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In Silico Structural Analysis of Malaria - Associated Single Nucleotide Polymorphisms in Human Genes *TLR4*, *ICAM1*, and *IL22*

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ABSTRACT

Background Indonesia's ethnic heterogeneity contributes to substantial human genetic diversity, including variation in genes associated with malaria susceptibility and severity. Single Nucleotide Polymorphisms (SNPs) in host genes encoding immune receptors and adhesion molecules may influence malaria pathogenesis by modulating inflammatory signaling and parasite–host cell interactions.

Aims: This study aimed to evaluate the potential structural and functional impact of selected malaria-associated SNPs in human genes using a systematic *in silico* approach.

Methods: A literature-guided and database-driven screening (dbSNP and UniProt) was used to identify relevant SNPs previously reported to be associated with malaria infection and/or the severity of infection. Inclusion criteria were: (1) localization within coding regions, (2) prior evidence of clinical relevance, (3) resulting in a nonsynonymous amino acid substitution, and (4) annotated with a reference SNP ID (rsID). Selected SNPs were subjected to protein structural modelling. Native and mutant protein structures were compared using PyMOL, conformational changes and differences were quantified using Root Mean Square Deviation (RMSD).

Results: A total of 38 SNPs in *TLR4, ICAM1*, and *IL-22* gene with reported clinical relevance to infection were identified, of which 6 SNPs (*TLR4*: n=2; *ICAM1*: n=3; *IL-22*: n=1) met all inclusion criteria for malaria-associated variants. Five selected SNPs were located in coding regions and resulted in amino acid substitutions, several of which involved changes in residue polarity, whereas one SNPs was located in non-coding region. Structural comparison showed detectable but minimal conformational differences between the native and mutant proteins, with low RMSD values (maximum 0.014 Å in TLR4 variant rs4986790).

Conclusion:This *in silico* analysis suggests that the selected malaria-associated SNPs in *TLR4, ICAM-1*, and *IL-22* genes are unlikely to induce major structural rearrangements but may contribute to localized changes that affect protein interaction interfaces or signaling functions. Their potential contribution to malaria severity may therefore involve minor structural deviation rather than large conformational changes. This study provides a systematic computational framework for prioritizing host genetic variants for further functional validation, particularly in genetically diverse populations such as Indonesia.

Keywords: *Genomic Variant; In Silico; Host; Malaria; Single Nucleotide Polymorphisms (SNPs).*
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1. Introduction

Malaria is a parasitic disease caused by protozoa of the genus *Plasmodium* and transmitted through the bites of infected female *Anopheles* mosquitoes (Alruwaisan et al., 2021). Despite decades of global control efforts, malaria remains a major public health challenge (Sugiarto et al., 2022). The World Malaria Report 2022-2023 estimated 263 million cases worldwide in 2023 (González-Sanz et al., 2023; Lestari, 2025). In Indonesia, malaria continues to be a significant burden, with over 418,546 reported cases in 2025, representing an increase from the previous year (Widi, 2023; "Kasus Malaria di Indonesia," n.d.).

Malaria control strategies in Indonesia continue to prioritize early diagnosis, prompt treatment, vector control, and surveillance. However, the emergence of antimalarial drug resistance presents an additional and growing challenge (Ikfi Hidayati, 2019). In this context, integrating host genetic variation into current frameworks may enhance risk stratification by identifying individuals or subpopulations predisposed to severe disease, given the influence of genetic variation on drug metabolism, immune responses, and therapeutic efficacy (Putri & Wathon, 2019).

Clinical manifestations of malaria range from mild to severe, life-threatening complications, including cerebral malaria, severe anemia, and multi-organ dysfunction (Sirisabhabhorn et al., 2021). Individuals exposed to similar parasite strains and environmental conditions often exhibit different clinical outcomes. Single Nucleotide Polymorphisms (SNPs) in the host gene constitute as a key source of genetic variability, contributing to up to one-third of the observed risk of severe malaria (Kariuki & Williams, 2020). SNPs in immune related genes may influence protein structure and function, which affects pathogen recognition, inflammatory signaling, and parasite-host interaction (Penha-Gonçalves, 2019; Alruwaisan et al., 2021). Well-known established determinants include hemoglobin variants, glucose-6-phosphate dehydrogenase (G6PD), pyruvate kinase, and haptoglobin (Alruwaisan et al., 2021). SNPs in genes encoding inflammatory mediators, such *as MCP1, TGFβ1, TNFα, IL-4 VNTR, IL-6,* and *IL-10*, have been implicated in regulating of host immune responses during infection. In parallel, genes involved in parasite adhesion and host–pathogen interactions, including *TLR4, ICAM1,* and *CD36*, have emerged as contributors to malaria pathogenesis. While numerous studies have identified associations between specific SNPs and malaria outcomes, the functional implications of many variants remain insufficiently characterized.

