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# *In-silico* Analysis of Adiponectin Protein in Diabetic Retinopathy.

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**ABSTRACT**

Diabetes mellitus is a disease with significant negative implications for the patient. Diabetic retinopathy is a complication of diabetes and a leading cause of blindness. It occurs when diabetes damages the tiny blood vessels inside the retina, the light-sensitive tissue at the back of the eye. The two stages of diabetic retinopathy are nonproliferative retinopathy where microaneurysms occur and proliferative retinopathy where many blood vessels is blocked, depriving several areas of the retina with their blood supply. Several recent studies have provided evidence that good diabetes control is important to prevent diabetic retinopathy. However, some groups of patients develop diabetic retinopathy despite good control and others escape retinopathy despite poor control. This suggests the role of genetic factors in susceptibility to retinopathy. Adiponectin (ADIPOQ) located at 3q27 is one of the candidate genes contributing to the development of diabetic retinopathy. An in silico technique was initiated to characterize the properties and structure of the protein. The 3D structure was modelled using Swiss model workspace and the structure was validated.

**Keywords:** Adiponectin, Diabetic retinopathy, Swiss model workspace

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## INTRODUCTION

The role of genetic factors in diabetic complications has been known for many years. The high prevalence of diabetes, being responsible for the increase for blindness, renal replacement therapy, cardiovascular interventions and significant health problems. Large clinical trials have demonstrated that normalization of glycemia can greatly reduce the incidence of diabetic complications.

Diabetic retinopathy (DR), a microvascular complication of diabetes in the retina, is one of the leading causes of adult blindness worldwide. Among the leading causes of adult blindness, DR comes next to cataract, glaucoma and age related macular degeneration (AMD) [1]. Diabetic retinopathy can cause vision loss in different ways: Macular edema, proliferative retinopathy and vitreous hemorrhage .The presence of diabetic retinopathy is evidenced by the appearance of retinal microvascular lesions. Early changes include microaneurysms, hemorrhages, hard exudates, cotton wool spots, intraretinal microvascular abnormalities, and venous beading and characterize nonproliferative diabetic retinopathy (NPDR). The more severe state of proliferative diabetic retinopathy (PDR) is marked by the formation of abnormal fragile new blood vessels that are prone to hemorrhage. [2]. Nonproliferative stage develops first and proliferative is the advanced and also severe form of this disease. The people associated with the type 1 and 2 diabetes are at risk for this condition.

People with untreated diabetes are 25 times more at risk for blindness than the general population [3]. The longer a person has had diabetes, the higher the risk of developing diabetic retinopathy. Good glycemic control arrests the development and progression of DR and decreases the visual loss. Significant technological advances have taken place to improve the diagnostic accuracy of diabetic retinopathy.

Diabetic retinopathy (DR) is associated with both environmental and genetic factors. Several candidate genes have been so far implicated in the pathogenesis of proliferative diabetic retinopathy (PDR) in subjects with type 2 diabetes. The progression of diabetic retinopathy (DR) to PDR is a serious complication of diabetes [4]. Changes in dietary habits and lifestyles associated with rapid economic growth have dramatically increased the incidence of diabetes, obesity, and related vascular complications [5, 6]. Both type 1 and type 2 diabetes are associated with hyperglycaemia, oxidant stress, inflammation and significantly increased risk for macrovascular complications and microvascular complications [5, 6].

The advancement in the diagnosis and treatment of diabetic retinopathy is increasing on the other side it is the leading cause of blindness in developed and developing countries.

It is necessary to develop the proper means to prevent, identify and treat retinopathy in its early stage than to wait for its consequences. For the past few years, many steps have been taken to identify the genes involved in diabetic retinopathy.

Adiponectin ( AdipoQ ) is a protein encoded by the gene ADIPOQ. This gene is expressed in adipose tissue exclusively. It encodes a protein with similarity to collagens X and VIII and complement factor C1q. The encoded protein circulates in the plasma and is involved with metabolic and hormonal processes. Mutations in this gene are associated with adiponectin deficiency. It is a long polypeptide which has 244 amino acids. This protein has four regions, first is a short a short signal sequence that targets the hormone for secretion outside the cell , secondly a short region that varies between species, the third is a 65-amino acid region with similarity to collagenous proteins; the last is a globular domain. Overall this gene shows similarity to the complement 1Q factors (C1Q). However, when the 3-dimensional structure of the globular region was determined, a striking similarity to TNFα was observed, and despite unrelated protein sequences. It is also involved in regulating glucose levels as well as fatty acid breakdown. Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism. Adiponectin is exclusively secreted from adipose tissue (also from the placenta in pregnancy) into the bloodstream and is very abundant in plasma relative to many hormones.. The hormone plays a role in the suppression of the metabolic derangements that may result in type 2 diabetes, obesity, atherosclerosis, non-alcoholic fatty liver disease (NAFLD) and an independent risk factor for metabolic syndrome. High-molecular-weight adiponectin was further found to be associated with a lower risk of diabetes with similar magnitude of association as total adiponectin. A low level of adiponectin is an independent risk factor for developing: Metabolic syndrome , Diabetes mellitus. Adiponectin (AdipoQ) plays an important role in the pathogenesis of diabetes mellitus and is

considered as an important candidate gene for type 2 diabetes mellitus (T2DM). So far, there have been many studies to investigate the association between the adiponectin polymorphisms and T2DM risk [7].

