

Original Research

In Silico Screening of AHSG Missense Variants and Exploring Their Interaction with BMP-10 in Cardiovascular Diseases

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Abstract

Fetuin-A, encoded by the AHSG gene, is a hepatokine that regulates metabolism, ectopic calcification, and cardiovascular homeostasis. Beyond mineral sequestration, it binds Bone Morphogenetic Protein (BMP) ligands, modulating their extracellular availability. BMP-10 supports endothelial integrity, vascular remodeling, and signaling through BMP receptor type 2 (BMPR2). Impaired BMP-10 signaling is linked to cardiovascular diseases (CVDs). Non-synonymous single-nucleotide polymorphisms (nsSNPs) in AHSG can alter fetuin-A structure and potentially disrupt its interaction with BMP-10. A total of 398 missense nsSNPs were analyzed using PredictSNP (functional impact), DUET, I-Mutant 2.0, and DynaMut2 (stability), ConSurf (conservation), and Project HOPE (structural effects). Further, the selected variants underwent 100-ns molecular dynamics simulations, and molecular docking was performed with BMP-10-BMPR2. Using the computational framework, seven high-confidence deleterious variants- I48T, I80S, I82T, E86G, R103S, V142G, F125S- plus two clinically relevant variants



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(M248T, S256T) from reported GWAS literature were identified. MD simulations showed significant deviations: S256T displayed the greatest conformational shift from wild type, while E86G increased solvent exposure and flexibility. Docking investigations predicted a stable interaction between wild-type fetuin-A and the BMP-10-BMP2 complex. Most variants showed reduced predicted binding interactions relative to wild type. These computational findings provide hypothesis-generating evidence of a structural basis for how AHSG variants may impair BMP-10 regulation, potentially contributing to cardiovascular dysregulation. The proposed framework may help prioritize variants for targeted experimental and genotype-phenotype studies to determine their functional and clinical relevance.

Keywords

Fetuin-A; AHSG gene; BMP-10; single nucleotide polymorphism (SNP); structural bioinformatics; molecular dynamics simulation

1. Introduction

Fetuin-A, also known as alpha-2-Heremans-Schmid glycoprotein (AHSG), is a ~64 kDa circulating glycoprotein primarily synthesized by the liver and encoded by the AHSG gene. It plays a vital role in systemic metabolic regulation and cardiovascular homeostasis. Reduced circulating fetuin-A levels are often associated with increased risk of cardiovascular disease (CVDs) [1, 2]. One of its most important physiological functions is acting as a negative inhibitor of ectopic calcification. Fetuin-A acts as a primary circulating mineral chaperone and binds calcium and phosphate to form soluble fetuin-mineral complexes (FMCs), which stabilize calciprotein particles (CPPs) and remove excess mineral from the circulation [3]. FMCs help prevent spontaneous deposition and precipitation of calcium phosphate and protect soft tissues from pathological mineralization [4]. A study reported that AHSG-knockout mouse models exhibit spontaneous soft tissue and vascular calcification, highlighting the protective role of fetuin-A in ectopic calcification [5]. It has been well documented that low levels of fetuin-A are significantly associated with CVD, chronic kidney disease (CKD), and metabolic disorders [6-9].

The AHSG gene, which encodes fetuin A, is located at chromosome position 3q27.3 and spans a sequence of approximately 8.2 kb. Fetuin A is a member of the cystatin superfamily, a subgroup of proteins that are primarily responsible for protease inhibition and extracellular regulatory functions [10, 11]. Serum expression of fetuin-A is regulated by various transcriptional regulators, such as CCAAT enhancer-binding proteins (C/EBP)- β and nuclear factor (NF)-1. Serum levels of circulating fetuin-A are influenced by alterations in the AHSG gene, which has been well documented, and the significant impact has been reported in various conditions such as metabolic, cardiovascular, and renal disorders, impaired bone mineralization, ectopic calcification, and certain neurological and neurodegenerative conditions [12-15]. Further, a genome-wide association study (GWAS) reported two single nucleotide variants (rs4917 and rs4918) to be associated with coronary artery calcification, atherosclerotic plaque, and valvular heart disease [16, 17]. While these variants correlate with disease susceptibility, the molecular mechanism remains elusive. Most investigations

focus on circulating protein levels; however, missense variants may impart functional toxicity by altering the protein's tertiary fold or its susceptibility to post-translational modifications (PTMs) [18].

Beyond mineral sequestration, fetuin-A functions as an extracellular signaling modulator, acting as a natural antagonist of BMP/TGF- β family ligands by limiting their receptor engagement, and independently regulating insulin receptor signaling in metabolic disorders [19, 20]. The structural and functional impact of AHSG missense variants on fetuin-A interactions with extracellular signaling partners remains poorly understood, particularly with respect to how they may predispose to CVDs. Fetuin-A may modulate cardiovascular homeostasis by influencing ligand availability and receptor engagement. While Bone Morphogenetic Protein (BMP)-2 is well explored for its osteogenic role in vascular calcification, cardiovascular tissues also depend on BMP ligands with more specialized regulatory functions [21]. Emerging evidence highlights BMP-10, a heart-specific circulating morphogen primarily expressed in the atria, as a key cardiovascular-specific BMP that regulates endothelial homeostasis, vascular remodeling, and BMPR2-mediated signaling. BMP-10 is highly expressed in cardiac and vascular tissues and signals primarily through the BMPR2 receptor complex, a pathway critically implicated in vascular integrity and CVD [22, 23]. Dysregulation of BMP-10-BMPR2 signaling has been associated with endothelial dysfunction, aberrant vascular remodeling, and pathological cardiovascular outcomes, positioning BMP-10 as a biologically relevant mediator at the intersection of vascular signaling and disease [22, 24].

Fetuin-A is known to interact with members of the BMP family, suggesting a potential role in modulating BMP ligand availability or receptor engagement and extracellular signaling molecules [19]. However, it is unclear whether fetuin-A structurally interacts with BMP-10 or the BMP-10-BMPR2 receptor complex, and how AHSG variants influence this interaction, which points towards a critical gap in understanding how SNPs may translate as a predisposing threat to cardiovascular risk.

Our study focuses on *in silico* investigations to understand the impact of deleterious AHSG missense variants on the structural conformation of their interaction with BMP-10 and the BMP-10-BMPR2 receptor complex. Using protein-protein docking, we identify amino acid residues that may govern binding interactions and variant-specific interactions. We hypothesize that variants may cause structural perturbations in fetuin-A and may alter their capacity to buffer or engage BMP-10 or its receptor complex, thereby influencing extracellular BMP-10 signaling.

2. Methodology

2.1 Collection of SNP Dataset

The SNP data of the AHSG (fetuin-A) gene (ENST00000411641.7) were obtained from the Ensembl genome browser (<https://asia.ensembl.org/index.html>). The amino acid sequence of the fetuin-A protein was retrieved from the UniProt database (<https://www.uniprot.org/>) (UniProt ID: P02765). A total of 398 missense variants underwent a multistep filtration process. The first level of screening aimed to find variants that could functionally change the protein. This was based on whether they were tolerated or deleterious, using consensus pathogenicity prediction tools. Variants predicted as damaging/deleterious underwent a second filtration step that segregated the SNPs based on their structural stability analysis and evolutionary conservation regions. This helped in prioritizing variants with the highest likelihood of altering protein structure and function (Figure 1).

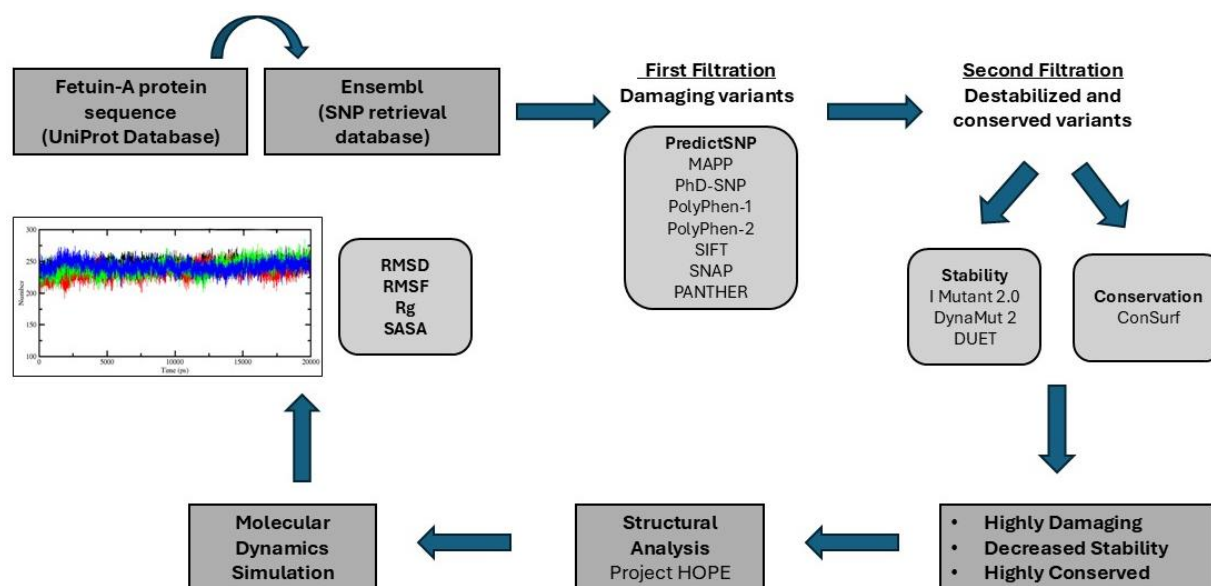


Figure 1 Schematic Workflow. This figure outlines the computational workflow used to identify potentially harmful missense SNPs in the fetuin-A (AHSG) gene. Variants were first screened for functional impact, effects on protein stability, and evolutionary conservation. The most damaging and conserved SNPs were then examined in detail using structural modeling and molecular dynamics simulations to understand how they might alter protein structure and contribute to disease.

