Volume : 3 | Issue : 4 | April 2014 • ISSN No 2277 - 8179

Research Paper

In-Silico Study of Neural Tube Defect in Relation to FOHL1 Gene

MEDICAL SCIENCE

KEYWORDS : Neural Tube Defect, FOLH1, GCPII, SNP, rs61886492.

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ABSTRACT

Neural tube defect is a neurological disorder occurs due to non closure of neural tube. To determine the role of FOLH1 gene in relation to Neural tube defect analysis of SNPs (single nucleotide polymorphism) associated with this gene was done. Alteration in genetic variants can lead to change in function of the gene products.

A total of 1778 SNPs are investigated for FOLH1. Bioinformatics tools were used to determine SNP (rs61886492). The amino acid change for rs61886492 is from histidine to tyrosine, i.e. from a basic amino acid having imidazole ring to an aromatic amino acid with polar charged group. Due to change in side chains of the amino acid residues brought about by the above SNP, affect the structure and function of the protein. The study helps in determining new insight for the genetic analysis of FOLH1 gene with neural tube defect.

Summary: In-silico structural and functional changes associated with FOLH1 gene due to polymorphism at H475Y in neural tube defect has been studied.

Bioinformatics tools were used to determine SNP (rs61886492). The amino acid change for rs61886492 is from histidine to tyrosine, i.e. from a basic amino acid having imidazole ring to an aromatic amino acid with polar charged group. Due to change in side chains of the amino acid residues brought about by the above SNP, affect the structure and function of the protein.

INTRODUCTION:-

Neural tube defects (NTDs) are among the most common congenital anomaly in human patients with an incidence of 1–2 infants per 1000 live births (Copp AJ et al, 2003). Neural tube development occurs in 3rd week of pregnancy leading formation of neural tube. When the neural tube does not close absolutely NTD develops. NTD is the prime cause of anguish and fatality in new borns. (Gos M et al, 2002), (Patrizia De Marco et al, 2006) It is non- infectious and is a non-syndromic disease. Neural tube closure defects (NTDs) occurs due to improper closure of neural tube during embryonic development (Hall et al, 1988). A low folate status is said to be associated with neural tube defect. Maternal supplementation with folic acid can lead to decrease in neural tube defect by about 70%. (Harding BN et al, 1997) Glutamate carboxypeptidase II (GCPII) gene conceal for three functionally definite splice modification namely, N-acetylated-α-linked acidic dipeptidase (NAALADase), folyl poly-γ-glutamate carboxypeptidase (FGCP) and prostate specific membrane antigen (PSMA), which are primarily asserted in brain, intestinal mucosa (Luthi-Carter et al, 1998) and prostate gland appropriately (Wright Jr. et al, 1995). GCPII is present on chromosome 11p11.2 (Maraj et al, 1998). Dietary folate exists in polyglutamate form and the conversion of polyglutamate to monglutamate is catabolised by folyl poly-γ-glutamate carboxypeptidase (Chandler et al, 1986). Polymorphism has been observed in exon 13 H475Y (1561C→T) in the catalytic domain of GCPII. The presence of this polymorphism is known to down regulate the FGCP activity, thereby block the intestinal absorption of folate (Halsted et al, 1998). This prompted us to focus on the systematic analysis of GCPII gene and its involvement in Neural tube defect using bioinformatics tools. Results obtained may be used in clinical investigation of NTD and will be beneficial to the researchers for understanding the role played by GCPII gene. The study is focused on SNP analysis and its association relative to change in structure of GCPII leading to neural tube defect.

Material and Method

Data source

Single nucleotide polymorphism database (dbSNP) (Smigielski et al, 2000), a public domain archive established by NCBI, to obtain the SNPs associated with GCPII gene. A total of 1778 SNPs were retrieved on 12th march 2014

Assessing the functional significance of SNPs

Selecting a SNP associated with neural tube defect lead to determination of H475Y. Sorting Intolerant from Tolerant (SIFT) was used to study sequence homology among related genes and domains across species to predict whether an amino acid substitution would affect protein function and hence, potentially alter the phenotype. SIFT helps in determining missense mutation (Johnson et al, 2005), (Ng and Henikoff, 2003). Using multiple alignments a tolerance index was calculated between 0 to1 for all possible substitution. Higher tolerance index has less functional impact a particular amino acid substitution was likely to have (Shen et al, 2006).The threshold for intolerance is 0.05.

FASTSNP server was also used to predict the functional consequence of SNPs. FASTSNP rates the phenotypic deleterious risks from 0 to 5 point scale. We observed SNP (rs61886492) having no any functional effect and no significant damaging score.

