

MTHFR 677 C< T and 1298 A>C polymorphisms increases the risk of recurrent abortion in the Iraqi woman

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Abstract

Background: The C677T and A1298C polymorphism mutations in the methylenetetrahydrofolate reductase (MTHFR) gene will be investigated in a multi-abortion study.

Aim: To determine mutation (SNP) in the methyl tetrahydrofolate reductase (MTHFR)

Gene with multiple abortion.

Methods: "We nominated two hundred patients for this study in three groups: the study group, The first group included 50 women with a history of 1 or 2 missed first trimester Abortions and fifty to control group the last group which consisted of one hundred Patients with a history more than two missed abortion. Anticoagulants human blood tests such as (protein C, protein S, and lupus) as well as general serum tests IgG and IgM for (Cytomegalovirus, Toxoplasma gondii, Rubella virus, Anti-cardiolipin antibodies and anti-phospholipid antibody) were performed. In addition, screening of the maternal MTHFR C667T and MTHFR a 1298C mutation was determined by PCR.

Result: all common serum test for study population (CMV, Toxo, Rubella, ACA and APL) IgG and IgM, also anticoagulants human blood test (protein C, protein S and Lupus) is negative. The frequency of heterozygous (genotype) A1298C and C677T was similar. The distribution of MTHFR, C677T and A1298C genotypes show significantly differences $P \leq 0.05$; OR= (95%CI) between patients with multiple abortions and control subjects.

Conclusions: the result suggestion MTHFR A>C 1298 and C< T 677 polymorphisms might be associated with multiple abortion in the examined population.

Introduction

The World Health Organization (WHO) defines miscarriage as the loss of embryo or fetus weighing less than 500 grams, which match up to approximately (20-22) weeks of pregnancy [1]. The classification of spontaneous abortion as early and late (<12 weeks) and (≥ 12 weeks) respectively [2]. In recent years, several studies have assessed the correlation of the methylenetetrahydrofolate reductase (MTHFR) and the hygienic risk of multiple abortions between C677T and A1298C polymorphisms [3,4].

Methylenetetrahydrofolate is a key enzyme of folate/homocysteine pathway [5,6] MTHFR which performs a vital role in folate-dependent homocysteine metabolic rate. Mutations in MTHFR have been reported as the major causes of hyperhomocysteinemia [7]. Hyperhomocysteinemia alone may pose a mild risk, but in conjunction with other factors causing vascular lesions it will increase the risk of disease. Encoding gene variants of the homocysteine (hcy) metabolism enzyme or, using up important substrates or cofactors for those enzymes, which include vitamin B12, vitamin B6 and folic acid\folate (FA), may result in the rise plasma homocysteine levels [8].

The active form of FA in an organism is derived from a reduced reaction of FA into tetrahydrofolate, tetrahydrofolic acid, (THF), with the latter being the true coenzyme of MTHFR. Metabolism of folate is

essential for proper cellular function. Within the folate track, methylenetetrahydrofolate reductase (MTHFR) reduces 5,10- methylenetetrahydrofolate to 5-methyltetrahydrofolate: a methyl donor for re-methylation of homocysteine to methionine” [9] as shown in figure1[10].

MTHFR stimulates irreversible transformation of 5,10-methyentetrahydrofolate CH₃-THF to 5- -CH₃-THF. The normal activity of MTHFR assistances to maintain methionine and folate in the bloodstream at steady levels, preventing Hcy aggregation [11]. The A1298C and C677T SNP of the MTHFR gene, in convinced conditions, may lead to an increase in homocysteineemia and plasma homocysteine, which can lead endothelial grievance in blood vessel. This may lead to increase thromboembolic possibility, which lead to stimulate an impediment of the placental vessels in pregnant women, which are the procreating outcomes in repeated abortions. Thus, Recurring PRLs are regarded as risk factors for hyperhomocysteinemia. Hyperhomocysteinemia may be demonstrated in women with RPLs. [12].

