

Review Article

The effects MTHFR gene mutation on vitamin B12 concentration in the blood; bioinformatics approach – Review

ABSTRACT

Introduction: The protein encoded by MTHFR gene catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. Genetic variation in this gene influences susceptibility to occlusive vascular disease; neural tube defects, colon cancer and acute leukemia. Also, mutations in this gene are associated with methylenetetrahydrofolate reductase deficiency.

Results: Human MTHFR gene can be studied through [Pan Troglodytes (chimpanzee)]. Hyperhomocysteinemia leads to several health problems including cardiovascular disease, deep vein thrombosis (DVT), pulmonary embolism (PE) and Pregnancy complications.

Methods: NCBI bioinformatics database application is used to search for MTHFR gene. Protein sequences were converted into DNA and mRNA. Protein sequences were analysed into DNA and mRNA. It includes information about the accession number, the number of amino acid in protein product, the number of exons and the length of nucleotide. Also, nucleotide sequences and mRNA were included in FASTA format. Additionally, basic local alignment search tool (BLAST) is used to compare the sequences of this gene in Human with other organism like mouse.

Conclusion: There is a relationship between MTHFR gene and vitamin B12 levels. Mutation in MTHFR gene has negatively impact vitamin B12 levels and methylation pathway, which affects conversion process of homocysteine to methionine. Therefore, increased level of homocysteine observed that leads to several health complications.

Keywords: MTHFR gene, human, mouse, Vitamin B12 (cobalamin), Folate.

Introduction

The 5, 10-methylenetetrahydrofolate reductase enzymes (MTHFR), is crucial in converting homocysteine to methionine. Homocysteine is a chemical component in human blood; it is formed when amino acid methionine of protein is broken down, and then the body gets rid of it by excreting through urine. It can be recycled to be reused in building other proteins. Vitamins B12, B6, and folate are needed for recycling. Vitamin B12 (cobalamin) is important vitamin that comes from our diet. In case of low levels of Vitamins B12 and B6, the homocysteine accumulate in the blood and cause several complications. Polymorphisms in MTHFR, is responsible for decreasing enzyme activity and cause sequence changes, thus methionine will not convert to Homocysteine, and this can result in hyperhomocysteinemia (Moll and Varga,2015).

There are plenty of risk factors that associated with hyperhomocysteinemia. Elevated homocysteine levels are associated with increased risk of cardiovascular disease. Moreover, it leads to deep vein thrombosis (DVT). Pregnancy complications have been observed more frequently among women with elevated homocysteine levels, including preeclampsia, placental abruption, and recurrent pregnancy loss (Shah et al, 2016).

There are two MTHFR genes, one inherited from each parent. Individuals with mutation in one MTHFR gene are heterozygous. Should identical gene mutations occur in both genes, the mutation is considered a homozygous. Should the mutation occur on only one gene, it is called a heterozygous mutation. Two types of MTHFR mutations which are C677T and C1286A. The most common mutation is the MTHFR C677T. The mutation is extremely common in developed countries such as United States. It has been shown that 20% to 40% of white and Hispanic populations are heterozygous for MTHFR C677T. In addition, North America, Europe, and Australia showed that from 8% to 20% of the population have MTHFR C677T mutations (Moll and Varga, 2015).

Many individuals are living with mutation in MTHFR gene. This mutation contributes to several health issues. It impacts vitamin B12 levels and methylation pathway. Vitamin B12 and folate are essential cofactors in methylation cycle. The methylation cycle starts with folate then converted into its active form 5-methyltetrahydrofolate. MTHFR is responsible for creating active form of folate. However, in case of shortage MTHFR, this will definitely result on deficiency in active form of folate (5-methyltetrahydrofolate) and negatively influence conversion process of homocysteine to methionine. Low levels of (5-methyltetrahydrofolate) leads to homocysteine build in the blood and lower methionine in the body. Increased level of homocysteine can also be caused by decreased vitamin B12 levels since vitamin B12 is a cofactor of the enzyme responsible for converting homocysteine to methionine. Without sufficient levels of vitamin B12, homocystine build could result in worse if it combines with MTHFR mutation (Al-Batayneh et al, 2018).