2.2 Screening the Variants

To evaluate the potential functional impact of missense nsSNPs variants in the *AHSG* gene, we employed the PredictSNP web server for our first filtration step. PredictSNP is a consensus-based bioinformatics platform designed to classify amino acid substitutions as either neutral or deleterious [25]. The amino acid sequence of fetuin-A in FASTA format was added to the server (<https://loschmidt.chemi.muni.cz/predictsnp1/>), and individual point mutations were introduced manually by substituting the wild-type residues with the variant residues at that particular position. Each substitution was analysed independently to generate predictive scores.

PredictSNP brings together the predictive outcomes of eight well-established algorithms across MAPP, PANTHER, nsSNP Analyzer, PhD SNP, PolyPhen1, PolyPhen2, SIFT, and SNAP. This integrative approach within a consensus-based framework will facilitate increased accuracy and precise prediction of the functional impact. The outcome of this investigation will help in generating a probabilistic assessment of SNPs and confirm their impact on disruption of a protein's structure or function. The robust dataset generated by PredictSNP, which is based on a foundation of more than 43,000 experimentally confirmed mutations, ensures the creation of reliable models without any significant bias. Each variant is categorized as either damaging or neutral and is accompanied by a confidence score that represents the degree of agreement among the individual algorithms. This consensus-driven filtering method was applied as the initial screening step to identify potentially pathogenic variants of the fetuin A protein for further structural and functional characterization.

In the second filtration step, we employed four different web servers (DUET, I-Mutant 2.0, DynaMut2, and ConSurf) to further identify the most pathogenic SNPs in the fetuin-A protein. The amino acid sequence and AlphaFold-predicted structure of fetuin-A, provided in FASTA and PDB

formats, respectively, were uploaded to the respective bioinformatics servers. Relevant analysis parameters were selected based on each tool's algorithmic requirements to evaluate the structural and functional impact of the selected variants.

We utilized I-Mutant 2.0 (<https://folding.biofold.org/i-mutant/i-mutant2.0.html>), DynaMut2 (<https://biosig.lab.uq.edu.au/dynamut2/>) and DUET (<https://biosig.lab.uq.edu.au/duet/stability>) web servers to evaluate the stability of the protein in response to changes in the non-synonymous residues in fetuin-A [26-28]. If a substitution leads to a decrease in protein stability, it is classified as potentially pathogenic or deleterious. I-Mutant 2.0, DUET, and DynaMut2 are web servers designed to predict the effects of mutations on protein stability using distinct yet complementary algorithms. I-Mutant 2.0 employs machine learning techniques trained on a large dataset of mutations, providing predictions on whether a specific mutation will increase or decrease protein stability based on structural information.

DUET integrates multi-algorithm sequence/structure data, which helps improve prediction accuracy through confidence scores, whereas DynaMut2 applies molecular dynamics simulations and normal mode analysis to identify mutations that can affect both stability and flexibility over a period of time. Taken together, these servers provide a comprehensive understanding of the functional impact of genetic variants based on both sequence and structural data. Further, the evolutionary conservation of each amino acid residue has been determined using the ConSurf web server (https://consurf.tau.ac.il/consurf_index.php) [29]. The outcome of the ConSurf analysis is categorized into three levels based on the score between 1 and 9: variable (1-4), intermediate (5-6), and conserved (7-9). Any alteration located in a conserved domain may have a significant functional impact, and thus the full-length protein sequence of fetuin-A was submitted to ConSurf to identify residues subject to high evolutionary constraint.

2.3 Prediction of Structural Effect Upon Mutation

The HOPE (Have Your Protein Explained) web server (<https://www3.cmbi.umcn.nl/hope>) was used to evaluate the structural implications of screened missense variants in fetuin-A [30]. This server utilizes data from UniProt, available 3D structures, and homology models to interpret the impact of point mutations on the structural and functional role of the protein. The amino acid sequence of fetuin-A was submitted to the server for each variant. Project HOPE provides insight into the effects of mutations by integrating a comparative analysis of biochemical properties such as size, charge, polarity and hydrogen bonding along with the three-dimensional structural context of the protein in both wild type and mutant residues. This server also gives a MetaRNN value, a deep-learning predictor that integrates functional, conservation, and allele-based scores to provide a value between 0 and 1. A value closer to 1 corresponds to the likelihood of having a deleterious effect.

2.4 Screening of Variants Using Literature Survey

The final dataset contained the primarily screened variants and other fetuin-A mutations which were reported to be associated with various disease conditions. The UniProt database was utilized to select additional variants, which were further identified by systematic literature review using various platforms such as Google Scholar and PubMed. Inclusion criteria for the selection of variants were significant evidence of clinical involvement, with a focus on fetuin-A polymorphisms

associated with CVDs and vascular pathologies. Widely cited studies and validated associations were considered as key reference standards. This rational approach to increase selectivity facilitated inclusion of both well-characterized variants and those newly emerging with potential clinical significance. This therefore ensured a strong foundation for evaluating the role of fetuin-A in disease susceptibility and progression.

2.5 Protein Structure Retrieval and Preparation

The structure of fetuin-A was obtained from the AlphaFold protein structure database (ID: AF-P02765-F1-v4), and all the chains were retained for analysis. Confidence in the model was tested using per-residue pLDDT values that are reported in the B-factor column and the predicted aligned error (PAE) matrix. Global mean pLDDT was 76.1. The two cystatin fetuin-A-type domains (residues 27-255) as a structured core were predicted with high confidence (mean pLDDT 92.0), while the signal peptide (residues 1-18, mean 58.9), intrinsically disordered C-terminal 256-298, and C-terminal chain B segment (341-367) were of low confidence (mean pLDDT 35-64). Contiguous sequences with pLDDT values <70 were residues 1-25, 210-217, 252-309, and 321-354. Analysis of the PAE matrix revealed consistently low error for residues 1-255 and high error for all downstream residues relative to this region, implying that the core is anticipated as a single rigid unit, but the position of the C-terminal region relative to the core is not predicted. All eight substitutions in the core (I48T, I80S, I82T, E86G, R103S, F125S, V142G and M248T) are at positions of very high confidence (pLDDT 94-98); S256T is at the beginning of the disordered region and is at a position of very low confidence (pLDDT 33.4). The structure was imported into the Schrödinger Suite (Maestro v14.0.136) for subsequent processing [31]. Initial cleanup involved the removal of heteroatoms, if present.

Protein preparation was performed using the Protein Preparation Wizard of the Schrödinger Suite 2024-2 within Maestro. Missing side chains were completed, and appropriate bond orders were assigned using the Chemical Component Dictionary (CCD). Hydrogen atoms were added, disulfide bonds were formed, and missing loops were modeled using the Prime module [32].

The protonation states of titratable residues were assigned based on PROPKA predictions at pH 7.4 ± 2.0 [33, 34]. We optimized hydrogen-bonding networks. The prepared structure was then subjected to energy minimization using the OPLS4 force field [35]. Additionally, all water molecules located more than 5 Å from heteroatoms were removed to reduce non-essential solvent noise.

2.6 Mutagenesis

Single-point mutations were created in silico using the residue and loop mutation tool implemented in Maestro. The target residues were selected from the workspace and mutated to the desired amino acids (Figure 2). Each mutant structure underwent advanced refinement, including gas-phase minimization of the entire protein to ensure structural relaxation post-mutation. This particular process was necessary to resolve any steric clashes and truncated geometry following residue substitution.