Among candidate genes, *TLR4, ICAM1*, and *IL-22* are of particular interest due to their roles in key pathogenic mechanisms. TLR4 (Toll-like receptor 4) is a pattern recognition receptor involved in innate immune activation and inflammatory signaling to parasites. Variation in *TLR4* may alter downstream NF-κB signaling and cytokine production. ICAM-1 (intercellular adhesion molecule 1) protein mediates cytoadherence of *Plasmodium falciparum*-infected erythrocytes to the vascular endothelium, a critical step in microvascular pathology that leads to cerebral malaria. IL-22 protein plays a role in immune regulation and tissue protection during inflammatory responses. SNPs in these three genes have also been associated with altered immune responses and clinical severity in malaria-endemic regions, particularly Southeast Asia (Rani et al, 2018; Sirisabhabhorn et al., 2021). This study aims to systematically evaluate selected malaria-associated SNPs in *TLR4, ICAM1*, and *IL-22* genes using *in silico* structural analysis. By focusing on coding variants with potential functional impact, this study aims to bridge the gap between genetic association data and molecular mechanisms.

2. Methods

Study Datasets

SNPs data on genes associated with susceptibility and severity of malaria, considering the allele frequency in Asia/South Asia/East Asia, were obtained through literature reviews. Publications from 2000 to 2023 were systematically screened. SNPs were included if they met the following criteria: (1) localization within coding regions, (2) prior evidence of clinical relevance (SNPs with ClinVar annotations, and SNPs with clinical variants related to malaria), 3) resulting in nonsynonymous amino acid substitution, and (4) annotated with a reference SNP ID (rsID). Identified variants were cross-referenced using public genomic databases, including dbSNP

(https://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgiand) and UniProt (https://www.uniprot.org/uniprotkb/O00206/entry#sequences), to confirm genomic position, transcript annotation, and consequences in their protein level.

Functional Prediction and Annotations

Variant annotation and functional prediction were performed using the Ensembl Variant Effect Predictor (VEP) tool (https://asia.ensembl.org/Tools/VEP). The potential impact of amino acid substitution was further assessed using two widely validated tools, the SIFT (Sorting Intolerant From Tolerant) and PolyPhen-2 (Polymorphism Phenotyping v2).

SIFT scores ≤ 0.05 were classified as deleterious while scores > 0.05 were considered tolerated. PolyPhen-2 predictions were interpreted as follows: score 0.00–0.15 (benign); 0.15–0.85 (possibly damaging), > 0.85 (probably damaging). In addition, physicochemical changes associated with amino acid substitution, including polarity, charge, and hydrophobicity, were evaluated to gather functional effect on protein stability, receptor-ligand interaction, and intercellular signaling.

Structural Modelling and Visualization

Protein structural analysis was conducted to compare the native (wild-type) and mutant variants. Reference protein sequences were obtained from UniProt (https://www.uniprot.org/uniprotkb/O00206/entry#sequences), and corresponding three-dimensional structures were retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/ and/or https://www.ncbi.nlm.nih.gov/snp/) when available. For a protein lacking a complete experimental structure, homology modelling was performed using SWISS-MODEL (https://swissmodel.expasy.org/interactive), with selected templates based on sequence identity and coverage.

Mutant structures were generated by introducing amino acid substitution into the native using PyMOL (version 2.6.2 Open-source; Schrödinger, LLC, 2023). The protein's structural alignment was performed using an algorithm in PyMOL. Visualization was used to qualitatively assess the local structural perturbations, particularly within the functional domains or ligand-binding regions.

RMSD Calculation and Interpretation

Differences between the native and mutant protein's structure were quantified using Root Mean Square Deviation (RMSD) values following the superimposition. The value calculation is based on backbone atom alignment to evaluate global conformational changes. RMSD interpretation was defined as follows: RMSD < 0.5 Å indicates negligible structural deviation; 0.5–2.0 Å indicates minor to moderate structural changes; and > 2.0 Å suggest potentially significant structural alterations. Given the sensitivity of RMSD to global structure, additional qualitative assessment of local residue environments was performed to interpret minor numerical differences.

Data Analysis

All identified SNPs from the human genes (*TLR4, ICAM1, and IL22*) and associated annotations were systematically recorded and curated using Microsoft Excel. The analysis was mainly descriptive, summarizing the distribution, predicted functional impact, and structural effects of selected variants. No inferential statistical analyses were performed.

3. Results

Candidate SNPs

In this study, candidate SNPs were identified through a structured literature review focusing on human genetic variation and its impact on malaria outcomes. Three of the most frequently reported malaria-associated SNPs in human genes, particularly in the Asian population, including South Asia and East Asia, were selected for further analysis (Table 1).

