteine levels.

In another study, three groups consisting of PD patients treated with L-DOPA, PD patients treated with L-DOPA and a COMT inhibitor, and controls were examined[72]. Plasma levels of homocysteine were significantly increased in both groups of PD patients compared with controls. When compared with each other, those treated with a COMT inhibitor in addition to L-DOPA had significantly lower levels of homocysteine compared with those who received L-DOPA only. There were also fewer patients in this group that had pathologically high (over 20 μmol/l) levels of homocysteine, with only 5 % compared with 30 % of those in the L-DOPA only group (the control group had none). An important consideration and potential confounding factor in this study was that folate concentrations were found to be significantly lower in the group treated with L-DOPA alone. As discussed previously, low levels of folate are associated with high homocysteine levels. Additionally, other studies have found no impact of L-DOPA on homocysteine levels, suggesting that L-DOPA may be interacting with other factors, such as folate deficiency or the duration of the disease, to elicit effects on homocysteine[33]. This theory is supported by further research that has proposed that the extent of elevated homocysteine in PD patients can be influenced by their folate status[66]. However, the researchers performed additional analysis that claimed that the homocysteine elevations observed in L-DOPA-alone group were due to the absence of a COMT inhibitor, rather than decreased folate levels[72]. Nevertheless, it will prove important to design future studies to control for differences in B-vitamin status, along with other potential confounding factors, in order to determine the precise roles of each on PD development and progression. Overall, these findings suggest that while treatment with L-DOPA probably has an impact on homocysteine levels, it cannot be ruled out entirely that factors such as PD contribute to observed elevations. L-DOPA also interacts with the transsulfuration component of one-carbon metabolism. Specifically, with the active form of vitamin B_6, pyridoxal-5'-phosphate (P5P), it sequesters P5P from P5P-dependent enzymes including CBS, potentially resulting in a functional B_6 deficiency[73,74].

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Mechanisms through which folate metabolism may influence Parkinson's disease onset and progression

To better understand how one-carbon metabolism may impact PD onset and progression, researchers have turned to *in vitro* and *in vivo* model systems. Summaries of the study details are listed in Table 5. *In vitro*, folate deficiency in cell culture has been shown to elicit neurodegeneration and increase reactive oxygen species (ROS) production[75]. Direct exposure of homocysteine to cells also produced the same effects, while inhibition of homocysteine formation was effective at suppressing increases in ROS. Such results reinforce the inverse relationship between folate and homocysteine. In another study, homocysteine exposure in combination with the pesticide rotenone or Fe led to increased membrane depolarisation in the mitochondria of human DA cells[76]. Mitochondrial ROS levels also increased with rotenone or Fe exposure and were further exacerbated by homocysteine but were suppressed by treatment with an antioxidant or an inhibitor of DNA damage.

In rodent studies, when folate-deficient diets have been combined with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exposure, they result in reduced numbers of surviving DA neurons and increased motor impairment compared with a control diet[76]. Direct infusion of homocysteine into the SNc has also been shown to produce these same effects[76]. Because these observations were seen only when MPTP was administered, and not when homocysteine was administered on its own, this may imply that folate deficiency and elevations in homocysteine act to increase vulnerability to neurodegeneration, rather than triggering cell death directly. However, other experiments have found that homocysteine on its own can prove toxic for DA neurons[77,78]. In a study investigating the effects on rat primary mesencephalic cells, homocysteine enhanced cell death in response to MPTP in a dose-dependent manner[78]. Cells with intracellular dopamine were also more vulnerable to homocysteine's toxic effects than other cells. Similarly, Lee *et al.*[77] found that homocysteine decreased tyrosine hydroxylase immunoreactivity as well as locomotor activity in mice after it was administered chronically over a period of 36 d. These inconsistent findings underline the need for further investigation regarding this topic.