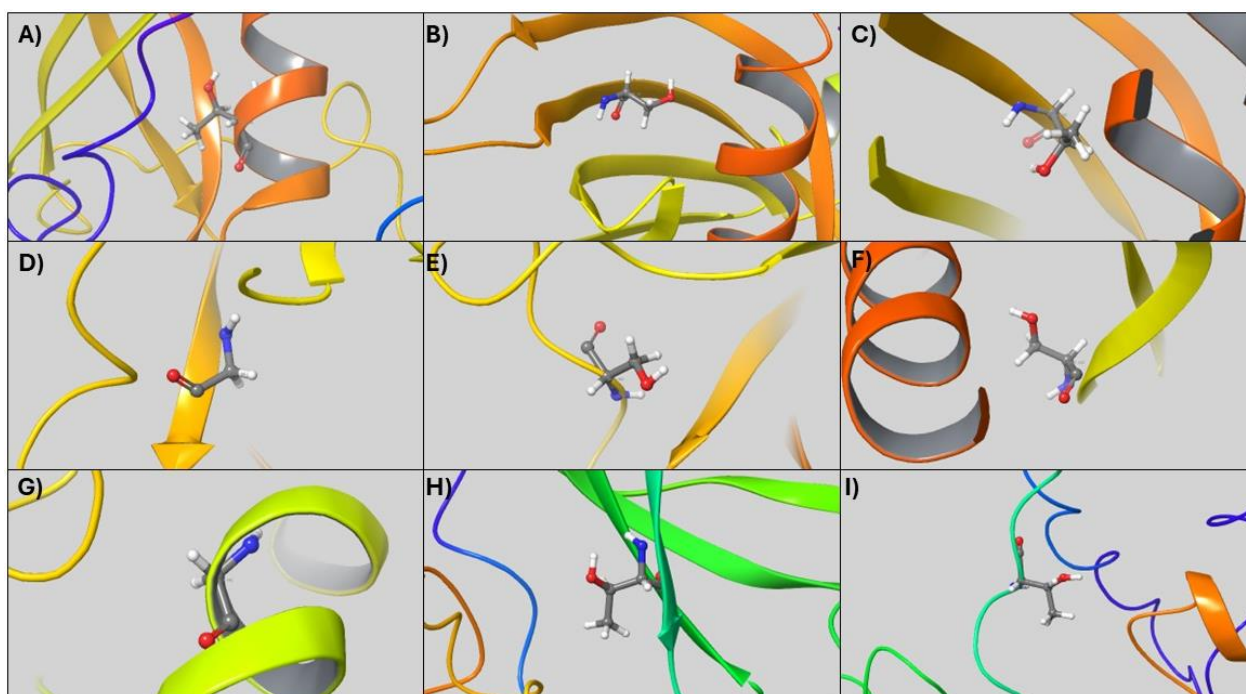


Figure 2 Single Point Mutations in Wild type to generate variants. This figure shows the 3D structural changes introduced into fetuin-A after single amino acid substitutions modeled using Schrödinger Maestro. Each panel (A-I) highlights one mutation, where the original amino acid was replaced with the specified variant. The mutated residue is displayed in ball-and-stick form to clearly visualize its position, while the overall protein structure is shown in ribbon format to illustrate its structural context. (A) Isoleucine at 48th position replaced by Threonine; (B) Isoleucine at 80th position replaced by Serine; (C) Isoleucine at 82nd position replaced by Threonine; (D) Glutamate at 86th position replaced by Glycine; (E) Arginine at 103rd position replaced by Serine; (F) Phenylalanine at 125th position replaced by Serine; (G) Valine at 142nd position replaced by Glycine; (H) Methionine at 248th position replaced by Threonine; (I) Serine at 256th position replaced by Threonine.

2.7 Molecular Dynamics Simulation

The Structural stability and dynamic nature of both wild-type and mutant forms of fetuin-A were investigated by molecular dynamics (MD). The Desmond simulation package developed by D. E. Shaw (<https://www.deshawresearch.com/>) was applied to assess all simulations [36], as implemented in the Schrödinger Suite (Maestro v14.0.136). The complete workflow was designed in two distinct stages. In the first stage, system setup was performed using the System Builder tool in Maestro. Each protein structure was solvated in an orthorhombic box with a $10 \times 10 \times 10$ Å buffer distance using the simple point charge (SPC) water model. Fourteen sodium (Na^+) ions were added to neutralize each system, followed by the addition of 0.15 M NaCl to mimic physiological conditions. The number of atoms varied slightly between systems: the wild-type system consisted of 105,111 atoms, whereas the mutant systems contained 101,428 (I48T), 101,782 (I80S), 101,926 (I82T), 101,559 (E86G), 101,514 (R103S), 101,670 (F125S), 101,310 (V142G), 101,757 (M248T), and 101,706 (S256T) atoms, respectively. In the second stage, production MD simulations were run for 100

nanoseconds under NPT ensemble conditions at a constant temperature of 300 K and pressure of 1.01325 bar [37]. Long-range electrostatic interactions were treated using Desmond's periodic electrostatics implementation, while short-range non-bonded interactions employed a 9.0 Å cutoff. We used the RESPA integrator with a 2-fs time step. The Nosé-Hoover chain thermostat with a relaxation time of 1 picosecond and the Martyna-Tobias-Klein barostat with a 2 picosecond relaxation time were applied for temperature and pressure coupling, respectively, along with a random seed of 2007 [38, 39]. No positional restraints were applied during production. An energy recording interval of 1.2 ps was applied for non-bonded interactions. Simulation trajectories were recorded every 100 picoseconds, generating 1,000 frames per simulation. Structural stability and convergence were assessed from the temporal behaviour and plateauing of protein backbone RMSD, residue-wise RMSF, Rg, and SASA. Post-simulation, trajectory data were analyzed using the Simulation Interaction Diagram tool in Desmond to evaluate structural deviations and fluctuations over time, focusing on parameters such as root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), and solvent-accessible surface area (SASA) to characterize the comparative stability and flexibility of each protein variant relative to the wild-type structure.

2.8 Protein-Protein Docking

BMP-10 and BMPR (PDB ID: 7PPA) were prepared following the same protocol as section 2.5. The Protein-Protein Docking Wizard from the Schrödinger Suite, which utilizes PIPER, was used for the docking of BMPR2 with BMP-10, followed by docking of the BMPR-BMP-10 complex with fetuin-A (wild type and mutant) and BMP-10 with fetuin-A (wild type and mutant). Standard mode of docking was utilized, generating 30 outputs after the refining process, along with a number of ligand rotations set to 70000. No attraction/repulsion, residue-specific, or distance restraints were applied, allowing the proteins to be docked without imposing a predefined binding interface. Following standard practice for PIPER-based docking, the primary parameter is the population (cluster size) of the top-ranked cluster, since cluster population reflects the breadth of the sampled basin around a binding mode and is more robust than the score of any single pose.

2.9 Analysis of MD Trajectories

Both WT fetuin-A and mutants underwent a single 100 ns MD simulation, generating 1000 trajectory frames per individual run. RMSD, RMSF, Rg, and SASA values were observed to study the conformational behavior of each individual system. As frames from a single trajectory are temporally correlated, the data are presented descriptively as mean $\pm$ SD.

3. Results

3.1 Retrieval of SNP Substitution

The Ensembl database contains comprehensive information regarding polymorphisms in the fetuin-A gene. Within this database, a total of 3,875 variant alleles have been reported for fetuin-A, including 2,861 located in intronic regions, 398 missense nsSNPs variants, 153 synonymous SNPs, and the remainder classified into other categories (S1). In our study, we focused exclusively on missense variants, as their occurrence may lead to changes in the structure or function of proteins.

3.2 Screening of the Variants

To sort out the deleterious/damaging SNPs from the 398 missense SNPs, first we utilized the PredictSNP web server as the first filtration process. PredictSNP provides a probability score that indicates the likelihood of a single nucleotide polymorphism being neutral or damaging/deleterious to protein function. A cut-off value of 50 was established, with SNPs predicted to be the most deleterious identified as those having a prediction score exceeding 50. We identified 69 variants as highly deleterious based on their predictive consensus scores. Subsequently, these selected SNPs underwent a secondary filtration process focused on stability and conservation analysis (S2).

In the second filtration step, the effects of amino acid substitutions on protein stability were evaluated using three computational tools: I-Mutant 2.0, DUET, and DynaMut2. These tools were applied to the deleterious SNPs identified in the first filtration phase to predict changes in protein stability based on sequence- or structure-derived features. I-Mutant 2.0, a support vector machine (SVM)-based predictor, estimates changes in Gibbs free energy ($\Delta\Delta G$) upon mutation, with negative $\Delta\Delta G$ values indicating destabilization. DUET integrates mCSM and SDM algorithms to provide $\Delta\Delta G$ values, where negative values suggest a decrease in stability. DynaMut2 combines normal mode analysis with energy-based predictions to assess both flexibility and stability, also reporting $\Delta\Delta G$ scores and visualizing dynamic perturbations in the protein structure. In this analysis, variants with $\Delta\Delta G \leq -1.5$ kcal/mol (in at least two of the three predictions) were considered significantly destabilizing. Based on this threshold, a total of 17 variants were identified for further evaluation (S2).

Simultaneously, the ConSurf tool was used to predict evolutionary conservation sites. An evolutionarily conserved protein residue is usually considered damaging when compared with residues in non-conserved positions. Analysis of the ConSurf web server indicated a highly conserved nature of the SNPs, with the highest conservancy scores, e.g., 9 (Figure 3). Based on the parameters of protein stability and conservation, we prioritized variants using a combined assessment of conservation analysis and stability (Table 1). This filtering process identified seven variants-I48T, I80S, I82T, E86G, R103S, V142G, and F125S for further evaluation. Although I80S and I82T had ConSurf values of 4 and 6, respectively; they were selected because they had negative $\Delta\Delta G$ scores and PredictSNP scores. These selected variants were subsequently analyzed to assess their structural impact.

