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Type-2 Diabetes Mellitus and Glucagon-Like Peptide-1 Receptor toward Predicting Possible Association

Nabaa Kamal Alshafei1, Intisar Hassan Saeed2, Mona Abdelrahman Mohamed Khaier3*

1Department of Biochemistry, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan
2Department of Physiology, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan
3Department of Molecular Biology and Bioinformatics, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan
Email: *munakhaier@gmail.com

Abstract

Aim: This study aimed to investigate the effect of non-synonymous SNPs (nsSNPs) of the Glucagon-like peptide-1 Receptor (*GLP-1R*) gene in protein function and structure using different computational software. **Introduction:** The *GLP1R* gene provides the necessary instruction for the synthesis of the insulin hormones which is needed for glucose catabolism. Polymorphisms in this gene are associated with diabetes. The protein is an important drug target for the treatment of type-2 diabetes and stroke. **Material and Methods:** Different nsSNPs and protein-related sequences were obtained from NCBI and ExPASY database. Gene associations and interactions were predicted using GeneMANIA software. Deleterious and damaging effects of nsSNPs were analyzed using SIFT, Provean, and Polyphen-2. The association of the nsSNPs with the disease was predicted using SNPs & GO software. Protein stability was investigated using I-Mutant and MUpro software. The structural and functional impact of point mutations was predicted using Project Hope software. Project Hope analyzes the mutations according to their size, charge, hydrophobicity, and conservancy. **Results:** The *GLP1R* gene was found to have an association with 20 other different genes. Among the most important ones is the GCG (glucagon) gene which is also a trans membrane protein. Overall 7229 variants were seen, and the missense variants or nsSNPs (146) were selected for further analysis. The total number of nsSNPs obtained in this study was 146. After being subjected to SIFT software (27 Deleterious and 119 Tolerated) were predicted. Analysis with Provean showed that (20 deleterious and 7 neutral). Analysis using Polyphen-2 revealed 17 probably damaging, 2 possibly damaging and 1 benign nsSNPs. Using two additional software SNPs & GO and PHD-SNPs showed that 14 and 17 nsSNPs had a disease effect, respectively. Project Hope software predicts the effect of the 14

nsSNPs on the protein function due to differences in charge, size, hydrophobicity, and conservancy between the wild and mutant types. **Conclusion:** In this study, the 14 nsSNPs which were highly affected the protein function. This protein is providing the necessary instruction for the synthesis of the insulin hormones which is needed for glucose catabolism. Polymorphisms in this gene are associated with diabetes and also affect the treatment of diabetic patients due to the fact that the protein acts as an important drug target.

Keywords

Glucagon-Like Peptide-1 Receptor, Single Nucleotide Polymorphism, *Insilico* Analysis, Non Synonymous SNP, SIFT, Polyphen-2, GeneMANIA

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is caused by inability of the pancreatic beta cells to produce sufficient insulin hormone against insulin resistance [1]. It is a heterogeneous disorder of glucose metabolism characterized by both insulin resistance and pancreatic β-cell dysfunction considered as multifactorial due to many genetic and environmental factors involved together in its pathophysiology [2] [3] (Before the development of T2DM, individuals develop hyperproinsulinemia and the elevated level of proinsulinemia have been significantly associated with diabetes [4] [5]. Globally, type 2 diabetes mellitus was diagnosed in 537 million adults aged 20 - 79 years in 2021 and is projected to affect 783 million adults by 2045 [6].

Glucagon-like peptide-1 (GLP-1) is secreted chiefly by the intestinal L cells in the distal small intestine and proximal colon encoded by the proglucagon gene and it is a 30 amino acid polypeptide [7] [8]. GLP-1 specifically targets the GLP1R (GLP-1 Receptor). The human *GLP1R* gene is allocated to the long arm of chromosome 6 (chr 6p21) and contains 13 exons [9]. The gene is a member of the B1 family of G protein-coupled receptors, mainly shows expression within islet β cells and contains 463 amino acids containing seven trans membrane domains belonging to the family of G-protein coupled receptors. GLP-1 binds to *GLP1R* gene to activate adenylate cyclase, which activates cyclic adenosine monophosphate (cAMP) dependent second messenger pathways, including protein kinase PKA and Exchange Proteins Activated (Epac) by cAMP, thus increasing insulin release through β cells and the number of β cells that respond to glucose [10]. Guanine nucleotide-binding protein (G-protein) and G-protein-coupled receptor (GPCR) were involved in the development of T2 DM [11]. G protein activates adenylyl cyclase (AC), which generates "second messenger", cAMP, which regulates glucose homeostasis by regulating glucose uptake, insulin and glucagon secretion, synthesis and breakdown of glycogen through protein kinase A (PKA). The cAMP is one of the most important cellular signaling molecules in the regulation of insulin secretion by beta cells [12] [13].

