Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.52.008263

The Effects of Growth Disorder-Related Mutations on The Conformational Structure of Insulin-like Growth Factor (IGF1): An *In Silico* Study

Muhammad Adnan Shah Bukhari[1]*, Mubeen Ali Niaz[1], Syed Bilal Hussain[1] and Shereen Rubab[2]

[1]*Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan*

[2]*Faculty of Pharmacy, Bahauddin Zakariya University Multan, Pakistan*

***Corresponding author:** Muhammad Adnan Shah Bukhari, Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

ARTICLE INFO

Received: August 18, 2023

Published: August 28, 2023

Citation: Muhammad Adnan Shah Bukhari, Mubeen Ali Niaz, Syed Bilal Hussain and Shereen Rubab. The Effects of Growth Disorder-Related Mutations on The Conformational Structure of Insulin-like Growth Factor (IGF1): An *In Silico* Study. Biomed J Sci & Tech Res 52(3)-2023. BJSTR. MS.ID.008263.

ABSTRACT

Insulin-like Growth Factor-1 (IGF1) is a hormone that plays a vital role in human growth and development. It regulates cell proliferation and tissue growth. Any alterations in the structure or function of IGF1 can have profound implications for various functions and diseases, including cancer and growth disorders. In this study, a comprehensive computational analysis was performed to examine the impact of single amino acid substitutions on IGF1 tertiary structure. The study involved using *In Silico* tools for comparing the amino acid sequences of wild-type and three variant IGF1 proteins, as well as assessing their physicochemical properties. Computational tools were employed for predicting and evaluating secondary and tertiary structure of variant and wild-type IGF1. The results conclude observable changes in the secondary and tertiary structure among variants with single amino acid difference. These findings emphasize the significance of studying single amino acid substitutions on proteins conformational changes to understand the underlying molecular mechanisms associated with IGF1 and other important proteins. The data suggests that even subtle changes in IGF1's structure can have significant impacts on protein function and cellular processes. This contributes to our expanding knowledge regarding diseases related to IGF1 and opens up possibilities for potential therapeutic strategies.

Keywords: IGF1; Mutation Analysis; 3D Structure; Growth Disorder; Genetic Disease; *In Silico*

Abbreviations: IGF1: Insulin-Like Growth Factor I; GH: Growth Hormone; IP: Isoelectric Point; GRAVY: Grand Average of Hydropathicity; MD: Molecular Dynamics; NMR: Nuclear Magnetic Resonance

Introduction

Insulin-Like Growth Factor I (IGF1) is a peptide hormone that plays a vital role in human growth, development, and various physiological processes. It is commonly referred to as a "growth factor" because it can promote cell proliferation and tissue growth. The discovery of IGF1 has significantly advanced our understanding of how the body regulates growth and has opened up new avenues for research and medical applications (Puche, et al. [1]). The fascinating story of IGF1 dates back to the 1950s when researchers were exploring the role of the pituitary gland in regulating growth. They made an intriguing observation that the pituitary gland secretes growth hormone (GH), which stimulates the liver to produce a substance that influences growth. This substance was later identified as Insulin-Like Growth Factor I (IGF1) (Anisimov, et al. [2]). The gene responsible for producing IGF1 is located on chromosome 12 and consists of several exons and introns. The structure of the IGF1 protein comprises 70 amino acids and bears resemblance to insulin, hence its name "Insulin-Like" Growth Factor. Despite this similarity, IGF1 operates independently with its unique functions (Beattie, et al. [3]). IGF1 exerts its effects through two primary modes: endocrine function and paracrine function. In terms of endocrine function, IGF1 is secreted into the bloodstream by the liver in response to GH stimulation. Once in circulation,

it can travel throughout the body and impact distant tissues, facilitating growth and development. Additionally, IGF1 acts through a paracrine mechanism where it is produced locally in specific tissues.