Enzymatic activity of MTHFR may be minimize due to the Polymorphisms in the gene encoding it. The variation C677T leads to a replacement of a cytosine C into a thymine T at situation 677 in exon 4 of the MTHFR gene. In position 222, at protein level (p.Ala>Val), this genetic variant provides a path to amino acid substitution in place [13]. A further MTHFR polymorphism linked to a reduction in enzyme activity is an adenine replacement with cytosine (A1298C) in position 1,298 [14,15]. in position 429 at the protein level (p.429Glu>Ala) variant ignites do to the substitution of a glutamate G with an alanine [16]. It has been established that the A1298C and C677T polymorphisms in heterozygous genotype, laterally with a folate deficiency, initiate increased level of Hcy plasmatic [17]. However, many studies have endeavored to finding the relationship between the genetic variations of the thromboembolic risk and MTHFR gene, which in turn brings about risk of RPL in pregnant women. The aim is to investigate the correlation between the methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphism in multiple-abortion.

Materials And Methods

Two hundred women (suffer from recurrent miscarriages were recruited as the subject of this study and all of them visited the Alburaq medical laboratory from January 2018 to August 2020. They were ask about their medical history and undertook the physical examination of genital system. Group of study are divided in two groups: The first group involved 50 women with a history of one or two missed trimester abortions, wherever the reason was unidentified. The second study group involved 150 cases with a history of more than two missed abortions. The age of all the women in our study ranging between 18-40 years. All causes of abortions in the first trimester were made after 5, 10 me-THF excluding other possible causes of multiple abortion, such as a common serum tests IgG and IgM for (Cytomegalovirus, Toxoplasma gondii, Rubella virus, Anti-cardiolipin antibodies and anti-phospholipid antibody) besides an Anticoagulants human blood test such as (protein C, protein S, lupus) was carried out. After separating the blood serum from the patient study, PCR was used to determine the presence of the mutations of MTHFR C667T and A1298C. Different groups compared the results.

Genotyping

Qiagen DNA extraction kit (Qiagen, USA) was applied to extract genomic DNA from 2 ml peripheral venous anticoagulant blood, and then stored at – 80 °C. MTHFR genotyping was determined after PCR amplification. The procedure includes three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilization as an array of parallel lines, bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrate. The probes and primers were designed and synthesized by Applied VIENNA LAB kits (REF: 4-240, LOT: 10-ZW-20-039, 10-NE-19-039, 04-51-18-039 and 20-51-19-039). All polymerase chain reaction (PCR) amplification systems (Scientific PCR Thermal Cycler, REF: A73028, SN: 2280418091255) were conducted in a total of 25 µl, containing; 5 µl Taq Polymers (Thermo fisher, LOT [00434514 and 00468843] with 5U/uL, 5 µl of DNA, and 15 µl of A and B PCR Master Mix. The cycling protocol was included in an initial denaturation at 94 °C for 2 min, and then carried out 35 cycles of 94°C / 15sec, 58°C / 30sec and 72°C/30s with final extension 72°C/3min.

Human blood test that works in this study show in table 1.

No.	Blood test	Instrument	SN of instrument	LOT/ of kit	Company	Methodology
1	Toxo IgG/IgM	CTKBiotic, Inc	-	R0234C.		TLC
2	CMV IgG/IgM	VIDAS	IVD7001732	1231181680	France	ELFA
3	Rubella IgG/IgM	VIDAS	IVD7001732	1008181480	France	ELFA
4	Protein C / S	ELx800	193580	A17490252 WB 18241	USA	ELISA
5	Lupus	Genex thrombo	FL701492	121145	France	Coagulation
6	ACA/APL IgG/IgM	Alegria argentic	492000	2007407 2005625	Germany	ELISA
7	DNA Extraction	GIAGEN	-	163048160	USA	Manual
8	MTHFR gene mutation	VIENNA LAB	-	10-ZW-20-039 10-NE-19-039 20-51-19-039 04-51-18-039	Vienna, Austria	Revers-hybridization

Analysis of statistics

SPSS Version 20 was used to carry out statistical data analysis (SPSS Inc., Chicago, IL, USA). In the Pearson chi-square test and at a 95% confidence interval the mutation frequencies in MTHFR mutation between the case study and the control group have been analyzed (95 % CIs). P values < 0.05 considered significant.

