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In Silico Functional and Structural Characterization of rs141029929 Variant in ANGPT1 Reveals Reduced Protein Stability and Potential Impact on Angiogenic Pathways

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***Abstract*: This paper presents In Silico functional and structural characterization of rs141029929 variant in ANGPT1 Reveals reduced protein stability and potential impact on angiogenic pathways.**

***Keywords*: ANGPT1, rs141029929, Protein stability, Molecular dynamics simulation, Protein–Protein interaction.**

1. Introduction

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is a fundamental biological process required for embryonic development, tissue repair, and wound healing. This process is tightly regulated by a balance between pro-angiogenic and anti-angiogenic factors, and its dysregulation contributes to the pathogenesis of several diseases, including cancer, cardiovascular disorders, and chronic inflammatory conditions [1], [2]. The coordination of endothelial cell proliferation, migration, and vascular remodeling is mediated by multiple signaling pathways, among which the angiopoietin–Tie system plays a critical role.

The angiopoietin–Tie signaling pathway is essential for vascular maturation and homeostasis. Angiopoietin-1 (ANGPT1), a secreted glycoprotein ligand of the endothelial Tie2 receptor, promotes endothelial cell survival, enhances vascular stability, and reduces vascular permeability [3], [4]. Unlike vascular endothelial growth factor (VEGF), which primarily induces angiogenic sprouting, ANGPT1 is involved in the later stages of angiogenesis, including vessel stabilization and maturation [5]. In addition, ANGPT1 signaling has been shown to exert anti-inflammatory effects and maintain endothelial integrity, thereby contributing to vascular quiescence [6]. Given these critical roles, alterations in ANGPT1 structure or function may have significant implications for vascular biology.

Single nucleotide polymorphisms [SNPs] represent the most abundant form of genetic variation in the human genome and can influence gene function at multiple levels, including transcriptional regulation, mRNA processing, and protein activity [7]. Among these, non-synonymous SNPs (nsSNPs) are of particular importance because they result in amino acid substitutions that may alter protein folding, stability, and function [8]. Such structural and functional alterations can disrupt protein interactions and biological pathways, potentially contributing to disease susceptibility. However, a large proportion of nsSNPs remain poorly characterized, particularly those lacking clinical annotation.

With the rapid expansion of genomic data, in silico approaches have become indispensable for the functional characterization of genetic variants. Computational tools such as SIFT, PolyPhen-2, and SNAP2 utilize sequence conservation, structural features, and machine learning algorithms to predict the deleterious effects of amino acid substitutions [9]–[11]. Similarly, protein stability prediction tools and structural modeling platforms enable the assessment of mutation-induced changes in protein conformation and energetics [12], [13]. Integration of these approaches provides a comprehensive framework for identifying potentially pathogenic variants and understanding their molecular consequences.

The SNP rs141029929 in the ANGPT1 gene is a non-synonymous coding variant that leads to multiple amino acid substitutions at position 257. Despite its presence in population databases, its functional and structural implications have not been systematically investigated, and no definitive clinical significance has been assigned. Considering the essential role of ANGPT1 in vascular regulation, characterization of this variant may provide insights into its potential impact on protein stability and function.

Therefore, the present study aims to comprehensively evaluate the functional and structural consequences of the rs141029929 variant in ANGPT1 using an integrated in silico approach, including functional prediction, protein stability analysis, structural modeling, and protein–protein interaction assessment.

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2. Materials and Methods

A. Retrieval of SNP and Protein Sequence Data

The non-synonymous single nucleotide polymorphism (nsSNP) rs141029929 of the ANGPT1 gene was retrieved from the NCBI dbSNP database. The reference protein sequence of ANGPT1 (Accession No. AAI52420) was obtained from the NCBI protein database. Available structural data for ANGPT1 were explored using the Protein Data Bank (PDB), and relevant structural templates were considered for subsequent modeling analysis.

B. Functional Prediction of Amino Acid Substitutions

The functional consequences of amino acid substitutions resulting from rs141029929 (T257I, T257K, and T257R) were evaluated using multiple in silico tools.

SIFT (Sorting Intolerant From Tolerant) predicts the effect of amino acid substitutions on protein function based on sequence homology and conservation patterns, classifying variants as tolerated or deleterious [14]. PolyPhen-2 (Polymorphism Phenotyping v2) evaluates the potential impact of amino acid substitutions on protein structure and function using sequence- and structure-based features [15]. SNAP2, a neural network-based classifier, predicts the functional effects of mutations by integrating evolutionary and biophysical information [16].