The effects of L-DOPA treatment in mouse models of PD have also provided some insight into how it may affect homocysteine accumulation. Acute *v.* chronic treatment has been reported to increase homocysteine and reduce levels of SAM[79–81]. Furthermore, when animals were placed on a folic acid-deficient diet there was a more robust response to L-DOPA compared with control animals[80], suggesting an interaction between folate and L-DOPA metabolism. In non-MPTP- and MPTP-treated mice, chronic treatment with L-DOPA leads to significant increases in homocysteine in the SNc[82]. L-DOPA also significantly increased the level of homocysteine in the SNc in MPTP-treated mice compared with the non-MPTP mice that also received L-DOPA. Researchers also examined the effect that these treatments had on DA cells in the SNc. They observed a 51 % reduction in TH+ cells for the MPTP plus L-DOPA group *v.* 47 % for MPTP alone. Therefore, the significant increase in homocysteine in the former did not result in a significantly greater loss of DA cells in the SNc. This is in contrast to the results seen by Duan *et al.*[76], who observed an increase in neurodegeneration following MPTP when homocysteine was increased using folate-deficient diets or was directly infused[76]. These differences may be due to differences in MPTP dosages between the studies, or the length of time between the last MPTP treatment and euthanisation[76,82]. Of course, L-DOPA may also have had additional effects in the latter study that affected the degeneration of DA cells. However, another study also examined mice after more than 30 d of homocysteine injections, and observed significantly increased homocysteine levels in the striatum, impaired locomotor activity, and reduced tyrosine hydroxylase immunoreactivity in the SNc[77]. Mouse models have also been used to study L-DOPA's impact on methylation processes. Administration of L-DOPA increased the amount of SAH, decreased SAM, and decreased SAM:SAH ratios in one study[65]. The brain is capable of *de novo* synthesis of methyl groups; however, when animals are deficient in dietary folic acid combined with L-DOPA, this results in a significant decrease in methionine levels in brain tissue and serum[83]. Another study demonstrated that blocking COMT with the inhibitor Ro 41-0960 can prevent the L-DOPA changes, such as decreased SAM and increased SAH and homocysteine levels in a rat model[84]. Furthermore, methionine intake can reverse the impact of L-DOPA in methyl group depletion[79]. This lends support to work demonstrating the potential for L-DOPA to influence gene expression.

Despite the lack of clear evidence suggesting that B-vitamin intake reduces risk of PD, the effects of B-vitamin supplementation on PD pathology have also been investigated. In one study, the effect of supplementation using several B-vitamin doses in several combinations was examined both in terms of behaviour and effect on levels of homocysteine in a rat model of PD[85]. 6-Hydroxydopamine, a well-characterised model of DA degeneration, was administered to the striatum. Rats that received ten times the folate normally given in a minimum essential medium diet had improved performance on rotational behaviour testing and rotarod[85]. The groups receiving five times the combination of B-vitamins also performed at levels close to control on the rotarod. Interestingly, levels of homocysteine were not reduced in any of the groups, and were in fact elevated in comparison with controls[85]. This unexpected finding indicates that such supplementation is providing beneficial effects through means other than decreasing homocysteine concentrations. Another study examined the effect of supplemental folate in a *Drosophila* model of early-onset familial PD[86]. Researchers introduced a novel recessive allele for the parkin gene, which resulted in reduced mRNA and null amounts of the Parkin protein. Parkin has a role in the degradation of unfolded proteins and is linked to normal mitochondrial function. The absence of Parkin resulted in numerous deficiencies in homozygous flies, including increased pupal lethality, decreased progression to later life stages and impaired motor function[86]. It also increased oxidative stress, while reducing antioxidant activity and mitochondrial functionality, as evidenced by lower levels of ATP[86]. Individuals given a 10–250 μM effective dose of folic acid showed at least partial recovery. Reductions in lethality, greater numbers of flies transitioning to later life stages, and improved

https://doi.org/10.1017/S0954422419000106 Published online by Cambridge University Press

motor function were observed. Oxidative stress decreased and the amount of ATP present increased, indicating improved mitochondrial function. These observations suggest that folate supplementation may be useful for attenuating some of the effects of DA degeneration in PD.

