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The Role of Crucial Biochemical Parameters and Key Gene Polymorphisms in the One-Carbon Pathway in Parkinson's Disease: Case-Control Study

Tek Karbon Yolağındaki Önemli Biyokimyasal Parametrelerin ve Gen Polimorfizmlerinin Parkinson Hastalığındaki Rolü: Olgu Kontrol Çalışması

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ABSTRACT Objective: Parkinson's disease (PD) is the second most common neurodegenerative disease. The cause of PD is shown to be the decrease or degradation of dopaminergic activity in the brainstem region, which is thought to be triggered by environmental factors, aging, infectious agents, and other pathological conditions. Abnormalities in the folate-mediated one-carbon pathway may play a role in the pathophysiology of PD as it increases total homocysteine (Hcy) levels. We aimed to show possible associations between folate, Hcy, cysteine (Cys), and vitamin B_{12} levels, which function in the one-carbon pathway, and PD risk. Furthermore, the effect of genetic polymorphism of *MTHFR* and *MTR*, which are involved in the one-carbon pathway, on PD risk was investigated. **Material and Methods:** 108 patients diagnosed with PD and 97 healthy volunteers participated in this study. The biochemical parameters were measured by ELISA, and genetic polymorphisms analyzed by polymerase chain reaction-restriction fragment length polymorphism. **Results and Conclusion:** After adjustment for confounding factors such as age, smoking habit, and gender, there was a statistically significant increase in the risk of PD as folic acid levels increased and as vitamin B_{12} and Hcy levels decreased. These findings suggest that extra folic acid intake in the patients' diets may have been the cause of these findings. Higher Hcy levels were observed in PD patients with the *MTHFR C677T TT* genotype and the *MTR A2756G GG* genotype. However, this difference was not significant. No effect of Cys levels on PD risk was observed. *MTHFR C677T* and *MTR A2756G* gene polymorphisms were not found to be risk factors for PD.

Keywords: Folic acid; genetic polymorphism; homocysteine; one-carbon group transferases; Parkinson's disease

ÖZET Amaç: Parkinson hastalığı (PH) en yaygın ikinci nörodejeneratif hastalıktır. PH'nin nedeni, çevresel faktörler, yaşlanma, enfeksiyöz ajanlar ve diğer patolojik koşullar tarafından tetiklendiği düşünülen beyin sapı bölgesindeki dopaminerjik aktivitenin azalması veya bozulması olarak gösterilmektedir. Folat aracılı tek karbon yolağındaki anormallikler, toplam homosistein [homocysteine (Hcy)] seviyelerini artırdığı için PH patofizyolojisinde rol oynayabilir. Tek karbon yolağında işlev gören folat, Hcy, sistein [cysteine (Cys)] ve vitamin B_{12} düzeyleri ile PH riski arasındaki olası ilişkileri göstermeyi amaçladık. Ayrıca, tek karbon yolunda yer alan *MTHFR* ve *MTR* genetik polimorfizminin PH riski üzerindeki etkisi araştırılmıştır. **Gereç ve Yöntemler:** Bu çalışmaya 108 PH tanısı almış hasta ve 97 sağlıklı gönüllü katılmıştır. Biyokimyasal parametreler ELISA ile ölçülmüş ve genetik polimorfizmler polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi ile analiz edilmiştir. **Bulgular ve Sonuç:** Yaş, sigara alışkanlığı ve cinsiyet gibi karıştırıcı faktörler ayarlandıktan sonra, artan folik asit seviyeleri ve azalan B_{12} vitamini ve Hcy seviyeleri ile PH riski istatistiksel olarak anlamlı şekilde artmıştır. Bu bulgular, hastaların diyetlerinde fazladan folik asit alımının bu bulgulara neden olmuş olabileceğini düşündürmektedir. *MTHFR C677T TT* genotipine ve *MTR A2756G GG* genotipine sahip PH hastalarında daha yüksek Hcy seviyeleri gözlenmiştir. Ancak bu fark anlamlı bulunmamıştır. Cys düzeylerinin PH riski üzerinde etkisi gözlenmemiştir. *MTHFR C677T* ve *MTR A2756G* gen polimorfizmleri PH için risk faktörü olarak bulunmamıştır.