Method:

NCBI database was selected to search for MTHFR gene because it is very comprehensive source of information considered by Online Mendelian Inheritance in Man OMIM (Omim.org, 2019). First of all, we entered the gene name (MTHFR) in NCBI then "gene" was selected from the navigation bar at the top of the NCBI start page, and then clicked on search. We got the links for all species, and [Homo sapiens (human)] was chosen. There was information about that gene such as genomic map, expression, sequence, protein function, structure and homology. The GenBank record in Related Information section on the lower right, gave us direct links to other databases with information on that query .We selected gene link and position cursor over the green bars in the Genomic region which gave us information about that gene such as gene's location in the genome and the chromosome number on which this gene can be found. On the Gene page, there are also additional links to examine a gene's structure and function. The total numbers of exons and introns in that gene was on the Gene page .Also we got FASTA format which representing either nucleotide sequences or peptide sequences, in which amino acids are represented using single-letter codes.

The next step after retrieving protein sequence is to perform a BLAST search. BLAST used to compare our protein product of human gene to protein product of any one of the living organism such as Mouse. First, we went to the BLAST home page and clicked "protein blast" under Basic BLAST. Then we pasted the sequence in the query box and entered the name of the organism of interest in the "Organism" box then Click the BLAST button, by this we then discussed the homologous of protein product of that gene to the protein product of other living organism (Chimpanzee) gene and wither it is possible to study the gene through Chimpanzee.

From above method and by using NCBI , BLAST and Uniprot database, we got the function of that gene which is catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and it is a co-substrate for homocysteine remethylation to methionine. An Accession number of the gene is NC_000001.11, the numbers of amino acids are 656 aa, the total numbers respectively are 13 and 12 for exons and introns .The length of nucleotide is 1,143 nt. This gene can be found in chromosome number 1 and the length of nucleotides is 20374 bp and the exact "nucleotide location" for this gene is (11,785,730..11,803,677).There are organisms other than human which also have this gene such as Zebra fish, Rhesus monkey and Norway rat.

Part of nucleotides sequence in FASTA format for MTHFR gene on figure (1)

```

FASTA ▾ Send to: ▾
Homo sapiens chromosome 1, GRCh38.p12 Primary Assembly
NCBI Reference Sequence: NC_000001.11
GenBank Graphics
>NC_000001.11:c11806103-11785730 Homo sapiens chromosome 1, GRCh38.p12 Primary Assembly
ATGACGATAAAGGCACGGCCTCAACGAGACCTGTGGGCACGGCCATGTTGGGGCGGGGCTCCGGTCA
CCCGCCCGGTGGTTTCCGCCCTGTAGGCCCGCCTCTCCAGCAACTGACACCTGCGCCGCGCCCTTCA
CTGCGTCCCCGCCCTGCAGCGCCACAGTGGTGGCCGGCCGGCCGAGCGTTCTGAGTCAACCGGGAC
TGGAGGTTGAGTGACGGCAGCGCCGGGGTCCCGGGAGGGAGATCTGGAGCCGGCAAAACCTCCCG
GGCAAGGACGTGCTGTGGCGGGGAGCGCTGGAGGCCGGCTGCCTCTCTTTGGGGGGGGTCCCG
CCTCCCTTGGCGACCCTTCGGGGATTAGTGTAACTCCCAATGGCTACCACTTCAGCGACCGCCAAACC
TCAAGCGAAGACTGACTTTGGCTCCCTGCCTGGACGGAGGGGCCCTGAGCCAGGGGTGACGATCCGCC
CCTCTGACCGGCCAAGGCCCGGTGTCTCGCCCATCGGTGACTCAGTGACCTGGTACTGGATTCTCGG
CCACTGGGCGCCGAGACGGCTTCCGGCTCCTGCCTTTTAAACTGCTCCCGCGCGATCACTGGAGAA
GAGCGTGGGCCCGGGGCACTGCGGTCCCTGGCGCCACTGCGTCCCGCTGCGCACGGGGTCCGCCGG
ACCTTTCTGGGAGTCTGAGGCTTAGTATCCAGTGTGGCGCAGACTAGTTGTTCAAGTGGCAGAG
GCTTATTTGAGAGAGTGGCAGCAGCTGGCCCTTGGCGCTCAGTGAATGTTGGCTATCAGCGTGTGCCA
AACTCTGGGGATACCCAGGAGGACACCGGTCTGTCTCAGGGAAGTGGGAAAGAGAAAGGAGACAGG
CCTTTTCAACCACAGTTACAACCCAGGGTGTATGGGAGTCCAGCTGATAACGGATAAATCGTGGGAGT
.....