The objective of this study is to predict the nsSNPs in the *GLP1R* gene and the effect they may impose on the protein structure and function using various computational software.

2. Material and Methods

2.1. Data Retrieval

Data was retrieved from the SNP database of the National Center for Biotechnology Information (dbSNP) (http://www.ncbi.nlm.nih.gov/snp). The NCBI SNP database was used to access the SNPs of the *GLP1R* gene (accessed June 2022). The primary sequence of the protein (accession number: P43220) encoded by the *GLP1R,* human gene was obtained from UniProtKB database (accessed June 2022).

2.2. Gene MANIA: (http://www.genemania.org)

It is a web interface that finds other genes related to an input gene, using a very large set of functional association data. Association data include protein and genetic interactions, pathways, co-expression, co-localization and protein domain similarity [14].

2.3. Sorting Intolerant from Tolerant (SIFT): (https://sift.bii.a-star.edu.sg/)

This is a tool that expresses whether a nsSNP at special position affects the structure and function of the protein based on sequence homology and the physiochemical characteristics of substituted amino acid. SIFT computes the normalized probability score (SIFT score) for each substitution. The SIFT score has a range of 0.0 to 1.0, the amino acid substitution with a score greater than or equal to 0.05 ($\geq$0.05) is predicted as tolerated polymorphism, whereas a score less than 0.05 (<0.05) is predicted to be damaging ones [15].

2.4. Protein Variation Effect Analysis (PROVEAN): (provean.jcvi.org/)

This is another sequence homology-based predictor. It is used to assess the possible functional influence of nsSNPs on a protein. It predicts the variation as deleterious or natural, if the functional impact score is less than or equal to −2.5 ($\leq$−2.5) it is estimated as a deleterious; score above − 2.5 (>−2.5) is estimated as neutral [16].

2.5. Polymorphism Phenotyping Version2 (PolyPhen-2): (genetics.bwh.harvard.edu/pph2/)

It is a combination of protein 3D structure and multiple homolog sequence alignment-based method. It predicts the potential consequences of single amino acid substitution on both protein function and structure. The prediction is provided as benign, possibly damaging and probably damaging according to the po-

sition-specific independent count (PSIC) scores difference between 2 variants (wild amino acid (aa1) and mutant amino acid (aa2)). PSIC score has a range of 0.0 to 1.0. The amino acid substitution with a score of 0.0 to 0.49 is predicted as benign, with a score of 0.5 to 0.89 is predicted as damaging and with a score of 0.9 to 1 is predicted as probably damaging [17] [18].

2.6. SNPs & GO (Single Nucleotide Polymorphism & Gene Ontology)

SNPs & GO is an accurate method that, starting from a protein sequence, can predict whether a mutation is disease-related or not by exploiting the protein functional annotation. SNPs & GO collects in unique framework information derived from protein sequence, evolutionary information, and function as encoded in the Gene Ontology terms, and outperforms other available predictive methods [19].

2.7. PHD-SNP: (Predictor of Human Deleterious SNP) (snps.biofold.org/phd-snp/phd-snp.html)

Predictor of human Deleterious Single Nucleotide Polymorphisms (PhD-SNP) is a support vector machine (SVM) based server. This server determines whether a certain amino acid substitution is related to disease or neutral by protein sequence information, protein structure, conservation and solvent accessibility. The output is a probability index with a score of 0.0 to 1.0, when the score is higher than 0.5, the substituted amino acid is pathogenic [16] [20].

2.8. Protein Stability Prediction

Two software were used to predict the effect of a missense mutation on the protein's stability.

2.8.1. I-Mutant 2.0 https://folding.biofold.org/cgi-bin/i-mutant2.0.cgi
(http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi)

This software offers the opportunity to predict automatically protein stability changes upon single-site mutations starting from protein sequence alone or protein structure when available. Moreover, it can predict deleterious Single Nucleotide Polymorphism starting from the protein sequence alone [20].

2.8.2. MUpro
(mupro.proteomics.ics.uci.edu): MUpro uses the Support Vector Machine (SVM) to assess the variation in the stability of the protein consequent to amino acid substitutions. The output is a confidence score among −1 and 1. A confidence score < 0 indicates the substituted amino acid decreases the stability and a score > 0 indicates the substituted amino acid increases the stability [21].