Anahtar Kelimeler: Folik asit; genetik polimorfizm; homosistein; tek karbon grup transferazlar; Parkinson hastalığı

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Parkinson's disease (PD) is a lifelong and progressive neurodegenerative condition that develops with bradykinesia, rigidity, postural imbalance, and tremor.[1] It is among the most common degenerative diseases of the central nervous system. About 3% of the population suffers from PD up to the age of 65, and 5% of people over the age of 85, and it has a higher incidence in men than in women. According to 2016 data, 100,000 patients in Türkiye were diagnosed with PD, which increases by 10% annually.[2] Aging, genetics, environment, immune status, and gender are essential factors in the development of PD.[3] Folate metabolism has a crucial role in the development of PD. Folate mediates an extensive series of transformations known as one-carbon metabolism.[4] Folate carries one-carbon units in various oxidation levels of formyl or methanol in the body.[5] The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme catalyzes the transformation of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a methyl donor in the methylation of homocysteine (Hcy) to methionine.[6] The enzyme methionine synthase converts Hcy to methionine with the help of vitamin B_{12}. Hcy, which is formed during the metabolism of methionine, is an amino acid containing sulfur. A constant elevation of Hcy in plasma or serum above the normal limits is called hyperhomocysteinemia. Excess Hcy increases excitotoxicity and causes death in neuronal cells.[5] One-carbon metabolites (Hcy, vitamin B_{12}, and folic acid) might be associated with PD risk.[7-9] Moreover, in folate metabolism, the *MTHFR* gene is a potential candidate for susceptibility to PD. *MTHFR* gene variants have been shown to reduce the function of the MTHFR enzyme, thereby causing metabolic degradation of Hcy.[10] However, studies on both one-carbon metabolites and gene polymorphism on the risk of PD give conflicting results.[11]

Hcy is involved in two critical pathways. First, it is a cofactor of the vitamin B_{12} remethylation pathway, which utilizes Hcy to form methionine, and second, the transsulfuration pathway, in which vitamin B_6 is used as a cofactor. Hcy is then metabolized to cysteine (Cys).[12,13] Due to Hcy's role in folate metabolism and its direct relationship with vitamin B_{12}, Cys, and MTHFR enzyme, we measured these biochemical parameters by Enzyme-Linked Immunosorbent Assay (ELISA). Since current studies have given inconsistent and contradictory results, Hcy and other one-carbon metabolites were involved in this study. Unlike those studies, possible associations between these metabolites and two significant gene polymorphisms of *MTHFR* and their impacts on PD risk were investigated. We analyzed *MTHFR* gene polymorphisms by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) to show whether there is individual susceptibility to PD.

MATERIAL AND METHODS

In total, this study included randomized 108 PD patients and 97 controls. Patients were diagnosed with PD based on their medical history, symptoms, and neurological and physical exams. The Ankara Numune Training and Research Hospital Clinical Research Ethics Committee approved this study (date: January 07, 2015, number: 378/2014), and written informed consent was received from all individuals before enrollment. The study was performed with the principles of the Declaration of Helsinki. None of the individuals had a family history of autoimmune diseases or inflammatory disorders. The inclusion criteria for all participants were being Turkish (Caucasian) since race may influence the results associated with genetic polymorphism and older than 18 years of age. The exclusion criteria for all participants were to have unequivocal cerebellar abnormalities, including cerebellar ataxia or cerebellar eye movement abnormalities, such as sustained gaze-evoked nystagmus.

DNA isolation

Peripheral blood samples were obtained in sterile EDTA tubes for DNA isolation. DNA was isolated by extraction with sodium perchlorate/ chloroform from the peripheral blood of each subject.[14]

Measurement of Serum Folate, Hcy, Cys, and Vitamin B_{12}

Serum samples were separated and used for measurements of one-carbon pathway metabolites. The serum folate, Hcy, Cys, and vitamin B_{12} levels were measured by ELISA with the manufacturer's instructions.