Its effects are limited to the immediate vicinity of its production, allowing it to influence particular tissues without affecting the entire body. The significance of IGF1 is particularly evident during periods of rapid growth, such as infancy, childhood, and adolescence (Yakar, et al. [4]). It plays a crucial role in promoting longitudinal bone growth and ensuring that children reach their full height potential. Beyond its impact on growth, IGF1 is involved in various other biological functions. For instance, it stimulates cell division, which is essential for tissue growth, repair, and maintenance. It also contributes to muscle development and repair, making it vital for athletes and individuals recovering from injuries. Moreover, IGF1 influences glucose metabolism and insulin sensitivity, thereby affecting how the body processes and utilizes energy (Racine, et al. [5]). IGF1's Role in Prenatal Development During the initial stages of pregnancy, precise signaling pathways are crucial for guiding the growth and differentiation of the developing embryo. IGF1, produced by both the mother and fetus, has a vital part to play in this complex orchestration. The placenta, a temporary organ that supports the growing fetus, produces IGF1 (Hellström, et al. [6]). This hormone helps regulate nutrient transport from the mother to the developing embryo, ensuring it receives essential building blocks for development. As the fetus continues to grow, it starts producing its IGF1 as well. This local production of IGF1 in fetal tissues fine-tunes growth processes and ensures proper organ development.

IGF1's Influence on Childhood Growth After birth, IGF1 remains a pivotal player in the growth and development of infants and children. Throughout childhood and adolescence, elevated levels of IGF1 orchestrate remarkable growth spurts (Hellström, et al. [6]). One of the most noticeable effects of IGF1 during childhood is its impact on longitudinal bone growth. In the growth plates of long bones, IGF1 stimulates chondrocytes, the specialized cells responsible for bone elongation by promoting their proliferation and differentiation. This process enables children to reach their genetically determined height potential. Additionally, during childhood, IGF1 contributes to muscle development and aids in repairing muscle tissues post-physical activity or injury. It promotes muscle fiber growth as well. Apart from bones and muscles, IGF1 also supports the growth and maturation of various organs such as the heart, lungs, and brain. Adequate IGF1 signaling is crucial for achieving functional organ development (Racine, et al. [5]). During puberty, IGF1 plays a pivotal role in the extraordinary transformations that occur as children transition into adolescents. Puberty is triggered by hormonal changes, with IGF1 intricately linked to these processes. The surge in IGF1 levels during puberty contributes to the growth spurt experienced by adolescents. Rapid bone growth and elongation of limbs are observed as the body prepares for adult height. IGF1 also influences the development of secondary sexual characteristics during puberty. It contributes to breast tissue growth in females and the development of facial hair and the deepening of voice in males.

Impact on Cognitive Development In addition to its impact on physical growth, IGF1 has implications for cognitive development and brain health. IGF1 supports neural growth and plasticity by facilitating the formation of new connections between neurons. This enhances learning and memory processes. Furthermore, IGF1 possesses neuro-protective properties that promote neuronal survival. It safeguards brain cells from damage caused by oxidative stress or injury. IGF1 not only plays a crucial role in the growth and development of humans but also shows promise as a potent defense against various diseases (Christoforidis, et al. [7]). Aging is a multifaceted process characterized by a gradual decline in physiological functions and an increased vulnerability to age-related diseases. IGF1 has emerged as a key player in the aging process, potentially influencing longevity and health outcomes associated with age. IGF1 actively participates in cellular repair and regeneration, promoting the maintenance and well-being of different tissues. By supporting the restoration of damaged cells, IGF1 may help alleviate the effects of cellular aging. IGF1 exhibits antioxidant properties that aid in counteracting harmful free radicals responsible for cellular damage and aging. Some studies suggest that maintaining optimal levels of IGF1 may be linked to healthier aging and a reduced risk of age-related diseases (Vitale, et al. [8]).

Materials and Methods

Obtaining Protein Sequences

Amino acid sequence of IGF1 protein and its three variants were obtained from Uni Prot. IGF1 is available at UniProt KB (Accession no: P05019) and variant viewer was used to select three variants, two pathogenic one of unknown significance, i.e., VAR_056113, VAR_075825 and VAR_013945.

Physicochemical Properties Analysis

Evaluation of physicochemical parameters is a fundamental aspect of protein analysis, as it aids in identifying variations between normal and variant proteins. The Expasy Protparam server (https://web.expasy.org/protparam/) (Gasteiger, et al. [9]) was employed to achieve this objective. This server accepts plain sequence input and provides crucial information, including molecular weight (MW), stability, GRAVY index (grand average of hydropathicity), and isoelectric point (PI) (Gasteiger, et al. [9]). These parameters offer valuable insights into diverse facets of a protein, enabling the detection of dissimilarities among protein variants.