Table 1. Malaria-associated candidate SNPs

No	Gene	SNP	Clinical Effects
1	***TLR4***	rs4986790	Reduces TLR4 signaling, and does not activate the inflammatory response efficiently
2	***IL22***	rs2227483	Protective role in malaria associated severe anemia, and IL-22 is increased in children infected with *P. falciparum*
3	***ICAM1***	rs5498	Affects malaria parasitemia levels. A>G mutations disrupt the binding properties of ICAM-1 to erythrocytes, thereby reducing the severity of malaria pathogenesis.

Clinical Annotation of SNPs

The selected SNPs were subsequently queried in the dbSNP database at NCBI (https://www.ncbi.nlm.nih.gov/snp/) and align with ClinVar to identify variants with clinical annotations. SNPs within the three target genes were then evaluated based on their genomic context, coding and non-coding regions (Figure 1).

SNP Effect Prediction

The pathogenicity effect analysis of SNP in *TLR4, ICAM1* and *IL22* genes using the SIFT, PolyPhen-2, and VEP showed a wide range of differences, from tolerated/benign to experiencing the effects of deleterious/damaging (Table 2).

Table 2. Comparison of Clinical Variant SNPs in dB SNPS using VEP Analysis

Clinical Variant of SNPs	***TLR4***		***ICAM1***		***IL-22***	
Searching SNPs in the Database	8.301		3.183		7.576	
Duplication Exclusion	8.165		3.103		7.354	
Clinvar at db SNP	18	0,22%	2	0,06%	18	0,24%
VEP SNPs Impact Prediction	21.653	2,65/SNP	44.123	14,21/SNP	4602	6,25/SNP
High Impact	124	0,57%	103	0,23%	33	0,72%
Low	590	2,72%	632	1,43%	206	4,48%
Moderate	1198	5,53%	974	2,21%	292	6,35%
Modifier	19741	91,17%	42414	96,13%	4071	88,46%
Clinvar SIFT Prediction	1.190	14,57%	971	31,29%	290	3,94%
Tolerated low confidence	25	2,10%	29	2,99%	0	0,00%
Tolerated	551	46,30%	488	50,26%	152	52,41%
Deletorious low confidence	33	2,77%	34	3,50%	0	0,00%
Deletorious	581	48,82%	420	43,25%	138	47,59%
Clinvar PolyPhen-2 Prediction	1.190	14,57%	896	28,88%	290	3,94%
Probably Damaging	336	28,24%	274	30,58%	102	35,17%
Possibly damaging	203	17,06%	151	16,85%	44	15,17%
Benign	651	54,71%	471	52,57%	144	49,66%

The table summarizes the comparison between SNPs with clinically annotated variants and those predicted by VEP, Shift, and Polyphen-2 tools. Notably, clinically annotated variants account for less than 1% of the total SNPs identified in the *TLR4, ICAM1*, and *IL22* genes, underlining the substantial proportion of variants that remain functionally uncharacterized. VEP-based annotations imply that individual SNPs may be associated with multiple predicted consequences, reflecting transcript diversity and variant context. Respectively, each SNPs in *TLR4, ICAM1,* and *IL22* genes associated with approximately 2, 14, and 6 predicted variant effects.

