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MTHFR Polymorphism and Folate Status of Korean Women of Childbearing Age*

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It should be concerned to the women with mutated genotype of methylenetetrahydrofolate reductase (MTHFR), C677T or A1298C, since they need more folate than those with wild genotypes. In this study, we evaluated the folate status of Korean women of childbearing age according to their MTHFR polymorpiysm. Dietary folate intakes, plasma and erythrocyte folate concentrations, plasma homocysteine concentrations, and urinary excretions of para-aminobenzoylglutamate (pABG) and para-acetoamidobenzoylglutamate (ApABG) of twenty-five subjects aged between 19 and 35 years old were determined. Folate intakes seemed to be inadequate, being only three-quarters of the Korean RDA of folate. More than one-quarter of the subjects was exposed to folate deficiency risk as determined by erythrocyte folate concentration and almost one-quarter of the subjects showed hyperhomocysteinemia, although they had normal plasma folate concentrations. Urinary excretions of pABG and ApABG seemed to be low and ApABG constituted more than 85% of total folate catabolites. There were no significant differences in dietary folate intakes, plasma concentrations of folate and homocysteine, and urinary excretions of pABG and ApABG among the geneotypes of both C677T and A1298C. However, the subjects with 1298AC genotype had significantly lower erythrocyte folate concentration than those with 1298AA. Erythrocyte folate concentration showed an inverse relationship with plasma homocysteine concentration and positive relationships with urinary excretions of pABG and ApABG. The results of this study imply that mutations of 677C→T and 1298A→C in the study were not associated with decreased plasma folate and raised plasma homocysteine concentrations. A1298C polymorphism might be, however, more influential on erythrocyte folate concentration than C677T polymorphism, and urinary excretions of folate catabolites, pABG and ApABG, might be reliable indexes of folate nutritional status like plasma homocysteine concentrations.

Key words: Folate, MTHFR, Polymorphism, Folate catabolites, Women of childbearing age

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INTRODUCTION

Folate plays an essential role as a major coenzyme in one-carbon metabolism, which is a vital process of biosynthesis of DNA and amino acids. Thus, adequate consumption of folate is important especially during periods of growth, pregnancy, and lactation. It is well known that folate deficiency during pregnancy may cause adverse results like neural tube defects (NTDs), megaloblastic anemia, abortion, and/or abruption placenta.[1] A couple of studies conducted in Korea showed that dietary intakes of folate of reproductive aged women were not sufficient[2,3] and a considerable proportion of the women was in marginal folate deficiency.[3,4] Since the neural tubes close during the very early stage of pregnancy[5] and approximately half of pregnancies are unplanned,[6] folate status of reproductive aged women is critical to prevent NTDs.

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in one-carbon metabolism, which catalyzes the reaction in which homocysteine is converted to methionine. The mutations of MTHFR, 677 base pair cytosine changes to thymine (677C→T)[7] or 1298 base pair adenine changes to cytosine (1298A→C)[8] induce a change of folate nutritional status. Recently the interrelationship between genetics and dietary folate adequacy has become a major interest in the field of folate nutrition. Because the activity of MTHFR is decreased by the mutated polymorphism, the women with the mutated genotypes should show additional concern regarding their folate status.

Several studies performed in western countries indicated a strong relationship between folate nutritional status and

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MTHFR polymorphism. The people with 677CT had a higher plasma homocysteine concentration and lower plasma folate concentration than the people with wild type, 677CC.[9] Mutation of C677T seems to be associated with an accumulation of formylated tetrahydrofolate in erythrocytes.[10,11] Moreover, plasma homocysteine concentration of the people with homozygous type, 677TT, was higher than those with heterozygous type, 677CT.[11,12] Mutation of A1298C, however, does show no agreement with these results; the people only bearing 1298AC did not show any significant changes of plasma folate nor homocysteine concentration.[13,14] On the other hand, one study presented that MTHFR specific activity was decreased markedly in A1298C mutation[15] and another study indicated that A1298C mutation affected plasma homocysteine concentration in the same way as the C677T mutation.[10] In addition, it appears that 677C→T and 1298A→C polymorphism could act synergistically, given that heterozygosity for both polymorphism is a critical cause of decreased MTHFR activity.[13-15] In Korea, the genotype frequencies of MTHFR of 677C→T[16] and 1298A→C[17] in healthy subjects and the relationships between MTHFR and colorectal cancer risk[18] were investigated. Folate status of the women of childbearing age were evaluated by their dietary folate intakes,[2,3] plasma folate concentrations, erythrocyte folate concentrations and/or plasma homocysteine concentrations.[2,3,19] These studies found that the women of childbearing age did not take sufficient folate and their folate status were inadequate. However, there were little studies on the folate status of the women of childbearing age related to MTHFR polymorphism.