Genotyping Analysis

Genotyping of both two gene polymorphisms was conducted by PCR-RFLP. 2 mM $MgC1_2$ (at 25 mM), 0.4 mM of dNTPs, two pmol of each primer, and 0.03U/µL *Taq* polymerase were used in PCR analysis. The primer sequences for *MTHFR* and *MTR* were *F*-TTTGAGGCTGACCTGAAGCAC; *R*-GACCTGAGAGGAGATCTGG and *F*-TGTTATCAGCATTGACCATTACTACAC and *R*-CCCTTTGTCCACGACTTTGTCA, respectively. Thermal cycling conditions were 94°C for 5 min, followed by 35 cycles of amplification (94°C for 30 s for denaturation and extension at 70 °C for 30 s) and final elongation at 72°C for 7 min. PCR products were incubated with *HinFII* and *HaeIII* restriction enzymes at 37°C for 16 hours for *MTHFR* and *MTR*, respectively. The digested fragments were separated by 2% agarose and the length of the resulting genotype fragments were 288 (*CC*), 288 bp, 242 bp, 47 bp (*CT*) and 241 bp and 47 bp for *MTHFR C677T* and 498 bp (*AA*), 498 bp, 345 bp, 153 bp (*AG*) and 345 bp, 153bp (*GG*) for *MTR A2756G.*[15]

STATISTICAL ANALYSIS

IBM SPSS Statistics 25.0 (IBM Corporation, Armonk, NY, USA) package program was used for data analysis. The Kolmogorov-Smirnov test was conducted to determine whether the tested values were normally distributed. Levene's test was used to check the assumption of homogeneity of variance between groups. Descriptive statistics were expressed as mean±standard deviation, median (minimum-maximum), or median (25^{th} percentile-75^{th} percentile) for continuous numerical variables and number of cases and (%) for categorical variables. The Hardy-Weinberg equilibrium for gene polymorphisms was evaluated by comparing the significance of the difference between the observed frequency and the expected frequency using the generic χ^2 test. he mean age difference between groups was analyzed by Student's t-test. In contrast, Pearson's χ^2 test was used to analyze the importance of the differences in gender distribution and the smoking habit. Univariate logistic regression analysis was used to investigate whether genotype profile and allele frequency were determinative in differentiating the control group from the PD group regarding gene polymorphisms. OR and 95% CI were calculated for each genotype and allele. Statistically significant differences in one-carbon metabolites were analyzed by the Mann-Whitney U test with two independent groups, and the Kruskal-Wallis test was used for more than two independent groups. Multivariate logistic regression analysis was performed to investigate whether gene polymorphisms and metabolites' measurements were determinative in differentiating the control group from the PD group when adjusted for age, gender, and smoking habit. The regression models used OR, 95% CI, and Wald test for each variable. Results were considered statistically significant for $p<0.05$ unless otherwise stated. However, Bonferroni correction was used to reduce Type I errors for all possible multiple comparisons.

RESULTS

Both groups' demographic characteristics and the PD group's clinical characteristics are presented in Table 1 and Table 2.

TABLE 1: Demographic characteristics of the control and Parkinson's disease groups.

	Control (n=97)	PD (n=108)
Age (years)	62.1±9.6	65.3±11.7
Gender		
Man	49 (50.5%)	60 (55.6%)
Woman	48 (49.5%)	48 (44.4%)
Smokers	48 (49.5%)	43 (40.2%)

PD: Parkinson's disease; Student t-test; Pearson χ^2 test.

TABLE 2: Clinical characteristics of the Parkinson's disease group.

	n=108
Stage*	2 (1-5)
Duration of disease (years)*	4 (0.2-33)
Body mass index (kg/m^2)**	27.7±5.0
Unified Parkinson's Disease Rating Scale*	38 (13-107)
Diabetes mellitus	
No	72 (79.1%)
Yes	19 (20.9%)
Parkinson's disease in the family	
No	85 (81.0%)
Yes	20 (19.0%)

Descriptive statistics were expressed as *median (minimum-maximum) or **mean±standard deviation.

ASSOCIATIONS OF GENE POLYMORPHISMS WITH PD RISK

The allele frequency for the *MTHFR C677T C* allele was 76.6% and 74.4%, in the control and PD groups, respectively. The genotype frequencies for *MTHFR C677T CC, CT,* and TT were 57.6%, 38%, and 4.4%, respectively in the control group and 53.4%, 42%, and 4.6%, respectively in the PD group.

The allele frequency for the *MTHFR A2756G A* allele was 74% and 80.1% in controls and patients, respectively, and the genotype frequencies for *MTHFR A2756G AA, AG,* and *GG* were 57.3%, 33.3%, and 9.4%, respectively in the control group and 65.9%, 28.4%, and 5.7%, respectively in the PD group.

