

Polycystic ovary syndrome and MTHFR gene C677T and A1298C polymorphisms

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Summary

In this study, it was aimed to determine the allele frequencies and genotype distributions of MTHFR gene C677T and A1298C polymorphisms in a Turkish study population with polycystic ovary syndrome. Genomic DNA was isolated from blood samples of 30 patients with polycystic ovary syndrome and 28 healthy controls. PCR-RFLP technique was used to analyze MTHFR gene C677T and A1298C polymorphisms. Products of PCR-RFLP technique were assessed with UV transilluminator by being exposed to agarose gel electrophoresis. According to MTHFR gene C677T polymorphism, TT genotype was found statistically higher ($p<0.05$) in the control group than in the patient group, but allele frequencies were similar between the two groups. Genotype distribution of MTHFR gene A1298C polymorphism between the groups did not show any difference, but according to allele frequencies A allele frequency was statistically higher ($p<0.05$) in the control group than in the patient group. As a result of this study, it can be concluded that MTHFR gene C677T and A1298C polymorphisms are not involved in polycystic ovary syndrome in this Turkish study population.

Key Words: MTHFR gene, C677T, A1298C, polymorphism, polycystic ovary syndrome.

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Polikistik over sendromu ve MTHFR geni C677T ve A1298C polimorfizmleri

Özet

Bu çalışmada polikistik overli Türk hastalarda MTHFR geni C677T ve A1298C polimorfizmleri alel frekansları ve genotip dağılımlarının belirlenmesi amaçlanmıştır. Genomik DNA polikistik overli 30 hasta ve 28 sağlıklı kontrolün kanlarından izole edilmiştir. MTHFR gen, C677T ve A1298C polimorfizmleri PCR-RFLP tekniği kullanılarak analiz edilmiştir. PCR-RFLP tekniği ürünleri agaroz jel elektroforezinde yürütülerek UV transilluminatör ile değerlendirildi. MTHFR geni C677T polimorfizmi açısından bakıldığında, TT genotipi kontrol grubunda hasta grubuna göre istatistiksel olarak daha yüksek ($p<0.05$) bulundu, ancak gruplar arasında alel frekansları birbirine benzerdi. MTHFR geni A1298C polimorfizmi genotip dağılımı gruplar arasında bir farklılık göstermezken, A alel frekansı kontrol grubunda hasta grubuna göre istatistiksel olarak daha yüksek ($p<0.05$) olarak belirlendi. Çalışmamızın sonucunda bu çalışmada yer alan Türk popülasyonunda MTHFR geni C677T ve A1298C polimorfizmlerinin polikistik over sendromunda yer almadığını söyleyebiliriz.

Anahtar Kelimeler: MTHFR geni, C677T, A1298C, polimorfizm, polikistik over sendromu.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous condition with unknown etiology and is considered to be the most common endocrine disease in women of reproductive age and is estimated to affect 5-10% of the population (1-5). Women with PCOS are more likely to develop components of the metabolic syndrome such as diabetes, obesity, hypertension and dyslipidemia, which are major risk factors for cardiovascular disease (5-12). Elevated plasma homocysteine levels are considered to be an independent risk factor for cardiovascular disease. Methylenetetrahydrofolate reductase (MTHFR) is an important regulatory enzyme in homocysteine metabolism. It converts 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, and provides the methyl group for homocysteine in methionine synthesis. The gene encoding MTHFR was mapped and a polymorphism of MTHFR C677T in which valine was substituted for alanine, and a polymorphism MTHFR A1298C, in which alanine was substituted for glutamate, showed a 20 – 30% reduction in enzyme activity as well as an association with an increased risk of hyperhomocysteinemia (13-15). Based on these findings, it was aimed at determining allele frequencies and genotype distributions of MTHFR gene C677T and A1298C polymorphisms in Turkish patients with polycystic ovary syndrome.

