



The Effects MTHFR Gene Mutations on Vitamin B12 Concentration in the Blood; Bioinformatics Approach – A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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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.

Methods: NCBI bioinformatics database application was 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 acids in the 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) was used to compare the sequences of this gene in humans with other organisms like mouse.

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.

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 the conversion process of homocysteine to methionine. Therefore, several health complications are caused by the observed increases in the levels of homocysteine.

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1. 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 the amino acid methionine of protein is broken down, and then the body gets rid of it by excreting it in 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 an important vitamin that comes from our diet. In case of low levels of Vitamins B12 and B6, homocysteine accumulates in the blood and cause several complications [1,2]. Polymorphisms in MTHFR is responsible for decreasing enzyme activity and causing sequence changes, thus resulting in methionine not being to convert to homocysteine, and this can result in hyperhomocysteinemia [3].

There are plenty of risk factors that are 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 [4].

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 homozygous [5-8]. Should the mutation occur on only one gene, it is called a heterozygous mutation. There are two common MTHFR mutations, known as C677T and A1298C. The most common MTHFR mutation is called the MTHFR C677T. This type of 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 have MTHFR C677T mutations in 8% to 20% of their populations [3].

Many individuals are living with mutation in their 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 and is then converted into its active form 5-methyltetrahydrofolate. MTHFR is responsible for creating active the form of folate [9-11]. However, in case there is a shortage of MTHFR, this will result in deficiency in the active form of folate (5-methyltetrahydrofolate) and negatively influence conversion process of homocysteine to methionine. Low levels of (5-methyltetrahydrofolate) leads to homocysteine buildup in the blood and lower methionine in the body [12,13]. 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, homocysteine buildup could result in worse conditions if it combines with MTHFR mutation [14].

The purpose of this study is to identify several health problems that associated by MTHFR gene mutation by using bioinformatics technique to study MTHFR gene in [Pan Troglodytes (chimpanzee)].

2. METHODS

NCBI database was selected to search for MTHFR gene because it is a 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 search was clicked. 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 positioned the 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 represents either nucleotide sequences or peptide sequences, in

which amino acids are represented using single-letter codes.

The next step after retrieving protein sequence was to perform a BLAST search. BLAST was 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 clicked 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 whether it was possible to study the gene through Chimpanzee.

From the above method and by using NCBI, BLAST and Uniprot database, we got the function of that gene which is to catalyze the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and to act as 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 are 13 and 12 for exons and introns respectively. The length of nucleotide is 1,143 nt. This gene can be found on 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 humans which also have this gene such as Zebra fish, Rhesus monkey and Norway rat.

When protein product of human gene was 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 (chimpanzee)], there are some differences as well (Table 1).

