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PREDICTION OF FUNCTIONAL AND STRUCTURAL EFFECTS OF SINGLE NUCLEOTIDE POLYMORPHISM ON VITAMIN B12 RECEPTOR *IN SILICO*

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Abstract
The aim of this study was to evaluate the functional and structural effects of single nucleotide polymorphism resulting in substitution of glycine by glutamic acid at position 145 of the protein sequence of Vitamin B12 receptor using computational methods. Data on Vitamin B12 receptor with accession no NP_ 0010722 was obtained from the database of National Centre for Biotechnology Information (NCBI) for the study. Prediction of the effects of single nucleotide polymorphism using Panther server indicated damaging effect of the amino acid substitution. A protein variation effect analyzer score of -6.88 was obtained using PROVEAN server indicating deleterious effect of the amino acid substitution. Polyphen 2 indicated damaging effect of the amino acid substitution... Mutpred 2 indicated that the molecular mechanism of structural changes in Vitamin B12 receptor included, altered transmembrane protein, gain of disulfide linkage, gain of catalytic site and altered metal binding. It can be concluded that single nucleotide polymorphism resulting in substitution of glycine by glutamic acid at position 145 of the protein sequence resulted in structural and functional changes in Vitamin B12 receptor. The findings suggested the potential use of single nucleotide polymorphism on Vitamin B12 receptor as a biomarker for genetic causes of Vitamin B12 receptor deficiency.

Keywords: Amino acid, polymorphism, provean score, vitamin B12 receptor

Introduction

Vitamin B12 receptor (cubilin) is the intestinal receptor for endocytosis of intrinsic factor-Vitamin B12 complex. (Renata *et al*., 1999). Vitamin B12 is an essential co enzyme in mammals which is transported across the intestine by gastric intrinsic factor and cubilin. Loss of function of any of these proteins results in Vitamin B12 deficiency (Christien *et al*., 2010). Intrinsic factor is produced in the gastric epithelium, it binds to dietary Vitamin B12, and the intrinsic factor-Vitamin B12 complex is transported to the distal portion of small intestine by the Vitamin B12 receptor (Christensen *et al*., 2002). Vitamin B12 deficiency result in megaloblastic anemia and severe degeneration of the central nervous system .The deficiency normally occur due to failure of gastrointestinal tract uptake mechanism of dietary Vitamin B12 (John *et al*., 2004). Cobalamin deficiency may result from selective malabsorption due to defects of the various components involved in cobalamin intake. Genetic and functional defects in Vitamin B12 receptor have been implicated in Vitamin B12 deficiency (John *et al*., 2004). Genetic variations in proteins involve in transportation, absorption and cellular uptake of Vitamin B12 is reported to influence tissue status of Vitamin B12 (Quadros,2010). Amino acid sequence and composition determine the 3D structure and functionality of a protein (Vasem *et al*., 2021). Structural alteration in a protein results in changes in allosteric sites and altered connections between elements within protein structure (Guarnera *et al*., 2017. The objective of this study was to evaluate the effects of single nucleotide polymorphism on the function and structure of Vitamin B 12 receptor using computational methods, in order to provide better understanding on the mechanisms of genetic causes of vitamin B 12 deficiency.

Materials and Methods

Data on VitaminB12 receptor with accession no NP_ 0010722 was retrieved from database of National Centre for Biotechnology Information (NCBI).

Predicting effects of single nucleotide polymorphism using Panther

Protein analysis through evolutionary relationship (PANTHER,version 15.0) was used for the analysis. The server estimates the likelihood that a non synonymous SNP will cause a functional impact on the protein as reported by Huaiyu *et al*., 2019.

Analysis of effects of SNP using Provean server

Protein variation effect analyzer was used to predict the effect of single nucleotide polymorphism in ovine interferon . A delta alignment score was compared for each supporting sequence , the scores are averaged within and across clusters to generate a final Provean score. Provean score equal to or below a pre defined threshold indicate a deleterious effect of the amino acid substitution. Provean score above the threshold indicate a neutral effect as described by Choi *et al*., 2012.