## MATERIALS AND METHODS

### Primary structure prediction

The FASTA sequence of adiponectin protein was retrived from Genbank database hosted by NCBI (http://www.ncbi.nlm.nih.gov). The primary structure prediction i.e., the physical and chemical parameters for a given protein sequence was computed with the Expasy ProtParam server (http://expasy.org/cgi-bin/protpraram). The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient [8], instability index [9], aliphatic index [10] and grand average of hydropathicity (GRAVY) [11].

### Secondary structure prediction

The Garnier-Osguthorpe-Robson (GOR) Method and Self - Optimized Prediction Method with Alignment (SOPMA) tools predicts the secondary structures of the protein using the FASTA sequence of adiponectin and describes the various secondary structures like alpha-helix, beta-sheet and coil.

### Protein functional sites

The signatures and the motif regions present in the sequence were predicted with the help of the tools InterProScan, FingerPRINTScan.

### Homology modeling and validation

Homology modeling was performed with fully automated protein structure modeling server, Swiss model and the modelled structure was validated using SAVES tool. Then the structure was visualized and analyzed by using Rasmol.

## RESULTS AND DISCUSSION

The sequence was subjected to similarity search with the help of BLAST (Basic Local Alignment Search Tool) and the result produced indicated maximum similarity to adiponectin isoform 1 (gorilla gorilla gorilla) (Table 1).

**Table 1: BLAST result of the ADIPOQ Protein**

| Accession | Description | Score | E value |
|---|---|---|---|
| XP 004038221.1 | Adiponectin isoform 1(gorilla gorilla gorilla) | 498 | 9e-177 |
| NP 001268483.1 | Adiponectin,C1Q and collagen domain containing precursor (Mesocricetus auratus) | 380 | 3e-130 |

### Primary structure prediction

The primary structures of adiponectin protein were predicted using Expasy's ProtParam server using the sequence and the results obtained are discussed here. The results showed that adiponectin had 244 amino acid residues and the calculated molecular weight was found to be 26413.6. Glycine was found to be highly concentrated with (16 %) and the least was tryptophan and cysteine (0.8 %). The total number of positively charged residues (Arg + Lys) was 19 and the total number of negatively charged residues (Asp + Glu) was 27. The calculated isoelectric point was computed to be 5.42 the computed value is less than 7 which indicates that the protein is acidic. The aliphatic index (72.70) indicates that this protein is stable for a wide range of

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temperature range because it if found high and the instability index (24.84) classifies the protein as a stable one. The Grand Average Hydropathicity (GRAVY) value is low -0.410, indicating better interaction of the protein with water.

**Secondary structure prediction**

The secondary structure is composed of alpha helix, beta strands and random coil and the secondary structures were predicted using GOR IV and SOPMA. Table 2 presents the comparative analysis of GOR IV and SOPMA from which it is clear that random coil is predominantly present when the structure was predicted both by SOPMA and GOR, followed by extended strand and alpha helix. The secondary structure prediction was done and random coil was found to be frequent followed by extended strand and alpha helix was found to be least frequent as shown in Figure 1.