FASTA Send to: ▾

Homo sapiens chromosome 1, GRCh38.p12 Primary Assembly

NCBI Reference Sequence: NC_000001.11

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

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>NC_000001.11:c11806103-11785730 Homo sapiens chromosome 1, GRCh38.p12 Primary Assembly
ATGACGATAAAGGCACGGCTCCAACGAGACCTGTGGGCACGGCCATGTTGGGGCCGGGCTTCCGGTCA
CCCGCGCCGGTGGTTTCCGCGCTGTAGGCCCGCTCTCCAGCAACTGACACCTGCGCCGCGCCCTTCA
CTGCGTTCCCGCCCTGCAGCGGCACAGTGGTGGCGCCGGCCGGCGAGCGTCTGAGTCAACCCGGGAC
TGGAGGGTGAAGTACGGCCGAGGCGGGGTCGCCGGGAGGGAGATCTGGAGCCGGCAAACTCCCGG
GGCAAGGACGCTGTGGGCGGGGAGCGCTGGAGCCGGCTGCCTCTCTTGGGGGGGCTGCCG
CCTCCCTTGGCACCCTTGGCGGATTAAGTGAATCCCAATGGCTACCACTCCAGCGACCACCAACCC
TCAAGCGAAGACTGACTTTGGCTCCCTGCCTGGACGGAGGGGCCCTGAGCCAGGGGTGACGATCCGGC
CCTCTGACGGCCAGGCCCGTGTCTCGCCCACTCGGTGACTCAGTGACCTGGTGAATGCTGGG
CCACCTGGGCGCCGAGACGGCTTCCGGCTCCTGCCTTTAAACCTGCCTCCCGCGCATCACCTGGAGAA
GAGGCTGGGCGCCGGGCACTGCGGTCTGCGCGCCCACTGCGTCCCGCTGCGCACGGGGTCCGCGGG
ACCTTCTGGGAGTGTAGGCTTAGTATCCAGTGTGGCGCAGACTAGTTGTTCAAGTGGCAGAG
GCTTATTTGAGAGAGTGGCAGCACCTGGCCCTTTGGCGCTCAGTGAATGTTGGTATCACCGTGTGCCA
AACTCTGGGATACCCAGGCAGGACACCGGTCTGTCTCAGGGAATGGGAAAGAGAAAGGAGACAGG
CCTTTTCAACCCAGTTACAACCCAGGGTGTATGGGAGTCCAGCTGATAACGGATAAATCTGGGAGTT
```

Fig. 1. Part of nucleotides sequence in FASTA format for MTHFR gene

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

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>NM_001330358.1 Homo sapiens methylenetetrahydrofolate reductase (MTHFR), transcript
variant 1, mRNA
GAGCCTCAGCCCTCCCTCCTCGCTGGAAGCCTTGCCCCGCCCCCTTGTGCTGGTGGAGCTCAAGCCT
CTTCTTTGTGCGAGCTCCGCCAGTTGAACACACCCGCTGGGGAAGGTGCCTCTGTTCCCTCCACAGC
ACTCTGGGCTGAGCTGACAGAGATGGACCATCGAAAAGCCAGGGTCTCCAGCTGGGCACTACTGCC
CTCGTAGGAATATGGGCTCGCAGGTGGCAGCGTGAGGTCTCTGTGCCACCTCCATCAGTAGGAAC
CCAGCCTGTTGAACGAAGCAGAGGAACAGCAGCCTCAACCCCTGCTGGAGGGCAGTGCAGCAGTG
GCAGTGAGAGCTCCAAGATAGTTCGAGATGTTCCACCCCGGGCTGGACCCGAGCGCATGAGAGACT
CCGGGAGAGATGAGGCGGCATTGGAATCTGGTGACAAGTGGTTCTCCCTGGAATTTCTCCCTCCTCGA
ACTGCTGAGGGAGCTGCAATCTCATCTCAAGGTTTGACCGGATGGCAGCAGGTGGCCCCCTACATAG
ACGTGACCTGGCACCCAGCAGGTGACCCCTGGCTCAGACAAGGAGACCTCCTCCATGATGATCGCCAGCAC
CGCCGTGAACACTGTGGCTGGAGACCCTGCACATGACCTGTGCCGTCAGCGCTGGAGGAGATC
ACGGCCATCTGCACAAAGCTAAGCAGCTGGGCTGAAGAATCATGGCGCTGCGGGGAGACCCAATAG
GTGACCAAGTGGGAAAGAGGAGGGAGGCTTCAACTACGACAGTGGACCTGGTGAAGCACATCCGAAGTGA
GTTTGGTACTTTGACATCTGTGTGGCAGGTTACCCCAAAGGCCACCCCGAAGCAGGGAGCTTTGAG
GCTGACCTGAAGCACCTGAAGGAGAAGGTGTCTGGGAGCCGATTTTCATCATCACGACAGCTTTTCTTTG
AGGCTGACACATTTCTCCGCTTTGTGAAGGCATGCACCGACATGGGCATCACTTGGCCCATCGTCCCGG
GATCTTTCCATCCAGGGCTACCACTCCCTTGGCAGCTTGTGAAGCTGTCCAAGCTGGAGGTGCCACAG
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Fig. 2. Part of mRNA sequence in FASTA format for MTHFR gene

Table 1. MTHFR gene in human and chimpanzee

	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

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 results, more than 100 studies related to vitamin B12 and MTHFR gene were found.

MTHFR gene, human, mouse, Vitamin B12 (cobalamin), Folate, and MTHFR gene mutation on vitamin B12 concentration. As a result 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 that were excluded including opinions, reviews, text studies or systematic reviews. Also, to avoid any factors that could 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 of 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.

Table 2. To make the results more accurate: The following keywords were used

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

Table 3. Five article studies the effects MTHFR gene mutation on vitamin B12 concentration in the blood

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 homocysteine.	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 an 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.	-homocysteine level decreased with vitamin B treatment. -no significant change in homocysteine level with placebo.	Iranian population provide evidence that plasma homocysteine level but not C677T mutation of the MTHFR gene is a significant risk factor in VT and measuring the level of homocysteine 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.

3. 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.

4. DISCUSSION

4.1 MTHFR and Vitamin B12

People who have mutation in MTHFR are commonly having problems with vitamin B12; they usually show signs and symptoms 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 the enzyme that is responsible for the process of conversion of folate into its active form 5-Methyltetrahydrofolate. Decreased levels of 5-methyltetrahydrofolate levels lead to a buildup of homocysteine and lower methionine in the body that can results 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.

4.2 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 affects the nervous system and could damage it leading to dementia, cognitive impairment and increased risk of Alzheimer's disease. High homocysteine 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.

4.3 Lab Test for MTHFR

A test called Methylentetrahydrofolate reductase DNA mutation analysis is used to find out if there is mutation in one of the two MTHFR genes which are C677T and A1298C. It is often used after other tests shows a high 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 the patient has, and whether he/she has one or two copies of the mutated gene. If results were negative, but the patient has high homocysteine levels, the health care provider need to order more tests to find out the cause.

People who have elevated homocysteine levels may be at high risk of developing premature cardiovascular disease (CVD) or thrombosis. However, many of those with *MTHFR* mutations may 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 [14].

5. CONCLUSION

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

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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