



Mutation Annotation, Peptide Designing and Targeting of V 383 Position in GLUT4, an Important Enzyme Involved in Glucose Metabolism

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Abstract:

Diabetes mellitus a well known disorder of current era has several direct and indirect causes for its onset. **Glucose transporter type 4, also known as GLUT4**, is a protein encoded by the *GLUT4* gene which is an insulin-regulated glucose transporter involved in insulin-regulated glucose transport into the cell. This protein is expressed primarily in muscle and fat cells which are the major tissues in the body that respond to insulin. The current work involves the analysis of the GLUT4 protein and its Mutational study. The work involves the targeting of the site 383 Valine in the protein sequence which is the most mutable site in the sequence as inferred from the literature. The site was replaced by all the other possible 19 amino acids using various insilico tools and softwares and the effect of these substitutions on the protein's stability and function have been analyzed. The results concluded that the substitution of Valine with Histidine or Tryptophan at 383 is probably more damaging when compared to the other 17 combinations. Due to the most deleterious effect of these two mutated peptides, the current work aimed at designing the 3D structure of the two followed by selecting a potent drug that can act against these two mutant peptides and can control the diseased condition. Docking studies were performed in order to select the best drug among the study ligands. The study also concluded that among the study chemical saxagliptin gave good docking results with lowest docking energy, indicating the importance of it in treating the condition.

Keywords: *GLUT4, Glucose Transporter, Mutational Analysis, Docking, Saxagliptin.*

Introduction:

Diabetes mellitus type 2

Diabetes mellitus type 2 occurs when the cells in the body do not respond to the insulin produced (insulin resistance) or sometimes combined with inadequate insulin production. It is also referred to as non

Insulin-dependent diabetes mellitus (NIDDM) or "Adult-Onset Diabetes". Some of the common causes known to all are: Living a sedentary lifestyle, obesity, junk foods and heredity. But most of the people are unaware of the actual causes of the condition [1] Approximately 90% of all cases of diabetes worldwide are of this type [2]. Some individuals may control the symptoms of their diabetes mellitus type 2 by following a healthy diet, losing weight, doing plenty of exercise, and monitoring their blood glucose levels. Overweight and obese people have a much higher risk of developing type 2 diabetes compared to those with a healthy body weight. The risk of developing type 2 diabetes is also greater with the onset of age. Men whose testosterone levels are low have been found to have a higher risk of developing type 2 diabetes. Major symptoms of type 2 diabetes include Frequent urination Disproportionate thirst Intense hunger, Unusual weight loss, Increased fatigue, Blurred vision.

Glucose Transporter Type 4 and Its Importance:

Type 2 diabetes has genetic and environmental factors that increase the chances of an individual of being attached. Among the environmental factors are high cholesterol and obesity, and among the genetic factors are genes involved in glucose metabolism or glucose intake signaling such as GLUT4 [3] The GLUT4 gene (aka SLC2A4) is a member of the solute carrier family 2 (facilitated **glucose** transporter) family and it encodes a 509 amino acid long protein called GLUT4 also termed as glucose transporter 4 that functions as an insulin-regulated facilitative **glucose** transporter [4]. There are several glucose transporters such as GLUT1, GLUT2, etc in the cell that help keep the blood glucose level low, but GLUT4 is the only one that responds to insulin. GLUT4 is in the family of solute carriers and is responsible for facilitating the transport of glucose into the cells in response to insulin [5]. For this reason, mutations in GLUT4

have been associated with Type 2 diabetes [6]. The GLUT4 gene has been mapped to the region 13 in the short arm of chromosome 17 (17p13) [7]. Also it has been determined that the gene is 6314 bp long and contains 11 exons.

Materials and Methods:

Sequence Retrieval: The sequence of the protein GLUT4 has been retrieved from the gene cards database. The data base is specific for the human genes and proteins. The sequence is further used for the analysis.