Results

Among 200 (150 miscarriage women, 50 control) women with a mean age of 29 years (range, 18-40 years), all of them had both IgM and IgG test results as common serum tests IgG and IgM for (Cytomegalovirus, Toxoplasma gondii, Rubella virus, Anticardiolipin antibodies, and anti-phospholipid antibody) also an Anticoagulants human blood test such as (protein C, protein S, lupus) are negative, The differences between Toxoplasma, Rubella, ACA, APL, and CMV IgM IgG rates were found to be statistically no significant". Similarly, the differences between protein C, protein S, and lupus differences rates were determined to be statistically no significant ($p < 0.01$). in addition, from the table (2) explain There are no significant differences between the studied groups) patient and control). This supports the fact that the studied groups are homogeneous .where when (sig) $p = 0,000$ this experiment is repeated 100 times, and every time the researcher rejects the null theory (the arithmetic mean is equal) there is no single decision of his decisions is wrong. This means that the data study is real

Table (2): Distribution of CMV, Rubella, ACA, APL, Toxo (IgG,IgM) Protein C, Protein S, Lupus according to group study.

ANOVA						
		Sum of Squares	df	ms	F	Sig.
CMV IgG	Between Groups	342.413	2	171.206	3.611	0.029
CMV IgM	Between Groups	5.924	2	2.962	0.671	0.512
Rubella IgG	Between Groups	116633.45	2	58316.725	0.660	0.518
Rubella IgM	Between Groups	272.172	2	136.086	12.285	0.000
ACA IgG	Between Groups	120.477	2	60.239	13.123	0.000
ACA IgM	Between Groups	106.637	2	53.318	14.681	0.000
APL IgG	Between Groups	5.387	2	2.694	0.669	0.514
APL IgM	Between Groups	6.877	2	3.438	1.108	0.332
Protein C	Between Groups	69.870	2	34.935	0.132	0.876
Protein S	Between Groups	88.535	2	44.267	0.117	0.889
Lupus	Between Groups	69.210	2	34.605	1.170	0.312
MTHFR	Between Groups	21.415	2	10.708	16.986	0.000
MTHFR_A	Between Groups	74.190	2	37.095	69.274	0.000
Toxo IgG	Between Groups	2.160	2	1.080	10.074	0.000
Toxo IgM	Between Groups	0.000	2	0.000		

MTHFR polymorphism genotypes of the 50 cases of miscarriage women in group A, 14 heterozygotes, and 1 homozygote genotype for C677T were identified, while 30 heterozygotes and 1 homozygote for A1298C

were identified. A total of 12 were compound heterozygotes, and 16 were without mutation. the 100 miscarriage women in group B, 11 homozygotes for C677T and 35 heterozygotes were identified. For the A1298C variant, 18 were homozygous and 64 were heterozygous. 15 subjects were compound heterozygotes genotypes. Table (3) show The analysis of allele distribution in group A for C677T showed that there were 16 cases with T and 84 cases with C in the case group, while in the control group, allele C was found in 97 cases, and T was found 3 in cases. Significant less allele C was observed in the case group (OR = 0.16, CL95= 0.04-0.57 P < 0.05).whereas distribution allele of A1289C show total A 68 in case group and 96 in control and C found 32 in cases and 4 in control this show high significant p=0.0001, CL=0.03-0.26).

Table (3): Distribution of genotypes and alleles of MTHFR gene C677T and A1289C of A group

Snpc677T	Patients(50)	Control(50)	P value	OR	(CI)95
CC ^a	35	48			
TT	1	1	0.67	0.72	(0.04-12.06)
TC	14	1	<0.0001*	0.05	(0.007-0.41)
total	50	50			
C	84	97			
T	16	3	0.001*	0.16	(0.04-0.57)
Snpc1289C	Patients(50)	Control(50)	P value	OR	(CI)95
AA ^a	19	47			
AC	30	2	<0.0001*	0.02	(0.006-0.12)
CC	1	1	0.50	0.40	(0.02-6.80)
total	50	50			
A	68	96			
C	32	4	0.0001*	0.08	(0.03-0.26)

*P ≤ 0.05; OR= (95%CI)

Whereas, statistically significant difference (P ≤ 0.05) was detected in the frequency of MTHFR SNPs C677T and A1289C in women as show in table (4). Combined heterozygosity and homozygosity of MTHFR polymorphisms for two SNP was a common phenomenon in the women suffer abortion more than two times was (29 %) of women .also combined heterozygosity for both SNPs in all studied groups was observed. Combined 677TT/1298AC ,677CT/1298CC or 1298CC/ 677TT genotypes, which contain three or four mutant alleles, were also identified in our study groups, 15% from the cases study show combined heterozygosity in both SNP This refer to the heterozygosis is more risk.