```

Figure (1)

Part of mRNA sequence in FASTA format for MTHFR gene on figure (2)

[GenBank](#) [Graphics](#)

>NM_001330358.1 Homo sapiens methylenetetrahydrofolate reductase (MTHFR), transcript variant 1, mRNA

```
GAGCCTCAGCCCTCCCTCGCCTGGAAGCCTTGCCCCGCCCTTGTGCTGGCTGGAGCTCAAGCCT
CTTCTTTTGTGCGAGCTCCGCCAGTTGAACACACCCGCTGGGGAAGGTGCCTGTTCCTCCCCACGC
ACTCTGGGCTGAGCTGACAGAGATGGACCATCGAAAAGCCAGGGTCTCCAGCTGGGCACTACTGCC
CTCGTAGGAATATGGGCTCGCAGGTGGCAGCGTGAGGTCTCTGTGCCACCTTCCATCAGTAGGAAC
CCAGCCATGGTGAACGAAGCCAGAGGAAACAGCAGCCTCAACCCCTGCTTGAGGGCAGTGCAGCAGTG
GCAGTGAGAGCTCCAAGATAGTTCGAGATGTTCCACCCGGGCTGGACCCGAGCGGATGAGAGACT
CCGGGAGAAGATGAGGCGGATTTGGAATCTGGTGACAAGTGGTTCTCCCTGGAATCTTCTCTCTCGA
ACTGCTGAGGGAGCTGTCAATCTCATCTCAAGTTTGACCGGATGGCAGCAGGTGGCCCCCTCATATAG
ACGTGACCTGGCACCAGCAGGTGACCTGGCTCAGACAAGGAGACCTCCATGATGATCGCCAGCAC
CGCCGTGAAGTACTGTGGCTGGAGACCATCTGCACATGACTGCTGCCGTCAGCGCTGGAGGAGATC
ACGGCCATCTGCACAAGCTAAGCAGCTGGCCTGAAGAACATCATGGCGCTGCGGGGAGACCAATAG
GTGACCAGTGGGAGAGGAGGAGGCTTCAACTACGCAAGTGGACCTGGTGAAGCACATCCGAAGTGA
GTTTGGTACTACTTTGACATCTGTGTGGCAGTTACCCCAAAGGCCACCCGAAGCAGGGAGCTTTGAG
GCTGACCTGAAGCACTTGAAGGAGAAGGTGTCTGCGGGAGCCGATTTATCATCACGAGCTTTTCTTTG
AGGCTGACACATTTCTCCGCTTTGTGAAGGCATGCACCGACATGGGCATCACTTGCCCATCGTCCCGG
GATCTTTCCATCCAGGGCTACCACTCCCTTCGGCAGCTTGTGAAGCTGTCCAAGCTGGAGGTGCCACAG
```

Figure (2)

When protein product of human gene compared to protein product of chimpanzee by using BLAST we found that the Identities is 99%, gaps is 0% and the Score is 4656, So it is possible to study the gene through chimpanzee model. Although there is a homologous in the protein product of human MTHFR gene and [Pan troglodytes

	Human	Chimpanzee
accession number	NC_000001.11	NC_036879.1
Exon	13	13
located	Chromosome 1	Chromosome 1
length of nucleotides	20374 bp	20055 bp
name Organism	Homo sapiens	Pan troglodytes

(chimpanzee)], there are some differences as well (Table 1)

Table (1): MTHFR gene in human and chimpanzee

In addition to bioinformatics application, search was done on January 16, 2021 using the following data resources: PubMed, Cochrane databases, Access Medicine and Google Scholar to find out studies and scientific information that focus on the effects of MTHFR gene mutation on vitamin B12 concentration in the blood. All the studies which are published in English from 2000 to 2020 were included in the search. As the results more than 100 studies related to vitamin B12 and MTHFR gene were found.

Table (2): To make the results more accurate: the following keywords were used:

Article title	Article 1: Genotype Prevalence and Allele Frequencies Of 5,10-Methylenetetrahydrofolate Reductase (MTHFR) C677T and A1298C Polymorphisms in Italian Newborns	Article 2 : ASSOCIATION BETWEEN MTHFR 677C>T POLYMORPHISM AND VITAMIN B12 DEFICIENCY: A CASE-CONTROL STUDY	Article 3: Reduced B Vitamin Therapy in MTHFR C677T/A1298C Patients with Major Depressive Disorder Clinical Response Correlates with Homocysteine Reduction: A Double-Blind, Placebo-Controlled Study	Article 4 : The Association Between Common C677T Mutation in Methylenetetrahydrofolate Reductase Gene and the Risk of Venous Thrombosis in an Iranian Population	Article 5 : Hyperhomocysteinemia, Low Folate, and Vitamin B12 Deficiency in Elderly Living at Home and Care Residences
---------------	---	---	--	---	---