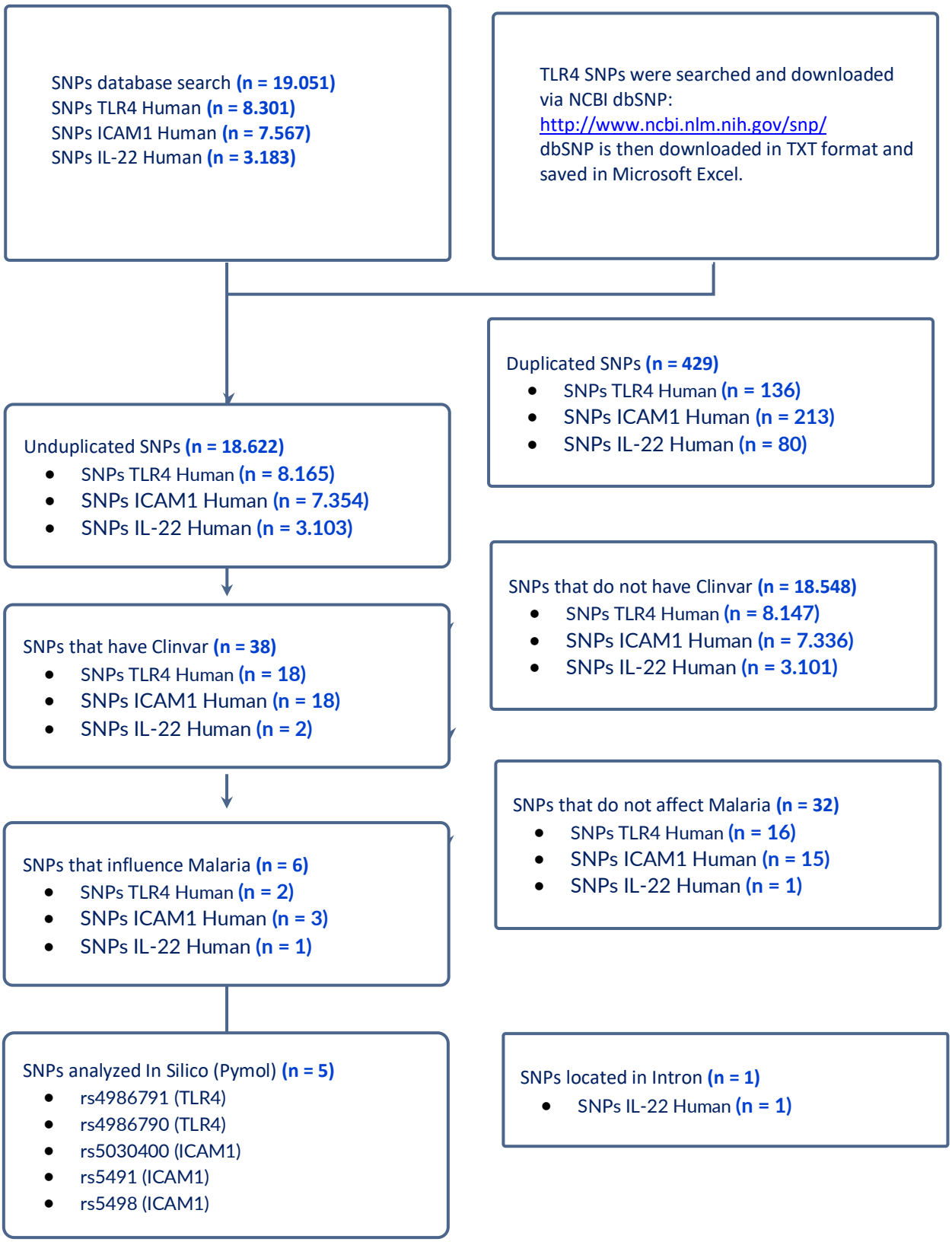


Figure 1. Data quering of Malaria-associated SNPs in *TLR4*, *ICAM1*, and *IL22* gene

Analysis of Protein Structure and Modeling

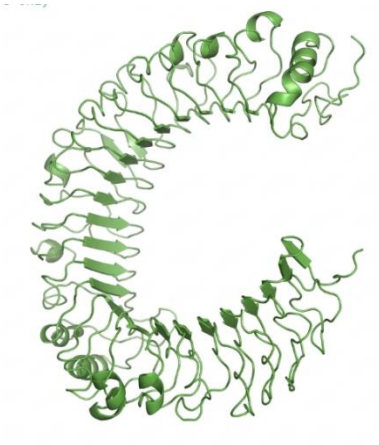
Six clinically relevant variants were selected for in-depth analysis, including rs4986791, rs4986790, rs2227483, rs5494, rs5491, and rs5498. Table 3 outline the amino acid substitution of selected SNPs, their physicochemical changes, and reported biological implications as described in previous studies.

Table 3. Physiochemical changes of Malaria-associated SNPs in *TLR4*, *IL22*, and *ICAM1* gene

No	Gene	Id_SNP	Position	Amino Acid Substitution	Physicochemical changes	Predicted Functional Effects
1	***TLR4***	rs4986791	exon 4	T (ACC) > I (ATC)	Polar → Non-polar	Alters glycosylphosphatidylinositol (GPI) binding and results in approximately 50% reduction in *TLR4* activity, leading to decreased production of pro-inflammatory cytokines that may influence malaria clinical outcome (Iwalokun et al., 2015).
		rs4986790	Exon 4	D (GAT) > G (GGT) D (GAT) > G (GTT)	Charged → Non-polar Charged → Non polar	Associated with reduced *TLR4* signaling, resulting in attenuated inflammatory response. It also affects the extracellular domain of the receptor and disrupts *TLR4* trafficking to the cell membrane (Rani et al., 2018).
2	***IL-22***	rs2227483	Intron	A > T (No Amino Acid substitution)	N/A	Located within the putative binding site of the aryl hydrocarbon receptor complex (AhR-ARNT), which interacts with specific ligands to regulate IL-22 production. The T allele has been associated with altered IL-22 expression (Aljarba et al., 2020).
3	***ICAM1***	rs5030400	Exon 7	R (CGG) > G (GGG) R (CGG) > W (TGG)	Charged → Non-polar Charged → Non-polar	The dbSNP database annotates the SNP as being associated with malaria, but the available literature does not provide strong evidence supporting its clinical relevance.
		rs5491	Exon 2	K (AAG) > R (AGG) K (AAG) > M (ATG)	Charged → Charged Charged → Non-polar	Known as *ICAM1* Kilifi, results in a Lysine to Methionine substitution at position 29. The allele has been associated with increased susceptibility to severe malaria (Gomez et al., 2013).
		rs5498	Exon 6	K (AAG) > E (GAG)	Charged → Charged	Involved in malaria parasite binding and influences parasitemia levels. It is also alters the binding properties of ICAM-1, particularly it interaction with parasite virulence protein PfEMP1, thereby contributing to severe malaria. Notably the G allele has been associated with lower *P. falciparum* parasite density (Sirisabhabhorn et al., 2021).