The distribution of 2 SNPs (*MTHFR C677T* and *MTR A2756G*) in both control (p=0.550 and p=0.187) and PD groups (p=0.326 and p=0.309) did not deviate from the expected Hardy-Weinberg equilibrium in genotype distribution. Our data indicated no association between the allele frequencies for two gene polymorphisms and the risk of PD (Table 3).

For the *MTHFR C677T* gene polymorphism, both *CT* (OR=1.192; 95% CI: 0.650-2.186 and p=0.570) and *TT* (OR=1.128; 95% CI: 0.369-2.593 and p=0.870) genotypes had no statistically significant effect on PD risk compared to the *AA* genotype. Similarly, having the *CT+TT* compared to the *CC* had no statistically significant effect on PD risk (OR=1.128; 95% CI: 0.658-2.136 and p=0.571) (Table 3).

As for *MTR A2756G,* either *AG* (OR=0.741, 95% CI: 0.391-1.405 and p=0.358) or *GG* (OR=0.527; 95% CI: 0.166-1.670 and p=0.276) genotype had no statistically significant effect on the risk of PD compared to the *CC* genotype. Similarly, the *AG+GG* genotype had no statistically significant effect on PD risk compared to the *CC* (OR=0.694; 95% CI: 0.381-1.262 and p=0.231) (Table 3).

When adjusted for age, gender, BMI, and cigarette pack-year, no statistically significant effect on PD risk was observed when analyzed according to gene polymorphisms.

ASSOCIATIONS OF BIOCHEMICAL PARAMETERS WITH PD RISK

Folic acid levels were higher in the patients than in controls (p<0.001) (Table 4). The levels of folic acid were adjusted for gender, age, and smoking habit in the PD group, and significance continued (p<0.001,

TABLE 3: Allele and genotype frequencies in the control and Parkinson's disease groups.

			p-value†	OR (95% CI)
MTHFR C677T Allele	Control (n=92)	PD (n=88)		
C	141 (76.6%)	131 (74.4%)	Reference	1.000
T	43 (23.4%)	45 (25.6%)	0.628	1.126 (0.696-1.822)
MTHFR C677T Genotype	Control (n=92)	PD (n=88)		
CC	53 (57.6%)	47 (53.4%)	Reference	1.000
CT	35 (38.0%)	37 (42.0%)	0.570	1.192 (0.650-2.186)
TT	4 (4.4%)	4 (4.6%)	0.870	1.128 (0.267-4.762)
CT+TT	39 (42.4%)	41 (46.6%)	0.571	1.185 (0.658-2.136)
MTR A2756G Allele	Control (n=96)	PD (n=88)		
A	142 (74.0%)	141 (80.1%)	Reference	1.000
G	50 (26.0%)	35 (19.9%)	0.163	0.705 (0.432-1.152)
MTR A2756G Genotype	Control (n=96)	PD (n=88)		
AA	55 (57.3%)	58 (65.9%)	Reference	1.000
AG	32 (33.3%)	25 (28.4%)	0.358	0.741 (0.391-1.405)
GG	9 (9.4%)	5 (5.7%)	0.276	0.527 (0.166-1.670)
AG+GG	41 (42.7%)	30 (34.1%)	0.231	0.694 (0.381-1.262)

†Univariate logistic regression analysis; OR: Odds ratio; CI: Confidence interval; PD: Parkinson's disease.

TABLE 4: The levels of folic acid, vitamin B_{12}, Hcy, and Cys in the control and Parkinson's disease groups.

	Control (n=97) Mean (Minimum-Maximum)	PD (n=108) Mean (Minimum-Maximum)	p-value†
Folic acid (ng/mL)	3.29 (2.54-4.98)	7.54 (5.39-9.67)	**<0.001**
Vitamin B_{12} (pg/mL)	413.0 (301.0-576.0)	201.0 (150.0-266.2)	**<0.001**
Hcy (µmol/mL)	23.8 (19.5-32.3)	17.3 (12.9-20.4)	**<0.001**
Cys (ng/mL)	79.4 (72.8-89.1)	77.6 (69.3-91.3)	0.602

PD: Parkinson's disease; Hcy: Homocysteine; Cys: Cysteine.

multivariate logistic regression analysis). As the levels of folic acid increased, the risk of PD also statistically significantly increased (Figure 1, Table 5).