MTHFR gene, human, mouse, Vitamin B12 (cobalamin), Folate, and MTHFR gene mutation on vitamin B12 concentration. As the results of using these keywords, the number of studies reduced to less than fifteen. For the purpose of quality and specificity of the search the studies were excluded if there are opinion, review, text studies or Systematic review and to avoid any factors that can affect the quality of the search and to increase its efficiency we filtered the studies that we obtained using PubMed search filter, Cochrane databases search filter and EMBASE search filter. As a result of using inclusion and exclusion criteria and other methods of filtering the number of studies reduced to six. These six studies were screened by examining the abstract, method and the results and finally the best five were selected based on the quality of the method and the results that have been found (Table 2). All the selected studies evaluate the effects MTHFR gene mutation on vitamin B12 concentration in the blood. To make sure that their methods are at the good quality we did appraise them using the JBI critical appraisal tools. The major type of checklist that is used is an experimental study checklist. By using this critical appraisal tool, we were able to find the strengths and weaknesses of each study and we concluded that the selected studies are at good quality to be used in any systematic review based on the JBI critical appraisal tools and inclusion and exclusion criteria.

Author	Bruno Zappacosta	Al-Batayneh	Farah at el,	Mohammad Soleyman Soltanpour	Mohammad Y Et al
Study aim	To evaluate the genotype and the allele frequencies of the polymorphisms C677T and A1298C of MTHFR in 104 newborns from central-southern Italy.	investigating the association between MTHFR polymorphisms and vitamin B12 deficiency in a Jordanian population	to evaluate the efficacy of vitamin B as monotherapy in lower plasma homocystine.	Investigate a possible association between fasting hyperhomocysteinemia and C677T mutation in the MTHFR gene with venous thrombosis.	examined the relationship of Hcy with vitamin B12 and folate levels in the studied population.
Population	104 newborns (57 males and 47 females)	100(45 male-55female)	330 adult patients	200 venous thrombotic patients	Healthy individuals >65 years old
Type of mutation	C677T and A1298C polymorphisms	C677C, C1286A	MTHFR C677T and A1298C polymorphism	MTHFR C677T	No gene mutation
outcome	- 677TT genotype associated with hyperhomocysteinemia, particularly in the presence of low folate levels. - 1298C allele is responsible for reduced enzyme activity	-B12 defect high in C677C group. - less deficient B12 level compared to C677C group.	-homocystein level decreased with vitamin B treatment. -no significant change in homocystein level with placebo.	Iranian population provide evidence that plasma homocystein level but not C677T mutation of the MTHFR gene is a significant risk factor in VT and measuring the level of homocystein is cheaper and more useful than the genetic test for the MTHFR mutation	Hyperhomocysteinemia and low folate was more prevalent in the ER than in the EH and younger individuals.

Table (3): five article studies the effects MTHFR gene mutation on vitamin B12 concentration in the blood

Results:

Human MTHFR gene can be studied through chimpanzee. Also, it has been found that patients with MTHFR mutations (C667T and A129C) have vitamin B12 deficiency that cause hyperhomocysteinemia which leads to several health problems including cardiovascular disease, deep vein thrombosis (DVT), pulmonary embolism (PE) and Pregnancy complications.

Discussion:

MTHFR and vitamin B12

People who have mutation in MTHFR are commonly having problems with vitamin B12; they usually show sign and symptom of vitamin B12 shortage. MTHFR mutations (C667T and A129C), influence the ability of the body to use vitamin B12. In case of MTHFR mutation, the body will have shortage of enzyme that responsible for the process of conversion folate into its active form 5-Methyltetrahydrofolate. Decreased of (5-methyltetrahydrofolate)

levels lead to a buildup of homocysteine and lower methionine in the body that can result in plenty of health problems. Moreover, active form of folate is needed for the utilization of vitamin B12 in the body. Increased level of homocysteine can also be caused by decreased vitamin B12 levels since vitamin B12 is a cofactor of the enzyme responsible for converting homocysteine to methionine.