Thus, this study was conducted to screen the mutations of 677C→T and 1298A→C in the gene of MTHFR in Korean women of childbearing age and to assess their dietary folate intakes, plasma and erythrocyte folate concentrations, plasma homocysteine concentrations, and urinary excretions of folate catabolites, para-aminobenzoylglutamate (pABG) and para-acetoamidobenzoylglutamate (ApABG), according to their MTHFR polymorphism.

MATERIALS AND METHODS

1. Subjects

A total of twenty-five subjects were participated voluntarily in this study with written consent. They were between 19 and 35 years old (23.9±5.2) and their height, weight, and body mass index were 159.0±4.7 cm, 52.4±6.1 kg, and 20.6±2.2 kg/cm^2, respectively. All subjects were non-smokers and not taking any oral contraceptives. They all had normal blood chemistry properties without any medical problems. They lived in either Gwangju or Suncheon, Korea.

2. Food Sampling and Folate Assay

Dietary intakes of nutrients of the subjects were determined by a weighed food record method for 3 consecutive days. Each subject weighed all foods consumed using a dietary scale (±1 g) and recorded them on the food diary form. One tenth of all foods consumed were collected for folate analysis. The collected food samples were homogenized with the same volume of 0.1 M potassium phosphate buffer (pH 6.3, 1% Na-ascorbate) and stored at -20 ℃ until analysis.

Food folate concentration was determined by a microbiological method using *Lactobacillus. casei* (ATCC 7469) as the test organism in 96-well plates[20] after the trienzyme treatment.[21]

3. Blood Sampling and Assays of Plasma and Erythrocyte Folate and Plasma Homocysteine

On the day after the 3-day dietary survey, about 5 mL venous blood from fasting subjects was drawn into the tubes containing EDTA. 100 µl of each whole blood sample was diluted with 900 µl of potassium phosphate buffer (pH 6.3, 1% Na-ascorbate) and stored at -20 ℃ for erythrocyte folate assay. Plasma was separated from the remaining blood and stored at -20 ℃ for folate and homocysteine assay.

Folate concentrations in plasma and erythrocyte were measured by the same microbiological method as mentioned above using *Lactobacillus. casei* (ATCC 7469) in 96-well plates.

Plasma homocysteine concentration was determined by HPLC with fluorescence detection according to the method of Araki and Sako[22] with some modifications.

4. MTHFR Polymorphism Assay

Portions of whole blood were used for MTHFR polymorphism assay. Genomic DNA was extracted from whole blood using AQUA PURE genomic DNA prep kit (Bio-rad Pacific, LTD., HongKong). Genotype was determined according to the method of Friedman *et al.*[13] with some modifications. Polymerase chain reaction (PCR) mixture used was a commercial premix (Bioneer, Korea). The primers for C677T PCR amplification were 5'-TGAAGGAGAAG GTGTCTGCGGGA-3' and 5'-AGGACGGTGCGGTGA GAGTG-3'. The primers for A1298C PCR amplification were 5'-CTTTGGGGAGCTGAAGGACTACTAC-3' and 5'-CACTTTGTGACCATTCCGGTTTG-3' (Bioneer,

Korea). *Hinf I* (Takara, Japan) and Mbo II (NEB, USA) were used for C677T and A1298C genotype determinations, respectively.

5. Urine Collection and Urinary pABG and ApABG Assay

For 3 consecutive days over the same period as the dietary survey, daily 24-h urine was collected into a PET bottle containing 3 g of sodium ascorbate. The bottles were kept refrigerated, as long as possible, during the collection period to prevent bacterial growth. Urine was homogenized throughly after total urine volume was measured and stored at -20 ℃.

