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A Novel Methodology for Structural, Functional and Toxicological Analysis of Mutant Angiogenin Protein in Human

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Abstract

Introduction: Angiogenin is a protein of 14.1 kDa encoded by ANG gene and belongs to a superfamily of pancreatic ribonuclease A. Angiogenin is an effective stimulator of new blood vessels formation. It plays a vital role in the pathological as well as physiological mechanisms by regulating cell proliferation, differentiation and invasion. Mutation in ANG leads to a disease called amyotrophic lateral sclerosis 9. Amyotrophic lateral sclerosis 9 is a motor neuron disease that causes the decease of neurons, which controls the voluntary muscles of body.

Material and Methods: The mutations F12S, P20S, Q36L, Y38H, K41E, D46G, S52N, R55K, C63W, K64I, I70V, K84E, P136L, V137I and H138R were selected for this study to investigate the single amino acid substitution effects on structure, function, stability and pathological impression on the protein.

Results: The study revealed that the mutations Q36L, C63W, K64I, P136L, V137R and H138R have strong functional, structural and conformational effects compared to F12S, P20S, Y38H, K41E, S52N, R55K and K84E suggesting a high rate of disorder tendency.

Conclusion: The *in silico* analysis of angiogenin identified several point mutations which may cause ALS9. The results of this study may be useful in planning and conducting clinical work on the ANG gene to find out, which mutation is responsible for the major cause of amyotrophic lateral sclerosis 9.

Keywords: Angiogenin; Amyotrophic lateral sclerosis 9; Cell proliferation; Disorder tendency

Introduction

Angiogenin (ANG) protein is a member of ribonuclease family induced by other angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (aFGF), acid fibroblast growth factor (bFGF) and epidermal growth factor (EGF) [1]. ANG plays important role in number of pathogenic conditions including cancer and neurodegenerative diseases by modulating cell growth and survival properties [2]. In contrast to being up regulated in cancer, ANG is down regulated in neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson and Alzheimer disease [3-5]. Role of ANG in neurodegenerative diseases have been documented due to function lose mutations found in patients [6,7]. ANG also facilitates the production of tiRNA (tRNA-derived stress induced RNA) [8,9] which suppress overall protein translation. tiRNA is important in stress mechanisms under adverse environments utilized by cell [10]. ANG and RNASE4 transcription is regulated by RNA polymerase III and a CCCTC binding factor (CTCF)-depending intragenic chromatin loop [11]. In malignancies, ANG is important proangiogenic and its higher expression is associated with a non-nuclear Maspin expression [12].

Studies have shown the critical role of ANG in tumor microenvironment for angiogenesis [13]. It was reported that ANG targets p53 and B-cell lymphoma 2 to accelerate cancer development through anti-apoptotic effects [14]. Sheng et al. explained a mechanism by which ANG stimulates rRNA transcription through an epigenetic activation of rDNA promotor [15]. Under androgen stimulation, ANG undergoes nuclear translocation in androgen-dependent prostate cancer cells, where it binds to rDNA promoter and stimulates rRNA transcription [16]. Ribosomal protein is mediated by ANG [2]. The P13k/Akt/mTOR signaling pathways is thought to be a central mediator in signal transduction pathways, which is frequently activated in different cancer types [17]. It was reported that ANG activates nitric oxide synthase (NOS) by interacting with cellular nucleus. Similarly NOS activity is stopped by blocking the P13k/Akt kinase signaling transduction cascade showing the importance of this pathway and ANG for NOS activity [18]. The experiments suggest that cross talk between ANG and protein kinase B/Akt signaling pathways could be essential for ANG-induced angiogenesis *in vitro* and *in vivo* [2,4].

ANG protein plays vital role in different pathways of human. All the reported mutations in this gene were selected for this study and using different bioinformatics and computational biology approaches analyzed their structure and their impacts and effects on its structure, stability and functionality.

There was a case study conducted for the investigation of MEN1 protein family but on low scale [19]. Therefore, until there is no such type of case study conducted on ANG. This case study will reveal facts about disease causing mutations and describing the information about disease severity with respect to mutation in ANG. This study will be helpful to conduct research on angiogenin at clinical scale to investigate which mutation leads to severity of disease. This case study also may

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helpful for mutation targeting in drug designing and helping in finding the mutation with neutral effect.