Protein sequence and mutation details were submitted to each tool using default parameters, and outputs were interpreted according to the respective scoring systems.

C. Protein Stability Analysis

The effect of the identified mutations on protein stability was assessed using I-Mutant 2.0 and MuPro.

I-Mutant 2.0 is a support vector machine (SVM)-based tool that predicts changes in protein stability upon mutation and provides ΔΔG values indicating the magnitude and direction of stability change (17). MuPro employs machine learning techniques, including SVM and neural networks, to predict the impact of amino acid substitutions on protein stability [18].

The ANGPT1 protein sequence along with mutation details was used as input, and ΔΔG values were interpreted such that negative values indicate decreased stability and positive values indicate increased stability.

D. Structural Modeling of Native and Mutant Proteins

Three-dimensional structures of the wild-type and mutant ANGPT1 proteins were predicted using the I-TASSER (Iterative Threading ASSEmbly Refinement) server. I-TASSER generates structural models by combining threading alignments with iterative structural assembly simulations (19).

For each sequence (wild-type and mutants), five structural models were generated, and the model with the highest C-score was selected for further analysis. Structural similarity between wild-type and mutant proteins was evaluated using TM-score and root mean square deviation (RMSD), where higher TM-scores indicate better structural alignment and lower RMSD values indicate reduced deviation.

E. Molecular Dynamics Simulation

Molecular dynamics (MD) simulations were performed using the GROMACS simulation package to evaluate the dynamic behavior and stability of the protein structures (20). The system was subjected to energy minimization followed by equilibration and production runs for a total simulation time of 500 ps.

Energy parameters, including potential and total energy, were monitored throughout the simulation to assess system stability. Additionally, the radius of gyration (Rg) was calculated to evaluate the compactness and conformational stability of the protein over time.

F. Protein–Protein Interaction Analysis

The interaction network of ANGPT1 was analyzed using GeneMANIA and STRING databases. GeneMANIA predicts gene function and interactions based on multiple data sources, including co-expression, physical interactions, co-localization, and shared pathways (21). STRING integrates known and predicted protein–protein interactions derived from experimental evidence, computational prediction methods, and public databases (22).

The ANGPT1 gene symbol was used as input, and default parameters were applied to identify interacting partners and functional associations.

3. Results

A. Characterization of rs141029929 Variant

The single nucleotide polymorphism rs141029929 in the ANGPT1 gene was identified as a non-synonymous coding variant with the highest reported minor allele frequency among coding SNPs lacking clinical annotation. This SNP is located at a functionally relevant position within the ANGPT1 protein and results in amino acid substitutions at position 257.

The reference allele (G) encodes threonine (Thr, T) at position 257, whereas alternative alleles give rise to three missense variants:

- T257I (Threonine → Isoleucine)
- T257K (Threonine → Lysine)
- T257R (Threonine → Arginine)

These substitutions involve changes in amino acid polarity and charge, suggesting potential alterations in local structural conformation and protein interactions.

B. Functional Impact Prediction of Amino Acid Substitutions

The functional consequences of the three amino acid substitutions were evaluated using SIFT, PolyPhen-2, and SNAP2. The predictions obtained from these tools demonstrated variability in assessing the deleterious potential of the variants.

PolyPhen-2 classified all three substitutions (T257I, T257K, and T257R) as probably damaging, with high confidence scores ranging from 0.991 to 0.996, indicating a strong likelihood of functional impairment. In contrast, SIFT predicted all variants to be tolerated, suggesting that the substitutions may not significantly affect protein function based on sequence

Table 1
Functional prediction of rs141029929 variants

Tool	T257I	T257K	T257R
PolyPhen-2	Probably damaging	Probably damaging	Probably damaging
SNAP2	Neutral	Neutral	Effect
SIFT	Tolerated	Tolerated	Tolerated

Table 2
Protein stability prediction of rs141029929 variants

Mutant	I-Mutant Stability	ΔΔG (kcal/mol)	MuPro Stability	ΔΔG (kcal/mol)
T257I	Decreased	−0.41	Decreased	−0.375
T257K	Decreased	−0.55	Decreased	−1.433
T257R	Decreased	−0.16	Decreased	−0.771

conservation alone. SNAP2 predictions identified only T257R as having a functional effect, while T257I and T257K were predicted to be neutral.