However, there was a substantial difference between the multiple abortion and control groups when the detected frequencies of the 677CT/1298AC and 677TT/1298AA genotypes were combined; this indicates that these genotypes are involved in multiple abortion pathogenesis.

Table (4): allelic Distribution of genotypes MTHFR gene A1289C and C677T of B group

Snpc677T	Patients(50)	Control(50)	P value	OR	(CI)95
CC ^a	54	45			
TT	11	1	0.01*	0.1	(0.01-0.87)
TC	35	3	0.0001*	0.1	(0.03-0.35)
total	100	49			
C	143	93			
T	57	5	<0.001*	0.13	(0.05-0.34)
SNPA1289C	Patients(50)	Control(50)	P value	OR	(CI)95
AA ^a	16	44			
AC	64	3	0.001*	0.01	(0.005-0.06)
CC	18	2	<0.001*	0.04	(0.008-0.19)
total	100	49			
A	96	91			
C	100	7	<0.0001*	0.07	(0.03-0.16)

*P ≤ 0.05; OR= (95%CI)

Discussion

It remains unclear how genetically modified substances contribute to the onset of multiple abortions. One of the key enzymes in folate metabolism, the MTHFR gene is found on chromosome 1 (1p36.3), which contains 12 exons [18]. MTHFR can influence gene expression via DNA methylation by participating in the methionine cycle. Dozen MTHFR gene mutations have been identified so far, there are still more, and more diseases linked to new mutations [19]. has been reported that C677T polymorphism of MTHFR gene is strictly related to birth defects hypertension, Alzheimer's, atherosclerosis, heart disease and hormone metabolism, miscarriage [20]. That attracted a lot of attention. The risk of these diseases can be reduced by exogenous folic acid supplementation.

"The MTHFR C677T allele frequencies and genotype distribution found in our patient group agree well with those reported in a previous investigation of many populations". But there is no previous research on the MTHFR C677T and A1298C polymorphism in Iraqi patient.

We observed a very high frequency of MTHFR 1298C and 677T alleles in the multiple abortion group this agreement with (Henrik, 2002) [21]. Frosst et al. found of CT heterozygote exhibited ~30% enzyme activity, which was significantly higher than TT homozygote that (almost 65% of normal enzyme activity) and CC homozygote (normal enzyme activity), MTHFR c.677C>T can have an effect on enzyme activity, according to this study. In addition, the c.677C>T allele of the MTHFR gene has been linked to mild hyperhomocysteinemia and RPL in some studies [22, 23].

The frequency of the c.1298 C allele in multiple abortion women was significantly higher than control group, and the risk factor for sensitivity was presented by the C allele.

Conclusion

Based on the current findings, it is evident in the first or second trimester that the MTHFR versions in C677T and A1298C influence miscarriage predisposition. Analyzing them for diagnostic purposes may be advantageous. The daily consumption of Folic acid however remains an important treatment for pregnant women to reduce, among other complications, the risk of increasing plasma homocysteine [17-18].

Our findings have a significant clinical significance for him: properly targeted folic acid supplements (active folate form) can help to prevent abortion. By catalyzing HCY re-methylation into methionine and thus lowering plasma HCY levels, targeted folate supplements could minimize thrombosis-related pregnancy loss.

Abbreviations

MTHFR: Methylene tetrahedral folic acid reductase enzyme; **Hcy**: Hyperhomocysteinaemia; **CMV**: cytomegaly virus; **ACA**: Anti cardiolipins anti body; **APL**: Anti phospholipids anti body; **Toxo**: Toxoplasma gondii; **PCR**: Polymerase chain reaction; **FA**: Folic acid; **RPL**: recurrent pregnancy loss; **TLC**: thin layer chromatography; **ELFA**: Enzyme Linked Fluorescent Assay; **SNP**: Single nucleotide polymorphism; **ORs**: Odds ratios.