Materials and Methods

Interaction and appearance of ANG genes

GeneMANIA is a user friendly online tool for the prediction of gene function. It classifies the functionally similar genes to a query gene list by using accessible proteomics and genomics data.

Mutational screening

Already reported mutation in ANG i.e. F12S, P20S, Q36L, Y38H, K41E, D46G, S52N, R55K, C63W, K64I, I70V, K84E, P136L, V137I and H138R were selected for this study. The selected mutation sequences were retrieved from UNIPROT [20]. In "Pathology and Biotech" section of UNIPROT database, data was available, showing that either the mutation is indel or substitution and also describes mutation involvement in disease.

Structural and functional impact prediction of SNPs

Effect of single amino acid mutation was studied by PredictSNP server which is used to predict either mutation is deleterious or neutral with percentage of expected accuracy [21]. The surface accessibility of selected mutations was examined by NetSurfp. NetSurfp not only predicts the accessible surface area and secondary structure of amino acids but also check the reliability for each prediction, in the form of a Z-score. The Z-score is related to the surface prediction and not the secondary structure. I-Mutant2.0 [22] is a support vector machine based web server. It was used for automatic prediction of protein stability based on single site mutation. It can predict the protein stability changes (more stability or de-stability) corresponding all possible mutations of a residue. I-Mutant2.0 gave DDG value (free energy change value). PROVEAN [23] (Protein Variation Effect Analyzer) was used to predict whether the single amino acid substitution, deletion or insertion affect the function of protein. It is very useful for analyzing protein variants and for identifying functionally important nonsynonymous mutations.

PolyPhen is a web server used for the prediction of probable impression of amino acid substitutions on the function and stability of human proteins via evolutionary comparisons. It provides functional annotations of single-nucleotide polymorphisms (SNPs) on the basis of gene transcripts maps coding, damaging effects, abstract protein sequences and attributes of structures [24].

Mutation3D is an online tool used for functional analysis and visualization of amino acid substitutions on three-dimensional structure of protein. This tool is intended to be utilized for the identification of groups of amino acid substitutions appearing from somatic cancer mutations across several patients in direction to determine fuel downstream suggestions and functional hotspots [25].

Protein 3d modeling and detection of hydrogen bonding and clashes

MODELLER was used to predict the 3D protein structure by using homology modeling. The end user offers a sequence alignment to be predicted with identified correlated structures, it automatically computes a model comprising all non-hydrogen atoms and implements homology protein structure modeling by consummation of spatial restraints [26]. Chimera was used to scan the 3D (three-dimensional) structure of specific protein and hence, changed the original amino acid with the mutated one to grasp the impact of the mutation. The outcome was graphic model depicting the mutation [27]. It is also used for localization of the mutated region and describing mutation in coil region are helix region and show hydrogen bonds, clashes and contacts of the mutant residue.

Disorder tendency analysis

PONDR is online available software used to predict disordered region in protein. Protein disorders were identified by individual predictor tool available at PONDR. PONDR-VLXT, PONDR-VL3 and PONDR-VSL2 were used for long as well as short protein disorder region of ANG [28]. For the confirmation of selected mutations in conserved region of ANG, Conserved Domain analysis of all selected mutations was carried out using CDD [29].