Prediction of SNP using Poly phen 2 (Polymorphism phenotyping 2)

Poly phen 2 (Version 2) predicts the impact of amino acid substitution on the function of a protein using physical and comparative consideration (Adzhubei *et al*., 2010). The Mutpred 2 server was used to predict the structural effects of single nucleotide polymorphism on Vitamin B12 receptor as described by Pejaver,2020..

Results

Result of prediction of the functional effect of single nucleotide polymorphism using Panther server is presented in table 1.

Table 1: Prediction of single nucleotide polymorphism using PANTHER

Substitution	Preservation time	Result	Pdel
G145E	455	**Probably damaging**	0.57

Preservation time (million years), Pdel- probability of deleterious effect

The result in table 1 indicates damaging effect of substitution of glycine by glutamic acid at position 145 of Vitamin B12 receptor sequence.

Table 2: Prediction of effect of single nucleotide polymorphism using PROVEAN

Variant	Provean score	Prediction
G145E	- 6.88	**DELETERIOUS**

The default threshold is -2.5, variants with score equal to or below the threshold are considered deleterious Provean score of -6.88 which is below the threshold of -2.5 indicate deleterious effect of the amino acid substitution.

Table 3: Prediction of effect of SNP using Poly phen 2

Protein	position	AA1	AA2	PREDICTION	score
VitaminB12 receptor	145	G	E	**0.7**	

Polymorphism phenotyping 2 score ranges from 0 -1 , SNP prediction is considered possibly damaging or probably damaging when prediction score > 0.5 or benign (< 0.5) as described by Pejaver,2020.. Prediction score of 0.7 indicate damaging effect of the amino acid substitution using poly phen 2

Prediction of molecular mechanism of structural effects of single nucleotide polymorphism on vitamin B12 using Mutpred 2 server is presented in table 4.

Table 4 : Prediction of structural effects of single nucleotide polymorphism using Mutpred2

Molecular mechanism	Probability	P value
Altered membrane protein	0.30	**2.5**
Gain of disulfide linkage	0.2	3.0
Altered metal binding	0.07	0.02
Gain of catalytic site at C141	0.14	0.02

The molecular mechanisms of structural changes include, altered membrane protein, gain of disulfide linkage, gain of catalytic site and altered metal binding as shown in table 4.

Discussion

Protein analysis through evolutionary relationship software relates protein sequence evolution to the evolution of specific protein function and biological roles (Huaiyu *et al*., 2019). The effect of non-synonymous substitution is calculated by length of time (in millions of years) a given amino acid has been preserved in the protein of interest. The longer the preservation time, the greater the likelihood of functional impact resulting from substitution of the amino acid. The protein variation effect analyzer work on the assumption that protein sequences survive natural selection, therefore, evolutionary conserved sequences are likely to be of functional importance and amino acid substitution in conserved positions will potentially result in deleterious effect on protein function (Choi *et al*., 2012). Amino acid variations which deviate from frequently occurring sequences are predicted as deleterious. The obtained provean score of -6.88 indicate deleterious effect of the amino acid substitution. Mutpred 2 predicts changes at atomic and molecular levels induced by amino acid substitution. It provides score of probability that a mutation is deleterious and a score of the structural and functional impacts of the amino acid substitution (Choi *et al*., 2012). It provide model of probability of variant impact on specific aspect of protein structure

and function and the potential molecular mechanism of structural change associated with the amino acid substitution. Structural alteration in a protein results in disruption of protein charge, geometry and protein interaction with other molecules (Shahadat *et al*., 2020). The 3D structure of a protein is determined by its amino acid sequence which also influences protein function. An alteration in amino acid sequence results in change of structure and stability of proteins (Vasam *et al*., 2021). These changes alter interaction of the protein with other molecules and overall function of the protein. A mutation resulting in structural changes in a protein may not alter functions of the protein. However, mutation at conserved residues can lead to structural and functional changes in a protein (Rignal *et al*., 2002). Structural changes in a protein result in variations in connections of elements within the protein structure and allosteric changes in functional sites (Guarnera *et al*., 2017).

Conclusion

It can be concluded that single nucleotide polymorphism in Vitamin B12 receptor resulting in substitution of glycine for glutamic acid at position 145 of the protein chain lead to structural and adverse functional effects on Vitamin B12 receptor

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