Structural information obtained by protein modeling

SWISS-MODEL, comparative modeling software was executed to build protein models from the templates obtained from either sequence similarity or fold recognition. Swiss model is a fully automated server for protein structure homology-modelling. It is accessible through expasy web server. (Arnold K. et al, 2006), (Kiefer F et al, 2009), (Guex N et al, 2009). Esypred 3D was used for modeling of native GCPII structure. (Lambert C et al, 2002)

3D protein structure visualization and RMSD calculation

The UCSF chimera version 1.5.3, a free molecular graphics program was used for viewing the modeled structures and for calculation of the root mean square deviation (RMSD) between the native and mutant structures. UCSF Chimera (Petterson et al, 2004) is a highly extensible program for interactive visualization and analysis of molecular structures and related data,

Volume : 3 | Issue : 4 | April 2014 • ISSN No 2277 - 8179

including density maps, supramolecular assemblies, sequence alignments, docking results, trajectories, and conformational ensembles. Match maker was used for superimposition of normal and mutant model of GCPII.

Result:

SNPs retrieved from NCBI SNP database

We used the NCBI SNP database to select SNPs of FOLH1 gene and we find that out of 1778 rs ids associated with FOLH1 gene; H475Y is associated with neural tube defect with rs61886492.

Table :1 SNPs identified by NCBI SNP database

dbSNP ID	Coding Type	Gene Name	AAPOS	Allele	Chromosome Position	Position relative to transcription start site	SNP CDS POS
rs61886492	coding	FOLH1	H475 Y	C/T	chr11:49186274	43948	1561

[H]Histidine [Y] Tyrosine, [C] Cytosine [T] Thymine

Non deleterious effect of SNPs predicted by SIFT

SIFT was used to identify the damaging effect of SNPs. SIFT program gives the effect on SNP by providing tolerance index score. The threshold for tolerance is 0.05. The SNP (rs61886492) with a tolerance index score of 1.00 is not predicted to be damaging, as shown in Table 2.

Table 2: Functional effect of SNP predicted by SIFT

SNP	Amino acid position	Damaging amino acid	Damage	Score
rs61886492	H475Y	Y	No	1.00

[H]Histidine [Y] Tyrosine

Analysis of 3D structure of native and mutant protein models

Esypred was executed to perform comparative modeling for constructing the native structure for protein GCPII (PM0078804) and swiss model was executed to model mutant structures for SNPs (rs61886492). The modeled structure of GCPII was refined using RAMPAGE and Errat server. Then the modeled structure was submitted to PMDB database The templates, 3bi1.1.A is found to be the best template candidate with 99.70% sequence identity is finally selected to construct the native and mutant protein models.

A RMSD value of 3.233˚A was found between the native GCPII protein and mutant protein structure of rs61886492. Evaluating superposition's across all 43 fully populated columns in the final alignment for RMSD of 01 mutant.pdb, chain A with PM0078804.pdb: 3.233 with Q score 0.003. Here, we have shown the native and mutant protein structures of SNP rs61886492 in Fig. 1, where histidine (His) residue in the native protein residing at 475 positions is replaced by tyrosine (Tyr) residue in the mutant protein.

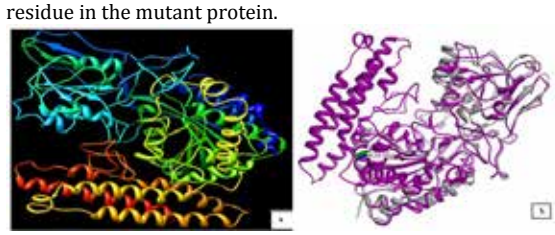

Figure 1: a. Native Modeled structure of GCPII protein. b. Superimposed structure of modeled native protein PM0078804 with modeled mutant protein for SNP rs61886492 having RMSD 3.23 ˚.

Discussion

We retrieved a total of 1778 SNPs associated with FOLH1 gene from the dbSNP Larsson *et al.* (2005) and identified SNP (rs61886492) by NCBI SNP database. SNP (rs61886492) was found to have significant non damaging tolerance index scores of 1.00, respectively, by SIFT. Lower the score more is the damaging impact Johnson *et al.* (2005), Shen *et al.* (2006). For rs61886492, the nucleotide change is in the 1561 position where G (Guanine) is changed to A (adenine) resulting in the substitution of amino acid H (histidine) to Y (tyrosine).

The functional consequence of these SNPs was assessed by utilizing FASTSNP server by identifying the high risk SNPs according to their phenotypic risks and putative functional effects Yuan *et al.*(2006). No damaging effect was observed using FASTSNP for SNP (rs61886492).

The 3D protein structures of both native and mutants were obtained by homology modeling after executing the Swiss model program. The native and mutant structures were superimposed and a RMSD of 3.23˚A between the native and mutant protein structures of rs61886492 as shown in Fig. 1. Hence, there might be a considerable change in the structure because of this SNP. The amino acid change found for rs61886492 is from histidine to tyrosine, *i.e.* from a basic amino acid having imidazole ring to an aromatic amino acid with polar charged group. Hence, due to the slight change in side chains of the amino acid residues brought about by these mutations, there seems to be a small change in structure. The 3D structure, shape and function of the protein are determined by primary structure of protein. The change in amino acid (from positive to neutral) leads to change in shape of the protein and thus its binding potential. It has been observed that H475Y polymorphism is associated with low plasma folate leading to increase in homocysteine level. This might be due to structural change due to above polymorphism leading to an increase in neural tube defect.

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