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Article

In-silico Study of Protein Modeling of ADAM33 Variants (SNP) in Asian Populations with Asthma

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Abstract: Asthma is a chronic respiratory disease with a prevalence that continues to increase, influenced by genetic factors and environmental changes. The ADAM33 gene has been consistently associated with susceptibility to asthma, but the functional impact of its variants on the protein remains unclear, especially in diverse Asian populations. This study aims to assess how single nucleotide polymorphisms (SNPs) in the ADAM33 gene affect the structure and function of the resulting protein, as well as their potential contribution to asthma pathogenesis. An in-silico approach was used: the relevant gene sequences were obtained, the ADAM33 catalytic domain was modeled using AlphaFold/ColabFold, and candidate SNPs were identified from UniProt. Protein stability and functional consequences were then analyzed using MuPro and PolyPhen-2. Results showed that all SNPs studied disrupted ADAM33 stability to varying degrees, with some causing significant conformational changes. Specifically, SNP rs41467948 produced the greatest destabilizing effect, while rs41534847 was predicted to be potentially deleterious despite its minimal impact on stability. These findings suggest that SNP-induced changes in ADAM33 may influence airway remodeling and disease mechanisms in asthma. They highlight the value of integrated computational methods for prioritizing specific prominent variants among understudied Asian populations for further functional analysis and therapeutic targeting.

Key words: ADAM33, asthma, single nucleotide polymorphism, protein modeling, in silico analysis.

1. Introduction

Asthma is a chronic lung disease characterized by inflammation of the airways, in which the airways become hyperresponsive to certain triggers such as exercise, viruses, and allergens (**Thomsen, 2015**). This hypersensitivity leads to the tightening of the muscles around the airways, causing episodes of airway obstruction – varying in duration, frequency and severity. The disorder is multifactorial and

polygenic, meaning that many factors, both genetic and environmental, contribute to its development amongst generations **(Thomsen, 2015).**

Globally, asthma is a major public health issue that impacts over 260 million people worldwide. While overall asthma prevalence in Asia is reported to be lower than in Western nations, the region's vast demographic and genetic heterogeneity means that prevalence rates vary widely, from as low as 1.4% in Bangladesh to 16.8% in the Philippines **(Rabe *et al.*, 2023).** Furthermore, with rapid urbanization, many Asian cities are experiencing a concerning rise in asthma, particularly among children and adolescents, with air pollution being a significant contributing factor. This increase, combined with a historical lack of research specifically targeting Asian populations, underscores a critical gap in our understanding.

During asthma, allergen-specific Th2 cells produce type 2 cytokines (interleukin (IL) IL-4, IL-5, IL-9, and IL-13) upon recognizing these triggers, leading to the overproduction of mucus, the accumulation of eosinophils in walls of the airways, and the synthesis of immunoglobulin E (IgE) by allergen-specific B cells **(Hammad and Lambrecht, 2021).** This disease affects people of all ages, with symptoms manifesting as breathlessness, wheezing, and coughing. Asthmatic episodes are typically reversible, either spontaneously or via appropriate asthma treatments such as the administration of a fast-acting bronchodilator (**Vos *et al.*, 2020**).

The investigation of biochemical factors contributing to asthma in Asian populations holds significant scientific value, due to the inherent genetic heterogeneity within Asian populations, encompassing diverse ethnicities with unique genetic backgrounds. This necessitates a focused study to uncover specific genetic variations associated with asthma susceptibility and severity within this diverse group. Several proteins involved in the pathogenesis of asthma in Asian populations include ADAM33 (**Shen *et al.*, 2017**) as discussed below; ORMDL3 which plays a crucial role in endoplasmic reticulum stress, strongly linked to asthma (**Moffatt *et al.*, 2007**); IL-13,a cytokine that acts as a key mediator of the type 2 inflammatory response characteristic of asthma **(Sahnoon *et al.*, 2025);** and TSLP, a cytokine involved in the activation of type 2 immune responses implicated in asthma pathogenesis **(Sahnoon *et al.*, 2025).**

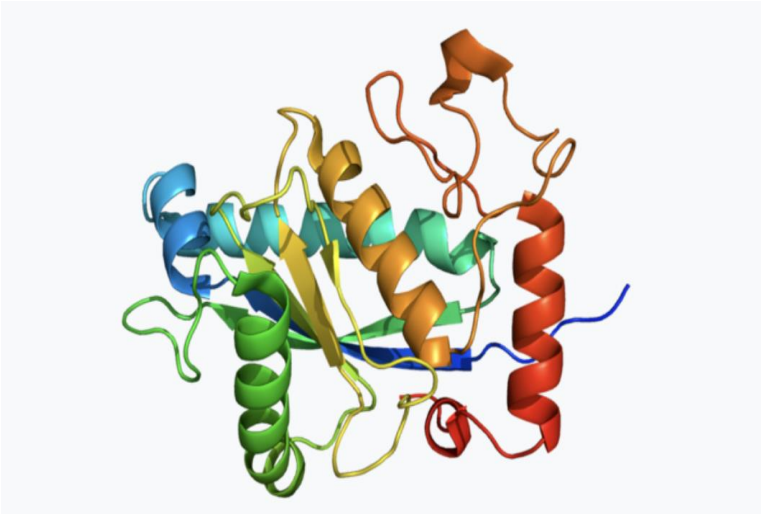

Figure (1). Predicted three-dimensional structure of the wild-type ADAM33 protein

ADAM33 (A Disintegrin and Metalloprotease 33), specifically, is an asthma susceptibility gene associated with bronchial hyperresponsiveness **(Slager *et al.*, 2012).** Focusing on this gene within the context of diverse Asian populations allows for the identification of specific genetic mutations that may

contribute to the unique asthma phenotypes observed within these populations. While environmental factors undoubtedly play a role in the development of asthma, studying genetic variations within Asian populations provides a complementary perspective that can provide valuable insight into the biochemical factors of asthma within Asia, enabling the identification of individuals at higher risk and facilitating preventive measures, paving the way for the development of new therapeutic approaches, such as gene therapy or personalized medications.