This discrepancy among prediction tools reflects differences in underlying algorithms and suggests that while sequence conservation may tolerate these substitutions, structural and physicochemical changes could still impact protein function.

C. *Effect of Mutations on Protein Stability*

To assess the impact of the identified substitutions on protein stability, I-Mutant 2.0 and MuPro were employed. Both tools consistently predicted a decrease in protein stability for all three variants.

I-Mutant analysis yielded ΔΔG values of −0.41, −0.55, and −0.16 kcal/mol for T257I, T257K, and T257R, respectively. Similarly, MuPro predicted ΔΔG values of −0.375, −1.433, and −0.771 kcal/mol for the respective variants. Among the three substitutions, T257K exhibited the most pronounced destabilizing effect.

The consistent prediction of reduced stability across both tools indicates that these substitutions may compromise the structural integrity of the ANGPT1 protein.

D. *Structural Modeling and Comparative Analysis*

Three-dimensional structural models of the wild-type and mutant ANGPT1 proteins were generated using I-TASSER. For each protein sequence, five models were obtained, and the model with the highest C-score was selected for further analysis.

The wild-type protein model exhibited a C-score of −1.84, a TM-score of 0.49 ± 0.15, and an RMSD of −11.7 ± 4.5 Å. In comparison, the T257I mutant showed a lower C-score (−2.69), reduced TM-score (0.40 ± 0.14), and increased RMSD (−13.9 ± 3.9 Å), indicating decreased structural confidence and increased deviation from the native conformation.

Although complete structural parameters for all mutants were not uniformly available, comparative analysis revealed that all substitutions led to structural perturbations, reflected by reduced model confidence and increased conformational deviation. These findings support the stability predictions and suggest that mutations at position 257 may alter protein folding and structural integrity.

Table 3
Structural parameters of wild-type and mutant ANGPT1

Structure	C-score	TM-score	RMSD (Å)
Wild-type	−1.84	0.49 ± 0.15	−11.7 ± 4.5
T257I	−2.69	0.40 ± 0.14	−13.9 ± 3.9

E. *Molecular Dynamics Simulation Analysis*

To further investigate the structural stability of ANGPT1 and its variants, molecular dynamics (MD) simulations were performed using GROMACS for a duration of 500 ps. The stability and conformational behavior of the system were evaluated using energy profiles and radius of gyration (Rg) analysis.

1) *Energy Profile Analysis*

The potential and total energy of the system remained relatively constant throughout the simulation period (0–500 ps), with only minor fluctuations observed (Figure 1). The absence of significant energy drift indicates that the system achieved equilibrium and maintained a thermodynamically stable state during the simulation. The consistently negative energy values further confirm that the protein structure remained energetically favorable under simulated conditions.

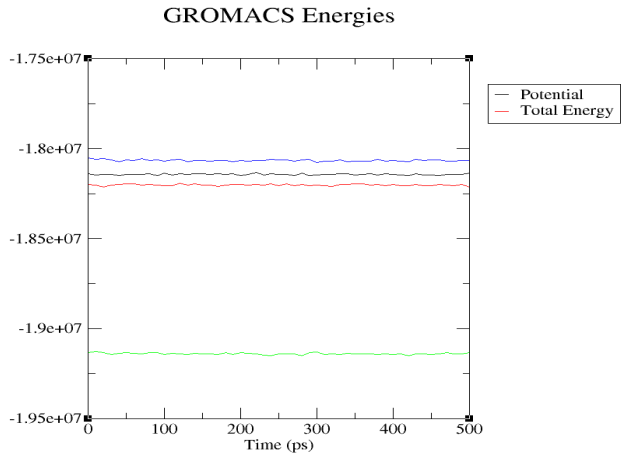

Fig. 1. Energy profile of ANGPT1 during molecular dynamics simulation

Potential energy and total energy of the ANGPT1 protein system plotted as a function of simulation time (0–500 ps). The relatively constant energy profiles with minimal fluctuations indicate system equilibration and thermodynamic stability throughout the simulation.