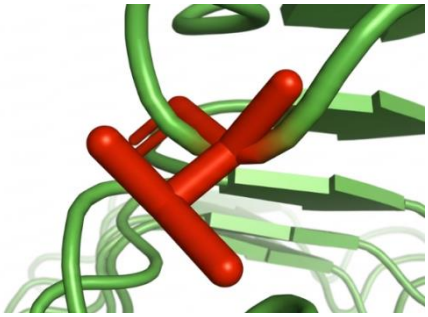
Among these 6 candidate SNPs, one SNP (rs2227483) is located in a non-coding intronic region, whereas the remaining variants are exonic and result in amino acid substitutions. The rs4986790 variant involves two nucleotide substitutions (A>G and A>T), both leading to a change from Aspartic Acid (D) to Glycine (G). Similarly, SNP rs5491 exhibits two alternative substitutions (A>G and A>T), resulting in amino acid changes from Lysine (K) to Arginine (R) and Methionine (M), respectively.

The functional impact of candidate SNPs was further evaluated using an *in silico* structural approach. Protein modelling and visualisation were performed to compare the wild-type and mutant structures. The 3-dimensional structure of each protein was visualized by Swiss-MODEL (https://swissmodel.expasy.org/). The protein models were constructed using a structural template obtained from the Protein Data Bank (PDB), with the resulting models presented in Figure 2. The generated models were subsequently aligned with the corresponding wild-type structures to evaluate the structural impact of candidate SNPs.

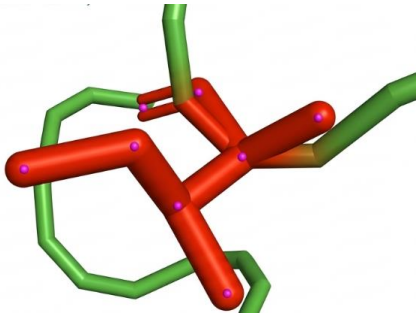


TLR4 Protein Structure

a) TLR4 protein

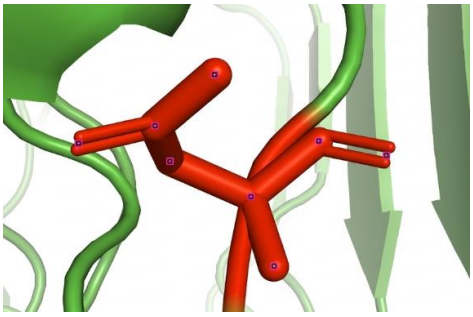


Wild-type

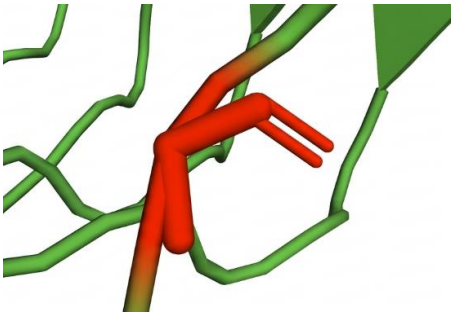


rs4986791 T (ACC) → I (ATC)

