IJBCM
International Journal of Basic and Clinical Medicine
Uluslararası Temel ve Klinik Tıp Dergisi

Research Article / Araştırma Makalesi

# Investigation of the Association of Homocysteine and MTHFR Polymorphisms and Treatment Options in Parkinson's Disease in Central Anatolian Region*

## Orta Anadolu'daki Parkinson Hastalarında Homosistein ve MTHFR Polimorfizmleri Arasındaki İlişkinin Araştırılması ve Tedavi Seçenekleri

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**Abstract**

**Aim**

In this study, we aimed to investigate the effects of MTHFR C677T and A1298C polymorphisms to homocysteine levels in patients with Parkinson's disease who were treated with levodopa and entekapone.

**Materials and Methods**

Plasma homocysteine (hcy), folic acid and vitamin B12 levels and MTHFR (C677T, A1298C) polymorphisms and treatment options were compared in 70 Parkinson's Disease (PD) patients who taking levodopa (n=26), dopamine agonist (n=11) and levodopa and entacapone treatment together (n=33) with 100 controls.

**Results**

Although no statistically significant difference was detected, hcy level of the patients was found higher compared to control group (patient 18.29 ± 9.22 µmol /l vs control 15.77 ± 7.58 µmol / l) and hcy level was highest in the patients receiving only levodopa (19.56 ± 10.77 µmol / l). The frequency of TT genotype in the patients was higher compared to the control group (11.4%, 6%). Especially, hcy level for levodopa-receiving patients with 677TT genotype became significantly higher level when compared with other genotypes of levodopa-receiving patients (respectively 677TT 36.28 ± 16.17, 677CT 13.5 ± 1.71, 677CC 17.2 ± 6.59). No statistically significant difference was detected between patients and controls regarding their folic acid and vitamin B12 levels and A1298C polymorphism.

**Conclusion**

Finally, both 677TT genotype and levodopa treatment might be jointly contributed to the increasing of the plasma hcy levels in PD patients and entacapone limitedly decreased hcy levels during levodopa treatment. It can be said that results need to be supported with larger sample sized comprehensive studies.

**Key words:** Parkinson's disease, MTHFR, levodopa, hyperhomocysteinemia

**Özet**

**Amaç**

Çalışmamızda levodopa ve entekapon kullanan Parkinson hastalarında MTHFR genindeki C677T ve A1298C polimorfizmlerinin homosistein düzeyine etkilerini araştırmayı amaçladık.

**Materyal ve Metot**

70 Parkinson (PD) hastasında; plazma homosisteini (hcy), folik asit, B12 vitamini seviyeleri, MTHFR (C677T, A1298C) polimorfizmleri ve tedavi seçenekleri karşılaştırıldı. 100 kişilik bir kontrol grubunun yer aldığı çalışmada, 70 hastanın 26'sı levodopa (n=26), 11'i dopamin agonisti (n=11) kullanırken, 33 hasta da levodopa ve entakapon tedavisini birliktealmaktaydı.

**Bulgular**

İstatistiki olarak anlamlı bir fark gözlenmese de, hastalardaki homosistein seviyesinin kontrol grubunda yer alanlara göre daha fazla olduğu tespit edildi (hasta 18.29 ± 9.22 µmol /l vs kontrol 15.77 ± 7.58 µmol/ l). Ayrıca homosistein seviyesinin en yüksek olduğu hasta grubunun sadece levodopa kullanan hastalar olduğu görüldü (19.56 ± 10.77 µmol / l). Hastalardaki TT genotipinin sıklığının da kontrol grubunda yer alanlara göre daha fazla olduğu görüldü (%11.4, %6). Özellikle, levodopa kullanan ve 677TT genotipine sahip olan hastalardaki homosistein seviyesi, levodopa kullanan ve diğer genotiplere sahip olan hastalardaki homosistein seviyesine göre anlamlı bir şekilde yüksek (sırasıyla 677TT 36.28 ± 16.17, 677CT 13.5 ± 1.71, 677CC 17.2 ± 6.59).