SMART Domain Analysis:

SMART is a tool used for the prediction of the Domains within a Protein Sequence. It is based upon the similarities within the selected proteins and their conservation upon which the domains are grouped.

Selection of the target site for the targeting of the protein:

Based upon the literature and the data base Gene cards it was found that the position 383 within the GLUT4 protein is the one that is most mutable and has effect on the functioning of the protein.

Effect of mutations on the Functionality of the protein:

Polyphen is the tool that would detect the effect of the user entered substitutions on the functionality of the protein n sequence. It would detect the complexity of a specific mutation on the protein's function. The tool has been employed to select those substitutions that have deleterious effect on the protein function and have a role in the disease occurrence.

I Mutant to detect the effect of mutations on the stability:

I mutant tool was used to detect the effect of all the other 19 combinations of amino acids at the 383 position on the stability of the protein. The tool would take the protein sequence and the target site as input long with he changed amino acid . The out put would be the effect of the specified change on the stability of the protein.

Construction of the mutant and wild peptides in the ARGUS LAB:

Argus lab is software used for the designing of the peptides or proteins three dimensional structures. The native and the mutant peptides were designed in the

Argus Lab and the energy's of the so built peptides were calculated.

Drug designing:

Based upon the peptides that have maximum deleterious effect the receptors were selected and were used for the targeting in the process of drug designing.

Compound Library Generation:

Pubchem is a chemical compound data base that is used for the collection of the chemicals specific or related to the search term, a disease or a receptor. Based upon the properties of the chemicals the best compounds can be selected.

Screening of the Ligands:

The chemical compounds from the data based have to be selected for the docking based upon certain criteria. There are three screenings which must be performed in series, **Primary screening** for the **Drug likeliness** based on the **Lipinski rule of five**. Once the compounds are selected for the drug likeliness they are subjected for the **secondary screening** based on the Toxicity studies using the tool **Mobyle ADME Tox**. In this the toxicity of the chemicals is tested. This is followed by the **Tertiary screening** for the Ambiguity check. The ambiguity check is performed using **Dundee Prodrgr server**.

Once the chemicals are selected after all the three screening strategies they are used for the docking with the selected receptor.

Docking of the compounds with the selected Receptors:

After screening all the chemicals for the three strategies they are subjected for the docking with the selected receptors in the software HEX. Hex performs flexible docking were there is no target site specified, rather it would be an automated mode for the binding site selection. Hex is also used for the calculation of the docking energy after the programme which can enable the selection of the best ligand.

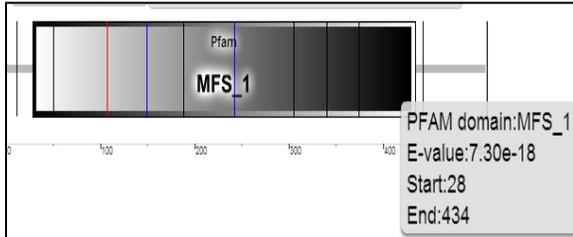
Results and Discussion:

The sequence of the GLUT4 protein has been retrieved from the database and is used for the analysis as hereunder.

SMART Domain Identification:

Using the SMART online tool the domain identification was performed for the GLUT4 protein. The result is shown below

Figure 1: Showing the Domain for the protein as shown by SMART



From the above figure 1 it can be inferred that the protein has only one domain starting from 28 and ending at 434 on the protein sequence. This region