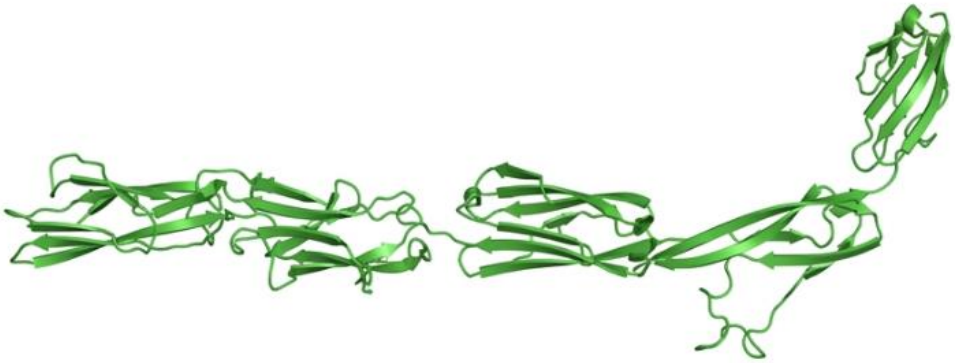
b) TLR4 protein



Wild-type

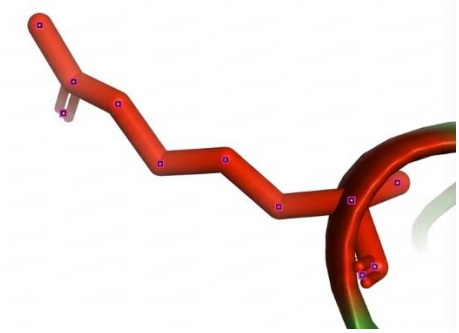


rs4986790 D (GAT) → G (GGT) or
D (GAT) → G (GTT)

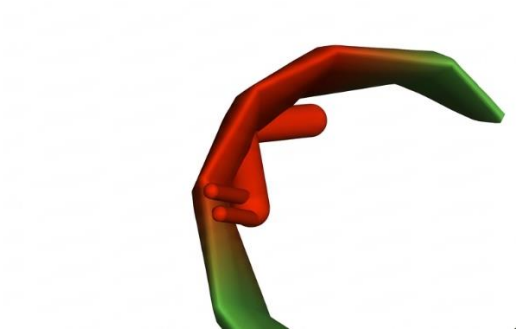


ICAM-1 Protein Structure

c) ICAM-1 protein

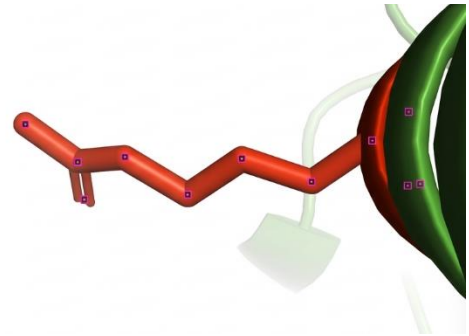


Wild-type

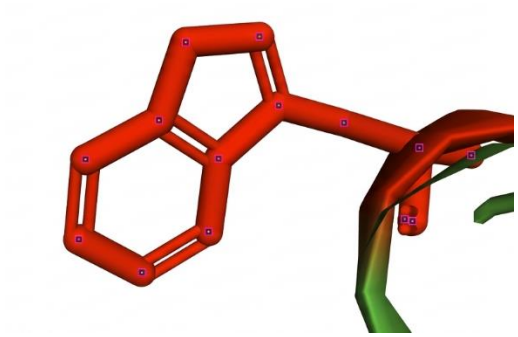


rs5030400 R (CGG) → G (GGG)

d) ICAM-1 protein



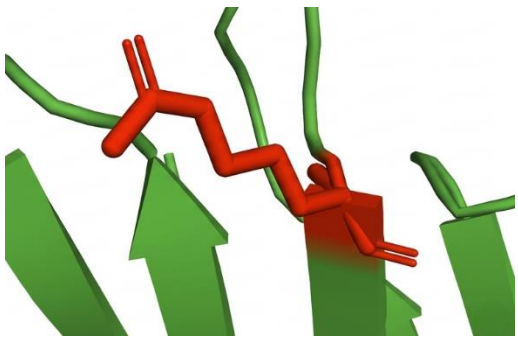
Wild-type



rs5030400 R (CGG) → W (TGG)