2) *Radius of Gyration (Rg) Analysis*

The radius of gyration (Rg) was analyzed to assess the compactness of the protein structure over time (Figure 2). The Rg profile exhibited an initial increase during the early phase of the simulation (0–150 ps), suggesting structural relaxation from the starting conformation. This was followed by stabilization of Rg values for the remainder of the simulation, indicating the attainment of a stable conformational state.

Axis-wise analysis (RgX, RgY, and RgZ) revealed minor directional fluctuations, with comparatively higher variation along the Y-axis during the initial phase. However, these variations diminished over time, and all components converged toward stable values. Overall, the absence of significant deviations in Rg suggests that the protein retained its compact structure without undergoing major unfolding.

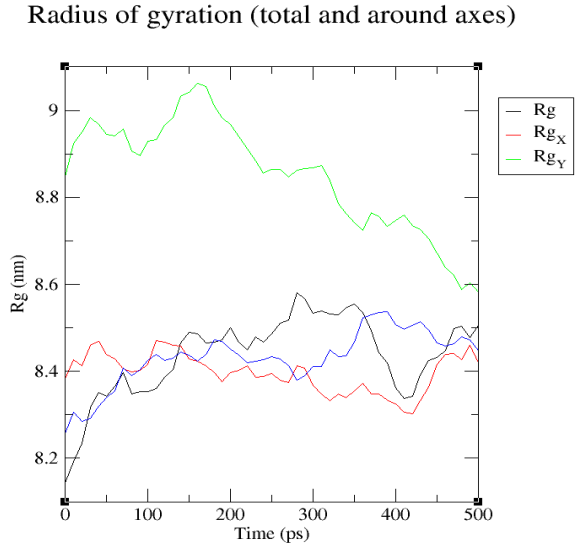

Fig. 2. Radius of gyration (Rg) analysis of ANGPT1

Radius of gyration (Rg) of the ANGPT1 protein plotted over the simulation time (0–500 ps), including total Rg and its components along X, Y, and Z axes. The initial increase in Rg followed by stabilization suggests conformational relaxation and maintenance of overall structural compactness without significant unfolding.

F. Protein–Protein Interaction Analysis

Gene interaction network analysis of ANGPT1 was performed using GeneMANIA to explore its functional associations and biological context. The generated network revealed that ANGPT1 occupies a central hub position, exhibiting extensive connectivity with multiple interacting genes (Figure 3).

A total of 20 functionally associated genes were identified in the interaction network. Among these, several key interacting partners included TEK (Tie2 receptor), ANGPT2, DOK2, GRB14, PTPRB, and SOS1, indicating strong involvement in signaling pathways related to vascular regulation and intracellular signal transduction.

The network demonstrated diverse types of interactions, including physical interactions, co-expression, co-localization, shared pathways, and predicted functional associations. Notably, ANGPT1 showed strong connectivity with TEK, its primary receptor, confirming the biological relevance and accuracy of the network. Additionally, ANGPT2, a closely related ligand within the angiopoietin family, displayed significant association, suggesting functional interplay within the angiopoietin–Tie signaling axis.

Several adaptor and signaling proteins, such as GRB7, GRB14, SOS1, and PTPN11, were also prominently connected within the network, highlighting the role of ANGPT1 in downstream signaling cascades. The presence of proteins like ACVRL1, TIE1, and PTPRB further supports its involvement in vascular development and endothelial cell signaling.

The network also indicated domain similarity between ANGPT1 and ANGPT2 and MFAP4, suggesting conserved structural and functional characteristics. The high degree of connectivity observed for ANGPT1 underscores its importance as a key regulatory molecule within protein–protein interaction networks.

Overall, the GeneMANIA analysis confirms that ANGPT1 functions as a central node in a complex interaction network, and structural alterations in ANGPT1, such as those induced by the rs141029929 variant, may have broader implications by potentially affecting multiple interacting partners and signaling pathways.