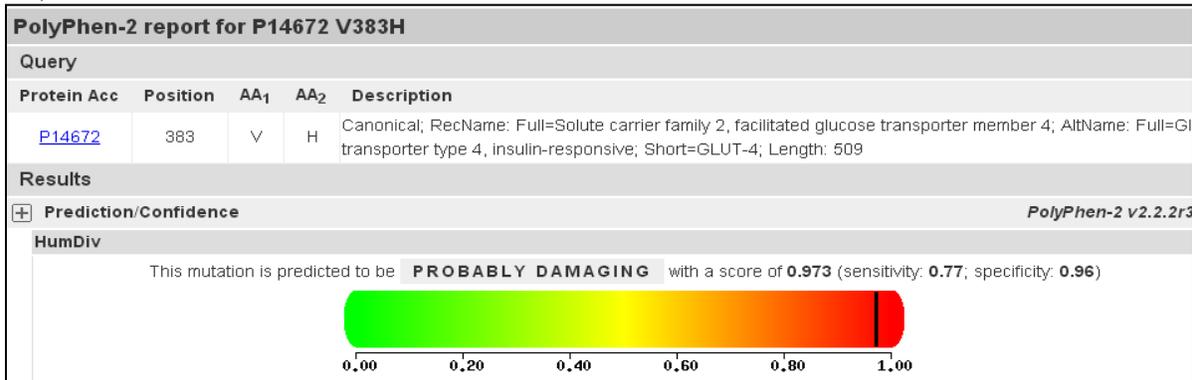
also includes the study site 383 Valine, supporting the importance of the site.

Mutational Analysis at 383 using PolyPhen:

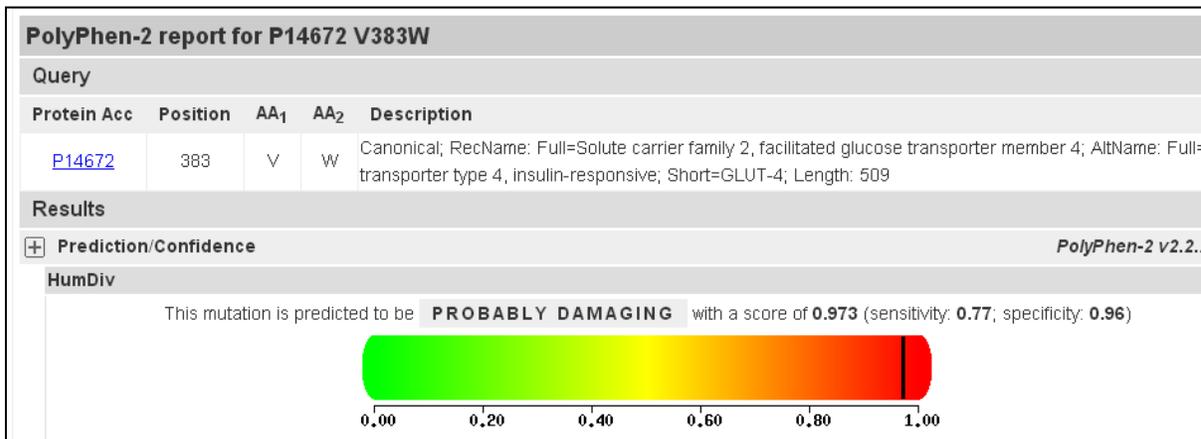
Polyphen would detect the effect of any substitution mutation on the functionality of the protein, it would indicate whether the specific substitution is benign, deleterious or Probably damaging. According to the results of Poly phen it can be concluded that out of the 19 possible combinations at the 383 position 7 substitutions namely, V383M, V383D, V383E, V383Q, V383W, V383H and V383Y. Out of the above mentioned 7 combinations the substitutions that have maximum deleterious effect are V383H and V383W as shown in the figure:

Fig 2: Polyphen results for V383H and V383W

A) V383H Substitution:



B) V383W Substitution:



From the above two results it can be inferred that the substitution of the amino acid Valine at the position 383 to either W or H would cause a probably damaging effect in the protein thus affecting the function in turn. Hence 424 these mutations can be of much importance in targeting the protein with any drug to treat the adverse conditions.

