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Research paper

Structural And Energetic Analysis of the p.Met1lle CFTR Variant

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Abstract: Cystic Fibrosis is a genetic disorder caused by mutations in the CFTR gene, leading to impaired epithelial ion transport and altered protein function. **Objective:** This study evaluates the structural and functional impact of the p.Met1Ile (c.3G>A) CFTR variant using in-silico approaches. **Methodology:** Comparative homology modeling and molecular docking were performed using Molecular Operating Environment (MOE). Wild-type and mutant CFTR structures were analyzed for docking scores, conformational stability, and protein–ligand interactions. Pathogenicity prediction tools were also applied to assess functional consequences of the variant. **Results:** The wild-type CFTR showed higher binding affinity (S = −8.5677 kcal/mol) compared to the mutant (S = −7.8977 kcal/mol). The mutant exhibited altered conformational energy (E_conf = −541.4630 kcal/mol) and disrupted hydrogen bonding patterns, indicating reduced structural stability and ligand interaction. **Conclusion:** The p.Met1Ile variant likely induces structural destabilization of CFTR, reducing ligand-binding efficiency. These findings support its potential pathogenic role and demonstrate the utility of computational docking in variant analysis.

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Introduction

Lung diseases affect the respiratory system, particularly the lungs impairing the exchange of oxygen and carbon dioxide. They are the leading cause of illness and death worldwide among cystic fibrosis is one of the major concerns (1). Cystic fibrosis is an autosomal recessive genetic disorder characterized by reduced or absent function of cystic fibrosis transmembrane conductance regulator CFTR. (2) . It was first distinguish from celiac disease in 1938. It was considered as a genetic disorder which was transmitted in an autosomal recessive pattern. cystic fibrosis results from reduced or absent function of the CFTR protein, a regulated anion channel located in the apical membrane of epithelia in multiple organs, including the lungs, liver, gastrointestinal tract, and pancreas (1). More than 2000 sequence variants of the CFTR gene (OMMIM 602421) have been identified out of which seven hundred have been shown responsible for the cause of disease (3). In a healthy epithelial cell CFTR protein is located in the apical membrane of epithelial cell in airways pancreatic ducts and the absorptive ducts of the sweat glands the discovery of CFTR gene in the late 1980s triggered a surge of basic research that enhanced the understanding of the pathophysiology and genotype phenotype relations of this variable disease (1). The three main regions particularly are in CFTR gene are the the membrane spanning domain (MSDs), the two nucleotide binding domains (NBDs) regulatory (R) domain. Abnormality occurs when the CFTR protein does not work properly because of mutation in delta f508 this mutation causes the CFTR protein to fold incorrectly this leads to thick mucus resulting in blockage of airways, trap bacteria and causes infection and breathing problem (3). Common symptoms observed in patients are persistent cough, salty tasting skin, poor growth or weight gain, fatigue, digestive systems may incline, greasy or bulky

stools, chronic diarrhea or constipation and abdominal pain or blotting (4). Diagnoses of CF are usually straightforward, but occasionally, they may prove difficult to make. This has led to the implementation of guidelines for diagnosis and to the development of assays, testing CFTR function in vivo and ex vivo . (2) Recently available treatments could only control symptoms and restrict the complications of cystic fibrosis .but the advancements of CFTR modulator therapies to address the basic defect of cystic fibrosis has been remarkable and the field is evolving rapidly but the CFTR modulator therapies are highly expensive which arouses the questions about the affordability of new treatments this thus created a gap between high income counties and low income countries (1). CFTR modulator therapies act by 2 mechanisms to enhance CFTR function. Potentiators, like ivacaftor, increase the probability that the protein channel is open, so chloride or bicarbonate can flow more easily through the cell membrane. correctors, like lumacaftor, tezacaftor, and elexacaftor, improve channel quantity at the cell surface by helping the protein fold properly, enabling transport to the cell surface. Severe variants such as F508del need both potentiators and correctors to improve channel quantity and function(4). The past six decades have been remarkable improvements in health outcomes for people with cystic fibrosis which was once fatal disease however the life expectancy for the people with cystic fibrosis has increased significantly, the disease continues to limit survival and quality of life and results in large burden of care for the affected people and their related families (3) . Furthermore, epidemiological studies have shown that high prevelance areas are European countries such as United States, Canada and Australia. In Pakistan cystic fibrosis is rare but causes do exist due to high rate of risk in consanguineous marriages. The present study aims to compile reported CFTR mutations and identify potential ligands to mitigate their functional impact. Using in-silico molecular docking, the study evaluates ligand-protein interactions to predict stabilization of CFTR, providing a computational framework for potential therapeutic intervention (4).