Declarations

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Authors' contributions

Emad Salaam Abood conceived and designed the experiment. Maryam Sabah Naser performed the data analyses and drafted the manuscript. Raheem Jabar Hameed participated in the sequence alignment. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset supporting the conclusions of this article is included within the article.

Ethics approval and consent to participate

All participants were provided with an explanation of the research and then gave written informed consent. Our study was approved by the ethics committee of the Affiliated Hilla University college.

Consent for publication

Not applicable.

Competing interests

The author declares that he has no competing interests.

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References

1. Mangalgi Ashutosh S, Pathak SA. Cytogenetics in recurrent abortions. *People's J Sci Res* 2008; 1: 5e8.
2. Zhou L, Gao X, Wu Y, Zhang Z. Analysis of pregnancy outcomes for survivors of the vanishing twin syndrome after in vitro fertilization and embryo transfer. *Eur J Obstet Gynecol Reprod Biology*. 2016;203:35–9.
3. Yang Y, Luo Y, Yuan J, Tang Y, Xiong L, Xu M, Rao X and Liu H: Association between maternal, fetal and paternal MTHFR gene C677T and A1298C polymorphisms and risk of recurrent pregnancy loss: A comprehensive evaluation. *Arch Gynecol Obstet* 293: 1197-1211, 2016.
4. Levin BL and Varga E: MTHFR: Addressing Genetic Counseling Dilemmas Using Evidence-Based Literature. *J Genet Couns* 25: 901-911, 2016.
5. Park WC and Chang JH: Clinical Implications of Methylenetetrahydrofolate Reductase Mutations and Plasma Homocysteine Levels in Patients with Thromboembolic Occlusion. *Vasc Spec Int* 30: 113-119, 2014.
6. Rai V. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and Alzheimer disease risk: a meta-analysis. *Mol Neurobiol*. 2017;54(2):1173–86.
7. Frosst P, Blom HJ, Milos R, Gayette P, Sheppard CA, Mattews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, Rozen R (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 10:111–113.
8. Trabetti, E. Homocysteine, MTHFR gene polymorphisms, and cardio-cerebrovascular risk. *J Appl Genet* 49, 267–282 (2008). <https://doi.org/10.1007/BF03195624>.
9. Kelly TL, Neaga OR, Schwahn BC, Rozen R, Trasler JM. Infertility in 5,10-methylenetetrahydrofolate reductase (MTHFR)- deficient male mice is partially alleviated by lifetime dietary betaine supplementation. *Biol Reprod*. 2005 Mar;72(3):667- 77. doi: 10.1095/biolreprod.104.035238. Epub 2004 Nov 17. PMID: 15548731.
10. Locasale, J. Serine, glycine and one-carbon units: cancer metabolism in full circle. *Nat Rev Cancer* 13, 572–583 (2013). <https://doi.org/10.1038/nrc3557>.

