Coexistence of the 677C>T and 1298A>C MTHFR polymorphisms and its significance in the population of Polish women

Znaczenie współwystępowania polimorfizmów 677C>T oraz 1298A>C genu MTHFR u kobiet w populacji polskiej

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Abstract

Objectives: *The aim of the study was to evaluate the frequency of the 677C>T and 1298A>C polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene, as well as the coexistence of both these genetic variants in women from the Polish population.*

Material and methods: *A total of 662 women from the Polish population were enrolled in the study group. The frequency of the investigated genotypes of the 677C>T and 1298A>C polymorphisms of the MTHFR gene was analyzed with the use of PCR/RFLP methods.*

Results: *The frequency of the 677CC, 677CT and 677TT genotypes in the studied population of women was 50.60%, 39.88% and 9.52%, respectively. As to the 1298AA, 1298AC and 1298CC genotypes, the obtained results were as follows: 42.75%, 47.88% and 9.37%, respectively (Tables II and III). Simultaneous analysis revealed the most frequent coexistence of 677CC/1298AC (28.85%), 677CT/1298AA (20.85%) and 677CT/1298AC (19.03%) genotypes. The coexistence of 677CC/1298AA (12.39%), 677CC/1298CC (9.37%) and 677TT/1298AA (9.51%) genotypes was observed less frequently. In the studied population of Polish women, the coexistence of 677CT/1298CC, 677TT/1298AC and 677TT/1298CC genotypes has been not observed.*

Conclusions: *The frequency and coexistence of genotypes of the 677C>T and 1298A>C MTHFR gene polymorphisms in the studied population of Polish women is similar to other North-European populations. Women carriers of the mutated variants of both, 677C>T and 1298A>C polymorphisms of the MTHFR gene should receive special perinatal care in order to prevent fetal defects and thrombosis-related complications during pregnancy. It is vital to emphasize the significance of proper education of folate supplementation, especially in pregnant patients and women of reproductive age.*

Key words: ***MTHFR*** / **genetic polymorphism** / **general population** /

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Streszczenie

Cel: *Określenie częstości występowania polimorfizmów 677C>T i 1298A>C genu kodującego reduktazę metylenotetrahydrofolianową (MTHFR) oraz ocena współwystępowania obydwu wariantów genetycznych u kobiet w populacji polskiej.*

Materiał i metoda: *Do grupy badawczej włączono 662 kobiety z populacji polskiej. Częstość występowania genotypów polimorfizmów 677C>T oraz 1298A>C genu MTHFR została zbadana metodą PCR/RFLP.*

Wyniki: *W badanej grupie częstość występowania genotypów 677CC, 677CT oraz 677TT wynosiła odpowiednio 50,60%, 39,88% oraz 9,52%. Natomiast genotypy 1298AA, 1298AC oraz 1298CC występowały z częstością 42,75%, 47,88% oraz 9,37%. Jednocześnie analiza współwystępowania genotypów pozwoliła na określenie najczęściej występujących genotypów – 677CC/1298AC (28,85%), 677CT/1298AA (20,85%), 677CT/1298AC (19,03%). Współwystępowanie genotypów 677CC/1298AA (12,39%), 677CC/1298CC (9,37%), 677TT/1298AA (9,51%) było obserwowane rzadziej. W badanej populacji nie zaobserwowano współwystępowania polimorfizmów 677CT/1298CC, 677TT/1298AC, 677TT/1298CC.*

Wnioski: *Częstość występowania i współwystępowania polimorfizmów 677C>T i 1298A>C u kobiet w populacji polskiej jest porównywalna do częstości występowania tych wariantów w innych populacjach północnoeuropejskich. Kobiety, nosicielki zmutowanych genotypów polimorfizmów 677C>T i 1298A>C genu kodującego MTHFR, powinny zostać objęte specjalną opieką perinatalną, z powodu wzrostu ryzyka wystąpienia wad płodu oraz powikłań zakrzepowych w czasie ciąży. Należy zwrócić uwagę na właściwą edukację i suplementację folianami kobiet, szczególnie w wieku rozrodczym oraz w czasie ciąży.*

Słowa kluczowe: ***MTHFR*** / **polimorfizm genetyczny** / **populacja ogólna** /

Introduction

Proper function of the folate cycle is directly related to optimal human development and functioning. Adequate folate concentration prevents hyperhomocysteinemia, deficit of the synthesis of purine and pyrimidine, and methylation failure. Folate deficiency may lead to cardiovascular diseases, neurodegenerative and mental disorders, and even some cancers [1, 2, 3, 4, 5]. One of the key-enzymes in the folate cycle is methylenetetrahydrofolate reductase (MTHFR). Proper functioning of that enzyme is especially important in pregnant women [6, 7, 8].