**Figure 1: Secondary structure predictions by GOR**

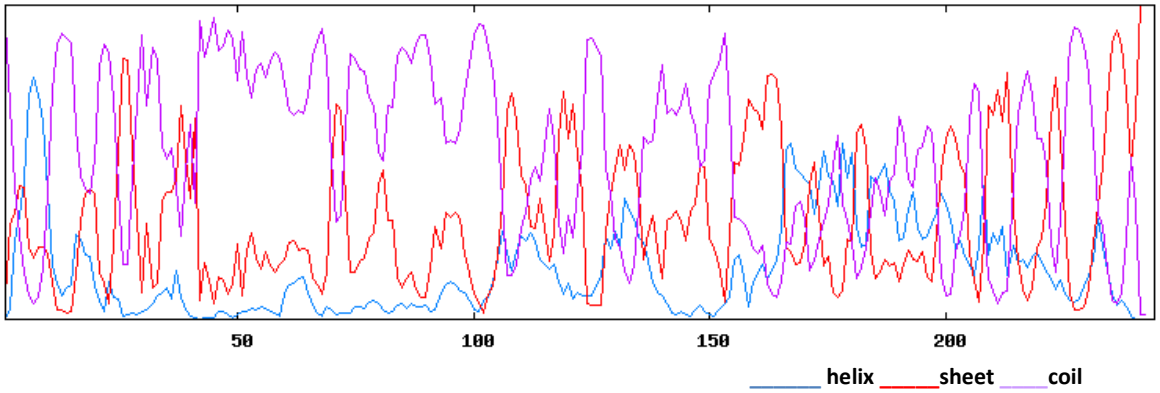
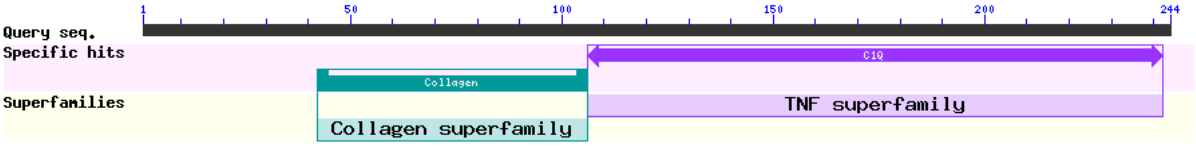



**Table 2: Secondary structures of ADIPONECTIN by SOPMA and GOR**

| Secondary Structures | SOPMA | GOR |
|---|---|---|
| Alpha helix | 6.97% | 6.97% |
| $3_{10}$ helix | 0.00% | 0.00% |
| Pi helix | 0.00% | 0.00% |
| Beta bridge | 0.00% | 0.00% |
| Extended strand | 24.18% | 29.10% |
| Beta turn | 6.15% | 0.00% |
| Bend region | 0.00% | 0.00% |
| Random coil | 62.70% | 63.93% |
| Ambigous states | 0.00% | 0.00% |
| Other states | 0.00% | 0.00% |
| Sequence length | 244 | 244 |

The domain search was done by Conserved domain search on the Blast site and it showed the presence of single domain –Collagen and TNF Superfamily (Figure 2).

**Figure 2 : Conserved domain in adiponectin protein**




The fingerprint scan of the sequence showed ten fingerprints having the motifs for each fingerprint in the sequence (Table 3).

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**Figure 3: Three dimensional structure of Adiponectin protein**