11. Sibani S, Leclerc D, Weisberg IS, O'Ferrall E, Watkins D, Artigas C, Rosenblatt DS and Rozen R: Characterization of mutations in severe methylenetetrahydrofolate reductase deficiency reveals an FAD-responsive mutation. *Hum Mutat* 21: 509-520, 2003.
12. Botto N, Andreassi MG, Manfredi S, Masetti S, Cocci F, Colombo MG, Storti S, Rizza A and Biagini A: Genetic polymorphisms in folate and homocysteine metabolism as risk factors for DNA damage. *Eur J Hum Genet* 11: 671-678, 2003.
13. Riso S and Choi SW: Gene-nutrient interactions and DNA methylation. *J Nutr* 132: 2382S-2387S, 2002.
14. Karimian M, Colagar AH. Association of C677T transition of the human methylenetetrahydrofolate reductase (MTHFR) gene with male infertility. *Reprod Fertil Dev.* 2016;28(6):785.
15. Forges T, Chery C, Audonnet S, Feillet F, Gueant JL. Lifethreatening methylenetetrahydrofolate reductase (MTHFR) deficiency with extremely early onset: characterization of two novel mutations in compound heterozygous patients. *Molecular Genetics & Metabolism.* 2010;100(2):143–8.
16. Stoccoro A, Tannorella P, Salluzzo MG, Ferri R, Romano C, Nacmias B, et al. The Methylenetetrahydrofolate Reductase C677T Polymorphism and Risk for Late-Onset Alzheimer's disease: Further Evidence in an Italian Multicenter Study. *J Alzheimers Dis* 2017;56:1451.
17. Parker SE, Yazdy MM, Tinker SC, Mitchell AA and Werler MM: The impact of folic acid intake on the association among diabetes mellitus, obesity, and spina bifida. *Am J Obstet Gynecol* 209: 239.e1-239.e8, 2013.
18. Greene ND, Leung KY, Gay V, Burren K, Mills K, Chitty LS and Copp AJ: Inositol for prevention of neural tube defects: A pilot randomised controlled trial - CORRIGENDUM. *Br J Nutr* 115: 1697, 2016.
19. Henrik Zetterberg, Björn Regland, Mona Palmér, Anne Ricksten, Lars Palmqvist, Lars Rymo, Demetrios A Arvanitis, Demetrios A Spandidos & Kaj Blennow.(2002). Increased frequency of combined methylenetetrahydrofolate reductase C677T and A1298C mutated alleles in spontaneously aborted embryos. *European Journal of Human Genetics* volume 10, pages113–118(2002).
20. Frosst P, Blom HJ, Milos R, et al. (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*1:111–113.
21. Nelen WL, Steegers EA, Eskes TK, et al. (1997) Genetic risk factor for unexplained recurrent early pregnancy loss. *Lancet*9081:861.
22. Jeddi-Tehrani M, Torabi R, Zarnani AH, et al. (2011) Analysis of plasminogen activator inhibitor-1, integrin beta3, beta fibrinogen, and methylenetetrahydrofolate reductase polymorphisms in Iranian women with recurrent pregnancy loss. *Am J Reprod Immunol* 2:149–156.
23. Abu-Heija A. (2014) Thrombophilia and Recurrent Pregnancy Loss: is heparin still the drug of choice?. *Sultan Qaboos Univ Med J*1:e26–e36.

Figures

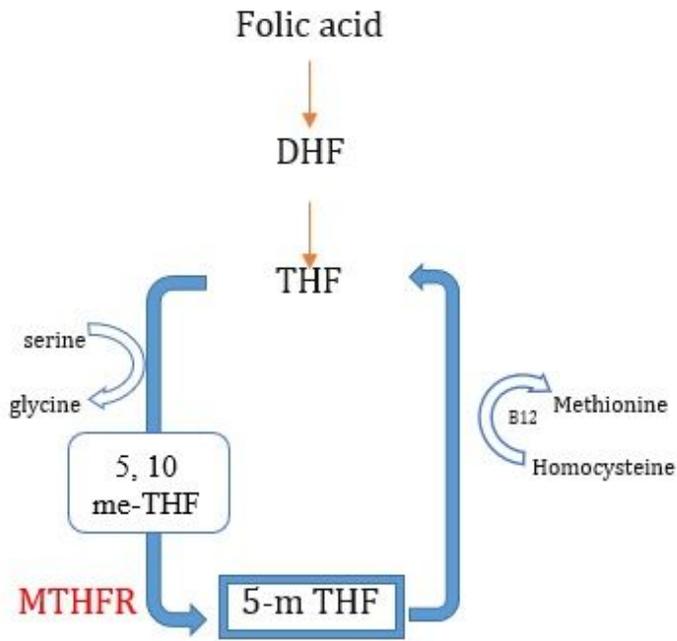


Figure 1

methyltetrahydrofolate (mTHF) by methylenetetrahydrofolate reductase (MTHFR). 5-mTHF is demethylated to complete the folate cycle by donating a carbon into the methionine cycle through the methylation of homocysteine (hCYS) by methionine synthase and its cofactor vitamin B 12”.

Supplementary Files

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