Methodology

Data retrieval

Gene disease association data was retrieved from UNiport and Genomad to identify genes for related Pulmonary disorders . Gene lists obtained from both databases were compared, and overlapping candidates were identi-fied using Venn diagram analysis performed with Venny. Among them CFTR was selected (3).

Pathogenicity prediction of the selected variant.

The functional impact of the CFTR variant was assessed using multiple silico prediction tools. MutationTaster was first employed to evaluate disease-causing potential based on evolutionary conservation, splice-site effects, and protein features. Variants consistently predicted as deleterious across these tools were prioritized for subsequent homology modeling, molecular docking, and molecular dynamics simulations (5).

Protein stucture and retrival preperation.

The 3D structure of CFTR was taken from the Protein Data Bank (PDB), its amino acid sequence was collected from UniProt, and a model was built using SWISS-MODEL based on similar known proteins. The best model was chosen using quality checks (6). Before docking, the protein was cleaned in Moe 2019.0102 by removing water molecules and other unwanted parts, then adding hydrogen atoms and performing energy minimization to make the structure more stable and accurate. The validated structure was subsequently used for molecular docking and molecular dynamics simulations (5).

Homology modeling

The amino acid sequence of CFTR was retrieved from the UniProt while the three-dimensional crystal structure was obtained from Protein Data Bank (PDB). Homology modeling was performed to predict and analyze the structural conformation of the CFTR protein and its mutant variants. The retrieved protein sequence was aligned experimentally determined template structures available in PDB database to identify structures of similarity. Based on sequence identity, coverage, and structural quality the most suitable template was selected for model generation. The generated homology model was further evaluated for stereochemical quality.

Ligand selection and preparation

Potential ligand molecules targeting CFTR were identified through literature review and chemical database screening. The three-dimensional structures of the selected ligands were retrieved from the Alpha fold in standard structure format. (8). The ligand structures were then imported into Moe 2019.0102 for preparation which included the removal of unnecessary atoms, addition of hydrogen atoms, and energy minimization to obtain stable con-formations. The prepared ligands were subsequently converted into appropriate formats required for molecular docking analysis (6).

Molecular docking

Molecular docking was performed to evaluate the binding affinity and interaction patterns between the CFTR protein and the selected ligands. The docking analysis was conducted using Alpha fold integrated within the Moe 2019.0102 platform. Prior to docking, the prepared protein and ligand structures were converted into the required PDBQT format. Dummies were then defined to encompass the active or predicted binding site of the protein. Singular docking conformations were generated, and the best binding pose was selected based on the lowest binding energy score and favorable interaction patterns. The docking results were further analyzed and visualized for intermolecular interactions between the ligand molecules and the CFTR protein (9).

Results

Mutation analysis

Mutational analysis of the CFTR gene was performed using uniport through pairwise sequence alignment of wild-type and mutant sequences. The analysis identified a single nucleotide substitution (G>A) at position N 000007.14:g.117480097G>A , corresponding to a coding change c.4 G>A, which results in a missense mutation p.Met1Ile at the protein level.

Pathogenicity assessment of NC_000007.14:g.117480097G>A

The identified variant NC_000007.14:g.117480097G>A, results in a missense mutation p.Met1Ile (ClinVar:ENST00000003084) present on chromosome number 7 . Pathogenicity predictions indicate a potentially deleterious effect. The mutation is not somatic and has been previously associated with adenomas and adenocarcinomas in large-scale studies. Cross-references are documented in the ClinVar: RCV000757844 database as a novel variant

Table 1: Genomic and clinical annotation of the CFTR missense variant associated with cystic fibrosis

featuren	Detail
Consequence	Misssence
Cytogenic band	7q31.2
somatic	NO
Acession	NC_000007.14:g
Consequence type	Misssence
Genomic location	NC_000007.14:g.117480097G>A,
Disease association	Cystic fibrosis
Source type	Large scale study
Cross reference	ClinVar: RCV000757844

Wild CFTR protein analysis of Ligand Binding Interactions

The interaction analysis identifies three primary hydrogen-bond-mediated contacts and one hydrophobic pi-H interaction between the ligand and receptor chain A. The strongest stabilizing force is a H-donor interaction between the ligand atom “0” and the side-chain oxygen (OD2) of ASP 924, characterized by a short distance of 2.85 Å and a favorable energy of –3.0 kcal/mol, indicating a strong polar anchor. Additional hydrogen bonds include a H-acceptor interaction with TRP 1145 (NE1) at 2.93 Å (–1.2 kcal/mol) and a weaker H-acceptor interaction with THR 1142 (CA) at 3.21 Å (–0.7 kcal/mol). A single pi-H interaction occurs between a 5-ring ligand moiety and VAL 345 (CG2) at a distance of 3.89 Å, contributing –0.5 kcal/mol. Overall, the binding is dominated by electrostatic hydrogen bonds, with ASP 924 serving as the key residue for ligand stabilization.