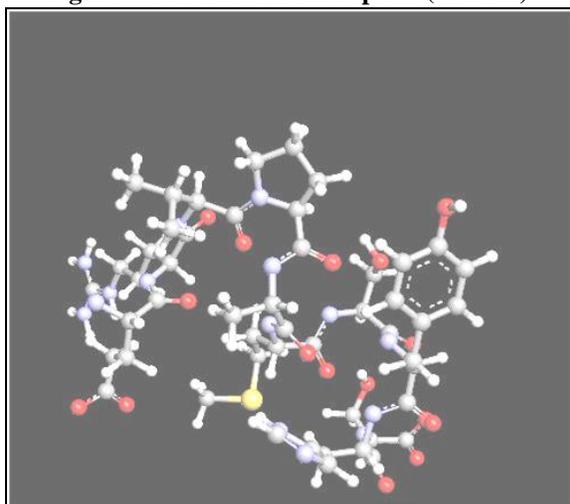
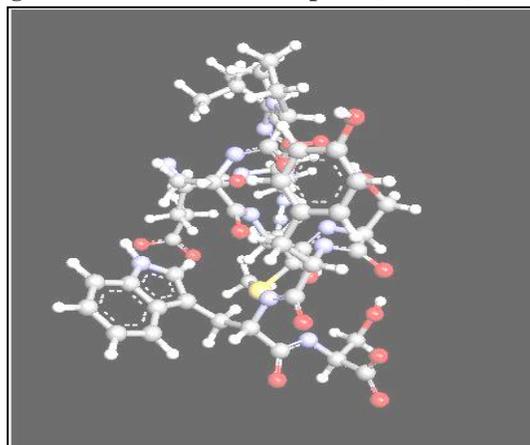
Figure 3: I Mutant to detect the effect of substitutions on the stability of the protein

Position	WT	NEW	Stability	RI	pH	T
383	V	L	Decrease	6	7.0	25
383	V	I	Decrease	9	7.0	25
383	V	M	Decrease	9	7.0	25
383	V	F	Decrease	9	7.0	25
383	V	W	Decrease	9	7.0	25
383	V	Y	Decrease	9	7.0	25
383	V	G	Decrease	10	7.0	25
383	V	A	Decrease	10	7.0	25
383	V	P	Decrease	8	7.0	25
383	V	S	Decrease	10	7.0	25
383	V	T	Decrease	10	7.0	25
383	V	C	Decrease	9	7.0	25
383	V	H	Decrease	10	7.0	25
383	V	R	Decrease	9	7.0	25
383	V	K	Decrease	9	7.0	25
383	V	Q	Decrease	10	7.0	25
383	V	E	Decrease	8	7.0	25
383	V	N	Decrease	9	7.0	25
383	V	D	Decrease	9	7.0	25

Inference: As shown in the above result all the 19 possible substitutions at the 383 position are responsible for a decrease in the stability of the protein. This can conclude that the 383 position is more important according to the stability and functionality and thus an important site for the study.

Construction of the peptides in Argus Lab:

After the above analysis two peptides were designed which are the mutant V383H (R1) and V383W (R2) respectively as follows.

Figure 4: Mutant V383H Peptide (domain)**Figure 5: Mutant V383W Peptide (domain)**

Ligand analysis:

In order to detect the best Ligands/ Drugs that can be used for targeting the mutant peptide thereby inhibiting the adverse conditions following method has been employed.