2.9. Prediction of Protein Modeling www.cmbi.ru.nl/hope/

Project Have your Protein Explained (HOPE) is a web server that was used for

the investigation of the impacts of a missense mutation on the native protein structure. HOPE will roll up and incorporate available information from UniProtKB, protein's 3D structure and DAS-servers. As regards the exact 3D-structures of some GLP1R protein isoforms are unknown; HOPE built the model of them based on homologous structures. HOPE processes the gathered data and produces a report, including schematic structures of the wild-type and the mutant amino acids, differences in the properties of wild-type and mutant amino acids and the impacts of a substituted amino acid on the protein structure along with figures and animations [22].

3. Results

In this study *GLP1R* gene was found to have an association with 20 other different genes. Among the most important ones is the GCG (glucagon) gene which is also a trans membrane protein (Figure 1 and Table 1).

Table 1. Gene description rank using *GLP1R* gene.

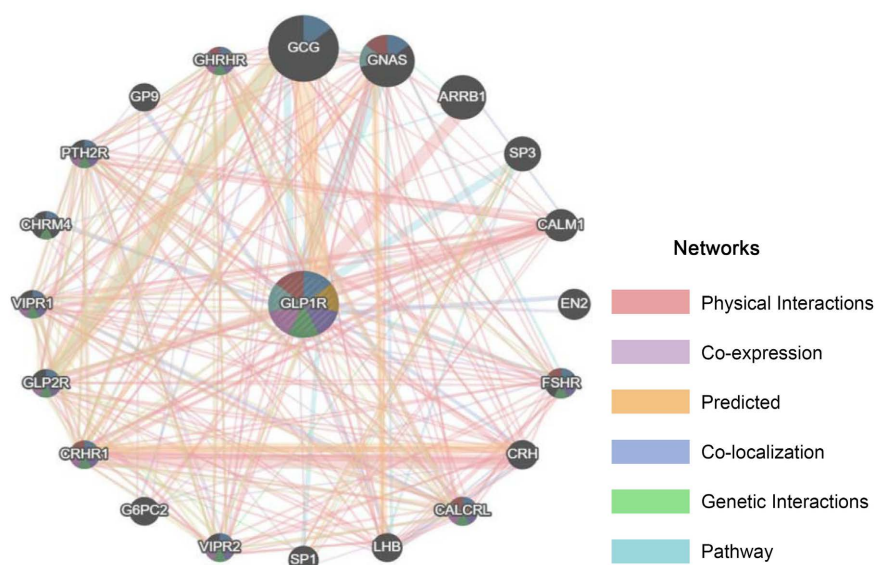| Gene	Description
GLP1R	Glucagon like peptide1 receptor
GCG	Glucagon
GNAS	GNAS complex locus
ARRB1	Arrestin beta1
SP3	Sp3transcription factor
CALM1	Calmodulin1
EN2	Engrailed homeo box2
FSHR	Follicle stimulating hormone receptor
CRH	Cortico tropin releasing hormone
CALCRL	Calcitonin receptor like receptor
LHB	Luteinizing hormone sub-unit beta
SP1	Sp1transcription factor
VIPR2	Vasoactive intestinal peptide receptor2
G6PC2	Glucose-6-phosphatase catalytic subunit 2
CRHR1	Cortico tropin releasing hormone receptor1
GLP2R	Glucagon like peptide 2 receptor
VIPR1	Vasoactive intestinal peptide receptor1
CHRM4	Cholinergic receptor muscarinic 4
PTH2R	Parathyroid hormone2 receptor
GP9	Glycoprotein I X platelet
GHRHR	Growth hormone releasing hormone receptor

Figure 1. GeneMANIA results for *GLP1R* Gen.

Overall 7229 variants were seen, and the missense variants or nsSNPs (146) were selected for further analysis. The nsSNPs obtained were subjected to SIFT software, 27 were predicted as deleterious and 119 were predicted as tolerated. Analysis with Provean showed that; 20 were predicted as deleterious and 7 as neutral, Table 3. Analysis using Polyphen-2 revealed that 17 were predicted as probably damaging, 2 possibly damaging and 1 as benign. By using software SNPs&GO there were 14 SNPs had a disease effect and 5 were neutral, while using PHD SNPs 17 SNPs had a disease effect and 2 were neutral, Table 2.

Disease-related mutations resulting from SNPs&Go were submitted to I-Mutant and MUpro software, results showed an effect on the protein stability with varied probabilities, Table 3 and Table 4.