According to the method of McPartlin *et al.*,[23] folate catabolites, pABG and ApABG, were determined using a reverse phase HPLC with octadecylsilica column (Ultramex C18, 5-μm particle size, 4.6 mm ID×250 mm; Phenomenex, Torrance, CA) after affinity chromatography.[24] A UV absorption detector (Diones AD 20) monitored at 280 nm. Quantification of pABG and ApABG was accomplished relative to standards prepared from commercial pABG (Sigma Chemical Co, St Louis, MO).

6. Statistical Analysis

SPSS, Windows version 10.0, (SPSS Inc, Chicago, IL) was used for data tabulation and statistical analyses. All data were expressed as means and standard deviations. For comparison of mean differences between each of the three genotype groups, a general linear model (GLM) with Fisher's Protected Least Significant Difference was used. For the relationships among the indexes of folate nutrition examined, Pearson's correlation coefficients were used and regression equations were tabulated. A p-value <0.05 was considered the level of significance. Since the data of plasma and erythrocyte folate, and plasma homocysteine concentrations did not show standard distributions, those were transposed into natural log before statistical analyses.

RESULTS AND DISCUSSIONS

1. MTHFR Genotypes

The frequency of subjects with MTHFR genotypes is presented in Table 1. In the mutation of C677T gene, the frequency of subjects heterozygous for the 677CT genotype was 36.0% and that of those homozygous for the type 677TT genotype was 12.0%. In the mutation of A1298C gene, the frequency of subjects heterozygous for the 1298AC genotype was 36.0% and that of those homozygous for the 1298CC genotype was 4.0%.

Table 1. Frequencies of MTHFR genotypes of the subjects

	C677T		A1298C	
Wild	CC	52.0 (13)	AA	60.0 (15)
Mutated	CT	36.0 (9)	AC	36.0 (9)
	TT	12.0 (3)	CC	4.0 (1)
Allele frequency	T	0.30	C	0.22

Values are % (n).

Table 2. Combinations of the genotypes of C677T and A1298C of MTHFR

		C677T		
		CC	CT	TT
A1298C	AA	20.0 (5)	32.0 (8)	12.0 (3)
	AC	24.0 (6)	8.0 (2)	0.0 (0)
	CC	4.0 (1)	0.0 (0)	0.0 (0)

Values are % (n).

The allele frequency of MTHFR 677T in this study, 0.30, was in a rough median of the data of Chinese (0.40 and 0.41),[25,26] Japanese (0.41),[27] Caucasian (0.27~0.37),[28-31] and Korean (0.39).[18] The allele frequency of MTHFR 1298C in this study, 0.22, was also in a rough median of the data of Chinese (0.17 and 0.19),[25,26] Japanese (0.19),[27] Caucasian (0.29~0.36),[28-31] and Korean (0.12).[18]

In the analysis of combined genotypes, only six sets of MTHFR polymorphism appeared among the nine sets that could be expressed, as presented in Table 2. The frequency of combined normal genotypes (677CC/1298AA), 20.0%, was higher than Chinese (16.4% and 18.7%),[25,26] Japanese (15.2%),[27] and Caucasian (10.8~18.4%)[28-31] but similar to the figure of Korean (24.5%).[18] These results indicate that the mutation of MTHFR gene is quite common. We found no double homozygote (677TT/1298CC) nor any one homozygote plus one heterozygote genotypes (677CT/ 1298CC and 677TT/1298AC). In the previous study conducted in Korea with considerably large number of subjects, there were no expression of the three combined genotypes.[18] However, 677CT/1298CC and 677TT/1298AC were reported in a study from China although the frequencies were very limited:[30] The frequency of the former was 0.2% and that of the latter was also 0.2%. The type of double homozygote (677TT/1298CC) has not been found yet all over the world. Beside the ethnic difference, the small number of subjects in this study could be one of the reasons that we did not found the type of one homozygote plus one heterozygote.

The results of this study confirmed that the prevalence of both C677T and A1298C polymorphism is very common but varies in different ethnic groups.

2. Concentrations of Plasma and Erythrocyte Folate and Plasma Homocysteine by MTHFR Genotypes

Concentrations of plasma and erythrocyte folate and plasma homocysteine according to MTHFR genotypes are presented in Table 3.