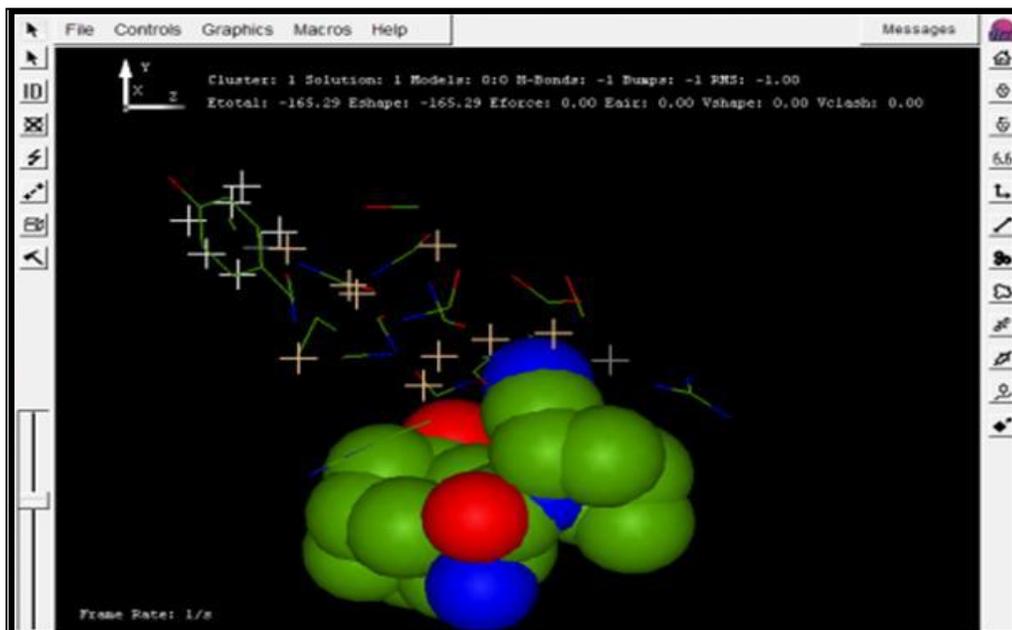
The compounds were collected from the Pubchem database and were screened for the Drug likeliness based on lipinsky's rule, Secondary screening was performed to check the toxicity using MOBYLE ADME TOX and final selected ligands were subjected for tertiary screening for ambiguity check using Dundee prodrgr server. After all the screening tests three forms of saxagliptin –saxagliptin(CID 66576989), saxagliptin (CID 44144606) saxagliptin, (CID 11243969) were selected as the best Liganands which must be used for docking with the designed peptides. The 2 most probably damaging form of GLUT-4 were docked with above mentioned 3 forms of Saxagliptin using HEX 5.1 and the following energy values were observed.

Table 1: Docking results of the three peptides with three ligands

S.No	LIGAND-RECEPTOR PAIR	E-TOTAL AFTER DOCKING
1	R1-L1	-165.29
2	R1-L2	-147.68
3	R1-L3	-165.97
4	R2-L1	-157.29
5	R2-L2	-152.69
6	R2-L3	-157.25

R1, R2, = mutant 1(V-H) and mutant 2 (V-W) respectively and L1, l2 and L3= Ligand 1, Ligand 2 and Ligand 3 respectively.

Figure 6: Docking of the Peptide R1 (V-H) with L1 Lignad Saxagliptin (CID 66576989) using HEX



Inference: From the above figure the docking energy for the R1 peptide with the selected ligand is -165.29.

From the *E total values* obtained above it can be concluded that the docking pair 1 and 3 are best, ie the mutant protein V383H when docked to the ligand (CID 66576989) gave the best E total (lowest) *Saxagliptin (CID 66576989) thus can be considered as the best ligand for inhibiting the adverse conditions of the mutation.* Lower the E total value higher would be the stability of the complex and lower are the side effects.

Conclusion:

From the above work it can be concluded that the (V->H) type of mutations at position 383 in GLUT-4 can be targeted with the ligand saxagliptin(CID 66576989). Saxagliptin being an inhibitor of the enzyme DPP-4, which breaks down incretin hormones that are responsible for the beneficial actions of Saxagliptin, including increasing insulin production in response to meals and decreasing the amount of glucose (sugar) that the liver produces. Because incretin hormones are more active in response to higher blood sugar levels (and are less active in response to low blood sugar), the risk of dangerously low blood sugar (hypoglycemia) is low with Saxagliptin. Thus saxagliptin could prove to be

of great help to the patients of Diabetes Mellitus Type-2.

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