When using five different software, (SIFT, Polyphen-2, Provean, SNPs & Go and PHD-SNP) for studying the functional and structural effects a total of 14 nsSNPs had a disease effect (Table 4). Regarding the effect on protein stability, 17 nsSNPs were predicted to decrease the stability of the protein when using I-Mutant 2.0. On the other hand, MUpro software showed 19 SNPs decrease the protein stability, Table 4.

The structural impact of the SNPs on protein structure and function was investigated using Project Hope. Fourteen nsSNPs were analyzed using Project Hope, Table 5.

rs201231115 (C46Y): showed that the mutant residue is bigger than the wild-type residue. The wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit. Mutation of a 100% conserved residue is usually damaging for the protein.

rs200765138 (R64W): the mutant residue is bigger than the wild-type and the size difference between wild-type and mutant residue makes that the new residue is not in the correct position to make protein structure.

rs201634613 (W87R): the mutant residue is smaller than the wild-type residue. The mutated residue is located in a domain that is important for the activity of the protein. The mutation can affect this interaction and as such affect protein function. There is also difference in the charge between the wild and mutant type.

Table 2. The results of different software.

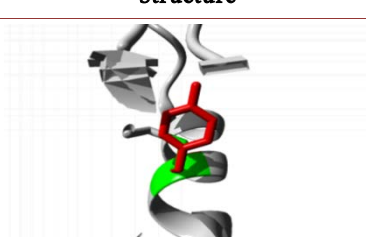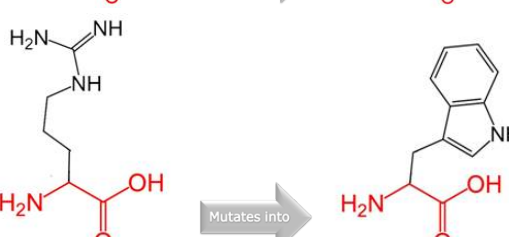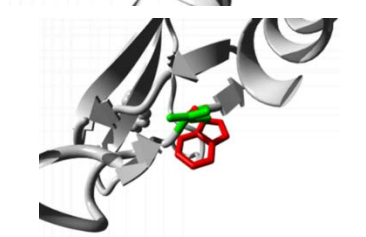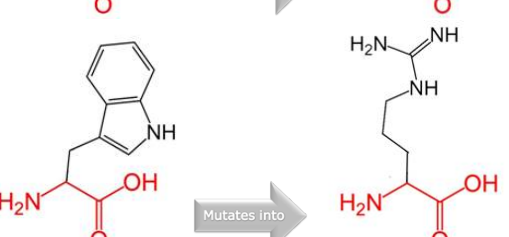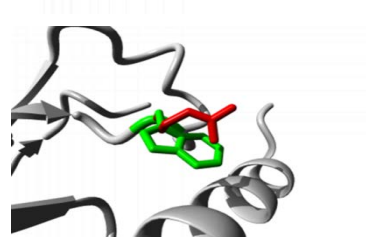| Software	Results
Retrieved SNPs	Total SNPs were 7229, 146 non-synonymous
SIFT	27 deleterious and 119 tolerated
Provean	20 deleterious and 7 neutral
Polyphen-2	17 probably damaging, 2 possibly damaging, and1 benign
SNPs & GO	14 nsSNPs had a disease association and 5 neutral
PHD SNPs	17 nsSNPs had a disease association and 2 neutral
I-Mutant	17 nsSNPs decrease the protein stability and 2increase it
MUpro	All the19 nsSNPs decrease the protein stability

Table 3. Results of SIFT, Provean and Polyphen-2 analysis.