Located on chromosome 20p13, the ADAM protein encodes a membrane-anchored metalloprotease, expressed in airway smooth muscle cells and fibroblasts **(Viyati *et al.*, 2023).** The protein's complex multi-domain structure includes a disintegrin domain involved in cell adhesion, and a metalloprotease domain responsible for its enzymatic activity.

Polymorphisms in ADAM33 have been linked to accelerated decline in lung function and airway remodeling—a key feature of asthma pathogenesis **(Yan *et al.*, 2021)**, playing a role in modifying airway smooth muscle proliferation and differentiation **(Slager *et al.*, 2012).** The rs2787094 polymorphism, specifically, has shown consistency with asthma risk in several studies – especially in Asian populations **(Liang *et al.*, 2013),** altered ADAM33 activity conceivably contributing to asthma development or progressions **(Viyati *et al.*, 2023).**

The exact biochemical mechanisms through which ADAM33 and its mutants cause asthma are still largely unknown. ADAM33's structure has been elucidated, but little is known about its asthma-causing mutants. In this work, we aim to leverage ColabFold **(Mirdita *et al.*, 2022)**, an open-source version of AlphaFold, to study how the structure of ADAM33's mutants, including rs2787094, which is strongly associated with increased asthma risk, vary from the wildtype structure in order to better understand how these mutants may result in asthma.

2. Methods

The gene sequences of the ADAM protein were obtained through the use of publicly-available genetic databases – primarily the 'Sequences & Isoforms' section of the Universal Protein Resource, also referred to as UniProt (**Mirdita *et al.*, 2022).** Created by the European Bioinformatics Institute (EMBL-EBI), the SIB Swiss Institute of Bioinformatics, and the Protein Information Resource (PIR), UniProt is a comprehensive domain with a catalog of experimentally characterized proteins, along with their sequences and clusters. The databases available in UniProt include UniProtKB (with sub-parts Swiss-Prot and TrEMBL), UniParc, UniRef, and Proteome.

After obtaining the amino acid sequence of the ADAM33 gene (UniProt ID: Q9BZ11) from these databases, we truncated the ADAM33 protein to focus on its catalytic domain, which is most relevant for biological action and known to play a key role in asthma-related processes. The protein was cut at residue 400, as the catalytic active site specifically ranges from residues 200 - 400.This region contains several key structural elements of importance to the functionality of the protein. This includes the catalytic zinc ion, needed for the protein's enzymatic activities coordinated by histidine residues His345, His349, and His355. Located near these histidine residues is Glu346, which acts as a base for proteolysis **(Orth *et al.*, 2004).**

The S1' pocket – large and mostly hydrophobic with some hydrophilic characteristics - is a feature required for substrate specificity. Met373 forms a "methionine turn" at the bottom of the zinc-binding site, a conserved feature in the metzincin family of metalloproteases. The β4 strand, its N-terminal loop, and the C-terminal wrapping loop (residues 375-378) form the substrate-binding groove **(Orth *et al.*, 2004).** Although this smaller region's structure can be affected by the structure of other regions of the protein, we truncated the protein for computational efficiency. To confirm that this truncation did not significantly alter the structure of residues 200-400, we also modeled regions of increasingly larger size; our predictions of these larger regions did not deviate significantly from the predictions for the 200-400 region alone.

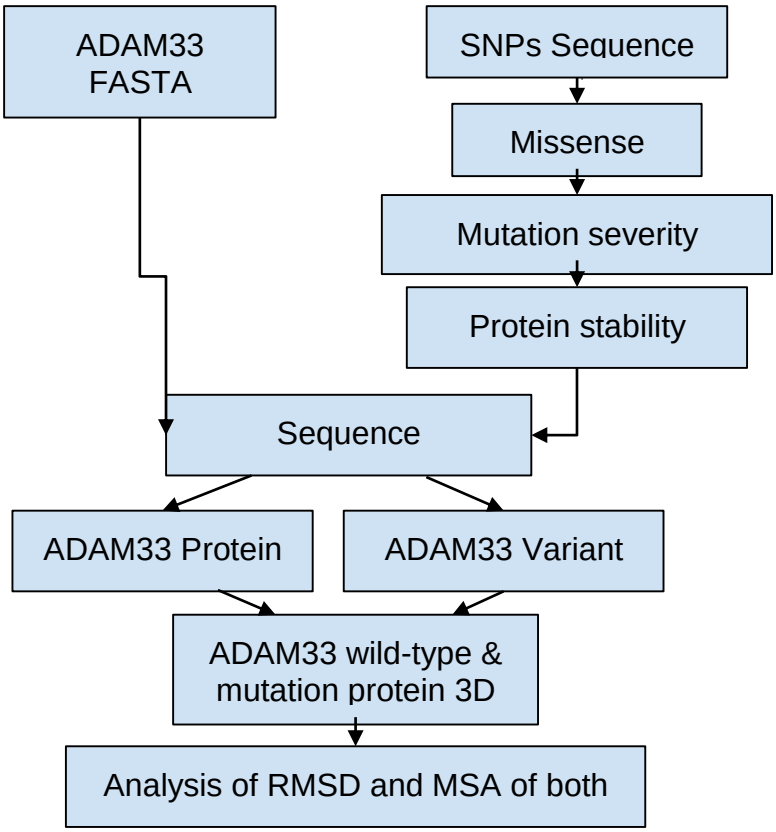

Figure (2). Methodology for in Silico Analysis of ADAM33 Protein and SNP Variants