Secondary Structure Prediction

The biological functions of a protein are intricately linked to its structural conformation. To gain insights into the secondary structure of the insulin-like growth factor 1 (IGF1) and its selected variants, we utilized the GOR4 server (Combet, et al. [10]). The GOR4 server employs information theory and Bayesian statistical analysis to make ac-

curate predictions for protein secondary structure. By inputting the amino acid sequences of IGF1 and its variants into the GOR4 server, (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_gor4.html), we obtained valuable predictions for their respective secondary structures.

Tertiary Structure Prediction

The Swiss model (https://swissmodel.expasy.org/) (Waterhouse, et al. [11]), an established and widely used tool, for predicting protein 3D structures was utilized to determine the conformational structures of IGF1 and its variant models. By applying parameters and methodologies four models were generated, including the wild type IGF1 and its variants. This approach guarantees consistency in our predictions enabling us to make comparisons and evaluations of the generated structures.

Tertiary Structure Refinement and Validation

The initial protein models were refined using a tool called the Galaxy Refine server (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE) (Heo, et al. [12]). This tool utilizes dynamics simulation to improve the quality of protein structures. Five refined models were generated by the Galaxy Refine server and the best model was chosen for analysis. To evaluate the quality of the 3D model we employed widely used web servers. These servers, MolProbity (http://molprobity.biochem.duke.edu/index.php) (Williams, et al. [13]), ProSA Web (https://prosa.services.came.sbg.ac.at/prosa.php) (Wiederstein, et al. [14]), ERRAT and ProCheck webserver (https://saves.mbi.ucla.edu/) (Colovos, et al. [15]) were used to analyze aspects of the protein models accuracy and stereochemical quality. MolProbity provides information about the geometry and interactions of atoms in macromolecular structures. ProSA Web assesses quality and reliability through potential energy calculations. ERRAT compares the model to electron density maps to evaluate its quality. Lastly ProCheck webserver applies validation criteria to identify errors or deviations from expected values, in the protein model. UCSF ChimeraX was utilized for visualization of 3D structures.

Results

Physicochemical Properties

Physicochemical analysis was performed to spot the differences between normal and mutated proteins. The resulting parameters are listed in the (Table 1). The results demonstrate no major differences in the physicochemical properties of normal and mutated peptides. Although slight changes can be seen in molecular weight GRAVY and instability index.

Table 1: Physicochemical properties of normal and variant IGF1 proteins.

Parameter	Normal	VAR_056113	VAR_075825	VAR_013945
Molecular weight	21841.19	21871.22	21871.22	21885.2
Theoretical pI	9.78	9.78	9.72	9.72
Instability index	64.11	63.12	61.86	64.11
Aliphatic index	51.59	51.08	51.59	51.08
GRAVY	-0.732	-0.745	-0.714	-0.759
Total number of atoms	3048	3052	3049	3051

Secondary Structure

GOR4 was used for predicting secondary structure. The details of predicted structures are provided in (Table 2). (Figure 1) presents the secondary structures in graphical form.

Table 2: Details of predicted secondary structures from GOR4.

		Normal	VAR_056113	VAR_075825	VAR_013945
Alpha helix	(Hh)	21.03%	21.03%	21.03%	18.46%
310 helix	(Gg)	0.00%	0.00%	0.00%	0.00%
Pi helix	(Ii)	0.00%	0.00%	0.00%	0.00%
Beta bridge	(Bb)	0.00%	0.00%	0.00%	0.00%
Extended strand	(Ee)	15.38%	15.38%	19.49%	15.38%
Beta turn	(Tt)	0.00%	0.00%	0.00%	0.00%
Bend region	(Ss)	0.00%	0.00%	0.00%	0.00%
Random coil	(Cc)	63.59%	63.59%	59.49%	66.15%
Ambiguous states	(?)	0.00%	0.00%	0.00%	0.00%
Other states		0.00%	0.00%	0.00%	0.00%