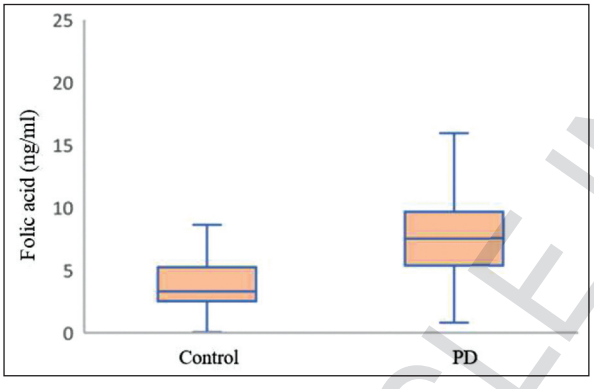

FIGURE 1: The levels of folic acid (ng/mL) in control and PD groups.
PD: Parkinson's disease.

The levels of vitamin B_{12} and Hcy were lower in patients compared to controls (p<0.001) (Table 4), and these low vitamin B_{12} and Hcy levels increased the risk of PD (Figure 2, Figure 3, Table 5).

Furthermore, the effect of Hcy measurements on PD risk was evaluated by multivariate logistic regression analysis when adjusted for gender, age, and smoking habit. The risk of PD decreased statistically significantly with an increase in Hcy levels (OR=0.892; 95% CI: 0.853-0.933 and p<0.001) (Figure 3, Table 6). As for Cys levels, no significant difference was found between the groups (p=0.602) (Figure 4, Table 4).

THE EFFECT OF BIOCHEMICAL PARAMETERS AND GENE POLYMORPHISM ON PD RISK

The combined effects of gene polymorphisms and biochemical parameters on PD risk were analyzed by multivariate logistic regression, adjusting for gender, age, and smoking habit. After adjustment, the most predictive factors in differentiating the control and PD groups were folic acid, vitamin B_{12}, and Hcy. Hcy (OR=0.916; 95% CI: 0.862-0.974, p=0.005) and vitamin B_{12} (OR=0.992; 95% CI: 0.990-0.995, p<0.001) levels decreased, whereas folic acid levels increased (Table 5). The risk of PD continued to increase statistically significantly by approximately 2-fold (OR=2.109; 95% CI: 1.624-2.738 and p<0.001).

TABLE 5: The gene polymorphisms and biochemical parameters on the impact of Parkinson's disease risk after adjustment for age, gender, and smoking habit.

PD group (n=108)	OR	95% CI	Wald	p-value
Age	0.998	0.950-1.049	0.007	0.934
Man factor	2.970	0.779-11.329	2.540	0.111
Smoking habit	0.885	0.237-3.297	0.033	0.855
MTHFR C677T CT	1.213	0.401-3.673	0.117	0.732
MTHFR C677T TT	2.496	0.221-28.140	0.547	0.459
MTR A2756G AG	0.807	0.235-2.769	0.117	0.733
MTR A2756G GG	0.805	0.088-7.381	0.037	0.848
Folic acid	2.109	1.624-2.738	31.333	**<0.001**
Vitamin B_{12}	0.992	0.990-0.995	31.235	**<0.001**
Homocysteine	0.916	0.862-0.974	7.959	**0.005**
Cysteine	1.006	0.964-1.051	0.080	0.777

Multivariate logistic regression analysis was applied; PD: Parkinson's disease; OR: Odds ratio; CI: Confidence interval.

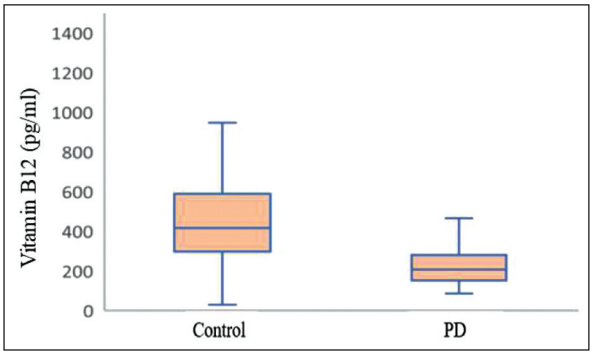

FIGURE 2: The levels of vitamin B_{12} (pg/mL) in control and PD groups.
PD: Parkinson's disease.