Once the appropriate residues were identified, the cut sequences were put into AlphaFold **(Senior *et al.*, 2020)** – an ML-based algorithm integrated into the Chimera X software that utilizes the amino acid sequences of proteins to predict their three-dimensional structures. UCSF's ChimeraX is an interactive molecular visualization program, successor to the original UCSF Chimera, developed by the UCSF Resource for Biocomputing, Visualization, and Informatics (RBVI) – providing a comprehensive suite of tools for researchers to explore the structure of biological molecules through simple visualization, molecular dynamic simulations, and publication-quality image & animation generations, amongst other functions.

ColabFold **(Mirdita *et al.*, 2022)** is an open-source platform built on the foundation of AlphaFold2 **(Jumper *et al.*, 2021)** and RoseTTAFold **(Baek *et al.*, 2021),** used to predict protein structures directly through Google Colaboratory. ColabFold integrates a rapid sequence search algorithm – MMseqs2 - to predict these sequences, with the capability for both single-chain predictions and complex modeling; this includes homo- and heteromeric protein complexes. Its batch processing capabilities for high-throughput studies exposes internal parameters for fine-tuning predictions, becoming a tool essential in structural biology research.

AlphaFold is the foundation behind ColabFold, developed by DeepMind. AlphaFold utilizes evolutionary data such as multiple sequence alignments (MSAs) along with deep learning to accurately infer the three-dimensional conformations of proteins. Its capabilities were proven during the CASP14 (Critical Assessment of Structure Prediction) competition confirming that AlphaFold can make predictions with near-experimental accuracy, and with accuracies far greater than alternative methods. Through the use of AlphaFold, over 200 million freely-available predicted structures have been made, enabling breakthroughs in protein engineering, allowing for furthering our understanding of the molecular mechanisms of diseases.

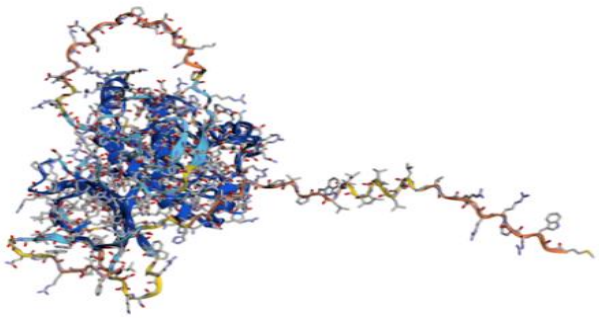

Figure (3). Detailed visualization of the ADAM33 protein variant (residues 0−400) and surrounding residues

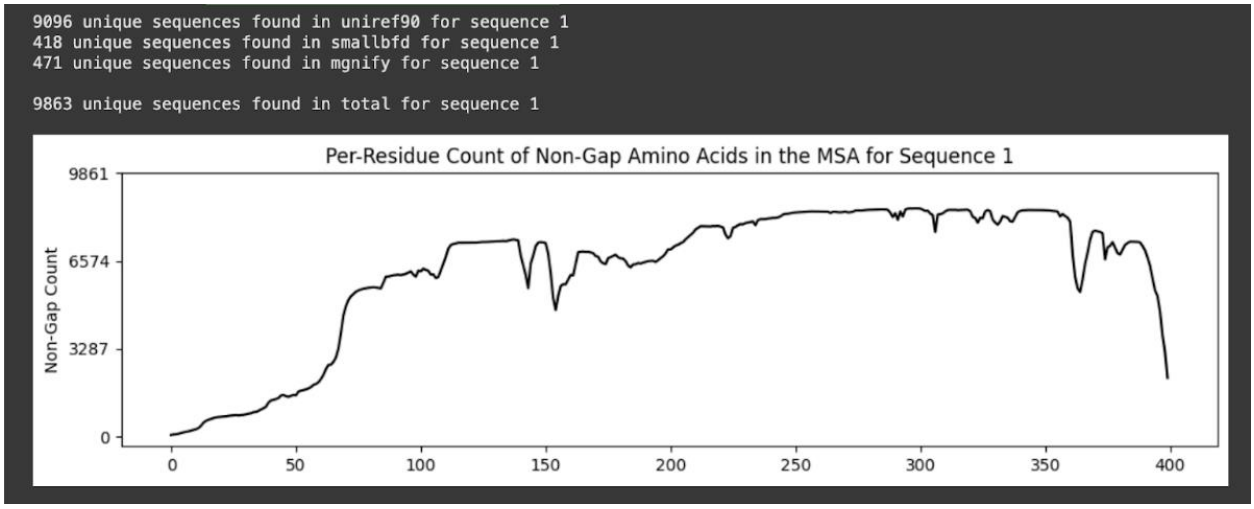

Figure (4). Per-residue conservation plot for ADAM33 residues 0−400 from Multiple Sequence Alignment (MSA)