MATERIAL and METHODS

Study population

This study involved 30 PCOS patients and 28 controls recruited from the department of Obstetrics and Gynecology, Private Muş Şifa Hospital in Muş, Turkey. Informed consent in accordance with the study protocol, approved by the ethics committee of Medical Faculty, Eskisehir Osmangazi University, Eskisehir, was obtained from each patient. PCOS was defined by the Rotterdam PCOS consensus criteria (16). Control subjects were consecutively selected among people without PCOS.

Genotype determination for MTHFR gene C677T polymorphism

DNA was extracted from 2 mL venous blood according to kit procedure (Vivantis, Malesia) and

stored at -20°C. DNA was amplified by polymerase chain reaction (PCR) in a thermal cycler (Amplitrionyx 4, USA). Allele-specific primers were used in the PCR. These primers were as follows: 5'- AGG ACG GTG CGG TGA GAG TG- 3' and 5'- TGA AGG AGA AGG TGT CTG CGG GA- 3'. 5 µl of DNA sample was amplified for 40 cycles with denaturation at 94°C for 60 s, annealing at 63°C for 60 s, and extension at 72°C for 60 s using a 50 µl PCR mixture containing a 10 pmol each primer, 10X PCR buffer, 2 mM dNTPs, and 5U Taq polymerase. The PCR products were separated by electrophoresis on 2% agarose gel containing 4 µl ethidium bromide and were visualized using a UV transilluminator (Nyxtechnik, USA) and photographed with a CCD camera (Clever, UK). After confirmation of an amplified fragment of the expected size (198 bp), the PCR products were digested with 1 unit of restriction enzyme Hinf I (Vivantis, Malesia) at 37°C for 1 hour. Digested PCR products were separated by electrophoresis on 2% agarose gel containing 4 µl ethidium bromide and were visualized using a UV transilluminator (Nyxtechnik, USA) and photographed with a CCD camera (Clever, UK) (Figure 1).

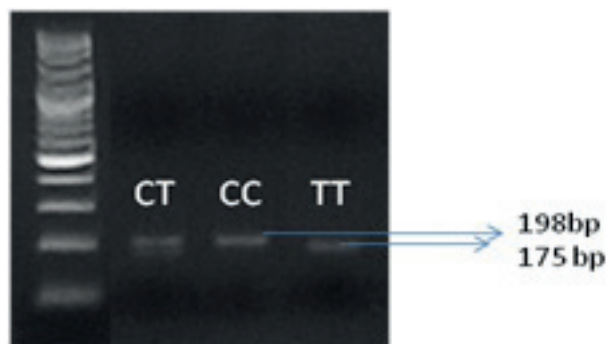


Figure 1. Gel image of MTHFR gene C677T polymorphism

Genotype determination for MTHFR gene A1298C polymorphism

DNA was extracted from 2 mL venous blood according to kit procedure (Vivantis, Malesia) and stored at -20°C. DNA was amplified by polymerase chain reaction (PCR) in a thermal cycler (Amplitrionyx 4, USA). Allele-specific primers were used in the PCR. These primers were as follows: 5'- CTT TGG GGA GCT GAA GGA CTA CTA C- 3' and R: 5'- CAC TTT GTG ACC ATT CCG GTT TG- 3'. 5 µl of DNA sample was amplified for 40 cycles with denaturation at 94°C

for 60 s, annealing at 63°C for 60 s, and extension at 72°C for 60 s using a 50 µl PCR mixture contained a 10 pmol each primer, 10X PCR buffer, 2 mM dNTPs, and 5U Taq polymerase. The PCR products were separated by electrophoresis on 2% agarose gel containing 4 µl ethidium bromide and were visualized using a UV transilluminator (Nyxtechnik, USA) and photographed with a CCD camera (Cleaver, UK). After confirmation of an amplified fragment of the expected size (163 bp), the PCR products were digested with 1 unit of restriction enzyme Mbo II (Vivantis, Malesia) at 37°C for 1 hour. Digested PCR products were separated by electrophoresis on 2% agarose gel containing 4 µl ethidium bromide and were visualized using a UV transilluminator (Nyxtechnik, USA) and photographed with a CCD camera (Cleaver, UK) (Figure 2).



