Research & Reviews in Biotechnology and Biosciences
Volume-1, Issue-2, Year-2014
PP: 1-6 ISSN No: 2321-8681

Bioinformatics Analysis of Methylene tetrahydrofolate Reductase (MTHFR) Enzyme

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Abstract

Methylenetetrahydrofolate Reductase (MTHFR) is an enzyme that is encoded by MTHFR gene on chromosome 1 location p36.3 in homo sapiens. Changes at DNA level like synthesis, repair and imprinting result in the conversion of homocysteine to methionine. This methylation is catalyzed by methylentetrahydrofolate reductase through reduction of 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate. Approximately 24 mutations in the MTHFR gene have been identified in people with homocystinuria. Two of the most identified are C677T (rs1801133) and A1298C (rs1801131) single nucleotide polymorphisms (SNP). Mutations at C677T and A1298C which confer amino acid substitution Ala222Val and Glu429Ala respectively with reduced activity. So this polymorphism and mild hyperhomocysteinemia are associated with neural tube defects in offspring, arterial & venous thrombosis, and cardiovascular disease etc. The MTHFR gene could be one of the factors of overall schizophrenia risk. Bioinformatics analysis was performed to predict the homologues relationship. The phylogenetic analysis of MTHFR was performed against ten taxa by using Mega4 software.

Key words : MTHFR, TAXA, GRAVY etc.

Introduction

The enzyme MTHFR activity is associated with polymorphism in methylentetrahydrofolate reductase (MTHFR) gene. An enzyme 5, 10-methylentetrahydrofolate reductase regulates the conversion of 5, 10- methylentetrahydrofolate to 5-methylentetrahydrofolate leads to the synthesis of methionine for DNA methylation. Because of mutations at C677T and A1298C, the amino acid substitution occurs at Ala222Val and Glu429Ala with reduced activity (Frosst et al., 1995).Two common allelic variants of the MTHFR gene, C677T (NCBI SNP ID: rs1801133) and A1298C (rs1801131), which lead to amino acid substitutions, Ala222Val and Glu429Ala, and result in the decreased enzymatic activity (Weisberg et al., 1998; Weisberg et al., 2001). According to some studies that methylenetetrahydrofolate reductase (MTHFR) polymorphism C667T has been associated with congenital malformation; this common missense mutation in the MTHFR gene may reduce enzymatic action, and may be involved in the etiology of congenital heart defects (CHD)(Yin et al.,2012). It has been reported that the elevated blood pressure (BP) observed in patients with cardiovascular disease who are homozygous for the 677C→T polymorphism (TT genotype) in the gene encoding methylenetetrahydrofolate reductase (MTHFR) was responsive to supplementation with riboflavin-the cofactor for MTHFR(Wilson et al., 2012). However, some individuals have a genetic deficiency in the methylene tetrahydrofolate reductase (MTHFR) gene that limits conversion of folic acid to its biologically active form, L-methylfolate. Several studies have identified a higher frequency of genetic variations in the MTHFR gene in depressed patients than in nondepressed ones (Lizer et al.,2011). Mutant form of MTHFR gene at C677T is also involved in Neural tube defects and cardiac anomalies.

The most well studied polymorphism related to the risk of neural tube defects changes a single DNA building block in the MTHFR gene. Especially it replace the nucleotide cytosine with the nucleotide thymine (T) at position 677(677C>T).It is unclear how variation in the MTHFR gene increase the likelihood of neural tube defects(Wang XW et al. 2012) .

Another, disease i.e. homocysteinemia also occurs due to MTHFR deficiency is a metabolic condition characterized by neurological problems, such as developmental delay, seizures, and microcephaly. It is inherited in an autosomal recessive fashion and is caused by mutations in the MTHFR gene. These mutations may cause a mild to severe loss of activity of the MTHFR enzyme and result in elevated levels of homocysteine in the blood (homocysteinemia) or urine (homocysteinuria). The most common MTHFR gene mutation is the C677T mutation (Ezzaher A. 2011).

In our present work, wild type MTHFR was computationally analysed. The various parameters like conserved motif & domain structures, orthologs and phylogenetic analysis were performed. Phylogenetic analysis was performed by using mega4 and clustalw. After homology modelling the putative ligand molecule was docked with MTHFR protein via viana autodock. Structure validations have been achieved with significant stereo chemical parameters.