As shown in the images above, Multiple Sequence Alignment (MSA) parameters identified a total of 9863 sequences for residues 0-400, drawn from several databases: 9096 from UniRef90[19], 418 from SmallBFD **(Steinegger *et al.*, 2019)** and 471 from MGnify **(Mitchell *et al.*, 2020).** These sequences formed the basis of the MSA used for the analysis of residues 0-400 of ADAM33. To reduce redundancy and computational cost, similar sequences within the MSA were automatically clustered by AlphaFold, grouping highly similar sequences together. AlphaFold's default recycling parameter of 3 was used, with the model refining its predictions three times to increase accuracy. AlphaFold's outputs included per-residue Local Distance Difference Test (pLDDT) scores, which provide a measure of confidence in the predicted structure at each amino acid position and the final predicted 3D protein structures.

Following residue identification, Single Nucleotide Polymorphism (SNP) selection and characterization were performed. Missense variants of the ADAM33 gene were selected from the UniProt database. This selection was restricted to missense mutations to focus on amino acid substitutions that directly impact the protein sequence. To predict the functional consequences of these

missense SNPs, computational profiling was performed using PolyPhen-2. Subsequently, MuPro was employed to assess mutation severity by calculating ΔΔG values, which quantify changes in protein stability. Elevated ΔΔG values indicate increased instability. These steps constitute for in-silico variant profiling and stability analysis. Each selected missense SNP was then put into AlphaFold for 3d modelling. The outputs are now ready to be used for structural analysis.

3. Results

Our hypothesis is that single nucleotide polymorphisms (SNPs) within the ADAM (A Disintegrin and Metalloproteinase) gene family that lead to specific amino acid substitutions will induce changes in protein stability and conformation. tested through investigating several ADAM missense mutations, specifically SNPs rs2280091, rs2280090, rs41467948, rs3918396/S1, and rs41534847. The methodology uses several computational protein structure prediction and analysis tools to quantify the impact of these mutations; MuPro for predicting the change in Gibbs free energy (ΔΔG) to assess protein stability, and ChimeraX, incorporating AlphaFold predictions, for structure visualization, analysis of predicted local distance difference test (pLDDT) scores, and calculation of alignment scores and Root Mean Square Deviation (RMSD) to explain resulting conformational changes at an atomic level.

SNP ID	Position	Mutation	ΔΔG (kcal/mol)	Stability Effect	Alignment Score	RMSD (Pruned)	RMSD (Full)
rs2280091	764	M → T (Methionine→Threonine)	-1.333	Destabilizing	4248.4	999	22.123
rs2280090	774	P → S (Proline→Serine)	-0.43	Mildly Destabilizing	4238.5	1.008	22.108
rs41467948	109	N → S (Asparagine→Serine)	-1.466	Destabilizing	4238.5	1.008	22.108
rs3918396	710	V → I (Valine→Isoleucine)	-0.74	Mildly Destabilizing	2944.6	614	3.524
rs41534847	272	T → M (Threonine→Methionine)	-47	Neutral	4226.7	967	21.743

Table (1). Predicted stability of selected ADAM33 SNPs, including position, mutation, ΔΔG values, stability effect, alignment score, and RMSD

Protein stability changes were quantified and presented as ΔΔG values (kcal/mol) generated by MuPro. These numerical values reflect the change in Gibbs free energy upon mutation, indicating the impact of each SNP on protein thermodynamic stability **(Bromberg and Rost, 2009)**. These SNPs can induce amino acid substitutions that alter this stability, with the change in Gibbs free energy (ΔΔG), calculated as ΔGmutant−ΔGwildtype, quantifying this impact **(Bromberg and Rost, 2009).** Negative values indicate a destabilizing effect whilst positive values suggest stabilization. The lower the Gibbs

score, the higher the destabilization. As presented in Table 1, the data shows that all analyzed SNPs are predicted to have a destabilizing effect. Specifically, SNP rs41467948, with a ΔΔG of −1.4657071 kcal/mol, showcases the most prominent destabilizing effect, suggesting a significant decrease in protein stability. Conversely, SNP rs41534847 shows the smallest change, with a ΔΔG of −0.046847693 kcal/mol, indicating a negligible impact on protein destabilization. The remaining SNPs (rs2280091, rs2280090, rs3918396/S1) display moderate stabilizing effects, ranging from −0.43028044 to −1.3330304 kcal/mol.

The SNPs are also presented through functional impact predictions from PolyPhen2 (Polymorphism Phenotyping v2), a widely recognized bioinformatics tool designed to predict the potential functional impact of single amino acid substitutions on human proteins **(Adzhubei *et al.*, 2013).** PolyPhen2 achieves this by analyzing various sequence- and structure-based features, including evolutionary conservation, the physicochemical properties of the substituted amino acids, and the proximity of the mutation to known functional or structural domains **(Adzhubei *et al.*, 2013)**. The output of PolyPhen2 are color-coded probability scores, ranging from (bright green, benign effect) to 1.00 (bright red, deleterious effect), visually representing the likelihood of each missense SNP affecting protein function. Along with these scores were sensitivity and specificity metrics, quantifying the predictive performance of PolyPhen-2 for each variant **(Adzhubei *et al.*, 2013).**