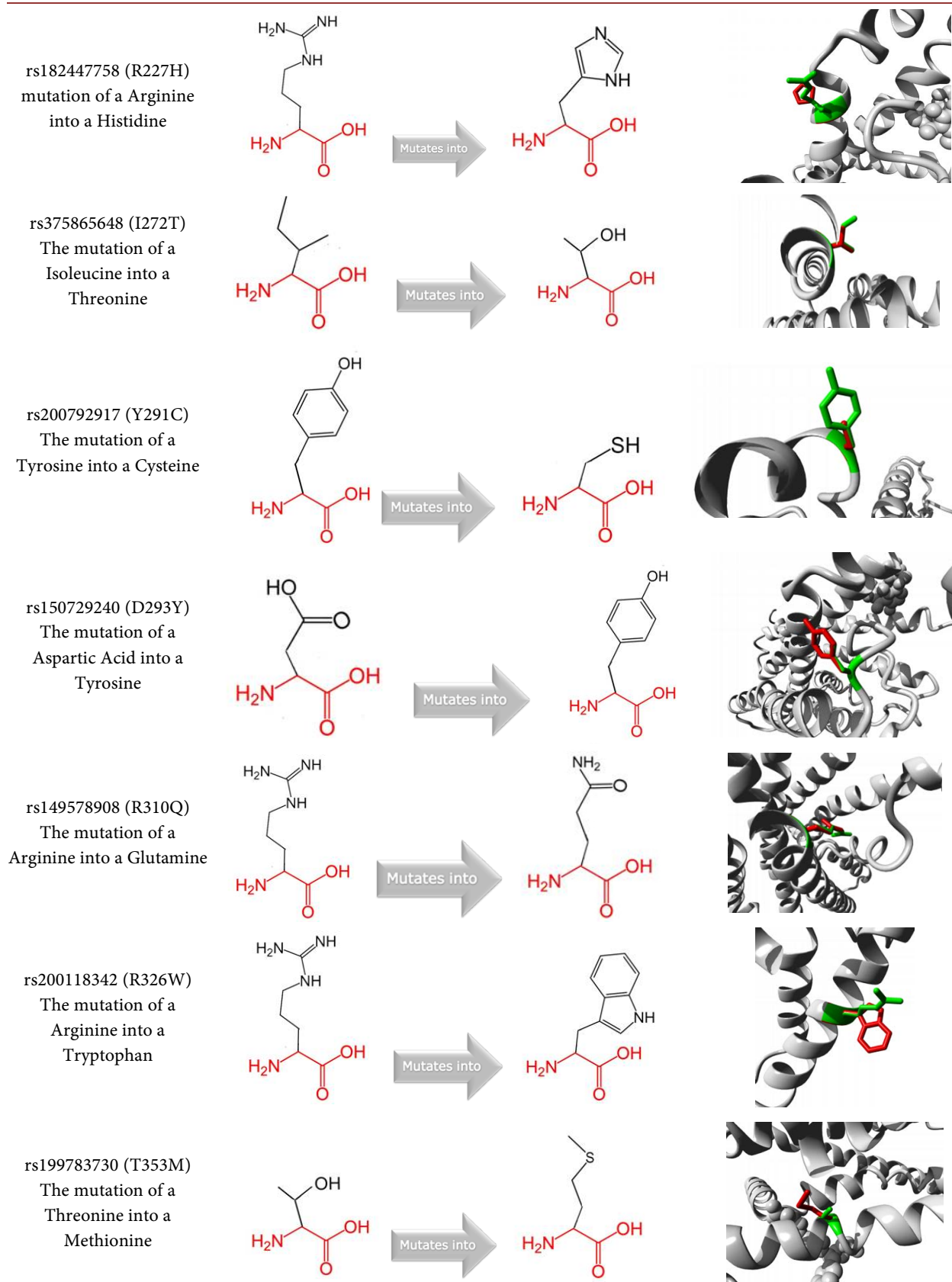| SNP	Amino acid change	Protein ID	SIFT Score	SIFT Prediction	Provean score	Provean Prediction	Polyphen-2 score	Polyphen -2 Prediction
rs201231115	C46Y	ENSP00000362353	0	Deleterious	−10.235	Deleterious	1	Probably damaging
rs200765138	R64W	ENSP00000362353	0.018	Deleterious	−5.814	Deleterious	1	Probably damaging
rs201634613	W87R	ENSP00000362353	0.002	Deleterious	−10.052	Deleterious	1	Probably damaging
rs182447758	R227H	ENSP00000362353	0.002	Deleterious	−4.495	Deleterious	1	Probably damaging
rs375865648	I272T	ENSP00000362353	0.003	Deleterious	−3.986	Deleterious	0.949	**Possibly damaging**
rs200792917	Y291C	ENSP00000362353	0.01	Deleterious	−6.321	Deleterious	0.999	Probably damaging
rs150729240	D293Y	ENSP00000362353	0.001	Deleterious	−4.24	Deleterious	0.625	**Possibly damaging**
rs149578908	R310Q	ENSP00000362353	0.018	Deleterious	−3.859	Deleterious	1	Probably damaging
rs200118342	R326W	ENSP00000362353	0.001	Deleterious	−6.585	Deleterious	1	Probably damaging
rs10305493	S333C	ENSP00000362353	0.003	Deleterious	−4.279	Deleterious	0.999	Probably damaging
rs202171972	D344E	ENSP00000362353	0.005	Deleterious	−4	Deleterious	0.996	Probably damaging
rs199783730	T353M	ENSP00000362353	0	Deleterious	−5.988	Deleterious	1	Probably damaging
rs201054657	I357T	ENSP00000362353	0.001	Deleterious	−4.682	Deleterious	1	Probably damaging
rs199818129	P358L	ENSP00000362353	0.019	Deleterious	−9.93	Deleterious	1	Probably damaging
rs201669667	L360P	ENSP00000362353	0.001	Deleterious	−6.677	Deleterious	1	Probably damaging
rs148543734	G361R	ENSP00000362353	0	Deleterious	−7.984	Deleterious	1	Probably damaging
rs200065197	R376W	ENSP00000362353	0.007	Deleterious	−3.742	Deleterious	0.999	Probably damaging
rs201617183	G377R	ENSP00000362353	0.002	Deleterious	−6.884	Deleterious	1	Probably damaging
rs201899163	Q394R	ENSP00000362353	0.008	Deleterious	−3.6	Deleterious	0.998	Probably damaging