In recent years, a few *MTHFR* gene polymorphisms have been described, including the *677C>T* and *1298A>C* as the most common. The *677C>T* polymorphism concerns transition of cytosine into thymine, resulting in substitution of alanine for valine in the catalytic region of the enzyme (position 222) [9]. The MTHFR activity is 60-70% lower in carriers of the homozygotic *677TT* genotype, which significantly increases the risk of hyperhomocysteinemia [10]. The *1298A>C* genetic variant is the effect of substitution of adenine for cytosine in exon 7, which results in the conversion of glutamine to alanine (position 429), and also decreases the MTHFR activity [11].

The differences in the frequency of the *677C>T* and *1298A>C* polymorphisms have been demonstrated to depend on race and ethnicity [12,13,14]. The mutated *677TT* variant concerns several to 15% percent of the Caucasian race. The mutated *1298CC* genotype is present in up to 10% of the Caucasian population (frequency of the *1298C* allele ranges from 27 to 36%) [12,13]. It has been shown that 15-20% of the Caucasian population are double heterozygotes (*677CT/1298AC*), and several percent are *677TT/1298AA* carriers [11]. The coexistence of *677TT/1298AA* and *677CT/1298AC* genotypes lowers MTHFR activity to a great extent, by 60-70% and 40-50%, respectively) (Table I) [11, 15].

In recent years, it has been shown that disturbances of the folate metabolism could be connect with decreased MTHFR activity and presence of the *MTHFR* polymorphisms. The consequences of the impaired folate metabolism in the general population are cardio-vascular, neurological, and psychiatric diseases [2,3,5]. In pregnant women, disturbed folate cycle constitutes a risk factor for neural tube defects, lip and palate cleft in the fetus, congenital heart and urinary tract defects, as well as Down syndrome. Also, it is the reasons for increased homocysteine level, leading to endothelium injury, thrombosis of the spiral arteries, and placental insufficiency. It may give raise to numerous obstetrical complications, including preeclampsia, recurrent miscarriages, fetal hypotrophy, preterm placental abruption, and intrauterine fetal death [6, 7, 8]. A correlation of the *677C>T* and *1298A>G MTHFR* polymorphisms with recurrent miscarriages has been suggested. It is also clear that the coexistence of both genetic polymorphisms has significant influence on the occurrence of the abovementioned disorders.

Objectives

The aim of the study was to evaluate the frequency of the *677C>T* and *1298A>C* polymorphisms of the *MTHFR* gene, as well as the coexistence of both genetic variants in Polish women.

Material and methods

Study group

A total of 662 healthy Polish women from the general population (Wielkopolska region) were enrolled. Mean patient age was 30.12±4.52 years, range 18-43 years. Women with cardiovascular, renal, and hepatic diseases, as well as with cancers or genetic disorders were excluded from the study. The analysis was performed at the Laboratory of Molecular Biology, Department of Perinatology and Women's Diseases, Poznan University of Medical Sciences, between 20011-2013. Written informed consent was obtained from all participants. Local Ethics Committee approved of the study.

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Table I. MTHFR activity [%] according to the presence of 677C>T and 1298A>C polymorphisms [Put, Weisberg].

Genotypes		677C>T MTHFR		
		CC (%)	CT (%)	TT (%)
1298A>C MTHFR	AA (%)	100	60-70	30-40
	AC (%)	70-80	50-60	-
	CC (%)	50-60	-	-

Table II. The frequency of genotypes and alleles of 677C>T MTHFR gene polymorphism.

Polymorphism	Genotypes	Observed value (%)	Expected value (%)
MTHFR 677C>T	CC	335 (50.60)	49.76
	CT	264 (39.88)	41.56
	TT	63 (9.52)	8.68
	Total	662 (100.00)	100.00
	Alleles		
	C	934 (70.54)	
	T	390 (29.46)	
	Total	1324 (100.00)	

Table III. The frequency of genotypes and alleles of 1298A>C MTHFR gene polymorphism.