Discussion

The present review assessed the role of one-carbon metabolism and homocysteine on the onset and progression of PD, as well as potential mechanisms of action. Our findings suggest that genetic polymorphisms in one-carbon metabolism may play a role in the risk of developing the disease, possibly through increased levels of homocysteine. Dietary levels of B-vitamins such as folate may be reduced in PD-affected patients. However, any benefit of B-vitamin supplementation for PD patients in general remains undetermined. To replenish the loss of dopamine, L-DOPA is frequently administered to PD-affected patients, leading to increased levels of homocysteine. For this reason, supplementation with B-vitamins may be beneficial for PD patients taking L-DOPA. Folate metabolism is also involved in generating methyl groups that are used for methylation reactions and gene expression. The literature suggests that there are methylation changes in PD-specific genes which may affect PD progression. The mechanisms through which folate metabolism makes an impact on PD progression remain undefined, but a consensus in the literature suggests that deficiencies in folate can increase vulnerability to damage.

Recent epidemiological data have suggested that homocysteine may be involved in the onset and progression of neurodegenerative diseases[87]. Using *in vitro* models, the link between homocysteine and neurodegenerative disease may be indicated by increased DNA damage[88,89], oxidative stress[90–93] and apoptosis[94]. The concentration of homocysteine used in many *in vitro* studies ranges from 0·5 to 300 μM[88,91,93]. It is important to note that the levels of homocysteine used in *in vitro* studies do not match the physiological levels (about 4 to 10 μM) present in living systems[31,32]. The association between high levels of homocysteine and PD is unclear[31,32,95]. Supplementation of B-vitamins has been reported to result in amelioration of symptoms associated with PD in both human[60] and model system studies[86]. In animal studies, there is a minimal reduction in homocysteine levels[85]. These reports suggest that homocysteine may be a marker for disease state and not the cause of disease onset and progression. Increased levels of homocysteine can be a result of deficiencies in folate metabolism, the nutrient choline, as well as reduced activity in the trans-sulfuration pathway.

PD is a complex disease and the cause of the loss of DA cells is not well understood. Both genetics[96] and dietary factors[17] have been implicated in disease pathology. Folates play an important role in the brain because of their functions in nucleotide synthesis, DNA repair, methylation, membrane lipid metabolism, second messenger signalling, ion channels and neurotransmitter and protein synthesis[20]. The elderly population is often deficient in B-vitamins, such as folate, riboflavin, vitamin B_{12} and vitamin B_6[97,98]. This is because of age-related changes in absorption of nutrients from food[99]. Supplementation with B-vitamins may be necessary for PD-affected individuals to sustain optimal levels of these vitamins. B-vitamins are also involved in the generation of methyl groups and SAM; therefore changes in gene expression may be affecting disease progression. Because of the many roles that B-vitamins have within the brain, low intake or status could render cells in the brain, specifically DA cells, vulnerable to degeneration. For PD patients with high levels of homocysteine, B-vitamin supplementation should be considered. This point is particularly important for PD patients taking L-DOPA, which can further increase homocysteine levels.

Conclusion and future directions

Our analysis of clinical and basic science studies suggests that the data still remain unclear; one-carbon metabolism and homocysteine may be involved in PD onset, pathology and progression, but the precise relationships have yet to be determined. Future research using more clinically relevant model systems that investigate mechanisms of action using environmental toxin PD models, as well as randomised controlled studies in human subjects to understand whether dietary supplementation is beneficial for PD patients, will provide more insight.

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Acknowledgements

The authors would like to thank Marc T. Avery for his useful comments on the manuscript.

This research was supported by a Canadian Institutes of Health Research studentship (L. K. M.) and a Natural Sciences and Engineering Research Council of Canada fellowship (N. M. J.).

Each author read and approved the final submitted manuscript. L. K. M. conducted the introduction, human bibliographic research and wrote the manuscript. N. M. J. conceptualised the study, conducted the model system bibliographic research, interpreted the data, supervised, reviewed and wrote the final draft of the manuscript.

There are no conflicts of interest.

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