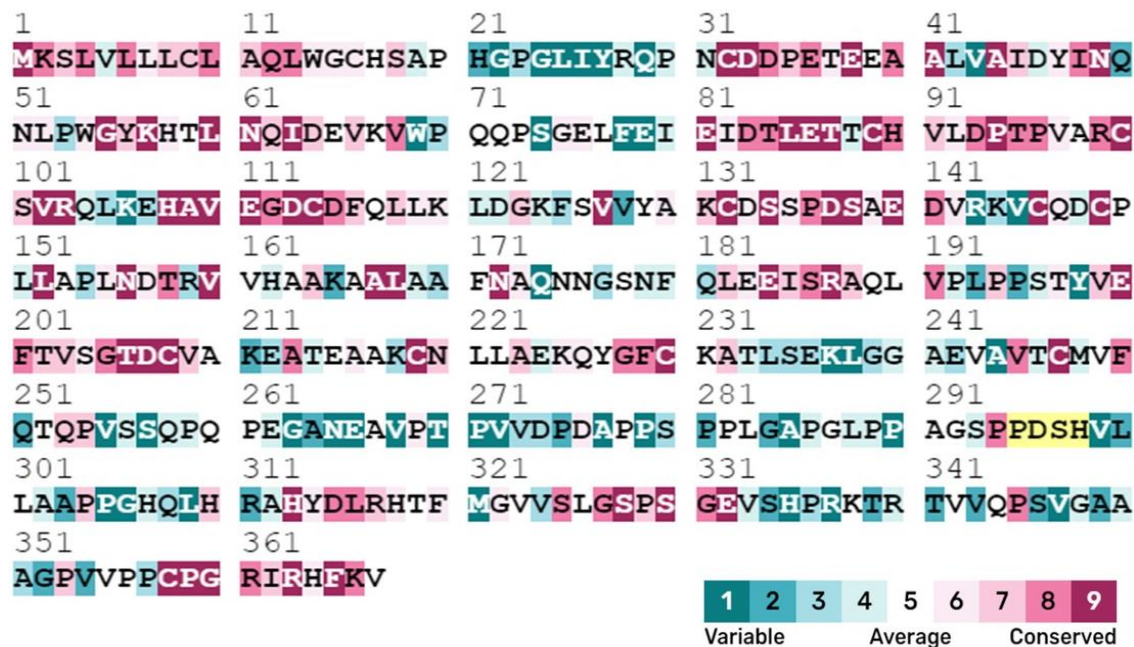


Figure 3 Conservation Analysis. This figure illustrates the evolutionary conservation pattern of the fetuin-A protein generated using the ConSurf server. Each amino acid is color-coded based on how conserved it is across related species-blue indicates variable residues, while magenta marks highly conserved and potentially functionally important positions. Yellow residues represent sites where a reliable conservation score could not be determined.

Table 1 Integrated computational prediction of prioritized variants in the AHS (fetuin-A) gene. The table presents the computational analysis of prioritized variants that met the functional and structural classification criteria. The results are depicted as the PredictSNP consensus score. High scores represent greater confidence in the variant’s deleterious impact. Correlation between each variant and protein stability ($\Delta\Delta G$ in kcal/mol) was predicted using DynaMut2, I-Mutant 2.0, and DUET, where negative values indicate destabilizing effects. Evolutionary conservation was analyzed using ConSurf (scale 1-9), where higher values reflect stronger conservation. MetaRNN applies a deep learning framework to predict pathogenicity, with scores closer to 1 indicating an increased likelihood that the variant can have a deleterious impact.

S. No	Variant	PredictSNP Score (%)	I-Mutant 2.0 ($\Delta\Delta G$ Kcal/mol)	DUET ($\Delta\Delta G$ Kcal/mol)	DynaMut2 ($\Delta\Delta G$ Kcal/mol)	ConSurf Score	MetaRNN Score
1.	I48T	87	-4.43	-3.13	-2.71	8	0.76
2.	I80S	72	-1.63	-3.57	-3.06	4	0.58
3.	I82T	76	-1.77	-3.14	-2.52	6	0.85
4.	E86G	87	0.19	-2.62	-2.13	9	0.93
5.	R103S	87	-2.3	-2.70	-1.73	9	0.97
6.	V142G	87	-2.77	-2.89	-2.09	7	0.72
7.	F125S	51	-2.6	-2.88	-2.46	7	0.86

3.3 Analysis of Structural Effects of Mutations

To further screen the structural and functional impact of the selected variants, Project HOPE was used. This web-based tool combines sequence and structural information to assess how point mutations alter the key properties of proteins. Our analysis showed that substitutions caused marked changes in physicochemical properties such as size, polarity, and charge, indicating a mechanism by which stability and molecular interactions of proteins may be reduced. Further, we applied MetaRNN, which integrates multiple algorithms with structural and functional annotations to generate a score for each variant. The outcome of this comprehensive analysis indicated that several variants carry significant potential to affect the structural integrity and functional capacity of fetuin-A, considering that scores close to 1 indicate a high probability of functional impairment. The I48T substitution created a destabilizing void by replacing a core residue with a smaller polar alternative, leading to probable structural and functional impairment. Similarly, the E86G mutation disrupted proper protein folding by replacing the charged glutamate with a small hydrophobic glycine, abolishing critical H-bonds and eliminating polarity. Also, the R103S mutation destabilized ionic interactions and disrupted external interactions by eliminating salt bridges by replacing a large, polar, positively charged residue with a smaller, neutral, and polar residue. Four substitutions (I80S, I82T, V142G, and F125S) replaced bulky residues with smaller residues. Among these, the V142G mutation was particularly significant, as the newly introduced glycine acted as a flexible hinge that disrupted overall protein rigidity. According to MetaRNN predictions, all seven variants indicated a high risk of deleterious effects; however, E86G and R103S showed maximum scores (0.93 and 0.97), suggesting their crucial role in structural and functional impairment (Table 1).

3.4 Screening of Variants Using Literature Survey

The UniProt database, PubMed Central, and Google Scholar were screened to identify clinically relevant variants of fetuin-A. This analysis revealed four variants, M248T, S256T, R317H, and R317C, which were significantly associated with disease susceptibility. We chose to further analyze S256T (rs4918; allele frequency 0.6604) and M248T (rs4917; allele frequency 0.6646) variants among these four variants because the two variants have been specifically linked to CVDs and therefore would be relevant candidates for further analysis with BMP-10, as it is an emerging CVD biomarker.

3.5 Molecular Dynamics Analysis

Molecular dynamics (MD) simulations were performed to study the impact of each point mutation on the structure and dynamics of fetuin-A. Various key factors such as solvent-accessible surface area (SASA), radius of gyration (Rg), root-mean-square deviation (RMSD), and root-mean-square fluctuation (RMSF) were examined by MD simulation. We compared wild-type and variants to assess changes in compactness, stability, and flexibility (Table 2).

Table 2 The table presents molecular dynamics simulation results. Parameters calculated were the solvent accessible surface area (SASA), radius of gyration (Rg), root mean square deviation (RMSD), and root mean square fluctuation (RMSF) values for the protein backbone, C α atoms, and side chains across wild-type and variants of fetuin-A.

S. No	Variant	SASA (Å ²)	Rg (Å)	RMSD backbone (Å)	RMSD C- α (Å)	RMSD sidechain (Å)	RMSF backbone (Å)	RMSF C- α (Å)	RMSF sidechain (Å)
1.	WT	21,141.625 $\pm$ 47.32	24.73 $\pm$ 0.050	12.45 $\pm$ 0.082	12.57 $\pm$ 0.083	13.15 $\pm$ 0.084	3.97 $\pm$ 0.172	3.99 $\pm$ 0.175	4.38 $\pm$ 0.182
2.	I48T	22,005.029 $\pm$ 32.87	26.44 $\pm$ 0.024	11.96 $\pm$ 0.04	12.09 $\pm$ 0.040	12.93 $\pm$ 0.043	3.52 $\pm$ 0.136	3.53 $\pm$ 0.138	3.92 $\pm$ 0.145
3.	I80S	21,049.958 $\pm$ 41.40	24.86 $\pm$ 0.037	12.47 $\pm$ 0.052	12.61 $\pm$ 0.053	12.93 $\pm$ 0.043	3.47 $\pm$ 0.123	3.48 $\pm$ 0.126	3.90 $\pm$ 0.133
4.	I82T	21,442.112 $\pm$ 43.12	25.49 $\pm$ 0.051	12.61 $\pm$ 0.053	13.30 $\pm$ 0.060	13.70 $\pm$ 0.055	3.65 $\pm$ 0.163	3.00 $\pm$ 0.109	4.04 $\pm$ 0.169
5.	E86G	22,248.321 $\pm$ 24.72	26.86 $\pm$ 0.057	11.59 $\pm$ 0.053	11.64 $\pm$ 0.053	11.94 $\pm$ 0.050	4.03 $\pm$ 0.132	4.04 $\pm$ 0.133	4.44 $\pm$ 0.141
6.	R103S	21,314.493 $\pm$ 49.35	25.47 $\pm$ 0.053	12.80 $\pm$ 0.056	12.90 $\pm$ 0.056	13.28 $\pm$ 0.056	3.95 $\pm$ 0.180	3.97 $\pm$ 0.183	4.40 $\pm$ 0.192
7.	F125S	21,220.925 $\pm$ 44.10	25.86 $\pm$ 0.058	12.38 $\pm$ 0.057	12.46 $\pm$ 0.057	12.73 $\pm$ 0.055	3.16 $\pm$ 0.147	3.17 $\pm$ 0.150	3.56 $\pm$ 0.158
8.	V142G	21,255.571 $\pm$ 34.41	25.51 $\pm$ 0.051	12.24 $\pm$ 0.045	12.32 $\pm$ 0.045	12.70 $\pm$ 0.047	3.65 $\pm$ 0.130	3.66 $\pm$ 0.132	4.10 $\pm$ 0.140
9.	M248T	20,999.231 $\pm$ 31.91	25.36 $\pm$ 0.036	13.00 $\pm$ 0.046	13.13 $\pm$ 0.046	13.42 $\pm$ 0.046	2.98 $\pm$ 0.122	2.99 $\pm$ 0.125	3.42 $\pm$ 0.134
10.	S256T	21,726.003 $\pm$ 35.46	24.71 $\pm$ 0.037	14.61 $\pm$ 0.060	14.76 $\pm$ 0.060	15.49 $\pm$ 0.061	3.37 $\pm$ 0.151	3.39 $\pm$ 0.153	3.80 $\pm$ 0.159