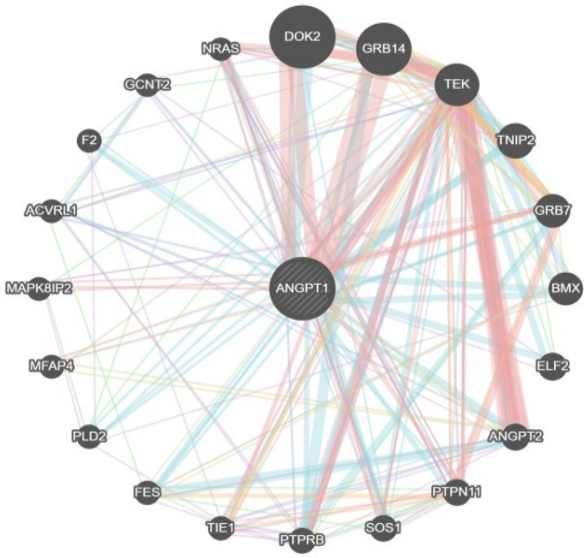

Fig. 3. GeneMANIA interaction network of ANGPT1

Gene interaction network of ANGPT1 generated using GeneMANIA. Nodes represent genes, and edges indicate functional associations, including physical interactions, co-expression, co-localization, shared pathways, and predicted interactions. ANGPT1 is centrally positioned, interacting with key proteins such as TEK, ANGPT2, DOK2, and GRB14, highlighting its role in vascular signaling and protein interaction networks.

To further elucidate the functional associations of ANGPT1 at the protein level, a protein–protein interaction (PPI) network was constructed using the STRING database. The generated network revealed a highly interconnected cluster of angiogenesis-related proteins, with ANGPT1 occupying a prominent and well-connected position within the network (Figure 4).

The STRING analysis demonstrated strong interactions between ANGPT1 and key receptor tyrosine kinases, particularly TEK (Tie2 receptor), which is its primary binding partner. Additional interactions were observed with TIE1, further supporting the involvement of ANGPT1 in endothelial signaling pathways. Notably, ANGPT1 also exhibited close associations with other members of the angiopoietin family, including ANGPT2 and ANGPT4, indicating coordinated functional roles within the angiopoietin signaling axis.

The network further included several critical regulators of

vascular growth and signaling, such as KDR (VEGFR2), FLT1 (VEGFR1), and FLT4 (VEGFR3), highlighting crosstalk between the angiopoietin–Tie and VEGF signaling pathways. Additionally, interactions with proteins such as EGFR, PDGFRB, and NTRK1 suggest broader involvement in receptor-mediated signal transduction and cellular proliferation pathways.

The dense connectivity and multiple interaction edges observed in the network indicate a high level of functional integration. The presence of both experimentally validated and predicted interactions suggests that ANGPT1 participates in a complex signaling environment where alterations in its structure may influence multiple downstream pathways.

Overall, the STRING-based PPI network reinforces the role of ANGPT1 as a key regulatory node in angiogenesis-related signaling networks, and suggests that structural perturbations induced by the rs141029929 variant may have widespread functional implications by affecting protein–protein interactions.

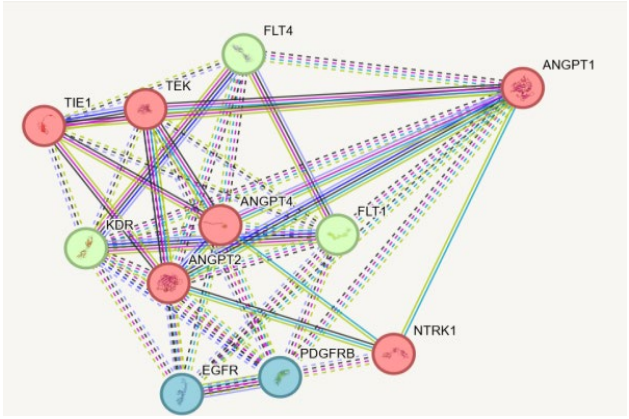

Fig. 4. STRING protein–protein interaction network of ANGPT1

Protein–protein interaction network of ANGPT1 generated using the STRING database. Nodes represent proteins, while edges indicate functional associations, including experimentally validated and predicted interactions. ANGPT1 shows strong connectivity with key angiogenesis-related proteins such as TEK, TIE1, ANGPT2, and VEGF receptors (KDR, FLT1, FLT4), highlighting its central role in vascular signaling pathways.

4. Discussion

The present study provides a comprehensive in silico characterization of the non-synonymous SNP rs141029929 in the ANGPT1 gene by integrating functional prediction, protein stability analysis, structural modeling, molecular dynamics (MD) simulation, and protein–protein interaction (PPI) network analysis. The results collectively indicate that this variant exerts a measurable impact on protein stability and structure, with potential implications for its functional role in vascular signaling networks.