DOI: 10.26717/BJSTR.2023.52.008263

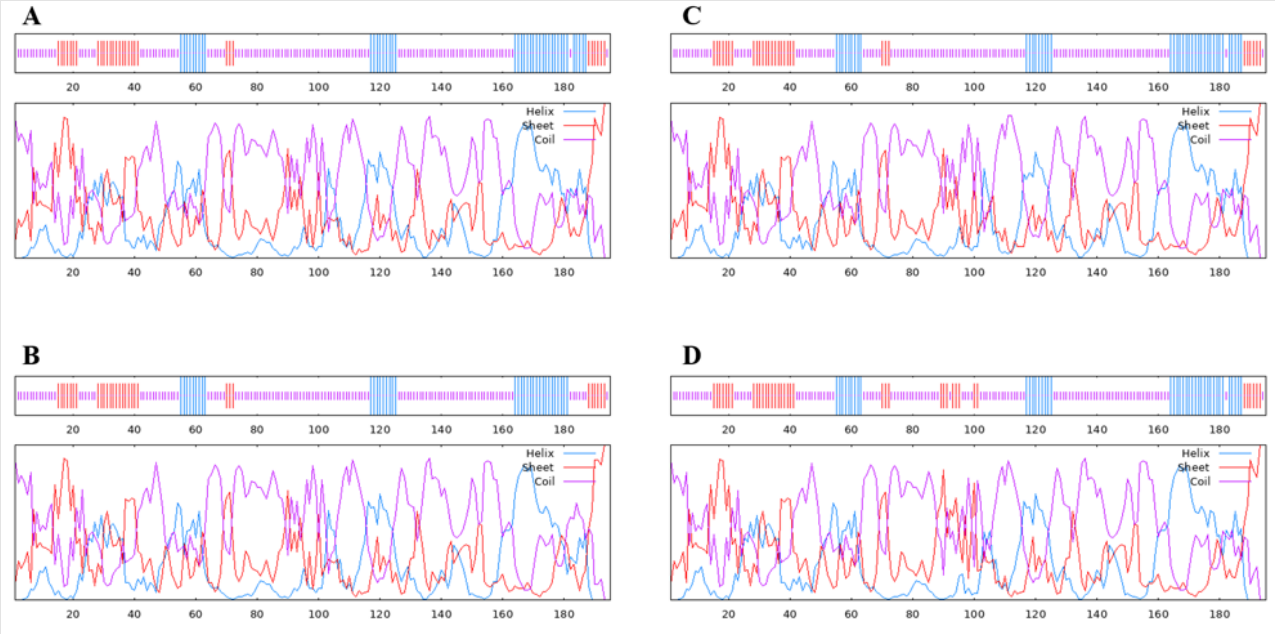

Figure 1: Graphical representation of GOR4-predicted secondary structure
A. Normal
B. VAR_013945
C. VAR_056113
D. VAR_075852.

Table 3: MolProbity and ERRAT scores of initial and selected-refined models.

		IGF1 Normal				Var_013945				Var_056113				Var_075825			
		Initial		Refined		Initial		Refined		Initial		Refined		Initial		Refined	
All-Atom contacts	Clash score, all Atoms:	0.65		3.59		0.65		3.26		0.65		2.93		0.66		3.27	
	Clash score is the number of serious steric overlaps (>0.4A) per 1000 atoms.																
Protein Geometry	Poor rotamers	11	6.59%	1	0.60%	11	6.55%	0	0.00%	11	6.55%	0	0.00%	11	6.59%	2	1.20%
	Favored rotamers	147	88.02%	165	98.80%	148	88.10%	167	99.40%	148	88.10%	166	98.81%	147	88.02%	165	98.80%
	Ramachandran outliers	15	7.77%	0	0.00%	15	7.77%	0	0.00%	15	7.77%	0	0.00%	15	7.77%	0	0.00%
	Ramachandran Favored	149	77.20%	190	98.45%	150	77.72%	191	98.96%	149	77.20%	191	98.96%	149	77.20%	188	97.41
	Rama distribution Z-score	-1.92±0.61		0.18±0.49		-1.95±0.60		-0.16±0.48		-1.96±0.61		-0.32±0.48		-1.98±0.61		-0.21±0.49	
	MolProbity score	2.11		1.15		2.1		1.12		2.1		1.08		2.11		1.29	
	Overall Quality Factor (ERRAT)	77.8		90.8		78.6		94.2		77.1		90.9		77.8		92.5	