SNP ID	Mutation (AA Substitution)	PolyPhen-2 Prediction	Score	Sensitivity	Specificity
rs2280091	M → T (Methionine → Threonine)	Benign	81	0,93	0,85
rs2280090	P → S (Proline → Serine)	Benign	36	0,94	0,82
rs41467948	N → S (Asparagine → Serine)	Benign	426	0,89	0,9
rs3918396	V → I (Valine → Isoleucine)	Benign	69	0,94	0,84
rs41534847	T → M (Threonine → Methionine)	Probably Damaging	1	0	1

Table (2). Predicted functional effects of selected ADAM33 SNPs based on PolyPhen-2 analysis, including mutation, prediction category, score, sensitivity, and specificity

As seen in Table 2, PolyPhen predicted that four out of the five SNPs (rs2280091, rs2280090, rs41467948, and rs3918396) were benign, with probability scores ranging from 0.036 to 0.426. These values, situated toward the lower end of the scale, suggest that these substitutions are unlikely to severely impair protein function. By contrast, rs41534847 was uniquely classified as probably damaging, with a score of 1.000, indicating a high probability of functional disruption.

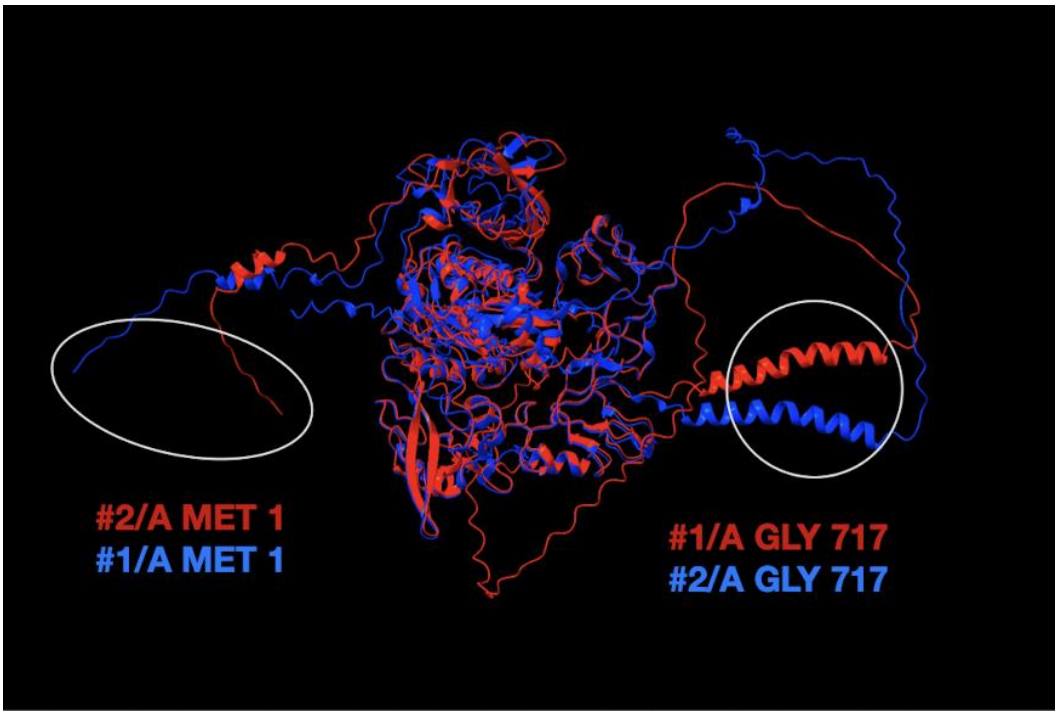

Figure (1). Structural superimposition of ADAM33 (wild type, blue) and rs2280091 (mutant, red), showing the impact of the M→T substitution on protein conformation