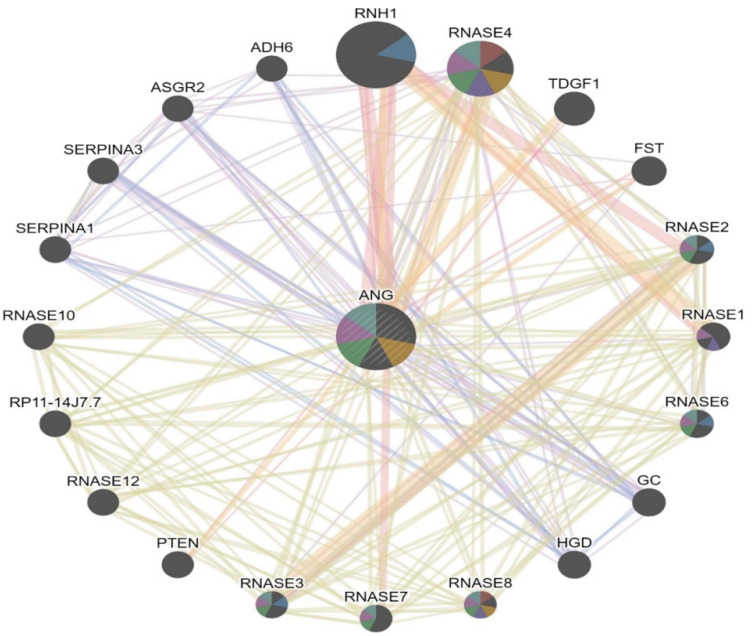
Results and Discussion

Interaction and appearance of ANG genes

GENEMANIA analysis revealed that ANG has active role in endoribonuclease activity, RNA catabolic process, endonuclease activity, Nucleic acid phosphodiester bond hydrolysis and ribonuclease activity. ANG gene possess physical interactions with RNH1 (ribonuclease/angiogenin inhibitor 1), RNASE2 (ribonuclease A family member 2) and RNASE7 (ribonuclease A family member 7) with 67.64% confidence score. ANG co-expressed and co-localized with ADH6 (alcohol dehydrogenase 6), ASGR2 (asialoglycoprotein receptor 2), (SERPINA1) serpin family A member 1, SERPINA3 (serpin family A member 3), GC (GC vitamin D binding protein) and HGD (homogentisate 1, 2-dioxygenase). RNASE-10 (ribonuclease A family member 10), RNASE12 (ribonuclease A family member 12), PTEN (phosphatase and tensin homolog), RNASE3 (ribonuclease A family member 3), RNASE7 (ribonuclease A family member 7) and RNASE8 (ribonuclease A family member 8) sharing common protein domains are shown in Figure 1.

Structural impact prediction of SNPs

Netsurp analysis revealed that the surface accessibility of mutated ANG was different as compared to wild-type ANG. Relative Surface Accessibility index (RSA) of a protein from 0-1 is a measure of residue solvent exposure. Higher RSA value depicts higher surface accessibility of protein. Comparison between RSA wild-type and RSA mutant is shown in Figures 2 and 3. Surface Accessibilities of F12S, Y38H, K41E, S52N, K64I, I70V, P136L, V137I and H138R were decreasing while P20S, D46G, R55K, C63W and K84E were increasing. PredictSNP server revealed that F12S, Q36L, C63W, K64I, P136L, V137I and H138R were deleterious with>70% expected accuracy while P20S, Y38H, K41E, D46G, S52N, R55K, I70V and K84E were neutral shown in Figure 4. I-Mutant protein stability prediction server provided the Free Energy change value (DDG) varying from positive to negative numbers. Positive values show that stability of protein is increasing whereas negative values confirm the decreasing stability of protein. DDG value of Q36L and H138R were positive while DDG values of all the other mutations were negative representing the decreased stability of protein with single amino acid substitution. Similarly, the results obtained from PROVEAN suggested that F12S, P20S, Q36L, Y38H, C63W, K64I, P136L and H138R were deleterious with -4.003, -2.990, -6.500, -3.179, -10.690, -7.995 and -7.449 (score higher than threshold -2.5) while K41E, D46G, S52N, R55K, I70V. K84E and V137I were found neutral with scores -1.003, -2.491, -0.957, 0.220, -0.476, -1.103 and -0.986 respectively (score less than threshold -2.5).