Methodology:

Gene location

The MTHFR gene is located on the short (p) arm of chromosome1at position 36.3. The MTHFR gene is located from base pair 11,769,246 to base pair 11,788,568 on chromosome1.

MTFR Sequence retrieval

Amino acid sequence of methylenetetrahydrofolate reductase was retrieved from SwissProt / Uniprot, database, which is an annotated database. It gives the description of a non redundant set of proteins, their function, motif and domain structure, posttranslational modifications,3D structure database, pathway databases, Gene Ontology(GO) and many other variants(Yin M et al. 2012). There are total 565 amino acid residues in Methylenetetrahydrofolate reductase sequence and molecular weight of the protein was 74596.5 as calculated from Emboss.

Their Domain and Motif were observed through various tools: Pfam, CDD, SMART and Prosite . The Molecular weight of MTHFR, theoretical PI , total number of negatively charged residues, total number of positively charged residues, Grand average of hydropathicity (GRAVY) score and aliphatic index was predicted by using ProtParam database.

Phylogenetic Analysis

Evolutionary relationship of MTHFR(NP_005948.3) with other 10 taxa betaine- homocysteine S-Methyl transferase1(NP_001704.2),cystathionine beta synthase(NP_000062.1), Neurogenic locus notch homolog protein 3(NP_000426.2) and other seven taxa of MTHFR from different species(Table 2) was preformed out through Mega 4 software and EBI (European Bioinformatics tool) server.

Number of aa	656
Molecular Weight	74596.5
Theoratical PI	5.22
Total number of negatively charged residues (Asp + Glu):	95
Total number of positively charged residues (Arg + Lys):	72
Ext. coefficient (M^{-1} cm^{-1}, at 280 nm measured in water)	119915
Aliphatic index	80.72
Grand average of hydropathicity (GRAVY)	-0.418

Table 1: ProtParam Result

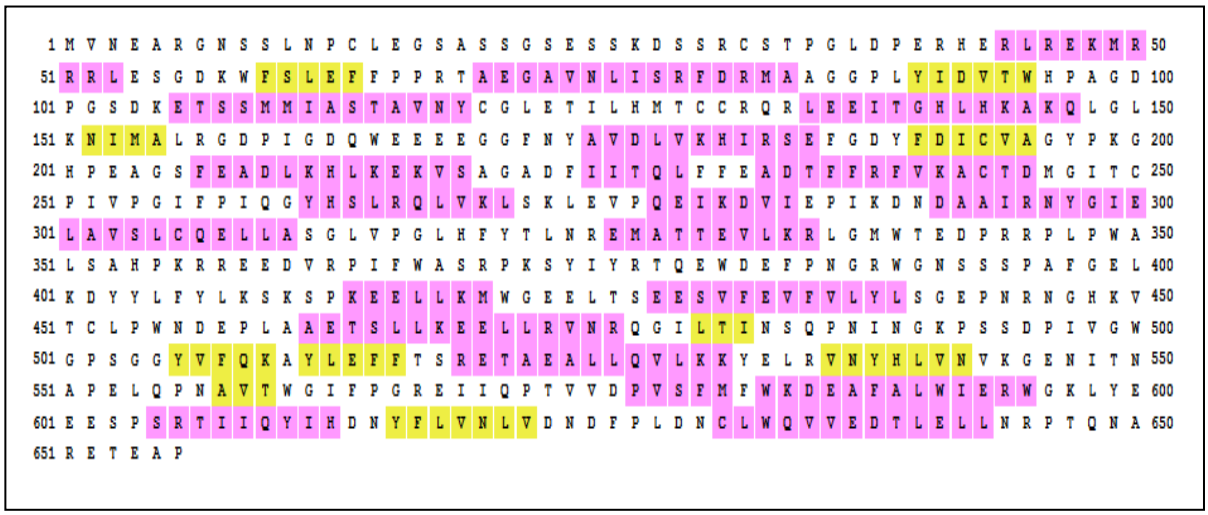

Fig 2: MTHFR Secondary structure result from PSIPRED database.

Result and Discussion

Bioinformatics analysis i.e. motifs & domain prediction, phylogenetic analysis, orthologus and mutation study of target protein Methylenetetrahydrofolate reductase reported in different diseases was the main motive of the present study.