3.5.1 Solvent Accessible Surface Area (SASA)

The wild-type protein became stable around a SASA value of $\sim 21,000 \text{ \AA}^2$ after reaching equilibration. Several mutants, including I80S, I82T, F125S, and V142G, had comparable SASA values, suggesting no major change in overall compactness. In contrast, I48T, E86G, and S256T maintained a higher SASA throughout the simulation, indicating greater solvent exposure; for E86G, this increase is likely due to the tendency of glycine to disrupt the local secondary structure. Only I80S and M248T showed slightly lower SASA values than the wild type. The R103S mutant presented SASA values similar to those of wild-type, suggesting no major change in overall compactness (Figure 4).

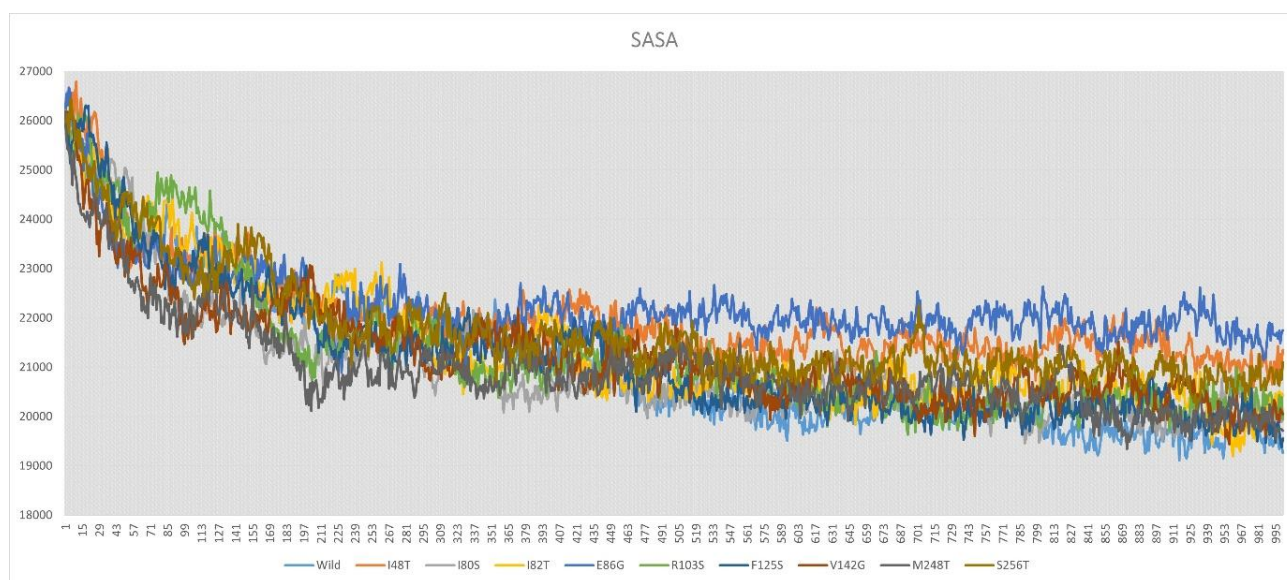


Figure 4 Solvent Accessible Surface Area (SASA) analysis. Solvent Accessible Surface Area (SASA) analysis of a 100 ns MD simulation ($n = 1$ independent MD simulation per system) of wild-type and mutant fetuin-A proteins. Comparative SASA profiles reveal differences in surface exposure and potential conformational changes among wild-type and variants of fetuin-A.

3.5.2 Radius of Gyration (Rg)

The radius of gyration was employed to evaluate the overall compactness of each system. The average Rg for the wild-type protein was $24.73 \text{ \AA}$ and the tight spread (SD $0.050 \text{ \AA}$) shows a steady overall dimension over the window studied. The highest expansion was observed for the variants E86G and I48T, with mean Rg values of $26.86 \text{ \AA}$ and $26.44 \text{ \AA}$, respectively, $2.13 \text{ \AA}$ and $1.71 \text{ \AA}$ higher than the wild type. F125S showed a modest but constant increase ($25.86 \text{ \AA}$), whereas I82T, R103S, V142G and M248T fell in an intermediate range of 25.36 - $25.51 \text{ \AA}$. I80S ($24.86 \text{ \AA}$) and S256T ($24.71 \text{ \AA}$) were closest to the wild type, the latter slightly below it. The increase in Rg for E86G is consistent with its increased solvent accessible surface area ($22,248 \text{ \AA}^2$, the highest of the set) and the higher local flexibility observed in the RMSF profile, and supports a more open conformation due to the substitution of a charged glutamate with glycine. The same is true for I48T, which has the second greatest Rg and the second highest SASA. No system had an Rg increase commensurate with

unfolding, suggesting that the substitutions affect packing at the local level rather than impairing the overall fold (Figure 5).

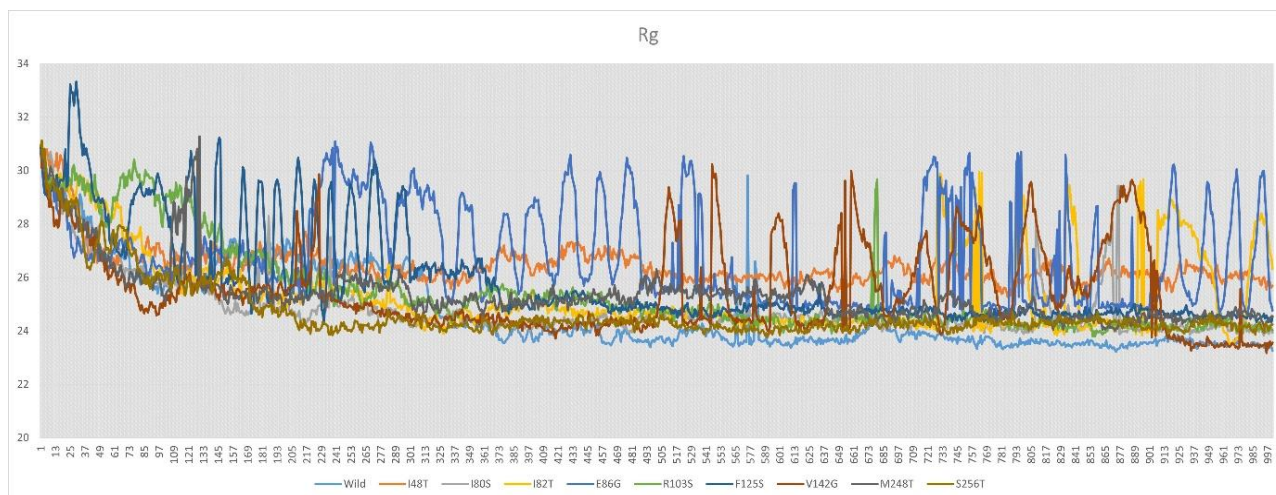


Figure 5 Radius of Gyration (Rg) analysis. Radius of Gyration (Rg) analysis of a 100 ns MD simulation ($n = 1$ independent MD simulation per system) of wild-type and mutant fetuin-A proteins. The Rg profiles illustrate the overall compactness and structural stability of wild-type and fetuin-A variants.

3.5.3 Root-Mean-Square Deviation (RMSD)

Backbone and C-alpha RMSD were utilized to quantify conformational drift from the initial structure. The wild-type protein displayed average backbone and C-alpha RMSD values of 12.45 Å and 12.57 Å, respectively. E86G had the lowest deviation of the set (11.59 Å backbone, 11.64 Å C-alpha) followed by I48T (11.96 Å and 12.09 Å), showing that the local rearrangements mentioned above took place without a corresponding rise in global backbone displacement. V142G (12.24 Å) and F125S (12.38 Å) were slightly lower than the wild type, while I80S (12.47 Å) and I82T (12.61 Å) were essentially indistinguishable from the wild type (0.21 Å or less in each case). R103S (12.80 Å) and M248T (13.00 Å) showed a slight increase.