Tertiary Structure

Tertiary structure was predicted using Swiss Model and refined using Galaxy-Webserver. MolProbity was used to evaluate refined models and select the best refined structure. (Table 3) MolProbity and ERRAT scores of initial and selected-refined models. presents the MolProbity results of initial and selected-refined models. ProSA Web and ProCheck webservers were also utilized for models' evaluation and constructing Ramachandran plots. Ramachandran plots and ProSA Webgraphs of refined models are presented in the (Figure 2) and (Figure 3) respectively. ProSA Web webserver was also used to evaluate the predicted 3D models. ProSA Web results take PDB files as input and present evaluation results in form of z-score. ProSA Web evaluation results of selected-refined structures are presented in the figure below.

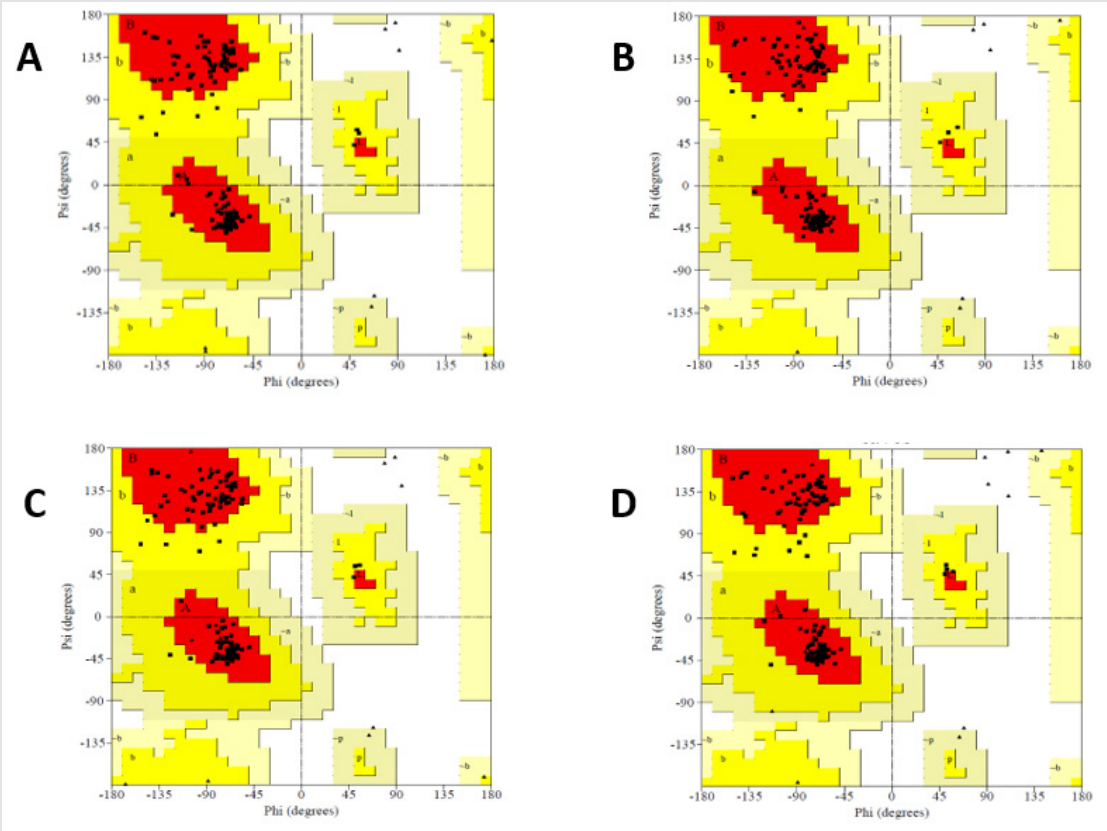

Figure 2: Ramachandran Plots of selected-refined models obtained from ProCheck Webserver:
A. IGF1 Normal
B. VAR_013945
C. VAR_056113
D. VAR_075825.