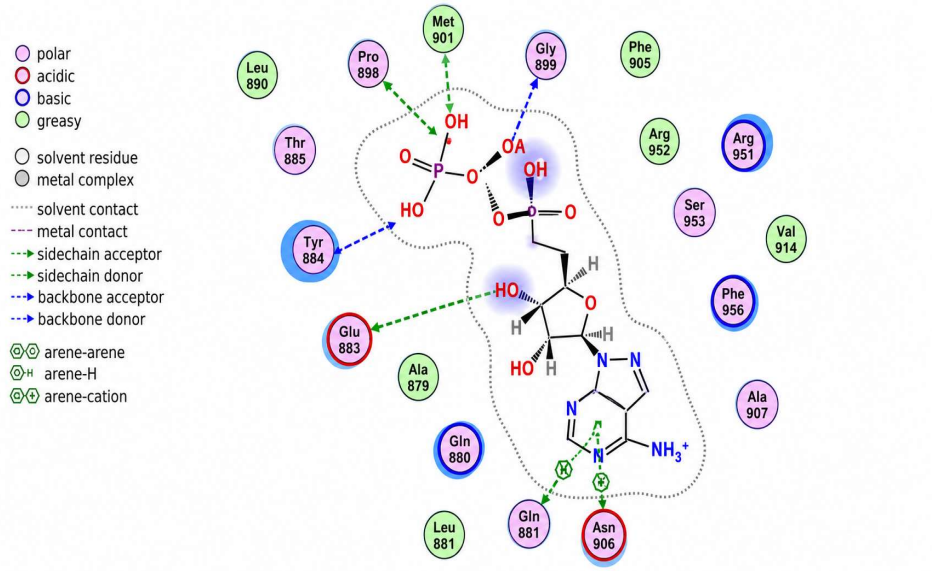

Figure 1: Showing key ligand inter-actions and binding orientation within the CFTR active site

Mutant CFTR protein analysis.

The mutant ligand-protein interaction analysis for Mut_CFTR reveals that the ligand interacts primarily with the amino acid Ile104 in the CFTR_1 protein through a hydrogen bond, where the nitrogen atom of the ligand acts as an H-acceptor and forms a hydrogen bond with Ile104. The distance of this hydrogen bond is measured at 2.85 Å, indicating a strong and favorable interaction. The energy associated with this interaction is -3.0 kcal/mol, reflecting a stable binding between the mutant ligand and the receptor residue (10).

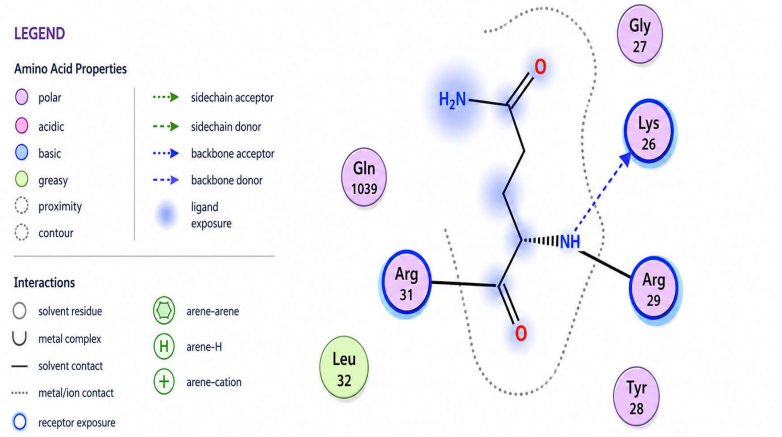

Figure 2: Two-dimensional (2D) protein–ligand interaction map showing the binding mode of the ligand within the active site of the target protein

Docking results of wild and mutant type

The molecular docking analysis was performed to evaluate the binding affinity of the ligand with both wild-type and mutant CFTR1 proteins. The first docking pose for the wild CFTR1-ligand complex showed a docking score (S) of –8.5677, with an RMSD refined value of 1.3719. The calculated conformational energy (E_conf) was –548.0458, while the placement energy (E_place) was –81.9769. Furthermore, the scoring parameters showed E_score1 = --12.0696 E_refine = - 48.6805, and E_score2 = -8.5677. In comparison, the first docking pose of the mutant CFTR1-ligand complex exhibit-ed a docking score (S) of –7.8977 with an RMSD refine value of2.0981. The conformational energy (E_conf) was -541.4630 and the placement energy (E_place) was – 73.1530 The scoring functions for this complex were E_score1 = -9.7609, E_refine = -35.8040, and E_score2 = -7.9877.