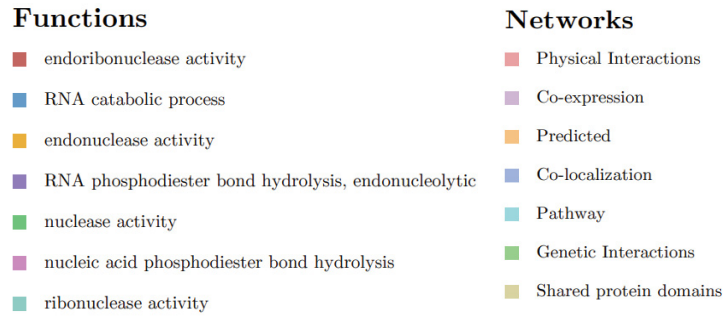


Figure 1: The interaction of ANG gene with other gene families and elaborating its major function involvement.

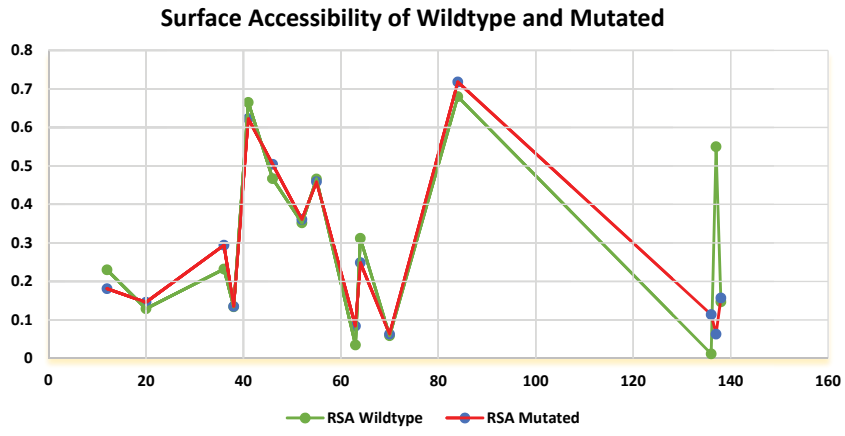


Figure 2: The comparison between RSA values of both wild-type and mutated.

Polyphen analysis revealed that F12S, P20S, Q36L, C63W, K64I, P136L, V137I and H138R have probably damaging effect on protein while Y38H, K41E, D46G, S52N, R55K, I70V and K84E have low damaging effect and considered as Benign shown in Table 1. Score ranges from 0-1, if score is higher than 0.5 then mutation has probably damaging effect and lower score revealed that mutation is Benign.

Mutation 3D analysis revealed that mutated amino acid was present either in cluster region or not. Red color sphere represents that mutation in cluster region while blue color sphere represents presence of mutated amino acid in covered region of angiogenin. Mutation in cluster region is more sensitive as compared to covered region. The results revealed that mutations i.e. Q36L, Y38H, I70V, P136L, V137I and H138R were in cluster region while remaining mutations were present in covered

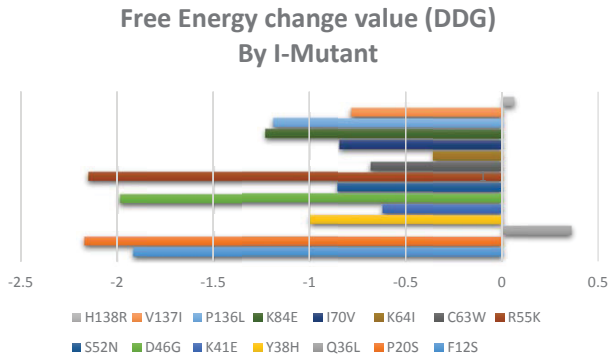


Figure 3: The Free energy change value DDG by I-Mutant Server. DDG value of Q36L and H138R showing positive value and remaining showing less than zero values that representing the stability of mutated protein decreasing with single amino acid substitution.