The most obvious outlier was S256T with backbone and C-alpha RMSD values of 14.61 Å and 14.76 Å respectively, which were 2.16 Å and 2.19 Å higher than wild type and at least 1.61 Å higher than all other variants. The same ordering was observed for the side-chain RMSD, where S256T reached 15.49 Å against 13.15 Å for the wild type. The side-chain values were larger than the backbone values in all ten systems, as expected from the greater mobility of the side chains relative to the backbone, with the relative ranking of the systems conserved between the two measures (Figure 6).

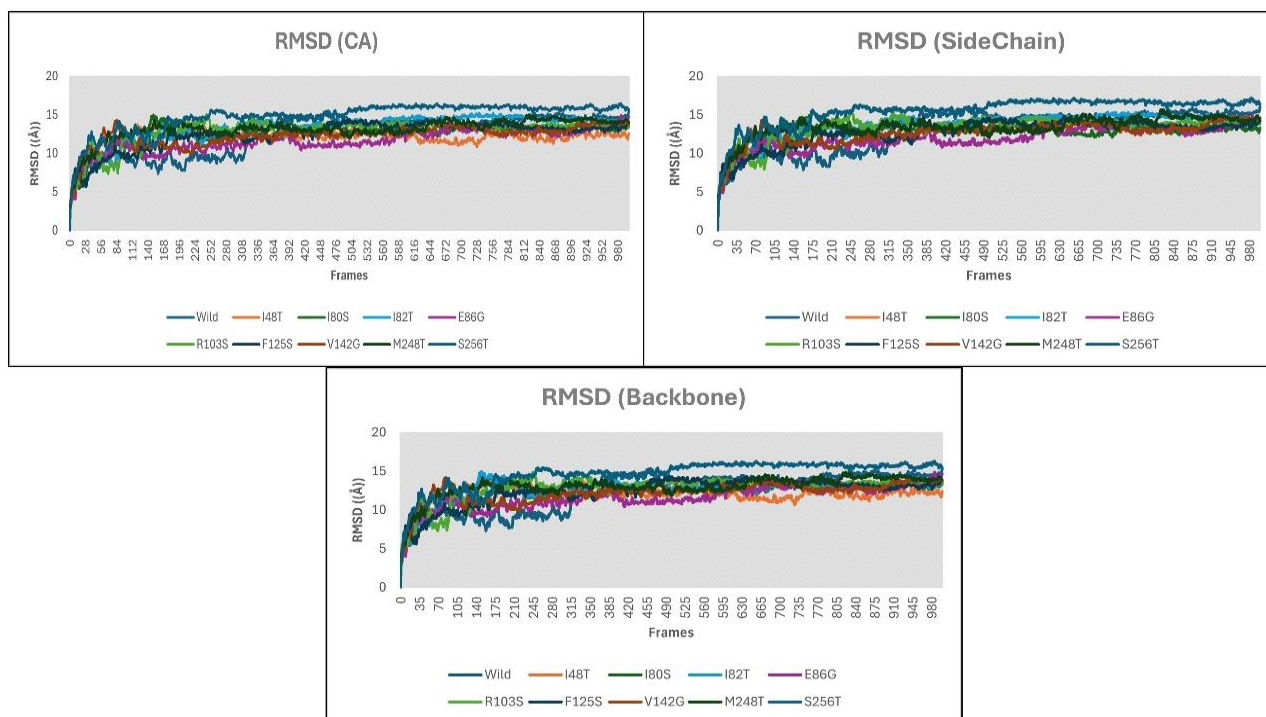


Figure 6 Root-Mean-Square Deviation (RMSD) analysis. RMSD analysis of 100 ns MD simulation ($n = 1$ independent MD simulation per system) of wild-type and mutant fetuin-A proteins. Structural deviations were assessed for C α atoms, side chains, and protein backbone across wild-type and variants of fetuin-A.

3.5.4 Root-Mean-Square Fluctuations (RMSF)

Most variants exhibited greater flexibility in the N-terminal and some loop regions, and the core β -strands remained relatively rigid. E86G showed higher fluctuations around residues 80-95, and R103S showed reduced flexibility around residue 103, which corresponds to the introduction of glycine at position 86 and the addition of new hydrogen bonding by serine, respectively. Variants such as I48T, I82T, and F125S showed decreased fluctuations in their local regions, indicating their role in stabilization of the surrounding structure. V142G, M248T, and S256T showed negligible changes in local flexibility compared with the wild type (Figure 7).

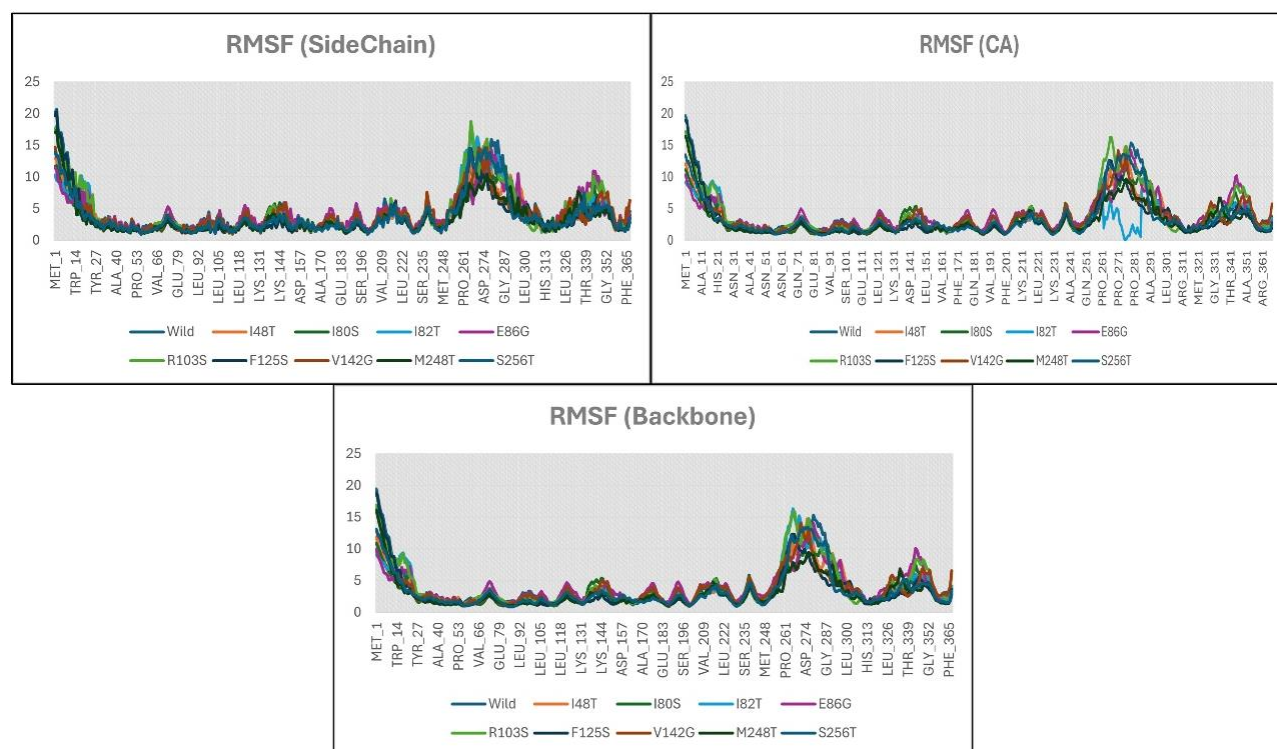


Figure 7 Root-Mean-Square Fluctuations (RMSF) analysis. RMSF analysis of 100 ns MD simulation ($n = 1$ independent MD simulation per system) of wild-type and mutant fetuin-A proteins. Residue-wise fluctuations were assessed for C α atoms, side chains, and protein backbone across wild-type and variants of fetuin-A.

3.6 Protein-Protein Docking Analysis

PIPER docking scores depicting residue-residue and residue-ligand interactions between BMP10 and fetuin fragments (Table 3). The docking of the BMPR2-BMP-10 complex (backbone) with fetuin A depicts that the wild-type (WILD) and F125S variants form a broad and contiguous interaction interface (Figure 8Aa). These observations are corroborated by their larger cluster sizes (132 and 88). Furthermore, the corresponding fingerprint indicates robust binding with the receptor's primary structural anchor hubs. Conversely, mutations such as I80S and I48T exhibit substantial interaction lapses, preventing the fetuin-A backbone from binding effectively to the receptor. This disruption reduces the cluster size to 76, indicating a less stable docking conformation in which the molecule fails to achieve a stable inhibitory orientation. The interaction of the BMPR2-BMP complex (side chains) with fetuin-A is presented in Figure 8Ab. Here, the R103S mutation shows a significant decrease in the side-chain fingerprint. These findings point to the fact that replacing arginine with serine breaks salt bridges that are essential for receptor complex binding. In contrast, F125S retains a dense side-chain interaction profile. This suggests that serine at this position does not disrupt local structure. Due to this, this variant is the most stable in the dataset; however, analysis of the BMP dimer backbone interaction with fetuin-A (Figure 8Ba) revealed a different pattern. The fragmented interaction pattern displayed by E86G underscores the critical role of residue 86 for effective fetuin-A binding to the BMP backbone. By comparison, I82T displays a denser interaction fingerprint than the baseline. These findings imply that, in the absence of a receptor, residues 82 and 125 of fetuin-A serve as the main mediators of growth factor sequestration. Finally, examination of the BMP

dimer (sidechain) (Figure 8Bb) interaction with fetuin-A provides further confirmation of the trends observed previously. The E86G and I48T variants show fewer interactions. In stark contrast, the I82T variant displays a significantly higher number of interactions. These findings indicate that threonine facilitates more favourable coordination with the BMP dimer as compared to the native isoleucine.