Table2: Comparison of molecular docking parameters between the wild-type and mutant protein–ligand complexes

Protein ligand complex	S	RSMD	E-conf	E-place	E-score1	E-Refine	E-score 2
Wild type	-8.56	1.3719	-548.0458	-81.9769	-12.0696	-48.6805	-8.5677
Mutant type	-7.8977	2.0981	-541.4630	-73.1530	-9.7609	-35.8040	-7.9877

Discussion

The computational analysis of the CFTR protein and its p.Met1Ile variant reveals significant structural and functional alterations that influence ligand binding affinity. In-silico pathogenicity prediction tools classify this missense mutation as potentially deleterious. The mutation is located on chromosome 7 at genomic position NC_000007.14:g.117480097G>A within the CFTR gene. Structural modeling indicates that substitution of Methionine with Isoleucine at the first codon may disrupt translational initiation or impair early protein folding stability. Molecular docking simulations further highlight these differences. The wild-type CFTR protein demonstrates strong ligand binding, with a docking score (S) of -8.5677 and a refined RMSD of 1.3719. Key stabilizing interactions include hydrogen bonds with residues such as Asp924 and Met96, with bond distances ranging from 2.63 Å to 4.15 Å. These interactions correspond to favorable binding energies between -2.0 and -0.8 kcal/mol, suggesting a stable and biologically relevant protein–ligand complex. In contrast, the mutant CFTR (p.Met1Ile) shows reduced binding affinity, with a docking score of -7.8977. Although hydrogen bonding is still observed—such as with Ile104 at a distance of 2.85 Å and energy of -3.0 kcal/mol—the overall conformational stability is compromised. The conformational energy (E_conf) shifts from -548.0458 in the wild-type to -541.4630 in the mutant, indicating a less stable structural configuration. Additionally, scoring parameters (E_score1 and E_score2) consistently show less favorable values for the mutant protein. These findings suggest that the p.Met1Ile mutation not only alters the primary amino acid sequence but also creates steric and electronic changes that hinder optimal ligand positioning within the binding site. The observed decrease in docking efficiency provides a molecular explanation for the reduced responsiveness to certain CFTR modulators seen in clinical settings. Overall, this study highlights the critical sensitivity of the CFTR protein to even single-nucleotide variations, particularly those occurring at the initiation codon, which can have profound effects on protein structure, stability, and function. This study demonstrates that the p.Met1Ile mutation in the CFTR gene significantly reduces the protein's binding affinity for therapeutic ligands compared to the wild-type form. Using homology modeling and molecular docking approaches, the mutant protein was shown to exhibit a distinct energetic profile and a disrupted hydrogen bonding network, notably involving the Ile104 residue. The observed decrease in docking scores, along with unfavorable shifts in conformational energy, indicates the formation of a less stable protein–ligand complex in the mutant structure. These structural and energetic alterations provide a molecular basis for the variability in patient responses to CFTR modulator therapies. Overall, these findings highlight the functional impact of the p.Met1Ile variant and emphasize the importance of personalized therapeutic strategies in the management of cystic fibrosis. This study thus contributes to the growing body of evidence supporting precision medicine approaches in cystic fibrosis treatment. The genetic architecture explored in this study centers on the CFTR gene, located on chromosome 7, with particular emphasis on a single nucleotide polymorphism involving a G>A transition. This substitution results in a missense mutation at the protein level, where the initiator Methionine is replaced by Isoleucine (p.Met1Ile). Notably, this alteration occurs at the translational start site, a critical component of gene architecture required for accurate initiation of protein synthesis. The CFTR protein consists of three major functional regions: the membrane-spanning domains (MSDs), which facilitate ion transport across the membrane; the nucleotide-binding domains (NBDs), responsible for ATP binding and hydrolysis; and the regulatory (R) domain, which modulates channel activity through phosphorylation. Although the p.Met1Ile mutation is located at the N-terminal start site rather than within these domains directly, its impact is upstream and fundamental, potentially disrupting proper translation and subsequent folding of all downstream structural regions. The variant is precisely mapped using the genomic coordinate NC_000007.14:g.117480097G>A, enabling accurate localization within the gene. Understanding this architectural framework is essential, as it governs the correct folding, stability, and anion channel function of CFTR in epithelial cells. Disruption at the initiation level can therefore have cascading effects on overall protein functionality and cellular ion transport. The novelty of this research lies in its focused investigation of the p.Met1Ile variant within the CFTR gene, a comparatively underexplored mutation relative to the