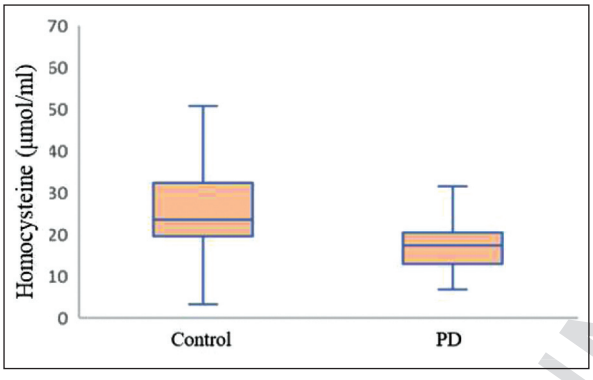

FIGURE 3: The levels of homocysteine (µmol/mL) in control and PD groups.
PD: Parkinson's disease.

TABLE 6: The effect of homocysteine measurements on Parkinson's disease risk after adjustment for age, sex, and smoking habit.

n=108	OR	95% CI	Wald	p-value
Age (years)	1.033	1.002-1.064	4.491	**0.034**
Man factor	2.174	0.984-4.802	3.686	0.055
Smoking habit	0.591	0.272-1.285	1.760	0.185
Homocysteine	0.892	0.853-0.933	25.136	**<0.001**

Multivariate logistic regression analysis; OR: Odds ratio; CI: Confidence interval.

FOLIC ACID-GENE POLYMORPHISMS

As for *MTHFR C677T*, individuals with *CC* and *CT* genotypes had significantly higher folic acid levels in patients than controls ($p<0.001$). Folic acid levels did not show a statistically significant difference regarding *MTHFR C677T* in the control and PD groups. As for *MTR A2756G,* the levels of folic acid did not change with this gene polymorphism.

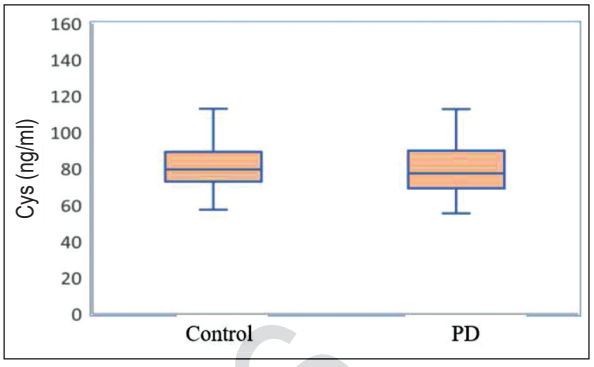

FIGURE 4: The levels of cysteine in control and PD groups.
PD: Parkinson's disease.

VITAMIN B_{12}-GENE POLYMORPHISMS

As for *MTHFR C677T*, individuals with *CC* and *CT* genotypes had significantly lower levels of vitamin B_{12} in the PD group compared to the control group ($p<0.001$). The vitamin B_{12} levels were not statistically significantly different related to *MTHFR C677T* gene polymorphism in either group. For *MTR A2756G*, individuals with *AA* and *AG* genotypes had ignificantly lower vitamin B_{12} levels in PD patients ($p<0.001$).

Hcy-GENE POLYMORPHISMS

Our results on the association of Hcy levels with gene polymorphisms were similar to the association of vitamin B_{12} with gene polymorphisms. In PD patients carrying *CC* and *CT* for *MTHFR* and *AA* and *AG* genotypes for *MTR*, PD patients had lower Hcy levels.

Cys-GENE POLYMORPHISMS

For the levels of Cys, there was no difference in terms of gene polymorphisms in both control and PD patients.

Regarding *MTR A2756G* gene polymorphism, a family history of PD was higher in patients carrying the *GG* compared to those with *AA* and *AG* ($p=0.023$ and $p=0.052$).

DISCUSSION

The one-carbon cycle is necessary for methylation reactions, which are essential to the body. Folate is a derivative of vitamin B, which has been linked to

neurodegenerative diseases; changes in its levels or deficiencies might have neurological consequences. High Hcy levels are noteworthy in individuals with neurodegenerative disease due to folate deficiency.[5] A limited number of studies evaluated possible relationships between biochemical parameters and gene polymorphism regarding individual susceptibility to PD. The present study selected vital biochemical parameters in Hcy metabolism and one-carbon pathway, which are considered to play a role in neurodegenerative diseases, and key enzymes in these pathways. Furthermore, individual susceptibility to PD using these polymorphic enzymes was evaluated.