Hastalar ve kontrol grubu arasında folik asit ve B12 vitamini seviyeleri ile A1298C polimorfizmi açısından anlamlı bir farka rastlanmadı.

**Sonuç**

Sonuç olarak; Parkinson hastalarında 677TT genotipinin ve levodopa kullanımının bir arada olmasının plazma homosistein seviyesini artırdığı, ayrıca entakaponun levodopa tedavisi esnasında sınırlı da olsa homosistein seviyesini düşürdüğü gözlemlenmiştir. Ancak sonuçların daha fazla örnek sayısı içeren kapsamlı çalışmalarla desteklenmesinin gerekli olduğu söylenebilir.

**Anahtar kelimeler:** Parkinson hastalığı, MTHFR, levodopa, hiperhomosisteinemi.

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**Article History / Makale Geçmişi:**

Date Received / Geliş Tarihi: 02.10.2015
Date Accepted / Kabul Tarihi: 09.12.2015

Int J Basic Clin Med 2015;3(3):98-105

*This study was presented as an oral presentation in Symposium of Genetic Diseases Seen in Adult Ages (6-7 December 2013 İstanbul)

## Introduction

Homocysteine (Hcy) is an amino acid with sulphur and is derived from the amino acid methionine[1,2]. Hcy level may increase due to genetic reasons such as C667T and A1298C polymorphisms in MTHFR gene [3]. MTHFR is a key enzyme in folic acid metabolism and catalyzes the reduction of 5, 10- methylenetetrahydrofolic acid to 5-methyltetrahydrofolic acid. 5-methyltetrahydro-folic acid is the carbon donor in the reaction of remethylation of hcy to methionine [4,5]. Cytosine in the 677$^{th}$ nucleotide of MTHFR gene is replaced by T, and A in the 1298$^{th}$ is replacedby C in C677T and A1298T polymorphisms of MTHFR gene respectively.

Enzyme activity decreases upon MTHFR 677 polymorphism in TT and CT genotypes, with the most decrease existing in TT genotype [6]. Although not as substantial as C677T, there is also a decrease in enzyme activity upon MTHFR A1298T polymorphism, too[7].Total hcy level is expected to be high in PD patients but there is no general consensus[8,9]. One of the factors that increase hcy level can be levodopa which is used in PD treatment[10].

Catabolism of levodopa by catechol-O-methyl transferase (COMT) enzyme takes place with tetrahydrofolic acid mediated methyl transfer and by this way, catabolism of excess levodopa leads to a decrease in the amount of methyl and thus might slow down the catabolism ofhcy[11]. In previous animal experiments, it was detected that COMT inhibitors such as entacapone or tolcapone prevented levodopa from increasing hcy levels by inhibiting the COMT[12,13].

The aim of this study is to investigate the effects of MTHFR C677T and A1298C polymorphisms on plasma hcy levels in Turkish patients receiving levodopa, levodopa / entacapone together, and only dopamine agonist.

## Materials and Methods

### Subjects

In this study, 70 patients (35 females and 35 males) with sporadic PD diagnosis and 100 healthy individuals (56 females and 44 males), all living in Turkey, were recruited in conformity with the diagnosis criteria of London Brain Bank. Average age is 65.89 ± 9.25 years for the patient group and 66 ± 5.85 years for the control group. The number of those who are receiving levodopa is 26, dopamine agonistis11 and levodopa and entacapone together is 33, respectively. Unified Parkinson's disease Rating Scale (UPDRSIII) score of the patients was calculated as 37.61 ± 15.57 and Hoehn Yahr score was calculated as 2.21 ±0.68.