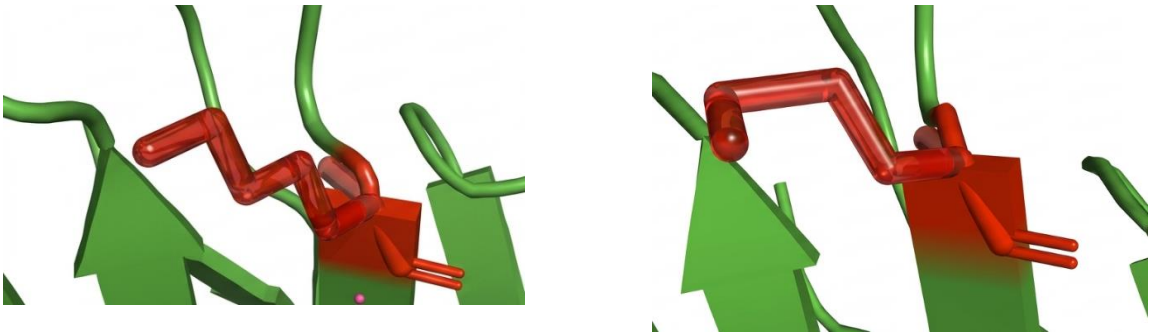
e) ICAM-1 protein

Wild-type



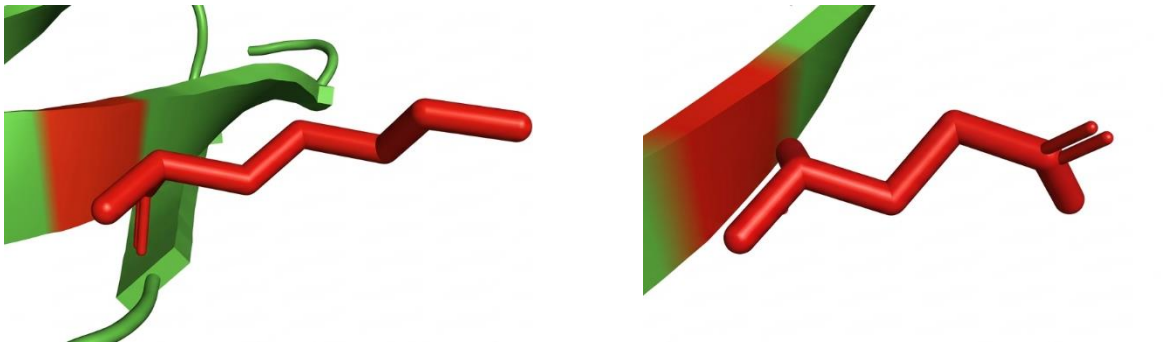
rs5491 K (AAG) → R (AGG)

f) ICAM-1 protein



Wild-type rs5491 K (AAG) → M (ATG)

g) ICAM-1 protein



Wild-type rs5498 K (AAG) → E (GAG)

Figure 2. PyMOL based structural comparison of wild-type and mutant TLR4 and ICAM1 proteins

a). Visualisation of amino acid changes at 399th position of TLR4 protein, Treonin to Isoleusin; b) Change of amino acid at 299th position, Aspartic acid to Glycine; c) Change of amino acid at 478th position, Arginine to Glycine; d) Change of amino acid at 478th position, Arginine to Tryptophan; e) Change of amino acid at 56th position, Lysine to Arginine; f) Change of amino acid at 56th position, Lysine to Methionine; g) Chnage in amino acid at 469th position, Lysine to Glutamic acid.

Quantification of Conformational Changes between wild-type and mutant

Structural deviation of candidate SNPs between their wild-type and mutant structures was quantified using the Root Mean Square Deviation (RMSD) analysis. Table 4 shows the alignment results for a total of five exonic SNPs in the *TLR4* and *ICAM1* genes that were assessed using PyMOL's structural modeling, consistently demonstrating very low RMSD values (<0.05 Å) and high structural similarity between wild-type and mutant forms.

Table 4. Protein Superimposing analysis of Candidate SNPs

No	Protein	SNPs ID	Position	Amino Acid Substitution	RMSD (A)
1	**TLR4**	rs4986791	399	T (ACC) --> I (ATC)	0.001
		rs4986790	299	D (GAT) --> G (GGT)	0.014
			299	D (GAT) --> G (GTT)	0.014
2	**ICAM-1**	rs5030400	478	R (CGG) > G (GGG)	0.002
			478	R (CGG) > W (TGG)	0.001
		rs5491	56	K (AAG) --> R (AGG)	0.001
			56	K (AAG) --> M (ATG)	0.003
		rs5498	469	K (AAG) --> E (GAG)	0.004

All analyzed SNPs exhibited RMSD values ranging from 0.001 to 0.014 Å. Based on the predefined criteria (RMSD < 0.5 Å, indicating negligible structural deviation), these results suggest that all variants fall within the category of minimal conformational change. Accordingly, these candidate SNPs are unlikely to substantially disrupt the overall three-dimensional folding of TLR4 and ICAM1 proteins.

Cumulative Result of SNPs Effect

Despite these minimal global structural changes, functional implications may differ between genes. Variants in the *TLR4* gene are more consistently associated with dysregulated inflammatory responses, which determines severe phenotype, whereas *ICAM1* variants are primarily associated with endothelial adhesion processes that contribute to microvascular complications in malaria and are also involved in parasite binding, thereby influencing malaria susceptibility (Table 5).