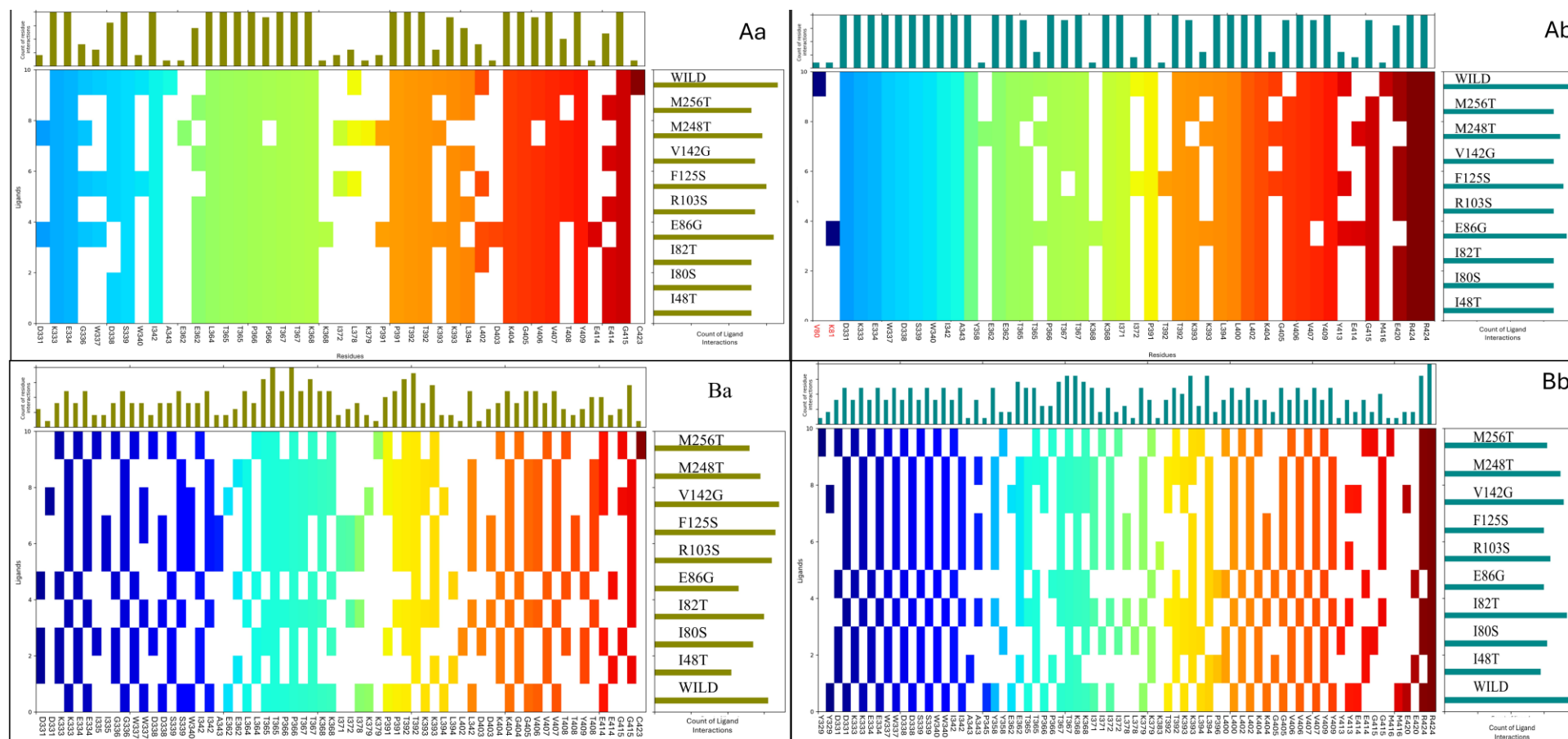


Figure 8 PIPER docking scores depicting residue-residue and residue-ligand interactions between BMP10 and fetuin fragments. The graph compares docking energies for backbone and side-chain interaction modes in different orientations. Aa represents BMPR-BMP10-FETUIN BACKBONE interaction, Ab represents BMPR-BMP10-FETUIN SIDECHAIN, Ba represents BMP10-FETUIN BACKBONE, and Bb represents BMP10-FETUIN SIDECHAIN.

Table 3 The table summarizes the cluster size, mean PIPER pose energy, and mean PIPER pose score for BMP-BMPR complexes and their variants, as determined by protein-protein docking. These parameters allow for a systematic comparison of putative interaction and clustering behaviour across wild-type and mutant complexes.

S. No	Name of Cluster	Cluster Size	Mean PIPER pose Energy	Mean PIPER pose Score
1	BMP10-BMPR	118	-697.53	-259.098
2	BMP10-BMPR-WT FET-A	132	-1343.94	-446.714
3	BMP10-BMPR-I48T	76	-1216.248	-397.55
4	BMP10-BMPR-I80S	76	-1166.938	-386.577
5	BMP10-BMPR-I82T	77	-1203.905	-383.475
6	BMP10-BMPR-E86G	79	-1294.696	-490.509
7	BMP10-BMPR-R103S	77	-1176.949	-376.724
8	BMP10-BMPR-F125S	88	-1348.50	-427.773
9	BMP10-BMPR-V142G	75	-1180.055	-391.662
10	BMP10-BMPR-M248T	126	-1253.985	-427.16
11	BMP10-BMPR-S256T	82	-1181.442	-390.603
12	BMP10-WT FET-A	123	-1310.635	-535.992
13	BMP10-I48T	98	-1059.95	-301.447
14	BMP10-I80S	90	-1273.91	-363.993
15	BMP10-I82T	82	-1408.818	-458.997
16	BMP10-E86G	99	-997.103	-289.302
17	BMP10-R103S	90	-1251.304	-372.53
18	BMP10-F125S	107	-1314.451	-398.056
19	BMP10-V142G	111	-1095.522	-481.253
20	BMP10-M248T	118	-1283.472	-506.939
21	BMP10-S256T	85	-1150.483	-428.274

4. Discussion

Fetuin-A is a hepatokine encoded by the AHSB gene. It plays a crucial role in maintaining stable levels of calcium and phosphate in the body. Fetuin-A strongly inhibits ectopic calcification by forming fetuin-mineral complexes (FMCs) with calcium phosphate crystals, which helps prevent these crystals from depositing in soft tissues. When fetuin-A is not properly regulated or is deficient, it has been linked to various disorders related to calcification, such as vascular calcification, chronic kidney disease (CKD), and atherosclerosis [1, 8]. Our previous study demonstrated that fetuin-A deficiency in patients with Rheumatic heart disease (RHD) may contribute to cardiac valve calcification. We reported that, in the Indian population, reduced serum fetuin-A levels were associated with cardiac valve calcification in RHD patients, suggesting its potential as a predictive biomarker in CVD [40]. Genetic variations, particularly single nucleotide polymorphisms (SNPs) within the AHSB gene, have been linked to changes in the expression, structure, and function of fetuin-A [41]. Bone morphogenetic protein-10 (BMP-10) is a ligand involved in cardiac and vascular tissues. It plays a key role in regulating endothelial stability, vascular remodeling, and BMPR2-

mediated signaling [22]. Given the ability of fetuin-A to bind and sequester extracellular ligands, it is plausible that genetic variation in AHSN could alter fetuin-A structure and its interactions, and how it behaves in the extracellular milieu. This can disrupt BMP-10 bioavailability and signaling potential, thereby modulating cardiovascular function and disease susceptibility.