### MTHFR genotyping

E.Z.N.A extraction kit (Omega bio tek) was used for DNA isolation from blood. In order to amplify MTHFR 677 gene region, 7 µl DNA was added to the mix containing 5 µl 10xPCRbuffer,0.4 µl dNTP mix (10mM), 4 µl primer F: 5' TGAAGGAGAAGGTGTCTGCGGGA 3', 4 µl primer R: 5' AGGACGGTGCGGTGAGAGTG 3, 4 µl (50mM) MgCI and 0.2 µl Taq pol. For PCR amplification, the mix was subjected to 5 minutes of initial denaturation at 94°C, followed by 35 cycles of 30 seconds of denaturation at 94°C, 45 seconds of annealing at 61°C and 30 seconds of elongation at 72°C, finally completing the amplification with 5 minutes of final elongation at 72°C. The digestion reaction

of Polymerase Chain Reaction (PCR) product by Hinf 1 restriction enzyme was performed in a mixture of 15 µl PCR product, 0.5µlenzyme,2.5 µl $R^+$ buffer and 7 µl of ddH O at 37°C for16 hours. MTHFR 1298 gene region was amplified using a PCR mixture of 5 µl 10 x PCR buffer, 0.4 µl dNTP mix (10mM), 4 µl Primer F: 5'CTTTGGGGAGCTGAAGGACTACTAC3', 4µl Primer R:5'CACTTTGTGACCATTCCGGTTTG 3', 4 µl(50mM) MgCI and 0.2 µl Taq pol, together with7 µl of DNA. The region was amplified by subjecting the mix to 5 minutes of initial denaturation at 94°C, followed by 35 cycles of45 seconds of denaturation at 94°C, 45 seconds of annealing at 57°C and 30 seconds of elongation at 72°C, lastly to 5 minutes of final elongation at 72°C. PCR product was digested using Mbo II restriction enzyme in a mixture of20 µl PCR product, 0.2 µl enzyme, 0.5µl $R^+$buffer and 4.3 µl dd$H_2O$ by incubating it at 37°C for 16 hours. Following digestion using Hinf 1 and Mbo II enzymes, the products were subjected to agarose gel electrophoresis in 2% agarose gels, followed by capturing gel images by gel imaging and documentation systems.

**Quantification of homocysteine, folic acid and vitamin B12**

Venous blood from control and tested groups were collected and exposed to starvation for a night. Vitamin B12 and folic acid were measured with an electrochemical luminescence immunoassay (ECLIA, Elecsys 2010 and Modular Analytics E 170, Roche Diagnostics, Germany). Hcy levels were detected by chemiluminescence method in the analyzer DPC- Immulite 2000 (Siemens -DPC Los eUSA).

**Statistical analysis**

Continuous data is presented as "average (±) standard deviation (SD)", whereas categorical data is presented as %. Student t test was used for data with normal distribution when number of groups is two. Mann-Whitney U test was used for data with non-normal distribution to compare pair of groups, whereas Kruskal-Wallis test was used for the analysis of multiple groups. Statistical Package for Social Science (SPSS) 15.0 statistical package software and Sigma stat 3.5 software were used in Windows environment in the statistical analysis of the findings of the study. Chi-Square analysis was used to compare qualitative data and to determine the differences between groups. Confidence interval of 95% and significance value of $p < 0.05$ were accepted as the criteria. No disequilibrium was found in Hardy-Weinberg tests.

**Results**

Although no statistically significant difference was detected between the patients and the controls regarding their C677T and A1298C allele frequencies, it is important that the frequency of 677TT genotype is higher in the patients than in the controls (Table 1), (11.4%, 6% ; $P < 0.05$). The most frequent compound genotype observed in the patients and the controls was 677CC / 1298AC (28.5% for patients, 28% for controls; p> 0, 05), (Table 1).

Risk estimation of genotypes for PD was not the point of view for this study. In our study, hcy level was found to be higher in the patients compared to control groups even though it is notstatistically significant (patient 18.29 ± 9.22 µmol/l vs control 15.77 ± 7.58 µmol / l; p> 0.05), (Table 1). No statistically significant difference was detected between the patients and the controls regarding their folic acid and

vitamin B12 levels (P > 0.05), (Table 1).