The FASTA format amino acid sequence of MTHFR proteins was retrieved from expasy server (www.expasy.org).Comparative modelling was performed by using ModBase database

The domain and motif present in MTHFR were observed through CDD(conserved domain database), SMART(Simple Modular Architecture Research Tool) and InterPro database. InterPro shows the Pfam domain(PF02219) 48-337 MTHFR, TIGRFAMs (TIGR00677) 59-341 fadh2_euk and GENE3D(G3DSA:3.20.20.220) 47-340 FAD-linked oxidoreductase-like. (Fig1). The X-ray analysis of E. coli MTHFR, reported in pfam, provides a model for the catalytic domain that is shared by all MTHFRs. This domain is a beta8alpha8 barrel that binds FAD in a novel fashion. Ala 177, corresponding to Ala 222 in human MTHFR, is near the bottom of the barrel and distant from the FAD (Fig1). The secondary structures present in MTHFR were predicted by using PSIPRED secondary structure prediction tool (Fig2). It categorizes the sequence according to Helices, coils, sheets and turns present in the structure. The Molecular weight of MTHFR was 74596.5, theoretical PI was 5.22 and Grand average of hydropathicity (GRAVY) score -0.418 was predicted by using ProtParam database(Table1).

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Fig1: (A) CDD (B) InterPro showing conserved domain

S.No.	Sequence name	Accession number	Length
1.	methylenetetrahydrofolate reductase [Homo sapiens]	NP_005948.3	656
2.	methylenetetrahydrofolate reductase [Mus musculus]	AAD20313.1	654
3.	cystathionine beta-synthase [Homo sapiens]	NP_000062.1	551
4.	betaine--homocysteine S-methyltransferase 1 [Homo sapiens]	NP_001704.2	406
5.	vang-like protein 2 [Homo sapiens]	NP_065068.1	521
6.	putative methylenetetrahydrofolate reductase	KEQ81700.1	666

	[Aureobasidium pullulans EXF-150]		
7.	methylenetetrahydrofolate reduct [Aureobasidium pullulans var. namibiae CBS 147.97]	KEQ75250.1	670
8.	methylenetetrahydrofolate reductase 1 [Neurospora crassa OR74A]	XP_961729.1	613
9.	methylenetetrahydrofolate reductase (NADPH) [Exophiala aquamarina CBS 119918]	KEF59224.1	642
10.	methylenetetrahydrofolate reductase [Trichophyton rubrum MR850]	EZF23942.1	587
11.	methylenetetrahydrofolate reductase (NADPH) [Trichosporon asahii var. asahii CBS 2479]	EJT52884.1	594

Table 2 : Different sequences with their Accession numbers for phylogenetic analysis

Evolutionary relationship was performed by using Mega4 (Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0.) The 11 different taxa were used for phylogenetic analysis by using the Neighbor-Joining method. The optimal tree with the sum of branch length = 5.94330518 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 384 positions in the final dataset (Fig3). In phylogenetic tree the different MTHFR taxas from different species are evolutionary orthologues. Whereas cystathionine beta-synthase and betaine--homocysteine S-methyltransferase showing close homology

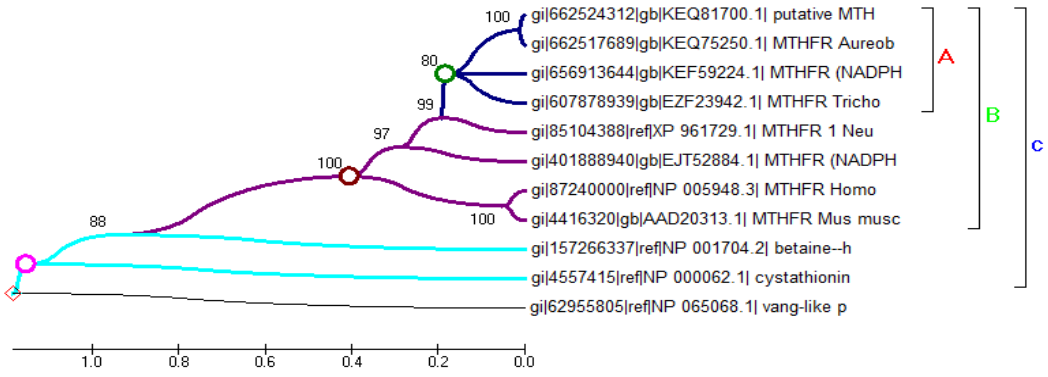

Fig 3: Evolutionary relationships of 11 taxa (linearized)

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