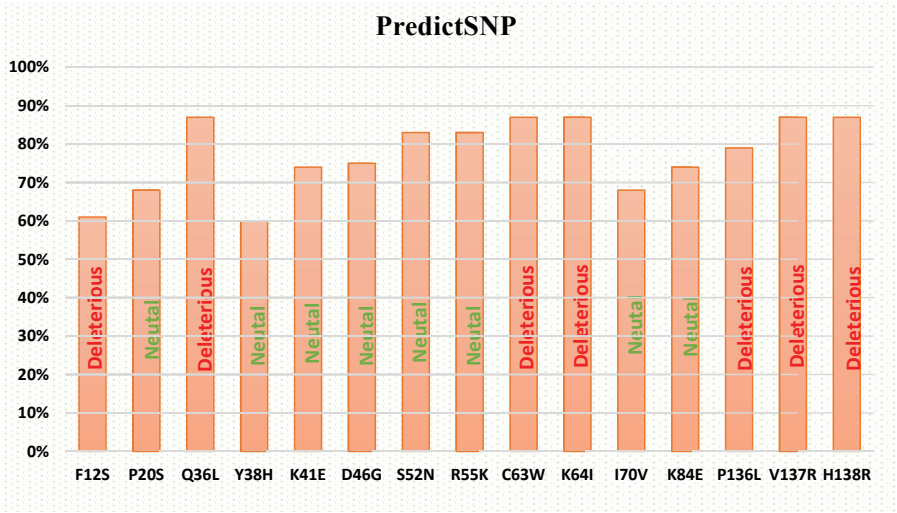


Figure 4: Single amino acid substitution is either deleterious or neutral with expected accuracy in percentage.

Mutant	Effect	Score
F12S	Probably damaging	0.987
P20S	Probably damaging	0.583
Q36L	Probably damaging	0.99
Y38H	BENIGN	0.303
K41E	BENIGN	0.037
D46G	BENIGN	0.005
S52N	BENIGN	0.011
R55K	BENIGN	0.002
C63W	Probably damaging	1
K64I	Probably damaging	1
I70V	BENIGN	0.246
K84E	BENIGN	0
P136L	Probably damaging	1
V137I	Probably damaging	0.993
H138R	Probably damaging	1

Table 1: showing the SNPs impact on protein function. Score rages from 0-1, if score is higher than 0.5 then mutation has damaging effect and lower the score reveal that mutation is Benign.

Mutant	QMEAN	Z-Score	Residues in most favored regions	Disallowed region	RMSD
F12S	0.497	-2.686	90.30%	0.00%	0.2130
P20S	0.493	-2.723	92.80%	0.80%	0.2140
Q36L	0.517	-2.467	90.30%	0.80%	0.2030
Y38H	0.496	-2.69	93.50%	0.80%	0.2100
K41E	0.514	-2.494	90.30%	0.00%	0.2110
D46G	0.519	-2.441	90.20%	0.80%	0.2030
S52N	0.52	-2.436	91.10%	0.00%	0.2000
R55K	0.501	-2.634	90.30%	0.00%	0.2080
C63W	0.475	-2.914	91.90%	0.00%	0.2060
K64I	0.554	-2.069	91.10%	0.00%	0.2010
I70V	0.529	-2.339	91.10%	0.00%	0.2030
K84E	0.532	-2.309	90.30%	0.80%	0.2040
P136L	0.483	-2.83	91.20%	0.80%	0.2150
V137I	0.519	-2.447	91.90%	0.00%	0.2110
H138R	0.567	-1.928	92.70%	0.80%	0.2050

Table 2: Representing the predicted mutated model assessment values.

region of protein except F12S and P20S. All the mutations were present in RNase A Domain from amino acid 26 to 140.

Protein 3d modeling and detection of hydrogen bonding and clashes

Angiogenin Protein 3D both wild-type and mutated models were predicted with the help of homology modelling by using MODELLER. Each mutation model represented almost 100% accuracy/confidence score. The Qmean score of predicted models of all mutations were in the range of 0-1, which signifies reliability of the mutated model data shown in Table 2. Mutated angiogenin model's evaluation by Procheck for stereo chemical quality showed that structures predicted by MODELLER are the best quality models and all models have>90% of residues in favored regions. These models were also validated using Swiss model and ProsA-web which gave a negative Z-score value representing a good quality of predicted models. Higher the negative value higher the quality of predicted models. RMSD values are calculated using Chimera, where RMSD value closest to zero shows the maximum deviation of mutated and normal angiogenin structure shown in Table 2. Chimera investigation manually checked whether the selected mutation was present in coiled region or helix region. In mutated models F12S, P20S, K41E, D46G, C63W and K64I mutation were found in coiled region while Q36L, Y38H, S52N, R55K, I70V, K84E, P136L, V137I and H138R were in helix region as shown in Figures 5 and 6. The mutation in helix region of protein is more sensitive than coiled region. UCSF Chimera exposed hydrogen bonds, clashes and contacts of the mutant residue as shown in Figure 7.