The prevalence of *MTHFR C677T* varies by ethnicity. The allele frequency was higher in Hispanics and Italians and lower in Africans and American Blacks. The frequency of *MTHFR CT* genotype distribution was highest in Hispanics and Italians. The CT genotype distribution among Europeans was highest in Italians and lowest in Germans.[16] In Britain, the percentage of the *CT* genotype distribution was approximately 13%. The *CT* genotype distribution ranged from 10 to 14% in countries such as Canada, the USA, Brazil, and Australia. The *CT* genotype distribution in Californians and Colombians was 25%.[17] The *CT* genotype distribution has been reported to be 11% for the Japanese population.[18] Our results regarding CT genotype distribution are similar to those of Spanish and Italian populations.

Smoking, an important confounding factor, has also been evaluated in studies on the development of PD. In our study, the percentage of smokers in the control group was 49.5%, and 40.2% in the PD group. An inverse relationship was shown in studies between smoking and the risk of PD, and we have also observed this inverse association, however, this was statistically insignificant.[19,20]

Studies investigating the relationship between *MTHFR* gene polymorphisms and PD risk show conflicting results. Wang et al. conducted a study in China to examine the impact of *MTHFR* and *MTR* gene polymorphism on PD risk.[21] They found the frequencies of *MTHFR C677T CC, CT,* and *TT* genotypes in the PD group as 19.1%, 44.9%, and 36%, respectively. The genotype frequencies of *CC, CT,* and *TT* in the control group were found to be 14.7%, 75.2%, and 10.1%, respectively. These results showed no significant difference between the groups, and they concluded that these gene polymorphisms had no impact on PD risk. Regarding the *MTR A2756G* gene, the *AA*, *AG*, and *GG* genotype frequencies were 67.3%, 32.7%, and 0%, respectively. In the control group, the frequencies of *AA, AG,* and *GG* genotypes were found to be 54.3%, 44.9%, and 0.8%, respectively, and no significant difference was found between the groups regarding *MTR A2756G* genotype distribution. Our results were consistent with Wang et al.'s study.[21] In a meta-analysis study, 256 articles were evaluated and investigated the association of the risk of PD with polymorphisms of the *MTHFR* gene. There was no link between *MTHFR C677T* polymorphism and PD risk.[10] Our findings are consistent with this meta-analysis. Zahra et al. investigated the effect of *MTHFR C677T* polymorphism on PD risk in 178 patients and 402 controls, but no significant association was found.[22] In another meta-analysis, 15 reliable studies were reviewed, and a significant association with predisposition to PD was found for *MTHFR C677T* polymorphism.[23] Subgroup analyses by ethnic group showed that the association between PD and *MTHFR C677T* polymorphism was present in the Asian and Caucasian populations. This meta-analysis supports that *MTHFR C677T* polymorphism is related to an increased risk of PD.[23] Most published studies have shown that *MTHFR C677T* polymorphism has no association with the risk PD, as in our study. However, *MTHFR C677T* gene polymorphism was only associated with increased PD risk in Asian and Caucasian populations. According to these findings, the risk of PD varies by ethnicity.

Vallelunga et al. investigated whether *MTHFR C677T* gene polymorphism affects the age of onset in PD, and they found that subjects with the *TT* genotype were at an earlier age compared to those with the *CC* genotype.[24]

Since the one-carbon cycle is required for methylation reactions, the presence and deficiency of folate in this cycle are associated with neurodegen-

erative diseases. Many studies have drawn attention to altered folate levels in individuals with neurodegenerative diseases. In a 2007 study in Greece in which vitamin B_{12} and folate levels of 111 patients treated with levodopa were compared with a healthy control group, patients with PD who also had symptoms of depression had lower serum folate levels than healthy volunteers.[25] Patients with PD and cognitive impairment signs had more deficient serum vitamin B_{12} levels than healthy volunteers.[25] We have also observed statistically significantly lower levels of vitamin B_{12} in PD patients. Low levels of vitamin B_{12}, which is involved in the folate-mediated one-carbon metabolism pathway, may cause an interruption in the metabolism pathway, leading to elevated levels of total Hcy and indirectly increasing the likelihood of PD. Low vitamin B_{12} may yield a more significant worsening of mobility, and low levels of Hcy might not cause cognitive decline. The use of vitamin B_{12} supplements by PD patients would contribute to the positive effect of Hcy on mental activity.