DOI: 10.26717/BJSTR.2023.52.008263

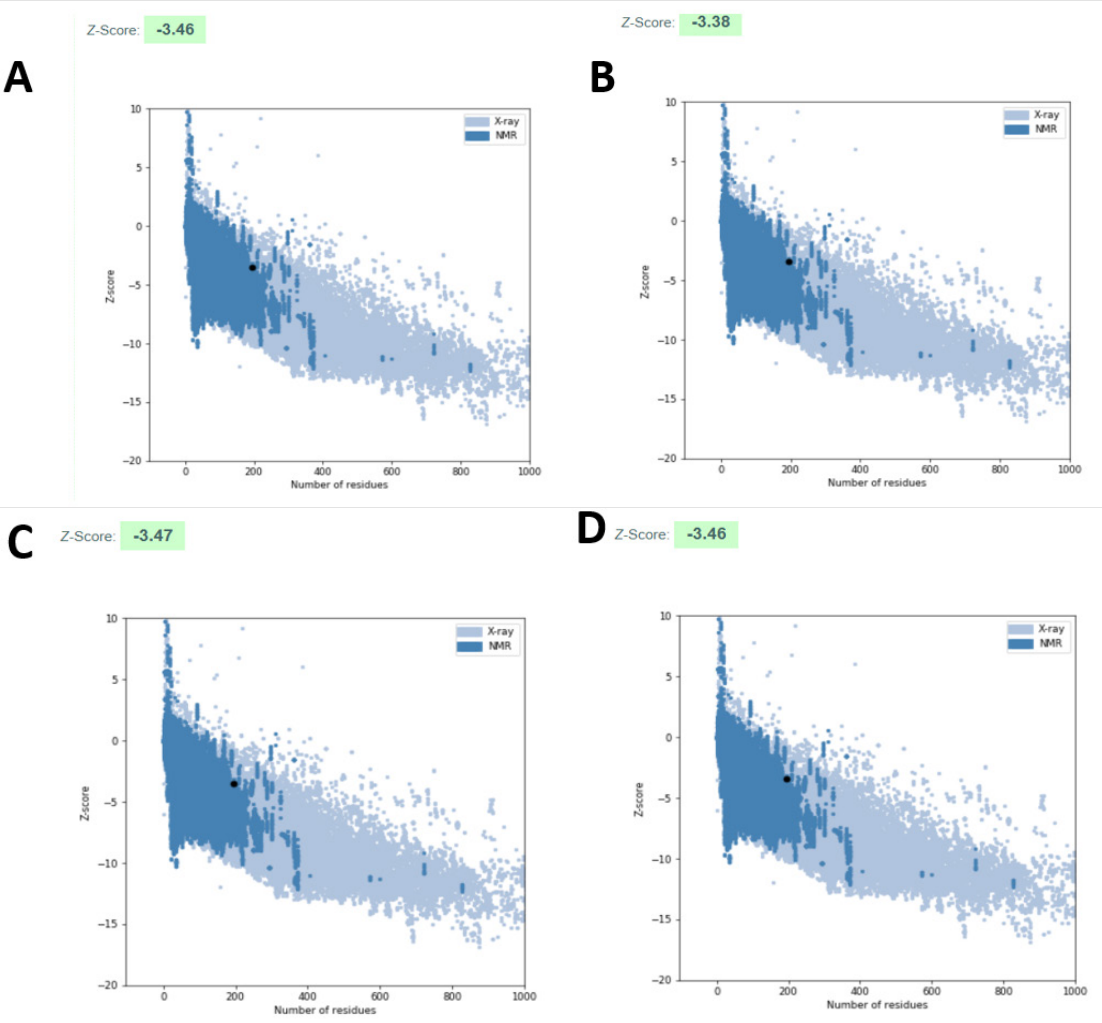

Figure 3: Z-score of selected-refined models obtained from ProSA Webserver
A. IGF1 Normal
B. VAR_013945
C. VAR_056113
D. VAR_075825.

Three Dimensional (3D) Structures Visualization

UCSF Chimera was used to visualize obtained 3D structures. All the structures including normal and variants of IGF1 were superimposed on each other to check any structural differences among them. (Figure 4) represents the visual results of selected 3D models and helps understanding inter-structural differences of the selected models. The results show that even a single amino acid substitution has cause changes among the 3D models of protein.