Plasma folate concentrations of the genotypes of C677T, CC, CT, and TT were 10.4±3.0, 11.4±4.0, and 9.3±3.0 ng/mL, respectively. Those of the genotypes of A1298C, AA, AC, and CC were 11.1±3.9, 10.1±2.3, and 8.8±0.0 ng/mL, respectively. Folate concentrations of erythrocyte of CC, CT, and TT were 184.8±82.8, 270.5±106.2, and 158.7±45.5 ng/mL, respectively and those of AA, AC, and CC were 234.7±74.6, 187.0±124.3, and 109.0±0.0 ng/mL, respectively. Plasma homocysteine concentrations of CC, CT, and TT were 13.3±3.9, 11.8±3.0, and 14.7±4.7 μmol/L, respectively. Those of the AA, AC, and CC were 12.4±3.5, 13.8±4.1, and 13.0±0.0 μmol/L, respectively. All subjects were within the normal range of plasma folate concentration (≥6 ng/mL) but, 28% were in deficiency (<140 ng/mL) and 8% were in border line deficiency (140 ~157 ng/mL) of erythrocyte folate concentration. These results show that more than one-quarter of the subjects in this study were exposed to folate deficiency risk. The result showing that 24% of the subjects were in hyperhomocysteinemia (≥15 μmol/L) also supported the proposal that the folate nutritional status of the subjects was inadequate.

Concentrations of plasma and erythrocyte folate and plasma homocysteine were not different among the three C677T genotypes. It is known that most homozygote individuals with low-normal plasma folate concentrations have an increased plasma homocysteine concentration.[32] The same trends were seen in this study, but they were not statistically significant; the group of 677TT genotype had the lowest plasma folate and erythrocyte concentration and the highest plasma homocysteine concentration among the C677T genotypes, but without statistical significance. Among the three A1298C genotypes, plasma folate and homocysteine concentrations were not different either, however, the erythrocyte folate concentration of 1298AC was significantly lower than that of 1298AA. The erythrocyte folate concentration of 1298CC seemed to be even lower than that of 1298AC, but it could not be concluded without statistical analysis. This was not like the result reported previously which showed that the effects of A1298C mutation on MTHFR activity and plasma folate concentration were weaker than those of C677T mutation.[13,30] In this study, among the seven subjects who were in folate deficiency, as evaluated by erythrocyte folate concentration, six had 1298AC genotype and only one had 677CT genotype. This implies that the mutation of A1298C might affect erythrocyte folate concentration more strongly than the mutation of C677T. The results in this study might confirm the theory that MTHFR activity decreases markedly in A1298C mutation[15] and that A1298C mutation affects folate status in a similar way to C677T mutation.[10] However, this cannot be concluded definitely due to the reason mentioned above. There were a couple of limitations in this study: one was the small number of subjects and the other was that there was only one subject who had 1298CC genotype. Since folate nutritional status by A1298C mutation has never been studied in Korea before, it should be investigated with a large population sample.

Table 3. Concentrations of plasma and erythrocyte folate and plasma homocysteine of the subjects by their MTHFR genotypes

		Folate		Plasma
		Plasma (ng/mL)	Erythrocyte (ng/mL)	Homocysteine (μmol/L)
C677T	CC	10.4±3.0[a]	184.8± 82.8[a]	13.3±3.9[a]
	CT	11.4±4.0[a]	270.5±106.2[a]	11.8±3.0[a]
	TT	9.3±3.0[a]	158.7± 45.5[a]	14.7±4.7[a]
A1298C	AA	11.1±3.9[a]	234.7± 74.6[a]	12.4±3.5[a]
	AC	10.1±2.3[a]	187.0±124.3[b]	13.8±4.1[a]
	CC	8.8±0.0	109.0± 0.0	13.0±0.0

Values are means±standard deviations.
Values with different superscripts in a row are significantly different at $p<0.05$ by GLM with Fisher's Protected Least Significant Difference.