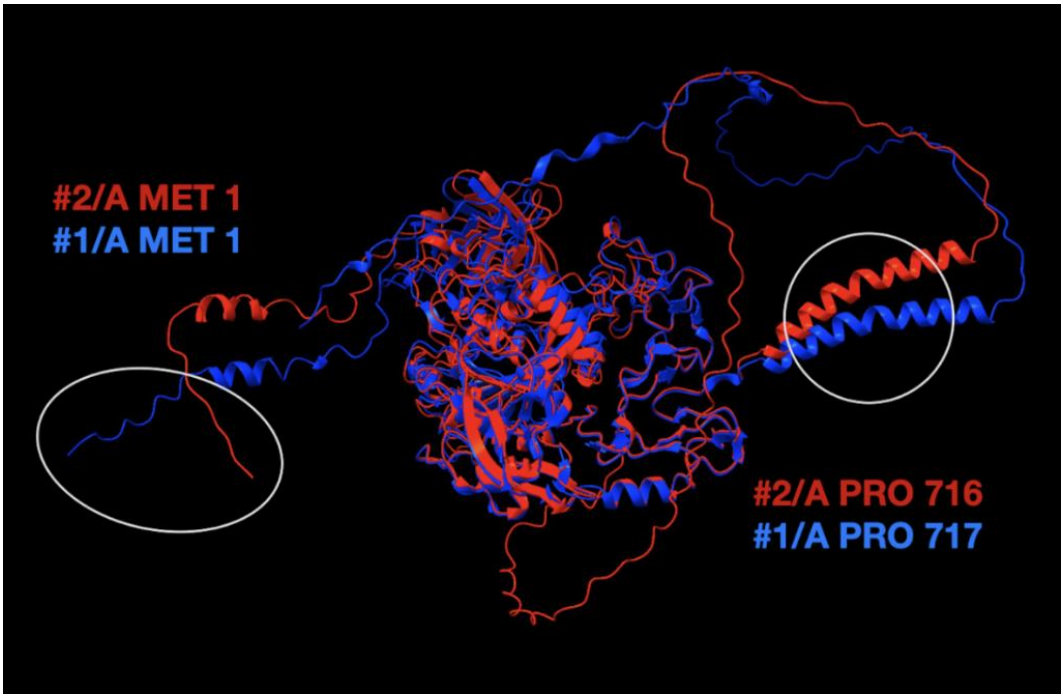

Figure (2). Structural superimposition of ADAM33 (wild type, blue) and rs2280090 (mutant, red), showing the impact of the P→S substitution on protein conformation.

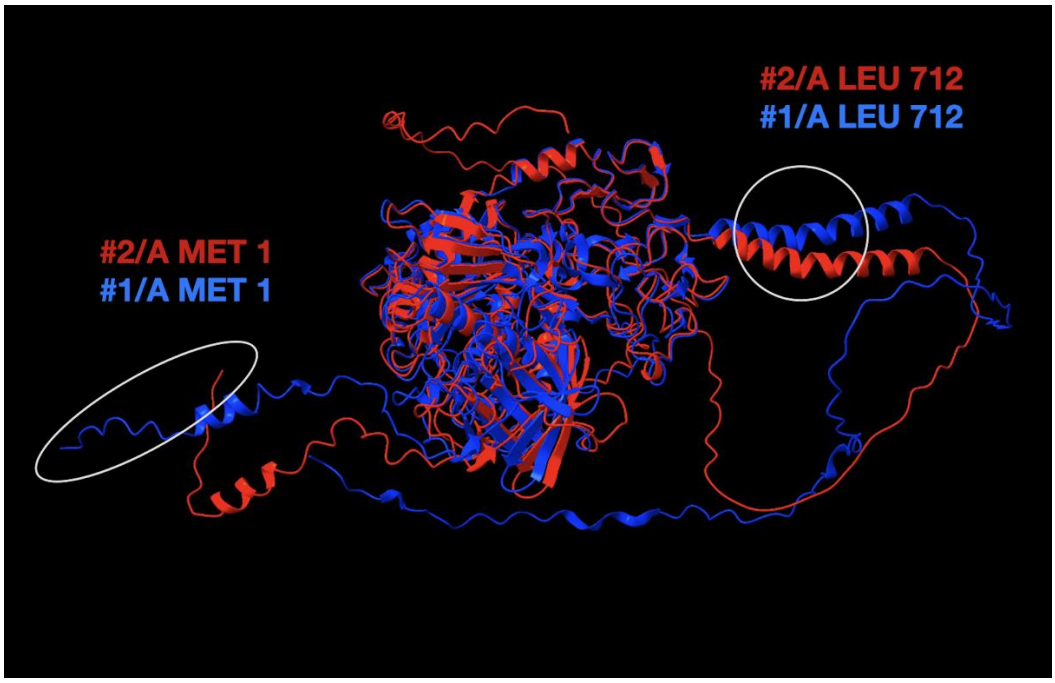

Figure (3). Structural superimposition of ADAM33 (wild type, blue) and rs41467948 (mutant, red), showing the impact of the N→S substitution on protein conformation

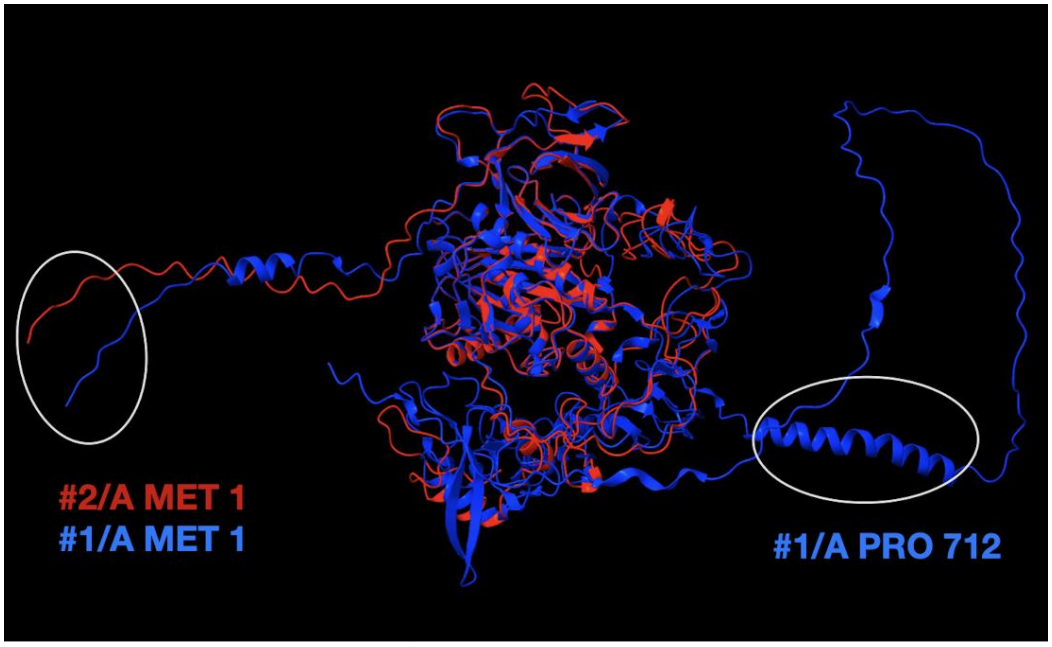

Figure (4). Structural superimposition of ADAM33 (wild type, blue) and rs3918396 (mutant, red), showing the impact of the V→I substitution on protein conformation.