ANGPT1 is a critical regulator of vascular homeostasis and exerts its biological effects primarily through activation of the Tie2 receptor (TEK), leading to endothelial cell survival, vascular stabilization, and suppression of permeability [23], [24]. In addition to its role in vessel maturation, ANGPT1 contributes to anti-inflammatory signaling and maintenance of endothelial integrity. Given its central position in angiogenic regulation, even subtle structural alterations may influence its interaction with binding partners and downstream signaling cascades.

The rs141029929 variant results in three amino acid substitutions (T257I, T257K, and T257R), each introducing distinct physicochemical changes at position 257. Functional prediction tools produced inconsistent results, with PolyPhen-2 indicating all variants as probably damaging, while SIFT classified them as tolerated and SNAP2 predicted only T257R as functionally significant. Such discrepancies are well documented and arise due to differences in algorithmic approaches, where conservation-based methods may fail to capture structural and contextual effects of amino acid substitutions [25], [26]. These findings reinforce the need for integrated computational approaches rather than reliance on single predictive tools.

In contrast, protein stability predictions using I-Mutant and MuPro demonstrated consistent results, indicating decreased stability for all variants. Protein stability is a critical determinant of folding efficiency and functional integrity, and even modest destabilization can alter conformational dynamics and interaction potential [27]. Among the variants, T257K exhibited the most pronounced destabilizing effect, suggesting a potentially greater impact on protein behavior. These findings highlight that structural destabilization may represent the primary mechanism through which rs141029929 influences ANGPT1 function.

Structural modeling further supported these observations, as mutant proteins exhibited reduced C-scores, lower TM-scores, and increased RMSD values compared to the wild-type structure. These changes indicate reduced structural confidence and increased deviation, suggesting localized conformational perturbations rather than complete structural disruption. Such localized effects are particularly important in proteins involved in signaling, where even minor conformational changes can influence ligand binding or receptor interaction [28].

Molecular dynamics simulations provided additional insight into the dynamic stability of the protein. The energy profiles remained stable throughout the simulation period, indicating that the system achieved equilibrium and maintained a thermodynamically favorable state. Similarly, radius of gyration (Rg) analysis demonstrated only minor fluctuations, with no evidence of large-scale unfolding or structural expansion. These findings suggest that although the mutations reduce intrinsic stability, the overall fold of the protein remains preserved under dynamic conditions. This highlights a key concept in structural biology: mutations may induce local destabilization without global structural collapse, thereby subtly affecting protein function while maintaining overall architecture [29], [30].

The interaction network analyses further emphasize the biological significance of ANGPT1. GeneMANIA identified ANGPT1 as a central hub interacting with multiple genes, including TEK, ANGPT2, GRB14, and PTPN11, reflecting its involvement in signaling pathways related to vascular function. Complementary STRING analysis revealed a dense protein–

protein interaction network involving ANGPT1 and key angiogenic regulators, including TEK, TIE1, ANGPT2, ANGPT4, and VEGF receptors such as KDR (VEGFR2), FLT1 (VEGFR1), and FLT4 (VEGFR3). These findings highlight significant crosstalk between the angiopoietin–Tie and VEGF signaling pathways, which are known to coordinate vascular development and remodeling [31], [32].

Furthermore, interactions with receptor tyrosine kinases such as EGFR, PDGFRB, and NTRK1 suggest that ANGPT1 may participate in broader signaling networks beyond classical angiogenic pathways. The high degree of connectivity observed in both GeneMANIA and STRING networks underscores the role of ANGPT1 as a central regulatory node, where structural perturbations could have cascading effects on multiple signaling pathways. Therefore, mutations such as rs141029929 may not only affect protein stability but also modulate interaction dynamics and signaling efficiency.

Taken together, the results of this study suggest that rs141029929 is a structurally relevant variant that primarily affects ANGPT1 through decreased protein stability and localized conformational changes. While the mutations do not induce global structural destabilization, their potential to alter protein dynamics and interaction networks may have important biological implications. These findings highlight the importance of integrating structural, dynamic, and network-level analyses for a comprehensive understanding of SNP effects.

However, certain limitations must be acknowledged. The study is based entirely on computational approaches, which, although powerful, require experimental validation. Functional assays, binding studies, and extended MD simulations would provide deeper insight into the biological impact of the identified variants. Additionally, the relatively short simulation time may not capture long-term conformational changes. Future studies integrating experimental and computational methods will be essential to validate and extend these findings.

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