Hcy can be converted to Cys using vitamin B_6 in the transsulfuration pathway. Cys is a non-sensorial amino acid essential for cell redox balance, signaling, and glutathione synthesis.[26] Therefore, an increase in plasma levels of Cys may have a positive effect in terms of scavenging radicals in PD and other diseases. We did not find any study investigating the relationship between PD and plasma Cys ratio. In our study, Cys levels and PD were not significantly associated.

In an Indian study investigating the possible impact of polymorphisms in the one-carbon pathway and total plasma Hcy levels on PD susceptibility, Parkinson's cases had elevated plasma Hcy levels compared to controls.[27] In a study conducted in the French population, total Hcy levels of subjects having the *MTHFR C677T TT* genotype significantly increased compared to the whole study population.[28] Emre et al. investigated the relationship between Hcy and *MTHFR* gene polymorphisms on PD risk.[29] They showed that the frequency of the *TT* genotype was higher in patients than in controls. They concluded that the *TT* genotype may impact the development of PD.[29] On the contrary, in our study, the frequency of *TT* genotype was similar in both groups. They also found elevated Hcy levels in PD cases, although this was not statistically significant. In addition, the PD group with the highest Hcy level was only levodopa users. The Hcy levels in patients using levodopa and having the *677TT* genotype were significantly higher than the Hcy levels in patients using levodopa and having other genotypes.[29] In another study, the levels of Hcy were found to be similar in the control and PD groups, similar to our findings. On the other hand, the *MTHFR TT* genotype was more frequent in PD patients than in controls.[30] Müller et al. investigated the impact of altered Hcy levels on patients using levodopa or decarboxylase inhibitors.[31] Plasma Hcy levels were not significantly different between untreated PD patients and healthy subjects. Still, plasma Hcy levels increased in treated PD patients. This increase was thought to be due to drug treatment.[31] In our study, serum Hcy levels of PD patients treated with L-dopa were lower than in the control group. In Gürsoy et al.'s study, the differences between folate, vitamin B_{12}, and Hcy levels of 46 PD patients and 30 volunteers were analyzed to study the effect of hyperhomocysteinemia on motor performance in patients with PD.[32] Significantly lower levels of vitamin B_{12} and folate and higher levels of Hcy were observed in the PD group compared to controls.[32]

CONCLUSION

In the present study, Cys did not affect PD risk, but low vitamin B_{12} levels may affect PD risk. The high levels of folic acid and low levels of Hcy in PD patients may be explained by the effects of the drugs used by the patients. Regarding *MTR A2756* gene polymorphism, having the *GG* genotype may increase the familial risk of PD.

Similar studies with enzymes and biochemical parameters that are effective on the one-carbon pathway will help to understand the etiology and pathogenesis of PD and to develop treatment modalities that can treat PD or delay the onset of the disease. Our study showed that polymorphisms in *MTHFR C677T* and *MTR A2756G* genes are not risk factors for PD. In the future, the association of *MTHFR C677T* and *MTR A2756G* genetic polymorphisms with PD can be evaluated in much more detail by noting the enzyme activities, gene polymorphisms, biochemical parameters, and prescribed medications

such as vitamin B_{12} and folic acid in larger study groups. Future studies will provide more data and information by including different genes and biochemical parameters that play significant roles in the folate cycle.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Bensu Karahalil;* ***Design:*** *Bensu Karahalil;* ***Control/Supervision:*** *Bensu Karahalil;* ***Data Collection and/or Processing:*** *Gürdal Orhan, Mevlüt Ulukaya;* ***Analysis and/or Interpretation:*** *Mevlüt Ulukaya;* ***Literature Review:*** *Mevlüt Ulukaya;* ***Writing the Article:*** *Bensu Karahalil, Aylin Elkama;* ***Critical Review:*** *Bensu Karahalil;* ***Materials:*** *Gürdal Orhan.*

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