Disorder tendency analysis

PONDR result showed disorder tendency by using three different disorder predictors as shown in Figure 6. Analysis of Conserved Domain of Angiogenin Protein CDD confirmed a domain of Pancreatic

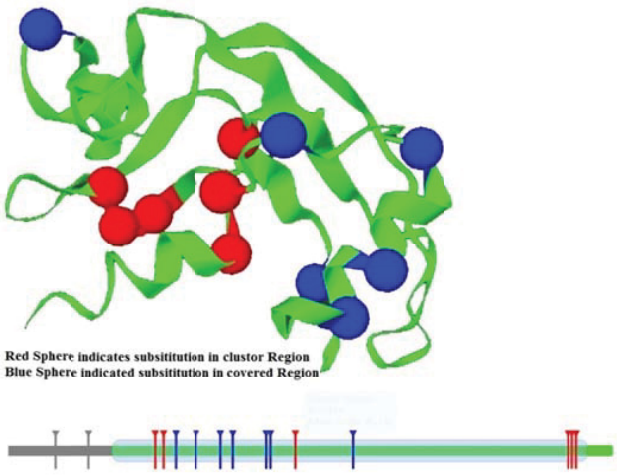


Figure 5: The selected mutation presence either in cluster region or non-cluster region.

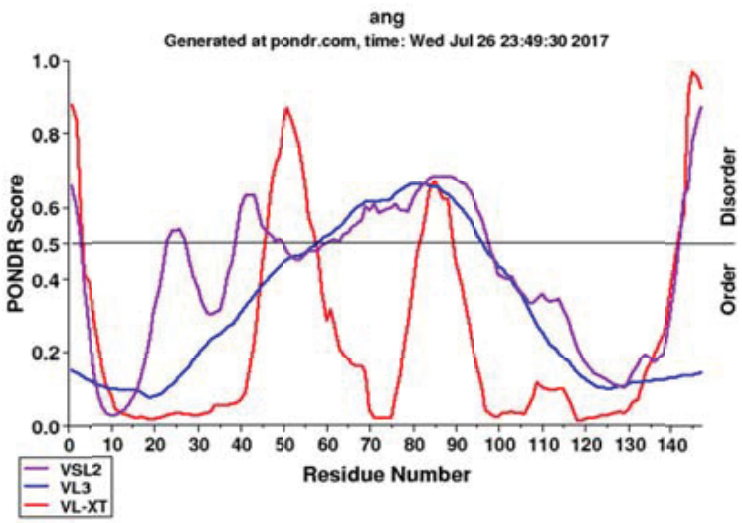


Figure 6: The disorder tendency of mutants. Higher the tendency of disorder touching the value to 0.5, lower the value means disorder tendency with low confidence score.