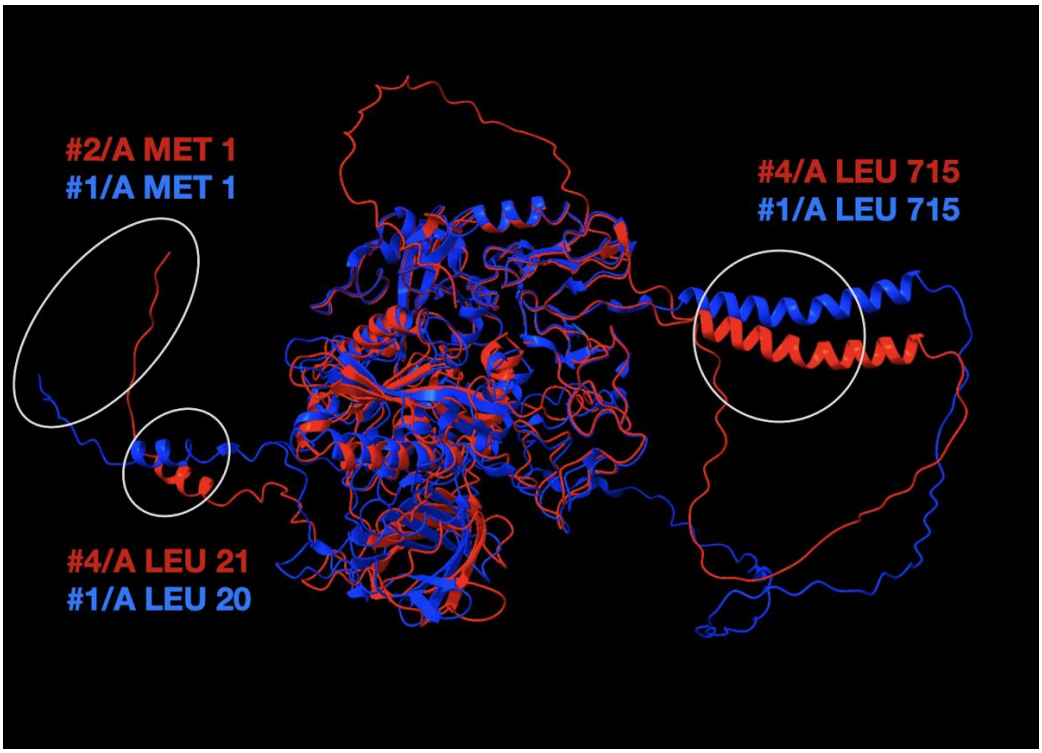

Figure (5). Structural superimposition of ADAM33 (wild type, blue) and rs41534847 (mutant, red), showing the impact of the T→M substitution on protein conformation

Lastly, the selected SNPS were visualized through the output of images generated by AlphaFold2, depicting the predicted three-dimensional models of the variant proteins, as seen in Figures 1 through 5. These images allow the analysis of direct visual comparisons of the structural alterations induced by each amino acid substitution. As mentioned, AlphaFold's default recycling parameter of 3 was used, with the model refining its predictions three times to increase accuracy **(Senior *et al.*, 2020)**. AlphaFold's outputs also include per-residue Local Distance Difference Test (pLDDT) scores which provide a measure of confidence in the predicted structure at each amino acid position & the final predicted 3D protein structures (**Senior *et al.*, 2020).**

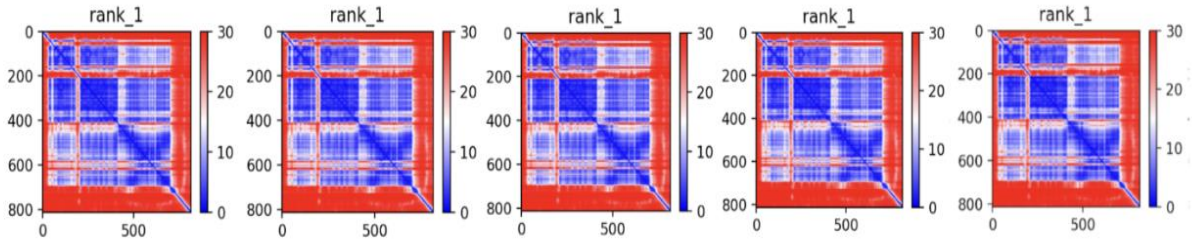

Figure (6). Heatmap of predicted ΔΔG values and stability classifications for ADAM33 SNPs. SNPs are arranged in the same order as in Table 1