3. Dietary Folate Intakes and Urinary pABG and ApABG Excretions by MTHFR Genotypes

Dietary folate intakes of the subjects according to MTHFR genotype are shown in Table 4. Folate intakes of the genotype of C677T, CC, CT, and TT were 215±61, 147±152, and 171±37 μg/day. respectively. Those of the genotype of A1298C, AA, AC, and CC were 194±76, 178±36, and 162±0.0 μg/day, respectively. No significant difference in dietary folate intakes was observed among

Table 4. Dietary folate intakes and urinary folate catabolite excretions of the subjects by their MTHFR genotypes

		Dietary folate	Urinary folate catabolite	
		(μg/d)	pABG (nmol/d)	ApABG (nmol/d)
C677T	CC	215± 61	10.3±3.4	85.2±16.1
	CT	147±152	11.2±3.3	87.5±24.7
	TT	171± 37	9.1±3.2	89.4±15.1
A1298C	AA	194± 76	11.2±3.7	89.2±17.6
	AC	178± 36	9.5±2.4	83.6±21.6
	CC	162± 0.0	8.8±0.0	71.4± 0.0

Values are means±standard deviations.
There are no values significantly different among the each genotypes.
pABG: para-aminobenzoylglutamate; ApABG: para-acetoamidobenzoylglutamate

the genotype groups of both C677T and A1298C. We did not find that the subjects with any mutated genotype consumed more folate.

All genotype groups did not meet the Korean RDA of folate for adult women, 250 μg/day. This result confirmed previous data that showed folate intakes of Korean women of childbearing age to be inadequate; previous data were 204±92,[2] 168±65,[3] or 140±57 μg/day.[33]

Urinary pABG and ApABG excretion according to MTHFR genotype are presented in Table 4. Urinary excretions of pABG of the genotype of C677T, CC, CT, and TT were 10.3±3.4, 11.2±3.3, and 9.1±3.2 nmol/day. respectively and those of A1298C, AA, AC, and CC were 11.2±3.7, 9.5±2.4, and 8.8±0.0 nmol/day, respectively. Urinary excretions of ApABG of the genotype of C677T, CC, CT, and TT were 85.2±16.1, 87.5±24.7, and 89.4 ±15.1 nmol/day, respectively, and those of A1298C, AA, AC, and CC were 89.2±17.6, 83.6±21.6, and 71.4 nmol/day, respectively. ApABG constituted more than 85% of total folate catabolites as reported by Gregory *et al.*[34] Excretions of pABG and ApABG of the subjects in this study were lower than those in other studies:[34-36] Non-pregnant women in the U.S. excreted 22 nmol/day of pABG and 94 nmol/day of ApABG when they consumed 450 μg of folate. Considering that folate catabolite excretion is responsive to changes in dietary folate intakes,[35] this could be understood by the fact that the folate intake of the subjects was not sufficient and their erythrocyte folate concentrations were inadequate.

No significant difference was observed in urinary excretions of pABG and ApABG among the genotype groups of both C677T and A1298C.

4. Correlations among the Indexes of Folate Nutritional Status

As shown in Figure 1, among the indexes of folate status examined, erythrocyte folate concentration was significantly related with plasma homocysteine ($r=-0.433$, $p<0.05$) and urinary excretions of both pABG ($r=0.432$, $p<0.05$) and ApABG ($r=0.428$, $p<0.05$). Since pABG and ApABG showed positive correlations with plasma erythrocyte folate concentration, total folate catabolite excretion (pABG+ApABG) also had a positive relationship with plasma erythrocyte folate concentration ($r=0.466$, $p<0.05$).

It is known that plasma homocysteine concentration is strongly regulated by folate status.[37] In this study, urinary folate catabolites, both pABG and ApABG, were correlated with erythrocyte folate concentration as well as plasma homocysteine concentration. These results confirmed that urinary excretions of pABG and ApABG reflect folate nutritional status like plasma homocysteine concentration.[36]