Table 4. Results of SNPs & GO, PHD SNP and I-Mutant software.

No.	Amino acids change	SNP&GO Prediction	SNP&GO RI	PHD SNP Prediction	PHD RI	I-Mutant Prediction	I-Mutant RI	MUpro
1	C46Y	Disease	6	Disease	1	Increase	4	Decrease
2	R64W	Disease	3	Disease	3	Decrease	7	Decrease
3	W87R	Disease	5	Disease	1	Decrease	8	Decrease
4	R227H	Disease	4	Disease	1	Decrease	7	Decrease
5	I272T	Disease	2	Disease	7	Decrease	9	Decrease
6	Y291C	Disease	6	Disease	8	Decrease	6	Decrease
7	D293Y	Disease	8	Disease	9	Decrease	1	Decrease
8	R310Q	Disease	4	Neutral	3	Decrease	8	Decrease
9	R326W	Disease	3	Disease	6	Decrease	6	Decrease
10	S333C	Neutral	7	Disease	2	Decrease	7	Decrease
11	D344E	Neutral	3	Neutral	3	Increase	0	Decrease
12	T353M	Disease	6	Disease	7	Decrease	3	Decrease
13	I357T	Neutral	1	Disease	4	Decrease	3	Decrease
14	P358L	Disease	6	Disease	8	Decrease	4	Decrease
15	L360P	Disease	7	Disease	9	Decrease	5	Decrease
16	G361R	Disease	7	Disease	9	Decrease	7	Decrease
17	R376W	Neutral	3	Disease	3	Decrease	8	Decrease
18	G377R	Neutral	0	Disease	6	Decrease	8	Decrease
19	Q394R	Disease	6	Disease	9	Decrease	6	Decrease

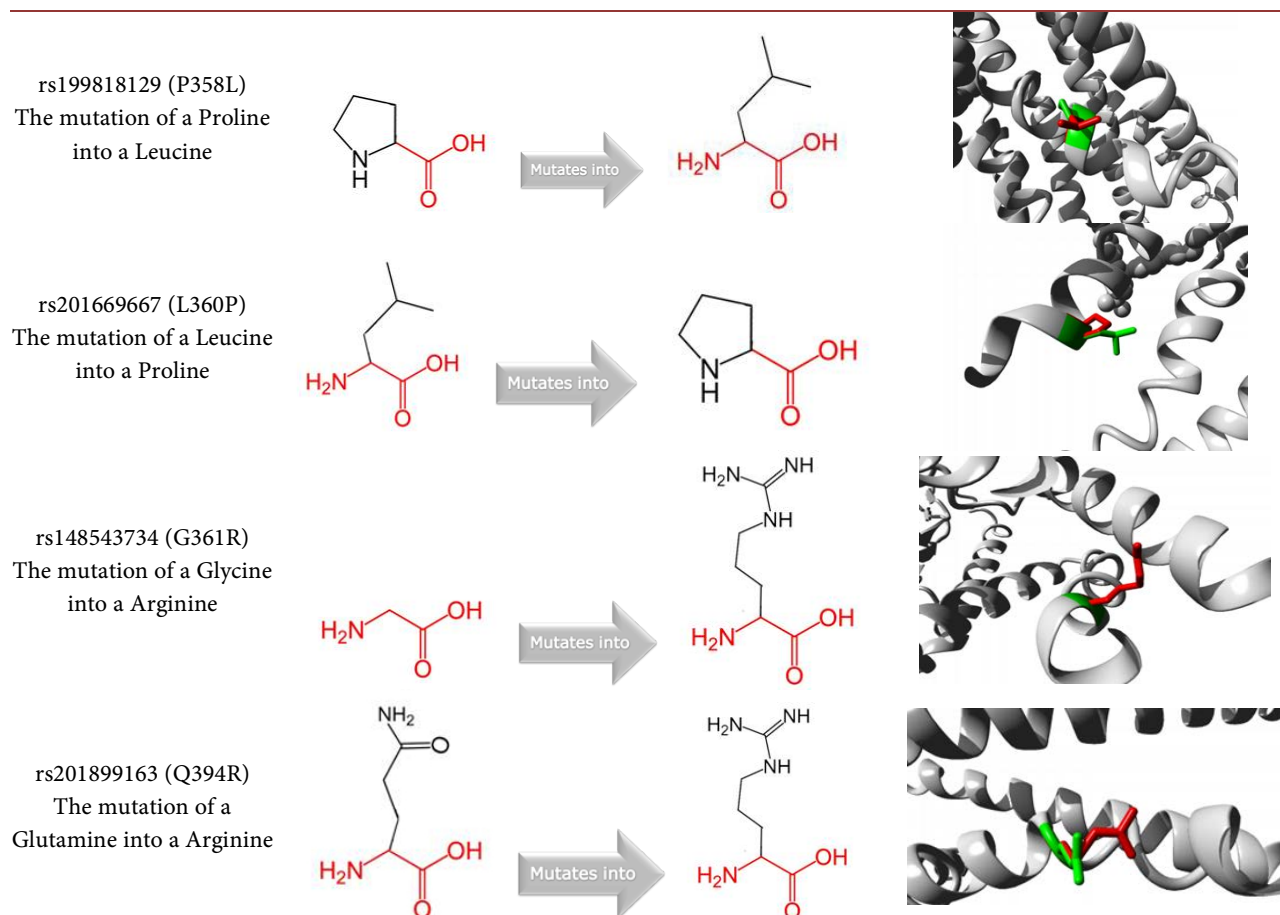**Table 5.** The effect of mutations on the protein using Project Hope software.