Figure 2. Gel image of MTHFR gene A1298C polymorphism

Statistical analysis

Data were analyzed using the statistical package for social sciences (SPSS ver.15). The values were expressed as means ± SE. Distribution of genotypes and allele frequencies between groups were compared by Pearson χ^2 -test. A p value of less than 0.05 is considered statistically significant.

RESULTS

MTHFR gene C677T polymorphism TT genotype was found statistically higher in the control group than in the patient group (Table 1), but allele frequencies were similar in the patient and control groups (Table 2). MTHFR gene A1298C polymorphism genotype distribution did not show any difference between the groups (Table 1), but A allele frequency was significantly higher in the control group than in the patients (Table 2).

DISCUSSION

Recent data suggest that T allele of MTHFR gene C667T polymorphism and C allele of MTHFR gene A1298C polymorphism are the cause of hyperhomocysteineima (13-15). Within this study, we analyzed the genotype distribution and allele frequencies of MTHFR gene C677T and A1298C polymorphisms in PCOS patients in Turkey. The present study indicates that MTHFR gene C677T polymorphism genotype distribution is significantly different between patients and controls. TT genotype of this polymorphism is found higher in the control group than in the patient group. However according to allele frequencies, there was no statistical difference between groups. When we look at MTHFR gene A1298C polymorphism genotype distribution, there was no significant difference between the

Table 1. Genotype distribution of MTHFR gene C677T and A1298C polymorphisms between PCOS patients and controls

Polymorphism	Control	Patient	p value
	(n=28)	(n=30)	
MTHFR C677T			
T/T	21	10	<0.05
C/T	1	16	
C/C	6	4	
MTHFR A1298C			
C/C	20	12	>0.05
A/C	1	3	
A/A	7	15	

Table 2. Allele frequencies of MTHFR gene C677T and A1298C polymorphisms between PCOS patients and controls

Polymorphism	Control	Patient	p value
	(n=28)	(n=30)	
MTHFR C677T			
T	13 (%23.2)	24 (%40)	>0.05
C	43 (%76.8)	36 (%60)	
MTHFR A1298C			
A	41(%73.2)	27 (%45)	<0.05
C	15 (%26.8)	33 (%55)	

patient and control group, but according to allele frequencies, A allele frequency was significantly higher in control group than in patients but these differences do not seem to be involved in the development of cardiovascular diseases in PCOS patients. In another study in Turkish population, Karadeniz et al. showed that homocystein levels were significantly different between controls and PCOS patients and MTHFR 677 CC genotypes had significantly higher proportions in the control group compared to the PCOS patients. They also indicated that the MTHFR gene C677T gene polymorphism does not influence homocystein levels of patients with PCOS, which is thought as one of the causes of cardiovascular diseases (17). In consistent with our results, Choi et al., and Orío et. al also did not show an association between MTHFR gene C677T polymorphism and PCOS in Korean and Italian populations (4,18). In a meta-analyses study, Bagos also found no evidence for association of MTHFR gene C677T polymorphism with PCOS (19). Palep-Singh et al. also compared MTHFR gene C677T and A1298C polymorphisms in South Asian and Caucasian populations with PCOS patients and found the frequency of homozygosity of TT677 and CC1298 7.2% and 4.9% in the Caucasians and 0% and 16.6% in South Asians. They have offered more comprehensive studies exploring the current theme in the PCOS group (20). Gene pools, life style, and gene– environment interactions vary between the populations, so the risk of PCOS development cannot be supposed to be similar with respect to genotypes in every populations. (21). Also genetic polymorphism studies with larger populations provide more meaningful results, so further studies with larger and even different populations, as well as including other genetic polymorphisms considered as a risk factor for PCOS to develop cardiovascular diseases will provide important validation to these results.

CONCLUSION

Consequently, it can be concluded that MTHFR gene C677T and A1298C polymorphisms are not involved in PCOS to develop cardiovascular diseases in this Turkish study population.

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