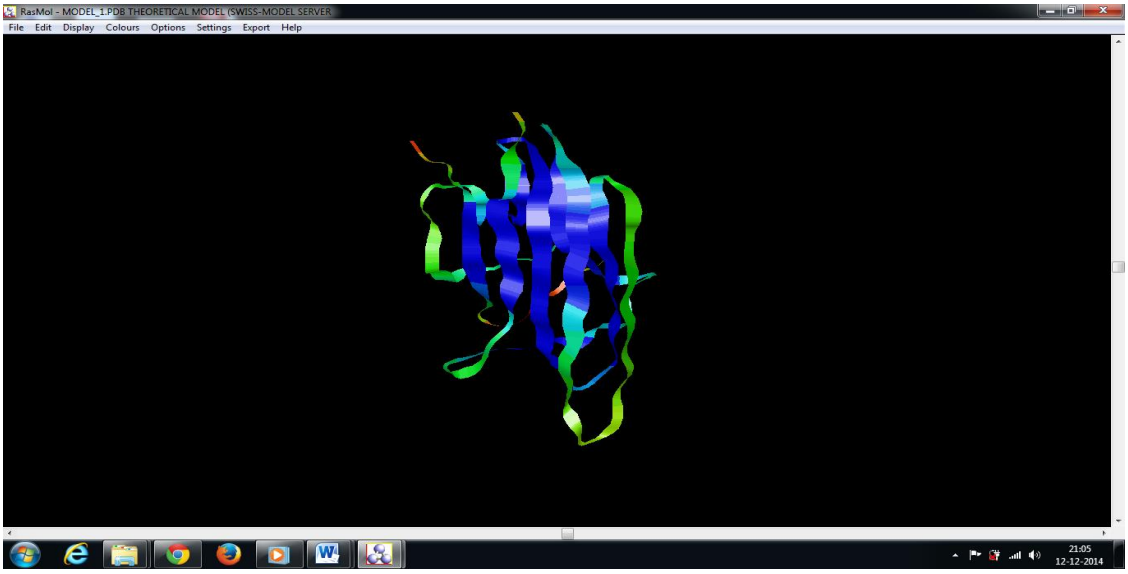


**Figure 4: Ramachandran plot**

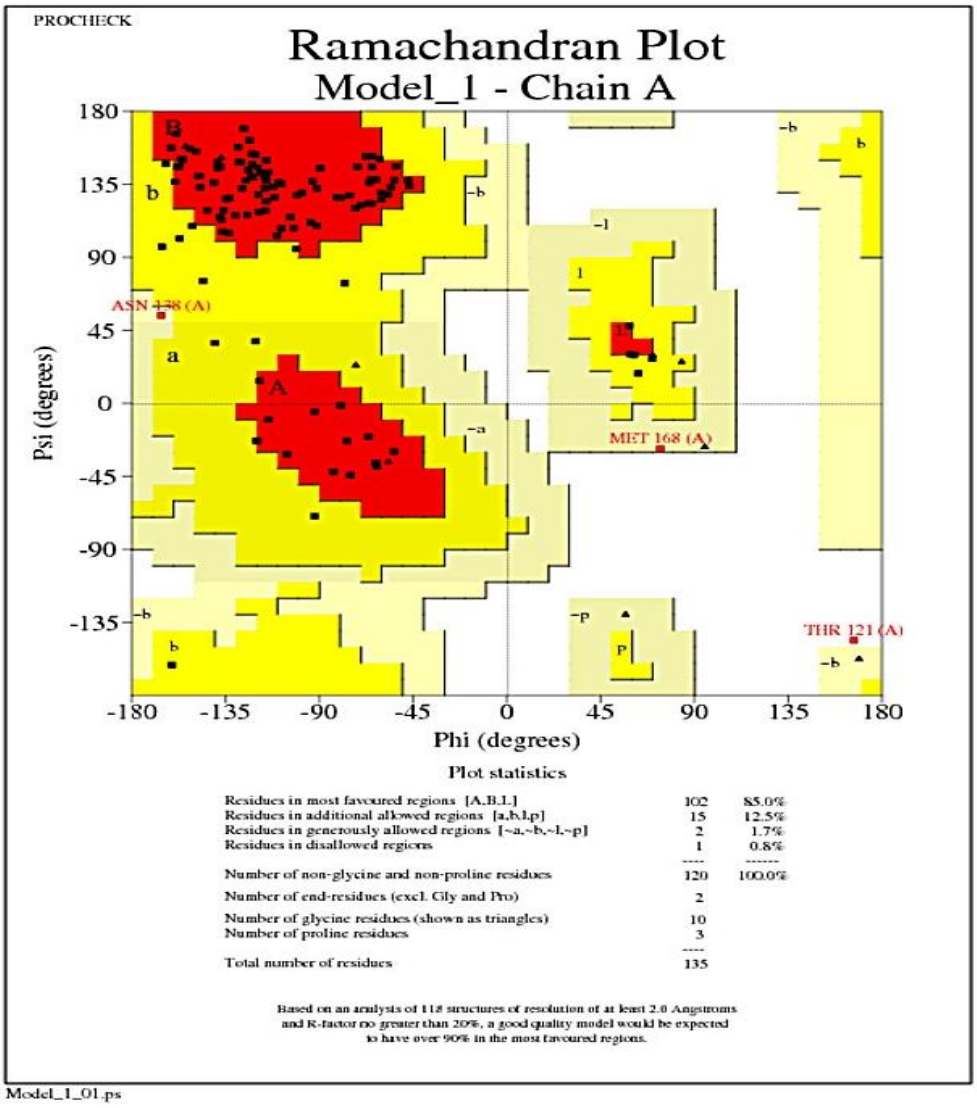

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**Table 3: Results of FingerPRINTScan**

| FingerPRINT | No. Of Motifs |
|---|---|
| COMPLEMNTC1Q | 4 |
| GLUTELIN | 2 |
| MYOSINHEAVY | 2 |
| AQUAPORIN11 | 2 |
| FLGPRINGFLGI | 2 |
| P67PHOX | 2 |
| GLHYDRLASE6 | 2 |
| INTRLEUKN1R2 | 2 |
| TAUPROTEIN | 2 |
| ANPHYLATOXNR | 2 |

The tertiary structure was modelled by Swiss model workspace (Figure 3) and the modelled structure showed 119 H bonds, 9 helices, 9 turns and absence of beta strands. The modelled structure was validated by SAVES tool and Ramachandran plot was plotted (Figure 4).

The analysis of Adiponectin protein showed sequence similarity mostly to adiponectin isoform 1(gorilla gorilla gorilla). The two domains were identified by conserved domain search. The modelled structure revealed that 85 % as the most favoured regions. Further research in this protein helps in preventing diabetic complications.

## CONCLUSION

The sequence and structural analysis of adiponectin protein was performed by various tools and softwares in this study. It could be concluded that further characterization of human adiponectin (ADIPOQ) gene is novel and will play an important role connected to diabetes mellitus.

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