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Computational Identification of Human TGFB1 Gene: SNPS and Prediction of Their Effect on Protein Functions of Preeclampsia Patients

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ABSTRACT: Growth factor (TGF-β1) belongs to the superfamily (TGF-β) as it has different roles and functions and participates in various physiological, biological, and pathological processes. The protein (TGF-β1) works to regulate cellular processes through pathways in which there is a connection to membrane receptors, which are of two types, I and II. Gene expression (TGF-β1) as well as its receptors can occur in the human placenta. This study aimed to identify some molecular parameters that could be considered accurate diagnostic parameters. Predictive bioinformatics techniques were used to predict whether the change occurring in the amino acid within the protein chain would affect the function of the protein in the future and thus the occurrence of the disease. The disease database, in which mutations were found, will be used and registered in the future. Mutations that occurred in the coding region that changed the amino acid in the protein were identified and tabulated in special tables. After that, the protein's sequence was obtained, and four predictive bioinformatics algorithms were applied. The results of this study showed that most of the mutations affect the natural protein. The effect may be on the physical and chemical properties, and it may be on the structural stability of the protein. Conclusions The candidate mutations in this study are mutations that can be considered diagnostic molecular indicators of preeclampsia.

INTRODUCTION

There is a condition specific to pregnancy known as preeclampsia. This condition is indicated by the appearance of some symptoms, namely high blood pressure and proteinuria. This condition may be diagnosed in the late stages of pregnancy1. Symptoms of preeclampsia, which are considered clinical diagnostic parameters. Preeclampsia, which clearly results from a defect in the lining of the mother's blood vessels 2-6. This disease may be called another toxicity, which is arterial hypertension, which is considered one of the causes of maternal death7-9. The disease can be diagnosed through high and clear differences in clinical and biochemical parameters from normal, in which case it is considered preeclampsia10. If the disease is not diagnosed early, this will lead to high blood pressure for late periods of pregnancy and even after birth, which will increase death for the mother and child 11-13. From a medical point of view, a defect in the epithelial lining of blood vessels is a major cause of high blood pressure in preeclampsia and many other diseases 14 - 16. Preeclampsia is a hereditary disease of three types. It is affected by environmental factors, genetic interactions, and gene-environment interactions17-20. The gene (TGF-β1) is one of the members of the superfamily (TGF-β) as it participates in many metabolic processes such as cell division20-22. It is possible that the study of preeclampsia is many, but using prediction algorithms in bioinformatics is almost rare. We conducted this study using modern biotechnology, where prediction algorithms were used to establish some molecular parameters that can help doctors accurately diagnose and confirm the disease. It is also possible that the study can be developed for familial prediction. With the presence of the disease and to open the way for researchers to use biotechnology, especially bioinformatics, in studying genetic diseases 23.

MATERIAL AND METHODS

Data collection:

Data on mutations (SNPs) of the TGFβ1 gene were collected through the use of the NCI-SNPB database (https://www.ncbi.nlm.nih.gov/snp/), where missense mutations that lead to changes in the amino acid were identified. It would be expected to affect the normal function of the protein. We obtained the protein sequence in FASTA format from the Uniprot database.

Prediction of deleterious nsSNPs: Using online tools, deleterious nsSNPs of the TGFβ1 gene were predicted.

1. Initially, **SIFT** performs multiple sequence alignment of related proteins and predicts whether a substitution will change the function of the protein or if it will be conserved [24:25]. Using sequence data, SIFT calculates a likelihood score for each new amino acid found in a location. A value between 0 and 0.05 is expected to be harmful and is thought to change the function of the TGFβ1 protein[26]. A number that is higher than the 0.05 limit is regarded as tolerant.

2. Using sequence, phylogeny, and structure-based characteristics of the variant amino acids, **PolyPhen v2** creates a conservation profile and assigns a prediction value, ranging from 0 (benign) to 1 (damaging), indicating whether the variation is likely harmful, maybe harmful, or benign. Other web-based tools were used to assess the SNPs that SIFT and PolyPhen v2 predicted to be harmful.
3. I-Mutant 3.0, for example, estimates the degree to which a variation might compromise the stability of a protein when compared to the wild-type amino acid [27]. The protein's stability changes in response to changes in either its primary amino acid sequence or its tertiary structure dictate the direction of the Delta Delta G: DDG value (folding free energy change) that the tool was taught to calculate [8,9]. Folding free energy (DG) is a critical protein stability metric that represents the free energy differential between folded wild-type and variant structures. I-Mutant 3.0 presents the results as DDG predictions, which are divided into three categories: neutral, denoted by values between − 0.5 and DDG ≤ 0.5 kcal/mol; typically stable, denoted by values > 0.5 kcal/mol; and predominantly unstable, denoted by values < − 0.5 kcal/mol.
4. PhD-SNP predicts whether nsSNPs are thought to be neutral polymorphisms or can be linked to a disease using a method based on support vector machines (SVMs) and sequence analysis 8.
5. To determine if SNPs are linked to illness, SNPs&GO uses the protein sequence, evolutionary conservation data, and functions listed in the GO database[28:29].
6.