**Table 1.** Biochemical data, MTHFR genotypes and allele frequency distribution of the patients and the controls values of continuous variables are mean ± SD

| | Patient (70) | Control (100) | P |
|---|---|---|---|
| Avarage age | 65.89 ± 9.25 | 66 ± 5.85 | |
| Female / Male | 35/35 | 56 / 44 | |
| **Biochemical data** | | | |
| Hcy (µmol / l) | 18.29 ± 9.22 | 15.77 ± 7.58 | P > 0.05 |
| folic acid (ng / ml) | 8.84 ± 3.31 | 7.59 ± 3.35 | P > 0.05 |
| Vitamin B12 (pg /ml) | 296.32 ± 214,09 | 379.41 ± 191,06 | P > 0.05 |
| **Genetic Data** | | | |
| **Allele Frequency** | | | |
| C677 | 0.75 | 0.72 | P > 0.05 |
| T677 | 0.25 | 0.28 | P > 0.05 |
| A1298 | 0.56 | 0.66 | P > 0.05 |
| C1298 | 0.44 | 0.34 | P > 0.05 |
| **Genotype Frequency** | | | |
| CC677 / AA1298 | 14 (20%) | 15 (15%) | P > 0.05 |
| CT677 / AA1298 | 4 (5,7%) | 22 (22%) | P > 0.05 |
| TT677 / AA1298 | 6 (8,5%) | 4 (4%) | P > 0.05 |
| CC677 / AC1298 | 20 (28,5%) | 28 (28%) | P > 0.05 |
| CC677 / CC1298 | 10 (14,2%) | 7 (7%) | P > 0.05 |
| CT677 / AC1298 | 11 (15,7%) | 20 (20%) | P > 0.05 |
| CT677 / CC1298 | 3 (4,2%) | 2 (2%) | P > 0.05 |
| TT677 / AC1298 | 2 (4,2%) | 0 | |
| TT677 / CC1298 | 0 | 2 (2%) | |
| **Genotype Frequency** | **Patient** | **Control** | **P** |
| TT677 / AA1298<br>TT677 / AC1298<br>TT677 / CC1298 | 8(11.4%) | 6(6% | **P<0.05** |
| CC677 / AA1298<br>CC677 / AC1298<br>CC677 / CC1298 | 44(62.8 %) | 50(50%) | P > 0.05 |
| CT677 / AA1298<br>CT677 / AC1298<br>CT677 / CC1298 | 18(25.7 %) | 44(44%) | P > 0.05 |

Hcy levels of patients with 677 TT / 1298 AA compound genotype was higher compared to other patient groups and the control group (27.33 ± 18.68 µmol / L, P > 0.05), (Table 2).

Hcy levels of patients receiving levodopa was higher compared to patients receiving levodopa and entacapone together and those receiving dopamine agonist even though the difference was not statistically significant (19.56 ± 10.77; 17.13 ± 7.69; 18.76 ± 9.94 µmol/L, respectively; P>0.05), (Table 3).

**Table 2.** Homocysteine levels and MTHFR distribution of patients and controls- Values of continuous variables are mean ± SD

| MTHFR genotype | Homocysteine level (µmol/l) | | P |
|---|---|---|---|
| | Patient vs Control | | |
| CC677 / AA1298 | 18.12 ± 9.56 | 11.95 ± 6.55 | P > 0.05 |
| CT677 / AA1298 | 15.23 ± 6.19 | 15.51 ± 7.57 | P > 0.05 |
| TT677 / AA1298 | 27.33 ± 18.68 | 23.65 ± 7.04 | P > 0.05 |
| CC677 / AC1298 | 16.83 ± 5.92 | 16.41 ± 6.37 | P > 0.05 |
| CC677 / CC1298 | 18.6 ± 6.42 | 16.74 ± 4.23 | P > 0.05 |
| CT677 / AC1298 | 16.83 ± 9.71 | 13.16 ± 7.13 | P > 0.05 |
| CT677 / CC1298 | 14.57 ± 3,5 | 25.9 ±0.42 | P > 0.05 |