Polymorphism	Genotypes	Observed value (%)	Expected value (%)
MTHFR 1298 A>C	AA	283 (42.75)	44.48
	AC	317 (47.88)	44.43
	CC	62 (9.37)	11.09
	Total	662 (100.00)	100.00
	Alleles		
	A	883 (66.69)	
	C	441 (33.31)	
	Total	1324 (100.00)	

Table IV. The frequency of coexistence of 677C>T and 1298A>C polymorphisms.

		677C>T MTHFR			
		CC	CT	TT	Total
1298A>C MTHFR	AA	82 (12.39)	138 (20.85)	63 (9.51)	283 (42.75)
	AC	191 (28.85)	126 (19.03)	0 (0.00)	317 (47.88)
	CC	62 (9.37)	0 (0.00)	0 (0.00)	62 (9.37)
	Total	335 (50.61)	264 (39.88)	63 (9.51)	662 (100.00)

Genetic analysis

Genetic analysis was conducted with the use of the polymerase chain reaction/ restriction fragments length polymorphism (PCR/RFLP) method. Genomic DNA was extracted from blood leucocytes using QIAamp DNA Blood Mini Kit (QIAGEN Inc., Germany). In order to determine the genotypes of the *677C>T* and *1298A>C* genetic variants, the previously described methodologies were used [9, 16].

Genomic DNA was amplified with primers (for the *677C>T* polymorphism: F5'-TGA AGG AGA AGG TGT CTG CGG GA-3', R5'- AGG ACG GTG CGG TGA GAG TG-3' and for the *1298A>C* polymorphism: F 5' CTT CTA CCT gAA gAg CAA gTC3' and R5` CAT gTC CAC AgC ATg gAg 3'). After amplification, the products were hydrolyzed with a *Hinf*I restriction enzyme (EURx, Poland) for the 677C>T polymorphism and with *Mbo*II (EURx, Poland) for the 1298A>C polymorphism.

The analysis of the digested fragments was conducted on 2% agarose gel by electrophoresis. The *677CC* genotype was identified in the presence of 198 base pairs (bp) long band, the heterozygous *677CT* genotype in the presence of 198, 175, 23 bp bands, and the mutated *677TT* genotype – of 175, 23 bp bands. The following genotypes were identified for the 1298A>C polymorphism: *AA* 176, 30, 28, 22 pz, *AC* 204, 176, 30, 28, 22 pz, *CC* 204, 30, 22 pz.

Table V. Co-existence of 677C>T and 1298A>C polymorphisms in different populations.

Author	Population	Study group	n	MTHFR 677C>T/MTHFR 1298A>C								
				N (%)								
				CC/AA	CC/AC	CC/CC	CT/AA	CT/AC	CT/CC	TT/AA	TT/AC	TT/CC
Isotalo i wsp., 2000	Canadian	newborns	119	17 (14.30)	42 (35.30)	9 (7.60)	14 (11.70)	23 (19.30)	-	12 (10.10)	2 (1.70)	-
van der Put i wsp., 1998	Dutch	women/men	403	62 (15.30)	105 (26.10)	38 (9.40)	81 (20.10)	81 (20.10)	-	36 (9.00)	-	-
De Re i wsp., 2010	Italian	women/men	454	24 (5.20)	90 (19.80)	38 (8.40)	98 (21.60)	137 (30.20)	3 (0.70)	57 (12.60)	7 (1.5)	-
		men	315	19 (60)	58 (18.40)	23 (7.30)	70 (22.20)	95 (30.20)	1 (0.30)	44 (14.00)	5 (1.60)	-
		women	139	5 (3.60)	32 (23.00)	15 (10.80)	28 (20.10)	42 (30.20)	2 (1.40)	13 (9.30)	2 (1.40)	-
Kumar Rai i wsp., 2005	Hindu	women	159	33 (20.80)	59 (37.10)	22 (13.80)	21 (13.20)	14 (8.80)	4 (2.50)	2 (1.30)	3 (1.90)	1 (0.60)
Rady i wsp, 1999	Ashkenazi/ Caucasian	women/men	186	33 (17.70)	50 (26.90)	20 (10.80)	19 (10.20)	39 (21.00)	-	25 (13.40)	-	-
		women/men	148	12 (8.10)	23 (15.50)	12 (8.10)	29 (19.60)	33 (22.30)	-	39 (26.40)	-	-
Sazci i wsp., 2005	Turkish	women/men	1684	178 (10.60)	401 (23.80)	146 (8.70)	411 (24.40)	363 (21.60)	17 (1.00)	146 (8.70)	22 (13.00)	-
Bodurogulu i wsp., 2005	Turkish	children	93	11 (11.80)	13 (14.00)	7 (7.50)	28 (30.10)	26 (28.00)	-	8 (8.60)	-	-
		fathers	72	3 (4.20)	10 (13.90)	10 (13.90)	28 (38.90)	27 (37.50)	-	2 (2.80)	-	-
		mothers	80	4 (5.00)	17 (21.25)	4 (5.00)	17 (21.25)	23 (28.75)	-	7 (8.75)	-	-
Ergul i wsp., 2003	Turkish	women	193	35 (18.10)	45 (23.30)	14 (7.30)	48 (24.90)	35 (18.10)	4 (2.10)	7 (3.60)	5 (2.60)	-