A comprehensive *in silico* analysis including functional prediction, protein stability assessment, evolutionary conservation analysis, molecular dynamics (MD) simulations, and protein-protein docking was performed for all reported missense nsSNPs in AHSN to understand how AHSN variants may alter regulation of BMP-10. This strategy helped successfully categorize high-confidence variants with a plausible role in pathogenicity from all missense nsSNPs of fetuin-A. Furthermore, it provided mechanistic insight into how specific amino acid substitutions may disrupt the fetuin-A structural integrity and intermolecular interactions. MD simulations highlighted structural perturbations across variants, the S256T variant showed the most pronounced difference as compared to wild type, with its notably high RMSD values indicating increased local conformational flexibility, however Rg remained close to wild-type suggesting flexibility is localized rather than global destabilization. The E86G substitution induced elevated solvent-accessible surface area (SASA) and greater local flexibility (RMSF) in the residues 80-95 region. Notably, this region lies near the 74S phosphorylation site, a known regulator of fetuin-A in a charged environment. The structural expansion observed in the E86G variant could plausibly impact kinase accessibility or phosphorylation efficiency at this site. If reduced, it might yield a hypo-phosphorylated protein with altered affinity for basic ligands. Likewise, the V142G variant adjacent to the 156N glycosylation site displayed a moderate elevation in SASA and radius of gyration, which may result in disrupted glycan attachment. In contrast, variants like M248T have a more compact and rigid structure. Despite its general stability, this rigidity may plausibly lead to reduced flexibility of the protein for molecular interactions. This suggests that even modest, non-unfolding structural perturbations like greater rigidity or flexibility might influence the interaction of fetuin-A during signaling; this remains to be tested experimentally [42].

Interaction between wild-type and mutant fetuin-A with the BMP-BMP2 complex and the BMP-10 dimer was studied to establish a link between structural perturbations and their functional impact. The wild-type interaction showed a stable and extensive binding interface, characterized by strong interaction energies and large cluster sizes. This stable binding interaction is consistent with the ability of fetuin-A to effectively inhibit BMP-10 signaling. The above findings show that the variant F125S efficiently interacts with BMP-10 while retaining protein functionality. These findings suggest that residue 125 may contribute to the putative interaction with BMP-10 and influence the predicted docking mode while maintaining protein function. By contrast, the I48T and I80S variants displayed fragmented interaction patterns and reduced cluster sizes, which indicates impaired backbone alignment with the receptor complex. Despite their structural compactness in simulations, these variants failed to align with the BMP2 complex and thus point to a mechanism whereby increased local rigidity likely constrains the flexibility required for fetuin-A to interact effectively with the BMP-10 dimer. Sidechain-specific docking showed that the R103S substitution altered key electrostatic interactions, which further weakened the fetuin-A-BMP-BMP2 interface. E86G variant had the weakest interaction with the BMP-10 dimer, marked by unfavourable pose energies and fragmented contacts. These results are in line with MD findings that showed increased local flexibility and increased solvent exposure at this position. Therefore, residue 86 may contribute to the putative BMP-10 interaction and influence the predicted docking mode. By contrast, the

enhanced binding density and favorable energetics of the I82T variant point to an important role for residue 82 in direct BMP-10 engagement when the receptor complex is absent. BMP-10 is a well-documented circulating biomarker for atrial stress and cardiac remodeling in chronic heart conditions. Therefore, these interaction patterns emphasize clinical relevance and are imperative to understand the cardiovascular profile and disease progression. It has shown prognostic value for negative cardiovascular outcomes, supporting BMP-10's role in pathological cardiac remodeling. Experimental studies also indicate that BMP-10 provides cardioprotective effects by promoting cardiomyocyte survival and reducing fibrosis [43, 44].

Collectively, the integrated MD and docking analysis demonstrate that specific AHSG variants, particularly E86G, I48T, I80S, and R103S, are most likely to compromise the ability of fetuin-A to limit BMP-10 activity. Under physiological stressors like inflammation and oxidative damage, problems with fetuin-A could plausibly contribute to unopposed BMP-10 signaling. This may lead to endothelial dysfunction, abnormal blood vessel remodeling, and disrupted cardiovascular balance. BMP-10 has been shown to regulate endothelial function and vascular signaling beyond development. Dysregulated BMP-10 and BMPR2 signaling is involved in vascular diseases. Also, changes in extracellular BMP signaling pathways are connected to issues with endothelial function and heart disease. This interpretation is compatible with clinical knowledge of these two types. M248T and S256T are the variants identified in our literature survey as associated with cardiovascular and metabolic phenotypes, and these associations have generally been attributed to altered circulating fetuin-A concentration and protein processing rather than to disruption of a defined binding interface. This view is supported by our observation that both variants affect the flexible C-terminal region and leave the predicted BMP-10 interface mostly untouched, mechanistically distinguishing them from cystatin domain variants E86G and R103S, which directly perturb the predicted interaction surface. The combined effects of genetic variations and physiological abnormalities may worsen the impact of structural changes in fetuin-A on BMP-10-mediated signalling pathways [24, 45]. Revealing the role of nsSNPs in this context may provide insight into the molecular mechanisms through which fetuin-A may alter, buffer, or regulate BMP-10-mediated signalling and cardiovascular homeostasis. This may impact BMP-10 bioavailability and receptor binding, which further may trigger a cascade leading to endothelial dysfunction, aberrant vascular remodelling, and increased risk of CVDs.

5. Limitations

The current study is designed as a computational approach to characterize missense variants of fetuin-A and generate hypotheses about their potential interaction with BMP-10; therefore, several limitations should be considered when interpreting the findings of the study.

Firstly, structural analysis should be interpreted in the context of post-translational modifications (PTMs). The protein model is without proteolytic processing, phosphorylation, or glycosylation, which influence the protein properties *in vivo*. As already mentioned above in the discussion, variants like E86G lie near the 74S phosphorylation site, and V142G lies adjacent to the 156N glycosylation site. Therefore, the predicted structural effects and docking interactions should be regarded as computational hypotheses requiring further experimental validation rather than definitive functional outcomes. Also, S256T lies within a low-confidence region of the AlphaFold model; therefore, predictions at this position should be treated accordingly. This can explain why

S256T showed the largest RMSD deviation among all variants yet only a modest change in docking stability. Regions with low pLDDT are intrinsically less well-defined structurally and tend to give a wider conformational space during simulation, which inflates RMSD independent of any specific functional consequence. The greater conformational value is independent of functional consequences.

Secondly, although present in our data from the Ensembl database, rs4917 and rs4918 were not selected by our filtration strategy but were still included due to their clinical relevance. Our study does not negate their clinical relevance. Still, we would like to address that biological systems are more complex and dynamic and involve multifactorial processes like PTMs and tissue-specific interactions in their microenvironment. These processes cannot be fully captured by sequence- or structure-based computational predictors alone, which makes this divergence expected rather than contradictory. We would like to state that this study is complementary to wet lab studies, as it will help identify plausible candidates to narrow the pool of selection for experimental validation.

Thirdly, this study is a systematic *in silico* screening of fetuin-A variants and aims to identify variants that might have clinical relevance. As part of the analysis, we propose an interaction between fetuin-A and BMP-10, based on the established precedent that other members of the BMP family interact with fetuin-A. Experimental validation is required to confirm the functional and clinical consequences of this interaction, warranting a targeted investigation to confirm the physical association. We acknowledge that experimental validation *in vivo* would give a clear understanding of the proposed hypothesis, but that is a future scope of the study, and the current study is entirely an *in silico*-based investigation. Additionally, the docking predictions should be interpreted as hypothesis-generating rather than experimentally validated structural models.

In conclusion, these findings highlight the significance of integrating computational methods in prioritizing genetic variants of interest and promise targeted selection of appropriate candidates for further experimental design. To confirm these computational predictions, rigorous experimental validation by population-based genotype-phenotype studies is required. Our findings present a framework to establish a significant association between AHSN genetic variants and BMP-10 regulation, which can lay the groundwork to study disease susceptibility. This underscores fetuin-A not only as a diagnostic marker but also its potential utility in studying disease predisposition.

Glossary

AHSG	Alpha-2-Heremans-Schmid Glycoprotein
CKD	Chronic kidney disease
CPP	Calciprotein Particles
CVD	Cardiovascular disease
FMC	Fetuin-Mineral Complex
MAPP	Multivariate Analysis of Protein Polymorphism
MetaRNN	Meta Recurrent Neural Network
nsSNP	Non-synonymous Single Nucleotide Polymorphism
nsSNP Analyzer	Non-synonymous Single Nucleotide Polymorphism Analyzer
PANTHER	Protein Analysis Through Evolutionary Relationships
PhD-SNP	Predictor of Human Deleterious Single Nucleotide Polymorphisms
PolyPhen	Polymorphism Phenotyping

PTM	Post translational modification
Rg	Radius of Gyration
RHD	Rheumatic Heart Disease
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
SASA	Solvent Accessible Surface Area
SIFT	Sorting Intolerant from Tolerant
SNAP	Screening for Non-Acceptable Polymorphisms
SNP	Single Nucleotide Polymorphism

Author Contributions

Aishwarya Rani: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing-original draft, Writing-Review & editing. Shalesh Gangwar: Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing-original draft, Writing-Review & editing. Neha Sharma: Supervision, Writing-original draft, Writing-Review & editing. Khalid Raza: Supervision, Resources, Writing-Review & editing. Sameer Gupta: Supervision, Writing-Review & editing. Devinder Toor: Project administration, Resources, Supervision, Writing-Review & editing.

Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and are provided in its Supplementary Material.

AI-Assisted Technologies Statement

The authors acknowledge the use of GPT-5, GPT-5.4 and GPT-5.5 to assist with the overall structuring of the manuscript and Grammarly for language editing and refinement. All scientific content, interpretations, and conclusions remain the responsibility of the authors.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Supplementary Material.

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