**Table 3:** Comparison of biochemical data and treatment options between patients and the controls values of continuous variables are mean ±SD

| | L | L – E - (DA) | L+ E+ | Control | P |
|---|---|---|---|---|---|
| Avarage Age | 70 ± 6.87 | 57 ± 10.16 | 65.03 ± 8.26 | 66 ± 5.85 | |
| Female / Male | 17 / 9 | 4 / 7 | 14 / 19 | 56 / 44 | |
| Hoehn Yahr | 2.52 ± 0.84 | 1.55 ± 0.15 | 2.2 ± 0.45 | | |
| Patient year | 8.96 ± 5.29 | 3.27 ± 1.27 | 8.06 ± 4.01 | | |
| Levodopa dose(mg / day) | 543.27 ± 310,13 | | 428.79 ± 167,25 | | |
| Entacapone dose(mg / day) | | | 478.79 ± 199,6 | | |
| Homocysteine ( µmol / l) | 19.56 ± 10.77 | 18.76 ± 9.94 | 17.13 ± 7.69 | 15.77± 7.58 | P > 0.05 |
| folic acid (ng / ml) | 8.93 ± 3.81 | 8.31 ± 2.45 | 8.93 ± 3.21 | 7.6 ± 3.35 | P > 0.05 |
| Vitamin B12 (pg / ml) | 295.14 ± 148,13 | 255.92 ± 153,63 | 289.34 ± 200,39 | 379.41± 191,06 | P > 0.05 |

Especially hcy level for levodopa-receiving patients with 677TT genotype became statistically significant level compared with other genotypes of levodopa-receiving patients (TT 36.28 ± 16.17, CT 13.5 ± 1.71, CC 17.2 ± 6.59; P < 0.01), (Table 4).

## Discussion

In our study, we investigated the association of hcy, C677T and A1298C polymorphisms in levodopa and/ or entacapone treated or only dopamine agonist treated patients of PD. In parallel with the former studies, hcy level wasfound higher in PD patients compared to controls even though no statistically significant level existed in our study 14,15. Although hcy level of the patients with 677 TT genotype and patients who received levodopa treatment separately had not statistically significant level according to the each of the compared groups. When these two parameters was jointly existed in the same compared group, hcy level of levodopa receiving patients together with 677TT genotype being higher compared to levodopa receiving patients with other genotypes became statistically significant level (p< 0.01).

**Table 4.**Comparison of MTHFR genotypes and treatment options between patients and controls-values of continuous variables aremean ± SD

| | L-dopa | L-E- | L+E+ | Control | P |
|---|---|---|---|---|---|
| | | | **C677T** | | |
| C/C | 17.2±6.59(18) | 16.09±6.58(6) | 18.5±8.15(20) | 15.12±6.42(50) | P>0.05 |
| C/T | 13.5±1.71(4) | 21.96±13.02(5) | 13.9±4.63(9) | 14.91±7.59(44) | P>0.05 |
| T/T | 36.28±16.17(4) | | 17.29±10.5(4) | 27.45±8.03(6) | P>0.05 |
| P | **P<0.01** | P>0.05 | P>0.05 | P>0.05 | |
| | | | **A1298C** | | |
| A/A | 25.97±16.59(8) | 18.1±7.83(3) | 16.65±9.22(13) | 15±7.74(41) | P>0.05 |
| A/C | 16.83±7.32(13) | 22.08±11.26(6) | 15.93±7.2(12) | 15.85±7.76(50) | P>0.05 |
| C/C | 20.6±7.05(5) | 9.79±1.7(2) | 19.68±5.74(8) | 18.78±5.45(9) | P>0.05 |
| P | P>0.05 | P>0.05 | P>0.05 | P>0.05 | |