RESULTS

One of the main pregnancy issues that jeopardizes the mother's and the fetus's health is PE. Due to uterine and placental insufficiency brought on by this issue, fetal growth is restricted and birth weight is low. Additionally, placental vascular diseases such as placental infarction arise from poor angiogenesis and inadequate spiral artery development [24]. Evidence indicated that the autophagy-deficient extra villous trophoblast (EVT) cell line exhibited both the trophoblast invasion and the impairment of placental vascular regeneration, which are the primary causes of preeclampsia [4].

Table 1: Nonsynonymous SNPs predicted with SIFT, Polyphen-2 and I-Mutant 30 programs.

SNP ID	Protein ID	Amino acid change	SIFT		Polyphen-2		I Mutant 30		
			SIFT prediction	SIFT score	Polyphen2 prediction	Polyphen2 score	SVM2 prediction effect	DDG	RI
Rs121918712	P36897	T200I	D	0.00	PD	1.000	Decrease	-0.20	2
Rs111854391	P36897	K232E	D	0.00	PD	1.000	Decrease	-0.53	2
rs121918710	P36897	S241L	D	0.00	PD	1.000	Increase	-0.24	0
rs121918710	P36897	D266Y	D	0.00	PD	1.000	Decrease	-0.89	7
	P36897	M318R	D	0.00	PD	0.985			
	P36897	D351G	D	0.00	PD	1.000	Decrease	-2.04	9
	P36897	T375R	D	0.00	PD	1.000	Decrease	-0.12	2
rs121918711	P36897	D400G	D	0.00	PD	1.000	Decrease	-1.14	8
rs113605875	P36897	R487P	D	0.00	PD	1.000	Decrease	-0.36	0
rs113605875	P36897	R487Q	D	0.00	PD	1.000	Decrease	-0.72	9
rs111426349	P36897	R487W	D	0.00	PD	1.000	Decrease	-0.40	6

*Deleterious: D ; *Probably Damaging :PD

The prediction is given a numerical value by the SIFT Polyphen-2 deleterious scores. Higher Polyphen-2 scores are indicative of harmful mutations, whereas lower or negative SIFT I-Mutant 30 values are indicative of harmful SNPs.

Table 2: Nonsynonymous SNPs predicted with PHD-SNPs and SNPs & Go programs; SNPs selected with a tolerance index of equal (0.009) and a PSIC SD range of 1–099

SNP ID	Protein ID	Amino acid change	PHD-SNPs		SNPs & Go	
			PHD-SNP effect	RI	SNPs & Go prediction	RI
Rs121918712	P36897	T200I	Neutral	1	Disease	5
----------	P36897	K232E	Neutral	4	Disease	9
Rs111854391	P36897	S241L	Neutral	4	Disease	8
----------	P36897	D266Y	Disease	1	Disease	8

rs121918710	P36897	M318R	Disease	4	Disease	2
----------	P36897	D351G	Disease	4	Disease	9
----------	P36897	T375R	Neutral	5	Disease	9
rs121918711	P36897	D400G	Neutral	1	Disease	9
rs113605875	P36897	R487P	Disease	6	Disease	9
rs113605875	P36897	R487Q	Disease	5	Disease	5
rs111426349	P36897	R487W	Disease	6	Disease	6