SNP ID	Wild and Mutant variation	Structure
rs201231115 (C46Y) The mutation of a Cysteine into a Tyrosine		
rs200765138 (R64W) The mutation of an Arginine into a Tryptophan		
rs201634613(W87R) The mutation of a Tryptophan into a Arginine		

Continued

rs182447758 (R227H) mutation of a Arginine into a Histidine		Mutates into	
rs375865648 (I272T) The mutation of a Isoleucine into a Threonine		Mutates into	
rs200792917 (Y291C) The mutation of a Tyrosine into a Cysteine		Mutates into	
rs150729240 (D293Y) The mutation of a Aspartic Acid into a Tyrosine		Mutates into	
rs149578908 (R310Q) The mutation of a Arginine into a Glutamine		Mutates into	
rs200118342 (R326W) The mutation of a Arginine into a Tryptophan		Mutates into	
rs199783730 (T353M) The mutation of a Threonine into a Methionine		Mutates into	

Continued

rs199818129 (P358L) The mutation of a Proline into a Leucine		
rs201669667 (L360P) The mutation of a Leucine into a Proline		
rs148543734 (G361R) The mutation of a Glycine into a Arginine		
rs201899163 (Q394R) The mutation of a Glutamine into a Arginine		

*Note: Grey colour is protein chains, green coloured atoms are the wild amino acid residues, while red coloured atoms are the mutated amino acid residues.

rs182447758 (R227H): the wild-type residue is smaller than the mutant residue. There is a difference in charge between the wild-type and mutant amino acid, this can cause loss of interactions with other molecules.

rs375865648 (I272T): the mutant residue is smaller and less hydrophobic than the mutant residue, this will cause a possible loss of external interactions and will cause an empty space in the core of the protein and this differences in hydrophobicity can affect the hydrophobic interactions with the membrane lipids.

rs200792917 (Y291C): the mutant residue is smaller than the wild-type residue. The mutant residue is more hydrophobic than the wild-type residue which might affect the function of the protein.

rs150729240 (D293Y): appeared that the mutant residue and wild residue are differing in size and charge. The mutant residue is more hydrophobic than the wild-type residue, so the interaction between these domains could be disturbed by the mutation, which might affect the function of the protein.

rs149578908 (R310Q): The mutant residue is smaller than the wild-type residue. This mutation might occur in some rare cases, but it's more likely that the mutation is damaging to the protein. The difference in charge will disturb the

ionic interaction made by the original, wild-type residue.

rs200118342 (R326W): indicated that the wild-type and mutant amino acids differ in size. The mutant residue is bigger than the wild-type residue. The residue is located on the surface of the protein. There is a difference in charge between the wild-type and mutant amino acid. Mutation of this residue can disturb interactions with other molecules or other parts of the protein. The mutation can affect this interaction and as such affect protein function.