Caccamo et al., in their study with 49 levodopa receiving PD patients and 86 healthy individuals, investigated the effects of folic acid/ vitB12, daily levodopa dose and MTHFR polymorphism on the progression of hyperhomocysteinemia in PD patients. They found that hcy level was significantly higher in the patients compared to the controls (16.3 ± 5.7 and 11.7 ± 2.7 µmol / L, P < 0.01). No significant difference was detected between the patients and the controls regarding their folic acid, vitB12 levels. However, they observed that the frequency of 677TT / 1298AA compound genotype was higher in the patients than the controls (32.5% and 17.4%, P < 0.05). Patients who are carriers of this genotypewere exhibited a mildhiperhomocystein-emia (22.1 ± 4.9 µmol / L) 15.In our study, it was also found that hcy level was higher in the patients and controls with 677TT / 1298AA compound genotype (27.33± 18.68 and 23.65 ± 7.04 µmol / L; p> 0.05).We suggested that 677TT /1298AAcompound genotype was considered to have an enhancing effect on plasma hcy level. It was observed that the 677TT allele had prominent effect on the elevation of hcy but 1298AA was combining the 677TT allele in compound genotype.

Yuan Ry. et al. studied the effects of levodopa and MTHFR C677T and A1298Cpolymorphisms on hcy level in 48 levodopa treated and 28 non-treated PD patients and 110 controls. They found that hcy level was remarkably higher in levodopa treated compared to non-treated and controls (p< 0,001). In levodopa treated group hcy level was higher in 677TT than in CT and in CC with a significant difference from TT (p< 0,014) but not differenced among A1298C genotypes. Likewise hcy was the highest in 677TT +1298AA; intermediate CT/ AA and the lowest in CC/ AA compound genotype in their study. They concluded that hcy elevation may be stemmed from levodopa administration, and further promoted by 677TT and 677 CT,but not A1298C genotypes10.

Todorovic et al. investigated the effects of MTHFR gene C677T polymorphism and levodopa on PD pathogenesis in levodopa treated, non-treated patients and controls (n: 83; 30 and 53, respectively). They found that hcy level was higher in both patient groups compared to the controls (P < 0.05). Additionally, hcy level

was found to be higher in all groups with TT genotype compared to other genotypes (P < 0.001). No statistically significant difference was detected between levodopa treated and non-treated groups interms of their hcy levels 16. However, hcy levelwas detected to be higher in the patients and controls with TT genotype. There was no statistically significant difference in our study when considering the levodopa receiving patients, hcy level of the cases with TT genotype became to be statistically significant level (respectively TT 36.28 ± 16.17, CT 13.5± 1.71, CC 17.2 ± 6.59 P < 0.01).

Zhao P et al. investigated the effects of entacapone on plasma hcy in PD patients treated with levodopa. They founded that the plasma hcy concentrations of 'Levodopa + Entacapone' group (15.1 ± 3.1 µmol/L) were lower than those of 'Levodopa' group (20.4 ±4.7 µmol/L), but still higher than those of 'Levodopa (-)' group (12.2 ± 2.4 µmol/L) and control group (9.1 ± 2.2 µmol/L). The Hcy concentrations of 'Levodopa (-)' group were also higher than those of control group. They concluded that entacapone increases the bioavailability of levodopa and simultaneously alleviates partially its resultinghyperhomo-cysteinemia17. In our study westated that entacapone with levodopa treated patients had lower hcy levels than only levodopa treated group (19.56 ± 10.77 and17.13 ± 7.69 µmol/L). We interpreted thatentacapone limitedly decreased hcy levels during levodopa treatment.

As a result, it might be interpreted that in our study the effects of 677TT genotype together with levodopa treatment were jointly increased the hcy level and entacapone limitedly decreased hcy levels during levodopa treatment. Additionally A1298C polymorphism was not associated with hcy level in PD.

**Acknowledgments**

The authors would like to thank to all patients and controls for their help for the study.

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