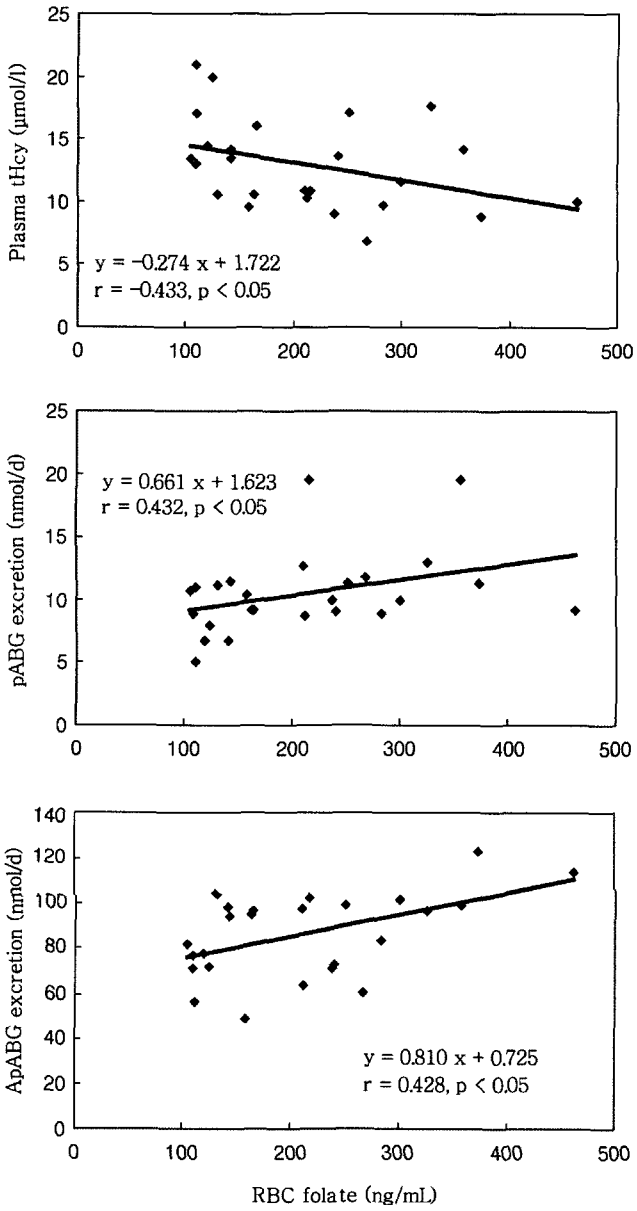

Fig. 1 Relationships between erythrocyte folate concentration and plasma homocysteine concentration and urinary excretions of pABG and ApABG.

tHcy: total homocysteine; pABG: para-aminobenzoylglutamate, ApABG: para-acetoamidobenzoylglutamate

SUMMURY AND CONCLUSION

This study was conducted to investigate MTHFR polymorphism and folate nutritional status of women of childbearing age in Korea. The twenty-five subjects were between 19 and 35 years of age and in non-pregnant, non-lactating status. MTHFR polymorphisms, C677T and A1298C, of the subjects were assayed and their dietary folate intakes, plasma and erythrocyte folate

concentration, plasma homocysteine concentration, and urinary excretions of folate catabolites, pABG and ApABG, were determined.

The allele frequency of 677C→T and 1298A→C in this study were 48.0% and 40%, which were in rough medians of the data from various ethnic groups, respectively. Among the nine combined genotype sets, only six sets were found. These results indicate that mutations of 677C →T and 1298A→C were very common with various allele frequencies among the ethnic groups. Concentrations of plasma folate of all subjects were above the normal level, but 28% of the subjects were in folate-deficiency and 8% of the subjects were in marginal folate-deficiency as evaluated by erythrocyte folate concentration. Also, 24% of the subjects showed hyperhomocysteinemia. There were no significant differences in plasma and erythrocyte folate concentration and plasma homocysteine concentration among the genotypes of C677T and A1298C. Only 1298AC genotype showed lower erythrocyte folate concentration than 1298AA. Folate intakes of all genotype groups were below the Korean RDA of folate and these were not different among each genotype of C677T and A1298C. Urinary excretions of folate catabolites, pABG and ApABG, were not different either among each genotype of C677T and A1298C. Erythrocyte folate concentration had an inverse correlation with plasma homocysteine concentration and positive relationships with urinary pABG and ApABG excreton.

These results indicate that the mutations of MTHFR, neither C677T nor A1298C are strongly associated with decreased plasma folate concentration and raised plasma homocysteine concentration, however, A1298C polymorphism might be more influential on erythrocyte folate concentration than C677T polymorphism. These results concerned that urinary excretions of folate catabolites, pABG and ApABG, are reliable indexes of folate nutritional status like plasma homocysteine concentration.

Considering the fact that those individuals who are in good folate status are not influenced by their genotype of MTHFR, it is important to maintain adequate folate status by consuming sufficient folate, to prevent the problems related to folate-deficiency, especially for those with a mutated genotype of MTHFR.

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