Symptoms of MTHFR mutation and vitamin B12 deficiency

Vitamin B12 deficiencies and MTHFR mutation combine and work together to cause increased levels of homocysteine, that affect nervous system and could damage it. Leading to dementia, cognitive impairment and increased risk of Alzheimer's disease. Hyperhomocysteine levels are related to elevated risk of cardiovascular issues such as heart attacks and stroke. Vitamin B12 symptoms include, Poor hair condition, Eczema or dermatitis, irritability, Anxiety, Tension, Lack of energy, Constipation, Tender or sore muscles, and Pale skin.

Lab test for MTHFR

Methylenetetrahydrofolate reductase DNA mutation analysis, test is used to find out if there is mutation in one of two MTHFR genes which are C677T and A1298C. It is often used after other tests showed a higher level of homocysteine levels in the blood. Conditions such as high cholesterol and dietary deficiencies can also increase homocysteine levels. An MTHFR test usually confirms whether or not the raised levels are caused by a genetic mutation. This test needs to be done if blood test showed higher than normal levels of homocysteine, close relative was diagnosed with an MTHFR mutation and close family members have a history of premature heart disease or blood vessel disorders. Results typically are reported as negative or positive for an MTHFR mutation. If positive, the result will show which of the two mutations patient have, and whether he has one or two copies of the mutated gene. If results were negative, but patient has a high homocysteine levels, health care provider more tests to find out the cause.

People who have elevated homocysteine levels may be at an increased risk of developing premature cardiovascular disease (CVD) or thrombosis, but many, including those with *MTHFR* mutations will never develop CVD or thrombosis. There are other causes of elevated homocysteine levels, including deficiency of vitamins B6, B12, and folate; these vitamins are required for homocysteine metabolism. The *MTHFR* mutation may not be present with these acquired, as opposed to inherited, causes of elevated homocysteine (Al-Batayneh et al, 2018).

Conclusion:

MTHFR mutations (C667T and A129C), impact the ability of the body to use vitamin B12. Moreover, the body will have shortage of enzyme that responsible for the process of conversion folate into its active form 5-Methyltetrahydrofolate. The test used to find out whether or not mutation in one of two MTHFR genes which are C677T and A1298C is Methylenetetrahydrofolate reductase DNA mutation analysis. There is a significant association between homozygous MTHFR variant and T vitamin B12 deficiency which effects conversion process

of homocysteine to methionine. Therefore, increased level of homocysteine observed that leads to several health complications.

Reference:

1. Al-Batayneh, K.M., Al Zoubi, M.S., Shehab, M., Al-Trad, B., Bodoor, K., Al Khateeb, W., Aljabali, A.A., Al Hamad, M. and Eaton, G., 2018. Association between MTHFR 677C> T Polymorphism and Vitamin B12 Deficiency: A Case-Control Study. *Journal of medical biochemistry*, 37(2), pp.141-147.
2. Gharaibeh, M.Y., Gahtan, R.A., Khabour, O.F. and Alomari, M.A., 2010. Hyperhomocysteinemia, low folate, and vitamin B12 deficiency in elderly living at home and care residences: a comparative study. *Laboratory Medicine*, 41(7), pp.410-414.
3. Mech, A. and DFAPA, A.F.M., 2007. Reduced b vitamin therapy in MTHFR C677T/A1298C patients with major depressive disorder—clinical response correlates with homocysteine reduction: a double-blind, placebo-controlled study. *depression*, 164(6), pp.861-7.
4. Moll, S. and Varga, E.A., 2015. Homocysteine and MTHFR mutations. *Circulation*, 132(1), pp.e6-e9.
5. Shah, J.H., Salagre, K.D., Sahay, R.N. and Anand, A., 2016. Heterozygous MTHFR A1298C Mutation causing Cerebral Venous Sinus Thrombosis. *The Journal of the Association of Physicians of India*, 64(11), pp.76-77.
6. Soltanpour, M.S., Soheili, Z., Pourfathollah, A.A., Samiei, S., Meshkani, R., Deyhim, M.R., Safa, M. and Ataei, Z., 2008. The association between common C677T mutation in Methylene tetrahydrofolate reductase gene and the risk of venous thrombosis in an Iranian population. *Laboratory Medicine*, 39(2), pp.97-100.
7. Zappacosta, B., Romano, L., Persichilli, S., Cutrone, L.A., Graziano, M., Vitrani, A., Di Castelnuovo, A., Giardina, B., Musumeci, S. and Mastroiacovo, P., 2009. Genotype prevalence and allele frequencies of 5, 10-methylene tetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms in Italian newborns. *Laboratory Medicine*, 40(12), pp.732-736.