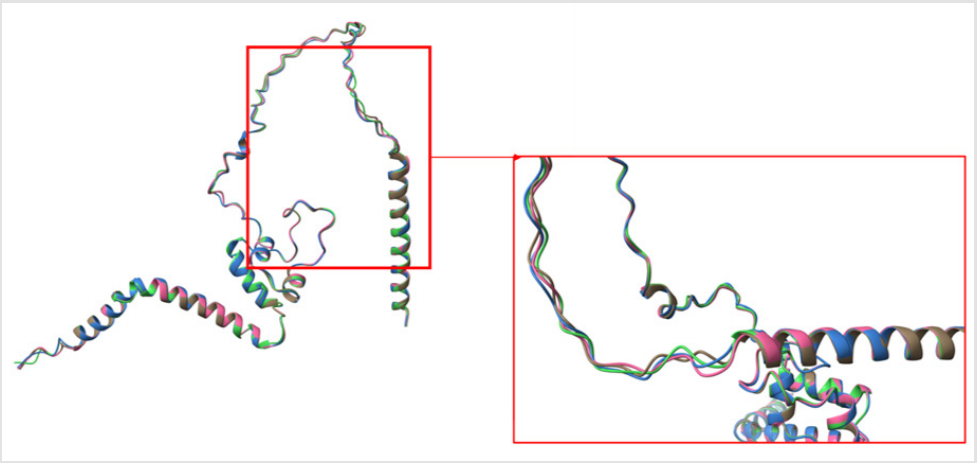

Figure 4: Visualization of structure differences among wild-type and variants of IGF1 protein.

Discussion

Insulin-like Growth Factor-1 (IGF1) is a peptide hormone that plays a vital role in regulating cellular growth, proliferation, and differentiation. Any changes in its structure or function can have important implications for various physiological processes and are linked to diseases such as cancer and growth disorders. To comprehend the molecular basis of these diseases, it is crucial to investigate the effects of single amino acid substitutions on IGF1. In this study, we conducted an extensive computational analysis to explore how these mutations impact the physicochemical properties, secondary and tertiary structures, and overall stability of IGF1. To begin our investigation, we obtained the amino acid sequences of both the wild-type and variant IGF1 proteins from the UniProt KB database. We performed a thorough physicochemical analysis using the Prot Param webserver to extract essential information about the protein sequences. Parameters like molecular weight, isoelectric point, extinction coefficient, aliphatic index, and grand average of hydropathicity (GRAVY) were assessed for both types of proteins. The results revealed subtle differences between the wild-type and variant IGF1 proteins in terms of their physicochemical properties. While these differences may not appear significant on a percentage scale, they could have functional implications due to their complex interplay in protein stability and folding. Particularly intriguing was the finding that all models displayed high instability indexes (>60). Notably, pathogenic variants showed a slight decrease in instability compared to normal and non-pathogenic variants of IGF1.

Further investigation is needed to understand the implications of this finding since stability plays a critical role in protein function. Next, we examined the secondary structures of normal and variant IGF1 proteins using the GOR4 server for secondary structure prediction based on machine learning algorithms. The results showed some variations among variants, but no clear pattern emerged when comparing them with wild-type proteins. This raises interesting questions about how secondary structure variations relate to the functional consequences of amino acid substitutions. To delve deeper into the structural differences, we utilized the Swiss Model webserver for tertiary structure prediction. This approach relies on known protein structures with high sequence similarity to predict the 3D structure of the target protein. The generated models were further refined using the Galaxy Webserver and Molecular Dynamics (MD) simulations to optimize accuracy. The refinement process resulted in five refined models for each IGF1 variant and wild-type protein. Selecting the best model is crucial as it represents the most likely conformation in its native state. We used validation criteria from MolProbity, ERRAT, ProCheck, and ProSA Web to assess model quality, accuracy, and reliability. Once we selected the best models for each variant and wild-type protein, we visualized them using UCSF Chimera software. This step allowed us to compare their 3D structures in detail. Despite only a single amino acid substitution, noticeable structural differences were observed among variants primarily in peptide turns.

This suggests that these regions may play a significant role in the functional divergence of IGF1 variants. These observed structural differences between wild-type and variant IGF1 proteins can have important implications for disease development. Single amino acid substitutions can disrupt protein-protein interactions, ligand binding sites or affect post-translational modifications leading to abnormal cellular signaling and pathological outcomes. By uncovering specific structural alterations caused by these mutations, our study contributes to a deeper understanding of molecular mechanisms underlying IGF1-related diseases.