rs199783730 (T353M): each amino acid has its own specific size, charge, and hydrophobicity-value. The original wild-type residue and newly introduced mutant residue differ in these properties. The mutant residue is bigger and more hydrophobic than the wild-type residue, based on this conservation information this mutation is probably damaging to the protein.

rs199818129 (P358L): the mutated residue is located in a domain that is important for the activity of the protein and in contact with residues in another domain. The wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit.

rs201669667 (L360P): the mutant residue is smaller than the wild type which will cause an empty space in the core of the protein and loss of hydrophobic interactions.

rs148543734 (G361R): showed that the mutant residue is bigger than the wild-type residue. The wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit. The mutation into another residue will force the local backbone into an incorrect conformation and will disturb the local structure.

rs201899163 (Q394R): the wild-type and mutant amino acids differ in size, based on conservation scores this mutation is probably damaging to the protein.

4. Discussion

Missense mutations resulting in the amino acid change disturb the potential protein structure, stability, and activity which can enhance the individual susceptibility to disease [23]. Investigating the impact of a missense mutation on proteins is a key step in understanding the effect of the mutation on the characteristics of the wild type protein and the resulting phenotype [24]. The major effects of GLP-1 protein include the potentiation of glucose-stimulated insulin secretion, suppression of appetite, and slowing of gastric emptying [25]. Five GLP-1 mimetic agents are approved for the treatment of type 2 diabetes, including exenatide, lixisenatide, liraglutide, dulaglutide, and semaglutide [26].

In this study, a total of 14 nsSNPs were shown to be deleterious, damaging, disease-related, and affecting the protein function due to differences in charge, size, hydrophobicity, and conservancy between the wild and mutant types using different software namely rs201231115 (C46Y); rs200765138 (R64W); rs201634613 (W87R); rs182447758 (R227H); rs375865648 (I272T); rs200792917 (Y291C); rs150729240 (D293Y); rs149578908 (R310Q); rs200118342 (R326W); rs199783730

(T353M); rs199818129 (P358L); rs201669667 (L360P); rs148543734 (G361R); and rs201899163 (Q394R) were not reported in ClinVar database and has not been previously reported in the *GLP1R* gene.

The rs1042044 of the *GLP1R* gene is a tag-SNP located in exon7 of the gene. This is a nsSNP resulting in (Phe260Leu) according to Hap Map location (http://www.hapmap.org). The nsSNPs of the *GLP1R* gene affect the in vivo response of GLP-1 and are considered to be the primary cause of the inconsistent clinical efficacy of GLP-1 analogs within T2DM patients. Anderson *et al.*, 2012 and Sathananthan *et al.* 2010 studied the effects of the *GLP1R* gene nsSNPs on insulin secretion from exogenous GLP-1 and found that two sites of *GLP1R*, rs6923761 and rs3765467, could change the insulin promoting effect of GLP-1 [27] [28]. In a clinical study for the same mutation indicated that patients with T2DM carrying the variant allele of rs3765467 showed a more robust response to the treatment by dipeptidyl peptidase inhibitors [29]. Also the rs3765467 and rs10305492 nsSNPs in the *GLP1R* gene showed to exert a critical effect on regulating insulin secretory capacity by β-cell and β-cell mass through leading to the dysfunction and apoptosis of β-cell, GLP1R rs3765467 and rs10305492 might also impair GLP-1 interaction with GLP1R [9] [30]. Another study found that the nsSNP rs367543060 is associated with T2DM susceptibility and, its expression can reduce the receptor affinity and intracellular signaling of GLP-1 [29].

When GLP-1R signaling pathway is disrupted, insulin secretion, insulin sensitivity and glucose effectiveness would be impaired, as in the case of GLP-1R mutation. So far, the N-terminal extracellular domain and three intracellular loops have been reported to be functionally important regions in terms of binding and signal transduction [9]. Some studies have shown that GLP-1 analog increases β-cell mass by both differentiation and neogenesis of precursor cells and by replication of pre-existing β-cells [30].

5. Conclusion

In this study, the 14 nsSNPs were highly affecting the protein function, which is providing the necessary instruction for the synthesis of the insulin hormones needed for glucose catabolism. Concluding that these nsSNPs are associated with T2DM and also affect the treatment of diabetic patients due to the fact that the protein acts as an important drug target. Thus, the findings of the present study provide a guideline for researchers to know the important role of these nsSNPs in the etiology of the complex diseases. In vitro research is needed to confirm these results.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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