Frequencies of the genotypes were compared by the chi-square test. The expected genotype frequencies were calculated from allele frequencies with the use of the Hardy-Weinberg equilibrium.

Results

The frequency of the *677CC*, *677CT* and *677TT* genotypes in the studied population of Polish women was 50.60%, 39.88% and 9.52%, respectively. As for the *1298AA, 1298AC* and *1298CC* genotypes, the obtained results were as follows: 42.75%, 47.88% and 9.37% (Tables II and III). Simultaneous analysis revealed the most frequent coexistence of the *677CC/1298AC* (28.85%), *677CT/1298AA* (20.85%) and *677CT/1298AC* (19.03%) genotypes. The coexistence of the *677CC/1298AA* (12.39%), *677CC/1298CC* (9.37%) and *677TT/1298AA* (9.51%) genotypes was observed less frequently. To the best of our knowledge, the coexistence of the *677CT/1298CC*, *677TT/1298AC* and *677TT/1298CC* genotypes has been not observed so far in studies on the population of Polish women (Table IV).

Discussion

Numerous studies have investigated the frequency of the genotypes and alleles of the *677C>T* and *1298A>C MTHFR* gene polymorphisms in various races and ethnic groups [14, 17, 18, 19, 20].

However, only a few analyses have focused on the coexistence of these genetic variants in different populations, and their correlation with MTHFR activity and folate level changes [21].

One of the first researches in this field was performed by van der Put et al., in the Dutch population. Coexistence of the *677TT/1298AA* and *677CT/1298AC* genotypes was observed in 9% and 20.1% the of studied population, respectively (50% decrease of MTHFR activity). That study also revealed a significant decrease of the MTHFR activity in case of coexistence of the *677TT/1298AA* genotypes (by approximately 70%). Other genotype combinations, such as *677CC/1298CC* (9.4% of the population) and *677CT/1298AA* (20.1% of the population) correlated with 40% less MTHFR activity. In the studied Dutch population, there was no coexistence of the *677CT/1298CC*, *677TT/1298AC* and *677TT/1298CC* genotypes [11].

Other results concerning the Italian population were obtained by de Re et al., in their study on 315 males and 139 females. These authors observed a higher frequency of the *677CT/1298AC* (30.2%) and *677TT/1287AA* (12.60%) genotypes, which correlated with decreased MTHFR activity [22].

A significant number of studies on the *MTHFR* gene polymorphisms were performed in the Turkish population. Sazci et al., performed an analysis involving 1004 women and 680 men. The coexistence of the *677CT/1298AC* genotypes was

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noted in 21.6% of the cases [23]. Also, Ergul et al., demonstrated a coexistence of the *677CT/1298AC* genotypes in 21.6% of the investigated Turkish population [24]. In a study by Bodurogulu et al., the coexistence of the *677CT/1298AC* genotypes was observed in 28.75% of the members of the Turkish population (the study group consisted of 245 subjects: 80 mothers, 72 fathers, 93 children) [25].

Another ethnic group which was analyzed was a population of 159 Hindu women. The coexistence of the *677CC/1298AC* genotypes was observed in 37.1%, whereas the coexistence of *677TT/1298CC* genotypes was significantly less frequent, only in 0.6% of the cases [26].