Conclusion

In conclusion, our extensive computational analysis provides insights into the potential impacts of amino acid substitution mutations on the structure and function of Insulin-like Growth Factor-1. The subtle differences in physical and chemical properties, as well as structural variations, observed between the original protein and its variants, may have significant implications for the normal functioning of IGF1 and its role in various diseases. This research contributes to the expanding knowledge base regarding the molecular mechanisms underlying IGF1-related disorders, setting a solid groundwork for future studies aimed at developing targeted therapies and personalized medicine approaches for affected individuals. Furthermore, interdisciplinary investigations will continue to enhance our understanding of the intricate relationship between protein structure, function, and disease, ultimately advancing our capacity to address complex medical conditions associated with IGF1 and beyond.

Future Prospects

When it comes to analyzing the effects of individual amino acid substitutions on IGF1, our computational analysis offers valuable insights. However, it is important to acknowledge the limitations of In Silico studies. To validate and enhance our findings, additional large-scale experimental studies using advanced techniques like Nuclear Magnetic Resonance (NMR) spectroscopy or X-ray crystallography are necessary. Furthermore, conducting functional assays to examine how these mutations affect cellular behavior and signaling pathways will be pivotal in understanding the exact role of specific amino acid substitutions in disease pathogenesis. It's crucial to combine computational analysis with experimental validation for a comprehensive understanding.

References

1. Puche JE, Castilla-Cortázar I (2012) Human conditions of insulin-like growth factor-I (IGF-I) deficiency. Journal of translational medicine 10: 1-29.
2. Anisimov VN, Bartke A (2013) The key role of growth hormone–insulin–IGF-1 signaling in aging and cancer. Critical reviews in oncology/hematology 87(3): 201-223.
3. Beattie J, Allan GJ, Lochrie JD, Flint DJ (2006) Insulin-like growth factor-binding protein-5 (IGFBP-5): a critical member of the IGF axis. Biochemical Journal 395(1): 1-19.
4. Yakar S, Adamo M L (2012) Insulin-like growth factor 1 physiology: lessons from mouse models. Endocrinology and Metabolism Clinics 41(2): 231-247.
5. Racine HL, Serrat MA (2020) The actions of IGF-1 in the growth plate and its role in postnatal bone elongation. Current osteoporosis reports 18: 210-227.
6. Hellström A, Ley D, Hansen-Pupp I, Hallberg B, Ramenghi LA, et al. (2016) Role of insulin like growth factor 1 in fetal development and in the early postnatal life of premature infants. American journal of perinatology 33(11): 1067-1071.
7. Christoforidis A, Maniadaki I, Stanhope R (2005) Growth hormone/insulin-like growth factor-1 axis during puberty. Pediatric endocrinology reviews PER 3(1): 5-10.
8. Vitale G, Pellegrino G, Vollery M, Hofland LJ (2019) Role of IGF-1 system in the modulation of longevity: controversies and new insights from a centenarians' perspective. Frontiers in endocrinology 10: 27.
9. Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, et al. (2005) Protein identification and analysis tools on the ExPASy server: Springer pp. 571-607.
10. Combet C, Blanchet C, Geourjon C, Deleage G (2000) NPS@: network protein sequence analysis. Trends in biochemical sciences 25(3): 147-150.
11. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, et al. (2018) SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic acids research 46(W1): W296-W303.
12. Heo L, Park H, Seok C (2013) Galaxy Refine: Protein structure refinement driven by side-chain repacking. Nucleic acids research, 41(W1): W384-W388.
13. Williams CJ, Headd JJ, Moriarty NW, Prisant MG, Videau LL, et al. (2018) Mol Probity: More and better reference data for improved all-atom structure validation. Protein Science 27(1): 293-315.
14. Wiederstein M, Sippl MJ (2007) ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic acids research 35(suppl_2): W407-W410.
15. Colovos C, Yeates TO (1993) Verification of protein structures: patterns of nonbonded atomic interactions. Protein Science 2(9): 1511-1519.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.52.008263

Muhammad Adnan Shah Bukhari. Biomed J Sci & Tech Res