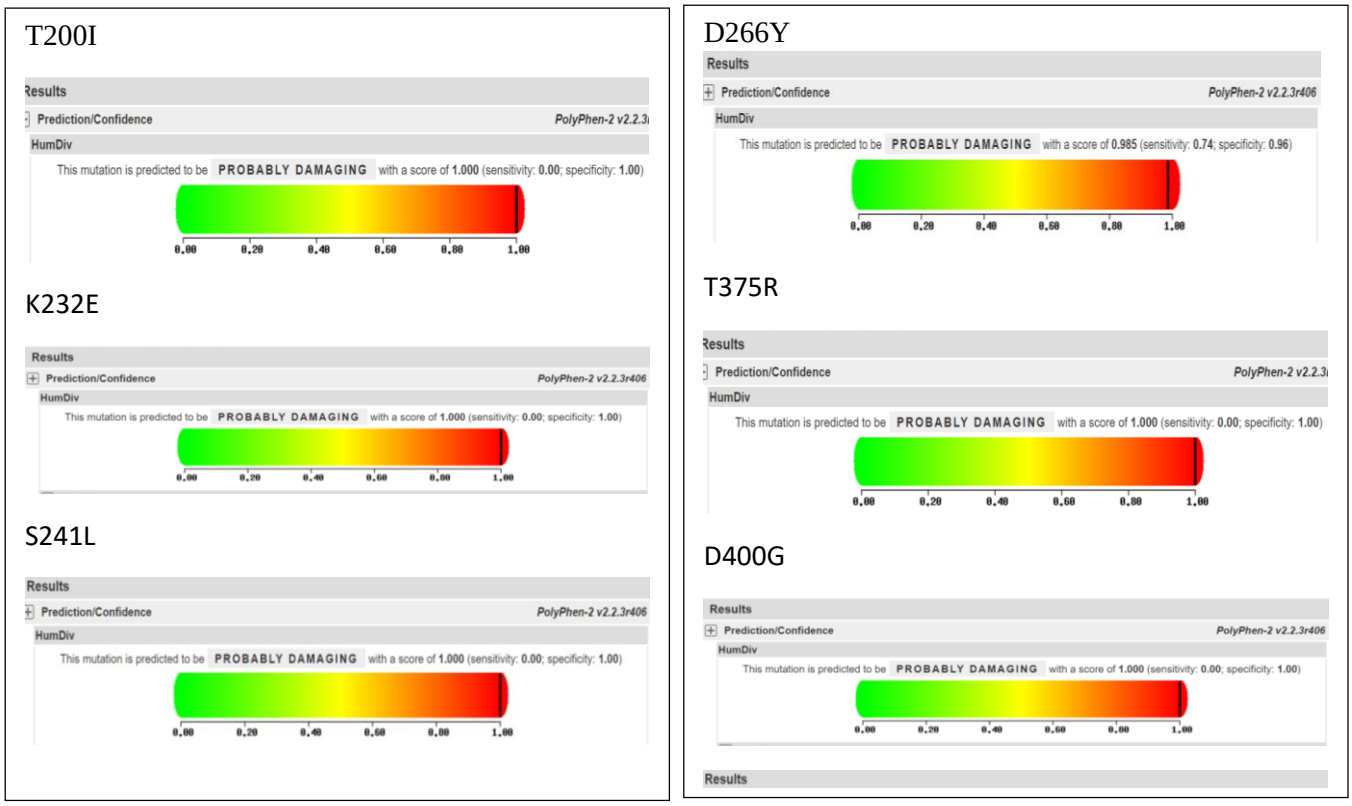

Figure:

The mutation is categorized as either a disease-related or neutral polymorphism by the PhD-SNP 2.0 and SNPs&GO programs. Six of the nsSNPs in the TGF1 gene set that were examined by PhD-SNP 2.0 were expected to be associated with disease, and all of the nsSNPs were identified as such by the SNPs&GO approach.

DISCUSSION

Five different systems that predict harmful non-synonymous polymorphisms (nsSNPs) were used to analyze the non-synonymous polymorphisms located in the TGFβ1 gene. The variations in the predictions produced by the systems point to the necessity of a combined analysis that could pinpoint precisely which nsSNPs are most detrimental to the TGFβ1 gene's function. In order to categorize the nsSNPs from the most detrimental to the most neutral, we pooled the findings from the five tools. Three programs with high concordance with two consensus prediction tools predicted most of the nsSNPs (11, approximately 62%) as harmful, detrimental, or disease-associated. Our combined analysis determined that the six nsSNPs that were deemed harmful by the five methods were the most harmful.

The results showed that these can be prioritized in future populational and laboratory studies, even though the majority of the most harmful nsSNPs described here were not examined by in vitro tests, and there is no information on the functional significance of these mutations in the MC1R protein. The most harmful mutations were chosen by analyzing the nsSNPs in several genes involved in biological processes using the method of using the predictions of various technologies.

CONCLUSION

These findings show that a variety of methods could be used to account for program variations and enhance the precision of the search for significant polymorphisms, disease occurrences, or phenotypic variations.

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