One of the most interesting investigations on that subject was performed by Rady et al. Their study involved two populations: Ashkenazi and Caucasian population from Texas, USA. In the Ashkenazi population, the most frequent genotype coexistence was *677CC/1298AC* and, surprisingly, a much less frequent *677CT/1298AA*, while in the population from Texas high frequency of the *677TT/1298AA* genotypes was observed (26.4%) [27].

In various studies on many European populations, the coexistence of mutated genotypes (four mutated alleles, *677TT/1298CC*), as well as the coexistence of homozygous and heterozygous genotypes (three mutated alleles, *677CT/1298CC* and *677TT/1298AC*) has been not observed [11], with the exception of the Turkish population, where a coexistence of *677CT/1298CC* as well as *677TT/1298AC* has been noted [23, 24]. Also, carries of the *677CT/1298CC* genotypes were found in the Hindu population (2.5%) [25]. Interestingly, the coexistence of three mutated *677TT/1298AC* alleles was also detected in the Canadian population (1.70%) [28]. The results of the abovementioned studies are presented in Table V. These results are consistent with those obtained in our analysis of Polish women. The frequency of the coexistence of the genotypes of the *677C>T* and *1298A>C* polymorphisms was comparable to those observed in the Dutch population [11]. As in their study, we also did not observe the coexistence of three or four mutated alleles of the investigated *MTHFR* polymorphisms in the Polish population. Contrary to our results, coexistence of the *677CT/1298AC* genotypes was observed most frequently (30.2%) in the Italian population (10% more as compared to the frequency observed in Polish women from the Wielkopolska region). Noteworthy, a coexistence of as many as three mutated alleles of the *677CT/1298CC* and *677TT/1298AC* polymorphisms in the population from the south of Italy was reported [22].

Our results suggested that environmental factors should be also considered in such analysis. The observed higher frequency of the coexistence of genotypes with a higher number of the mutated alleles suggested a significant role of diet rich in folate in the south of Europe (Italy, Turkey). Higher folate intake could promote survival of individuals with mutated genotypes of the *MTHFR* polymorphisms. Polymorphism distribution is also influenced by ethnic origin and folic acid demand. Increased seasonal availability of folate-rich food compensates for the negative effects of the *677T* allele. Proper folate intake is believed to increase the prevalence of the *677T* allele in the general population. High survivability of the *677T* embryos might result in an increased number of cardiovascular and neurodegenerative diseases [29].

Some authors also demonstrate the influence of latitude and ultraviolet radiation on the frequency of the *677C>T* polymorphism in many populations. Prolonged radiation time is correlated with increased frequency of the mutated alleles of the *677C>T* polymorphism. Elongation of the environmental UV radiation time in the periconceptional period decreases folate concentration, because ultraviolet light type A induces oxidative degradation of 5-methylenetetrahydrofolate (5-MTHF) in the skin, which results in decreased 5,10-MTHF cell concentration. In such cases, the 5,10-MTHF is more intensively used for DNA synthesis in *677T-MTHFR* embryos than in *677CC* embryos. As a result, *677T-MTHFR* embryos are in the group with a great chance of survival and the frequency of *677T* alleles increases [30].

The frequency of the mutated *MTHFR* gene variants and coexistence of the *MTHFR* genotypes are influenced by the interaction of the genetic-environmental natural selection [30,31]. All these facts show that increased folate intake should be recommended in female carriers of both, the mutated genotypes of *677C>T* and the *1298A>C* polymorphisms (*677TT, 1298CC, 677CT/1298AC* and *677TT/1298AA*). Both these genetic variants are correlated with changes in the MTHFR activity, higher homocysteine, and lower folate concentration.

Conclusions

1. The frequency of genotype coexistence of the *677C>T* and *1298A>C MTHFR* gene polymorphisms in the studied population of Polish women is similar to findings about other North European populations.
2. Female carriers of the mutated variants of both, *677C>T* and *1298A>C* polymorphisms of the *MTHFR* gene should receive special perinatal care in order to prevent fetal defects and thrombosis-related complications during pregnancy. It is vital to emphasize the significance of proper education about folate supplementation, especially in pregnant patients and women of reproductive age.

Oświadczenie autorów

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