Table 5. The Result of Cumulative SNPs Effect Prediction

Protein	SNP ID	Susceptibility	Severity
TLR4	rs4986790	Yes	Strong association
TLR4	rs4986791	Yes	Moderate–Strong association
ICAM-1	rs5498	Yes	Strong association
ICAM-1	rs5491	Possible	Context-dependent
ICAM-1	rs5030400	Possible	Limited evidence
IL-22	rs2227483	Possible	Limited evidence

4. Discussion

Malaria remains a major global infectious disease in which clinical outcomes are shaped by complex interactions among parasite biology, environmental exposure, and host genetic background. Increasing number of malaria cases highlights host genetic variation as a key determinant of disease severity, particularly through its role in modulating immune responses and parasite-host interactions (González-Sanz, Berzosa, & Norman, 2023). By integrating genetic association data with *in silico* structural analysis, this study provide insight on the potential functional implication of selected malaria-associated SNPs.

The present findings support the biological relevance of SNPs in *TLR4, ICAM1,* and *IL22* genes in malaria pathogenesis, namely rs4986790, rs4986791, rs5498, rs5491, rs5030400 and rs2227483, respectively. Recent studies show the role of the *TLR4* gene polymorphism in modulating susceptibility and disease progression. For instance, Abdulrahman A. Al Qahtani et al. (2021) demonstrated that SNPs such as rs4986790 are associated with altered signaling in the adaptive immune system and increased susceptibility to *Plasmodium falciparum* infection. More recent analyses suggest that *TLR4* gene polymorphism may influence parasitemia levels and inflammatory responses, supporting their role in innate immune signaling pathways (Ramirez et al., 2022; Ariff et al., 2023; Silva et al., 2025). Taken together, these finding align with this study, suggesting that although the observed structural changes are minimal, they may still influence functional effects on receptor activity.

Recent genomic and structural studies highlight the role of *ICAM1* mediating cytoadherence of infected erythrocytes to endothelial cells, a key process in severe malaria pathophysiology. *ICAM1* gene polymorphism, such as rs5498 has been associated with variability in adhesion efficiency and disease severity, depending on population-specific genetic background (Sirisabhabhorn, Chaijaroenkul, & Na-Bangchang, 2021). This supports the current finding, where minimal global conformation changes suggest that functional effects likely arise from localized structural perturbations affecting the binding interfaces.

Several studies indicate that *IL22* gene polymorphisms may influence cytokine expression and contribute to protection against malaria by modulating inflammatory balance (Marquet, S et al, 2017; Aljabra et al., 2020). The present finding extend this understanding bu suggesting that non-coding variants, such as rs2227483, may alter functional effect through transcriptional regulation rather than direct structural changes of the protein (Nikamo et al., 2016; Aljarba *et al*., 2020; Ali Agha et al., 2026).

Structural modelling in this study revealed only negligible global conformational differences between wild-type and mutant proteins, as reflected by low RMSD values. This observation is consistent with recent computational studies indicating that many protein function through subtle and localized changes (Damena et al, 2021). Consequently, reliance on RMSD alone may underestimate functionally relevant perturbations, especially those occurring at active or binding sites.

These findings support the emerging view that the functional impact of host genetic variation in malaria is mediated through molecular mechanisms rather than overt structural alterations. Importantly, such effects may be highly influenced by genetic background and environmental exposure. This is particularly relevant for Indonesia, where high ethnic diversity may result in specific genetic architectures that differ from those reported in African or South Asian populations (Sugiarto et al, 2022) hypothesis is that malaria-associated host SNPs exert functional effects through subtle mechanistic pathways rather than overt structural damage.

5. Conclusion

This study provides a structural assessment of malaria-associated SNPs in *TLR4, ICAM1, and IL-22* genes, highlighting that these variants are unlikely to exert their effects through large-scale protein destabilization. Instead, the consistently low RMSD values observed across variants support a model in which functional consequences arise from subtle, localized molecular perturbations that may influence immune signaling, receptor interactions, and inflammatory regulation. These findings underscore the limitation of relying solely on global structural metrics to infer biological impact and reinforce the need for integrative approaches that combine genetic, structural, and functional analyses. Importantly, the relevance of these variants within the Indonesian population remains to be established. Future studies therefore prioritize the integration of population-based genomic data with functional validation to elucidate the mechanistic role of host genetic variation in malaria. Such effort will be essential for translating genetic insights into clinically meaningful applications, including improved risk stratification and the development of precision-based therapeutic or preventive strategies.

Conflict of Interest

The authors declare no conflict of interest for the results.

Author's contribution

A.B.W. conceived and developed the idea. A.L. performed the experiments. V.M.S.S., A.J.K., A.B.W. supervised the experiments. V.M.S.S., A.L., A.B.W. wrote the manuscripts. All authors discussed and agreed on the manuscripts.

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