widely studied F508del mutation. While existing literature predominantly emphasizes folding defects within the nucleotide-binding domains (NBDs), this study uniquely examines the consequences of a start-codon mutation on ligand binding dynamics through an in-silico framework. A key contribution of this work is the comparative analysis of binding efficiency between wild-type and mutant CFTR protein structures. By incorporating advanced docking parameters such as conformational energy (E_conf), placement energy (E_place), and RMSD refinement values, the study quantitatively characterizes the energetic penalties introduced by the p.Met1Ile substitution. This enables a more precise understanding of how early translational disruptions propagate into altered molecular recognition and binding stability. Importantly, this work also contributes region-specific insight by contextualizing findings within the Pakistani population, where mutation spectra and therapeutic responses may differ from globally studied cohorts. As such, it adds a data-driven and population-relevant dimension to ongoing cystic fibrosis research, supporting the advancement of precision medicine strategies. A primary limitation of this study is its reliance on in-silico simulations without complementary in-vitro or in-vivo validation to substantiate the docking outcomes. Although the homology model was constructed using high-quality templates from the Protein Data Bank (PDB), it may not fully capture the dynamic conformational flexibility ("breathing") of the CFTR protein within a native lipid bilayer environment. Additionally, the study evaluates interaction with a single ligand, which does not reflect the complexity of intracellular conditions where multiple ligands, cofactors, and molecular chaperones coexist. The docking simulations are performed under static conditions, thereby overlooking critical physiological variables such as pH fluctuations, ionic strength, and membrane-specific effects that can significantly influence binding stability and protein conformation. Another important limitation is the absence of investigation into alternative translation initiation mechanisms. Given that the mutation affects the start codon of the CFTR gene, the potential utilization of downstream initiation sites and their functional consequences remain unexplored. Furthermore, the study focuses exclusively on a single variant (p.Met1Ile), which restricts the generalizability of the findings to other rare or common CFTR mutations. Expanding the analysis to a broader spectrum of variants would provide a more comprehensive understanding of genotype–phenotype correlations in cystic fibrosis.

References

1. Joshi R, Lazaro S, Purohit S, McKie K, Forseen C, Taskar V. Use of Registry Data to Improve Center Outcomes and Inform Global Care Standards in Adult Cystic Fibrosis. medRxiv. 2026:2026-04.
2. Bell SC, Mall MA, Gutierrez H, Macek M, Madge S, Davies JC, Burgel PR, Tullis E, Castaños C, Castellani C, Byrnes CA. The future of cystic fibrosis care: a global perspective. The Lancet Respiratory Medicine. 2020 Jan 1;8(1):65-124.
3. Tsui LC, Dorfman R, Crowdy E. Cystic fibrosis mutation database. Режим доступу http://www
4. Callebaut, I., Hoffmann, B., Lehn, P., & Mornon, J.-P. (2016). Molecular modelling and molecular dynamics of CFTR. Cellular and Molecular Life Sciences, 74(1), 3–22. https://doi.org/10.1007/s00018-016-2385-9
5. Jedidi, I., Ouchari, M., & Yin, Q. (2018). Autosomal single-gene disorders involved in human infertility. Saudi Journal of Biological Sciences, 25(5), 881–887. https://doi.org/10.1016/j.sjbs.2017.12.005
6. O'Ryan, L. P., Rosenberg, M. F., Hughes, G., Zhao, Z., Aleksandrov, L. A., Riordan, J. R.
7. Prasad, R., et al. (2023). A comprehensive review of cystic fibrosis in Africa and Asia. Journal of Personalized Medicine, 13(6), 950. https://pmc.ncbi.nlm.nih.gov/articles/PMC10258508/
8. Chen, Q., Shen, Y., & Zheng, J. (2021). A review of cystic fibrosis: Basic and clinical aspects. Animal Models and Experimental Medicine, 4(3), 220–232. https://doi.org/10.1002/ame2.12180
9. Farinha, C. M., & Callebaut, I. (2022). Molecular mechanisms of cystic fibrosis – how mutations lead to misfunction and guide therapy. Bioscience Reports, 42(7). https://doi.org/10.1042/bsr20212006
10. De Boeck, K., & Amaral, M. D. (2016). Progress in therapies for cystic fibrosis. The Lancet Respiratory Medicine, 4(8), 662–674. https://doi.org/10.1016/s2213-2600(16)00023-0