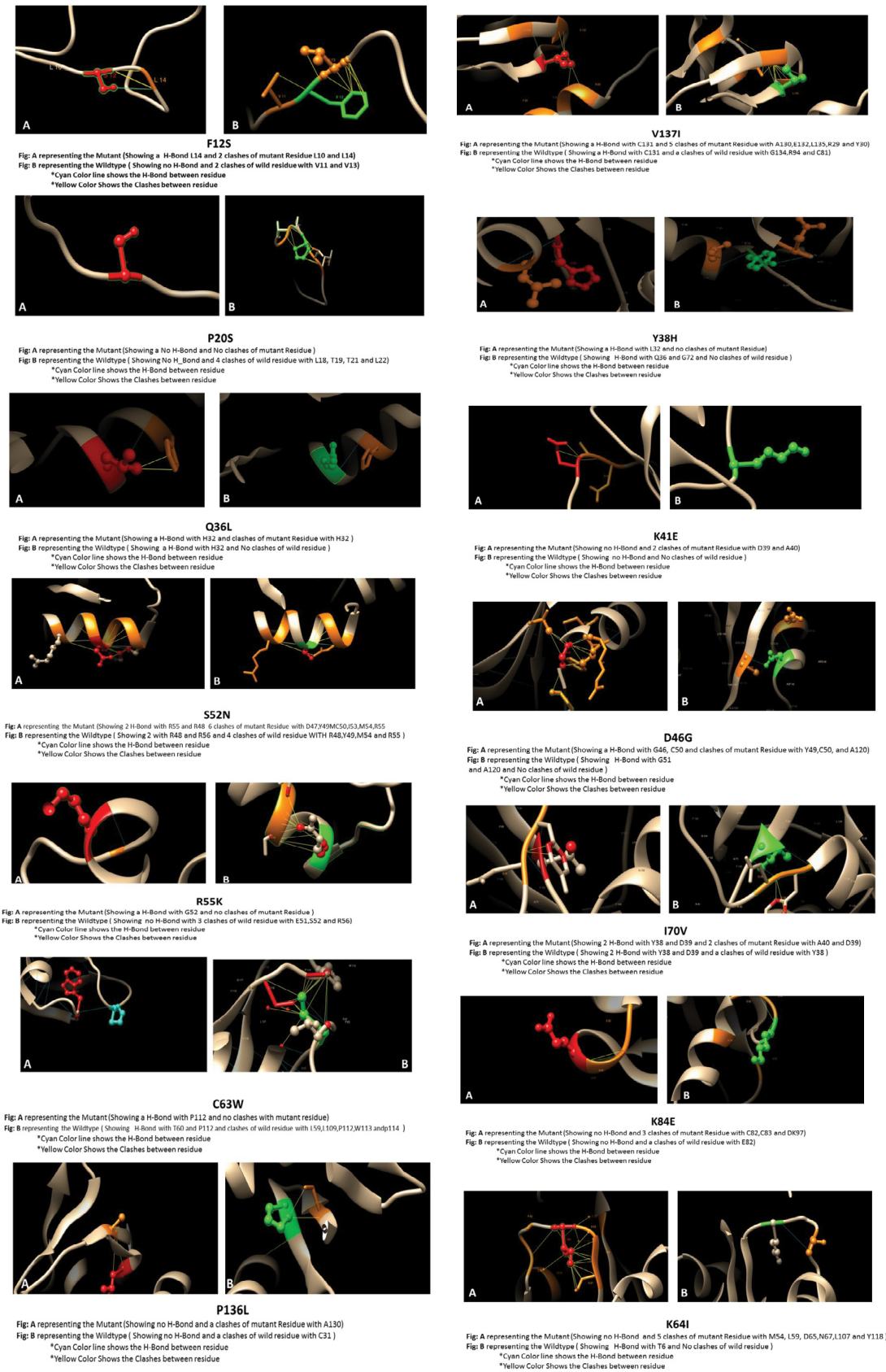


Figure 7: Selected mutation present in coiled region or helix region.

Ribonuclease A from amino acid to 26 to 140 with 2.24e-55. Pancreatic ribonuclease A possesses many Catalytic sites but mutation containing catalytic sites are from amino acid 33 to 38, 64 to 72 and 136 to 140. Disorder Tendency was checked which revealed the disorder tendency value as shown in Figure 6 higher than 0.5 describing the probability of disorder causing in protein.

Conclusion

This study hypothesized that selected missense mutations in angiogenin family Q36L, C63W, K64I, P136L, V137R and H138R have functional, structural and conformational effects with high confidence score in wild- type protein. Furthermore, these mutations i.e. Q36L, C63W, K64I, P136L, V137R and H138R disturb the stability and surface accessibility of protein. Consequently, these mutations are pathogenic in a high rate of disorder tendency, while F12S, P20S, Y38H, K41E, D46G, S52N, R55K, I70V and K84E also pathogenic to wild-type angiogenin, disturbing the structure and function but with low confidence score. These investigations may prove supportive to conduct clinical work on ANG family and to find out which mutation is responsible for the disease. This case study could be helpful for mutation targeting in drug designing and finding the mutation with neutral effect.

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