The pLDDT (predicted local distance difference test) score is a confidence metric used by AlphaFold to tell how well the predicted structure agrees with multiple sequence alignment data and PDB structure information. It ranges from 0 to 100, with higher values indicating more confident

predictions **(European Bioinformatics Institute, n.d.)**. Generally, regions with pLDDT scores greater than 70 are considered more reliable, as they indicate higher levels of confidence in the structure prediction **(European Bioinformatics Institute, n.d.)**. As observed in Figure 6, localized drops in pLDDT scores correspond to regions where the predicted structure is less confident, or where significant conformational changes are anticipated due to the mutation. In Figure 6, Rs41467948 shows more extensive regions with lower pLDDT scores, suggesting more widespread and less confident structural predictions, indicative of greater structural disruption **(Senior *et al.*, 2020)**

Further quantitative assessment of structural changes is provided by the Table 1 of Alignment Score & RMSD. The Alignment Score reflects the quality or confidence of the sequence alignment used for structural modeling, with higher scores indicating better alignment **(Altschul & Pop, 2017).** The Root Mean Square Deviation (RMSD) is a metric used to quantify the average distance between the atoms of superimposed protein structures. A higher RMSD value indicates a greater deviation between the two structures, implying a more significant structural change induced by the mutation **(Bagewadi *et al.*, 2023).** The table provides two RMSD values: one for "pruned atom pairs" (a subset of atoms considered most representative or stable) and another for "across all 813 pairs" or "across all 569 pairs," which represents the overall structural deviation **(Bagewadi *et al.*, 2023).**

Examining the RMSD values, SNP rs2280091 shows an RMSD of 0.999 Å for pruned atom pairs and 22.123 Å across all 813 pairs. SNP rs2280090 has similar RMSD values of 1.008 Å (pruned) and 22.108 Å (all 813 pairs). SNP rs41467948 also shows RMSD values of 1.008 Å (pruned) and 22.108 Å (all 813 pairs). The RMSD values for rs2280091, rs2280090, and rs41467948 are relatively high, suggesting substantial global structural rearrangements, despite the small changes in pruned atom RMSD. In contrast, SNP rs3918396/S1 shows a lower RMSD of 0.614 Å for pruned atom pairs and a significantly reduced 3.524 Å across all 569 pairs. This shows that rs3918396/S1 induces a considerably smaller overall structural change compared to the other SNPs. Finally, rs41534847 has an RMSD of 0.967 Å for pruned atoms and 21.743 Å across all 813 pairs, again suggesting a substantial structural change. The alignment scores are generally high across most SNPs, indicating good quality alignments for structural prediction, with the exception of rs3918396, which has a lower alignment score of 2944.6.

4. Discussion

The SNPs analyzed exhibit varying degrees of impact on protein stability and structure. Notably, rs2280091, rs2280090, and rs41467948 show significant destabilization ($\Delta\Delta G < -1$) combined with substantial structural deviations (high RMSD > 20 Å) despite very high alignment scores, indicating conservation of sequence but critical structural perturbations. These mutations likely affect essential structural sites or long-range interactions critical for protein folding, leading to global conformational rearrangements. Conversely, rs3918396 exhibits mild destabilization with low RMSD and reduced alignment score, suggesting this variant resides within a more flexible or less conserved region with minimal disruption to core structure. rs41534847 shows a near-neutral stability effect and moderate structural change, likely reflecting localized side-chain perturbations without broad functional consequences.

These findings underscore the complex interplay between sequence conservation, structural stability, and conformational integrity. High sequence similarity does not guarantee conservation of structure or function, especially when mutations occur at critical sites affecting folding dynamics or interaction networks.

The integrated analysis highlights that severe destabilizing mutations can produce extensive structural rearrangements, even when sequence similarity remains high, emphasizing the limitations of sequence-based assessments alone. Mild or neutral mutations in flexible regions tend to preserve structural integrity, reinforcing the need to combine $\Delta\Delta G$, RMSD, and alignment information for comprehensive mutation impact characterization. This combined approach aids in prioritizing variants for experimental validation and provides a framework for understanding SNP effects in protein function and disease relevance.

5. Conclusions

This study leveraged a computational approach to investigate the structural and functional consequences of asthma-associated single nucleotide polymorphisms (SNPs) within the ADAM33 gene, with a specific focus on variants prevalent in Asian populations. Our integrated analysis, employing AlphaFold/ColabFold for structure prediction, MuPro for protein stability, and PolyPhen-2 for functional impact, provides valuable insights into how specific amino acid substitutions may influence the protein's catalytic domain and overall conformation.

Our findings consistently demonstrate that all evaluated SNPs introduce varying degrees of protein destabilization, as evidenced by negative ΔΔG values. The most significant destabilizing effect was observed for rs41467948, while rs41534847 showed a comparatively minor impact on protein stability. Interestingly, despite its minimal effect on stability, PolyPhen-2 analysis uniquely classified rs41534847 as "probably damaging," suggesting a potential to significantly impair ADAM33 function. Structural modeling and Root Mean Square Deviation (RMSD) analyses corroborated these findings, revealing that certain variants induce notable conformational shifts, particularly in regions critical for catalytic activity.

These results collectively indicate that ADAM33 polymorphisms can lead to altered protein stability and conformation. Such changes may contribute to the airway remodeling processes central to asthma pathogenesis. The observed variability in predicted effects among the SNPs highlights the intricate genetic landscape of asthma susceptibility, particularly within diverse Asian populations. By integrating various in-silico structural biology tools, this study provides a mechanistic framework for understanding how specific genetic variants might increase disease risk. This approach serves as a cost-effective strategy for prioritizing variants for further investigation in future experimental and clinical research.

Data and Software Availability

https://www.uniprot.org/

https://www.cgl.ucsf.edu/chimerax/

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