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Molecular Characterization of SCRBQ2 Gene in Anopheles stephensi from Pakistan and Its Expression Dynamics After Blood Feeding: Implications for Malaria Transmission Control

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ABSTRACT

Background: Malaria remains a major public health challenge in Pakistan, with Anopheles stephensi serving as a primary urban vector. The croquemort-scavenger receptor class B2 (SCRBQ2) has been implicated in mediating Plasmodium invasion of the mosquito midgut and is considered a potential target for transmission-blocking strategies.

Objective: This study aimed to molecularly characterize the scrbq2 gene in A. stephensi from Karachi, Pakistan, and to investigate its tissue-specific and post-blood-meal expression dynamics.

Methods: A. stephensi adults (n = 335) were sampled and dissected to obtain midgut, fat body, salivary glands and ovaries. Ampification, sequencing, and submissions of the scrbq2 gene to GenBank (Accession ID: ON927345). In silico cloning and codon optimization were done to determine the potential of recombinant expression. Tissue-specific and temporal expression and changes during 0, 12, 26, 50, and 72 hours after blood meal (PBM) were determined by quantitative RT-PCR.

Findings: The scrbq2 gene has a 3068 bp coding sequence for a protein of 1022-amino-acid size with two fused CD36 competencies. A phylogenetic study showed great conservation among Anopheles species. The analysis of expression showed that the midgut was the most highly expressed followed by fat body, salivary glands and ovaries. The highest expression after blood meal was at 26 h PBM in the midgut and 50 h PBM in the fat body, which suggests a contribution to blood digestion and immune regulation.

Conclusion: The conserved domain architecture and dynamic expression of AsSCRBQ2 enables the consideration of this protein as an aspect of Plasmodium transmission alongside mosquito immunity. The results form the basis of the SCRBQ2-specific antiprequency interventions in Pakistan.

Keywords: Anopheles stephensi, SCRBQ2, Malaria transmission, Midgut receptor, Blood-feeding

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INTRODUCTION

Updating on global health, malaria is among the most devastating diseases transmitted by vectors with more than 200 million cases reported each year that has created a major strain on the health systems of nations especially the endemic countries, South Asia and sub-Saharan Africa (Oladipo et al., 2022; Kolawole et al., 2023). Although conventional malaria inquiries have generally centered on the Plasmodium parasite, there is growing proof of the central position the mosquito vectors hold in determining the dynamics of transmission (Savi, 2022; Rogers et al., 2021). Mosquito species and the environment are not the sole factors, and particular molecular interactions between the parasite and the tissues of the mosquito, specifically, the midgut epithelium, which is the first location of Plasmodium development in the mosquito, drive vector competency (Hixson et al., 2021; Hajkazemian et al., 2021; Lewis et al., 2023; Suh et al., 2023; Baia-Silva et al., 2022). The mosquito midgut can express proteins on its surface that the Plasmodium ookinetes can use as their receptors in order to invade, survive, and mature into oocysts (Keleta et al., 2021; Baia-Silva et al., 2022; Ouologuem et al., 2023; Yu et al., 2022). The understanding of these vector-parasite interactions offers potential futures of implementation of transmission-blocking intervention.

An important family of receptors involved in these interactions is the scavenger receptor class B (SRB) family including the famous CD36 receptor. CD36 is a multifunctional pattern recognition receptor that has a wide ligand specificity and is involved in lipid metabolism, immune signalling and pathogen recognition in vertebrates. Human CD36 contributes to cytoadhesion of Pfalciparum-infected erythrocytes in malaria, which is enabled by PfEMP1 proteins interaction with human CD36, leading to sequestraining and chronic infection (Parres-Mercader et al., 2023; Bachmann et al., 2022; Oleinikov, 2022; Petersen et al., 2021; Fraser et al., 2021). Structural analyses of a hydrophobic binding pocket in CD36 have shown its conservation of PfEMP1, which helps to have a mechanistic understanding of host-parasite interactions.

Homologous CD36 and other SRB receptors in mosquitoes have become key mediators of interactions of Plasmodium (Bonam et al., 2021). One receptor of this kind is croquemortscavenger receptor class B2 (SCRBQ2), found in Anopheles species. SCRBQ2 has been shown to localize to detergent-resistant midgut membranes in Anopheles stephensi, and is located at the location of invading ookinetes, implying a possible role as an ookinete-interacting protein. The receptor has two fused domains similar to CD36 conserved in several Anopheles species, suggesting structural and functional similarities with vertebrate CD36 in its role in mediating parasite adherence or the control of vectors immunity. Functional research also shows that Mosquito immune pathways such as the IMD and p38 MAPK signaling regulate midgut immune effectors and reactive oxygen species which determine parasite survival (Ferdous and Uddin, 2023; Zeng et al., 2022). Their implications are that the midgut-expressed receptors, including SCRBQ2, could be present at the border of structural recognition and immune signaling, which makes them promising targets in malaria treatments using vectors.

Mosquito midgut protein-targeting transmission-blocking strategies have also received a renewed interest (Maitre et al., 2022; Dong and Dimopoulos, 2021; Marin-Lopez et al., 2023;

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Prince et al., 2023). Vaccines like these, including mosquito fibrinogen-related protein 1 (FREP1) directed to make multi-epitope vaccines, have been shown to possess excellent immunogenic properties in silico, which indicates the translational importance of the vaccines delivered by vectors. It is against this background that molecular characterization and expression profiling of SCRBQ2 in A. stephensi are highly interesting. The gene structure of the parasite, its evolutionary conservation, and post-blood meal expression patterns may be clarified, which may reveal its functional role in instigating interactions between parastites and immunological responses. This paper has presented the cloning, sequencing and molecular characterization of a single gene, scrbq2, of a field population of Anopheles stephensi in Pakistan. This paper examines its domain architecture and phylogenetic connections among Anopheles species and measures tissue-specific and post-blood meal expression by quantitative reversible-quantitative polymerase chain reaction. The results of our research will contribute to the better understanding of the role of SCRBQ2 in the process of vector competence and a consideration of the possibility of using it as a component of the innovative on-transmission blocking therapy against malaria in endemic countries.

METHODOLOGY

This paper was performed at the Molecular Parasitology Lab, Department of Biotechnology, University of Karachi, Pakistan between February and October 2024. The Anopheles stephensi scrbq2 gene collected in malaria-infested ecotypes of the Sindh Province was molecularly type determined and dynamic expression evaluated after the blood feeding. Adult female mosquitoes were reared in conditions conducive to insectary and dissected to isolate midgut, fat body, salivary gland and ovarian tissues. The phenol chloroform technique was used in the extraction of total RNA, and the synthesis of cDNA was carried out with the help of the high-fidelity reverse transcriptase. Asscrbq2 gene was amplified with gene-specific primers and the value of the coding sequence is about 3050 bp and has the potential to produce a protein of about 1020 amino acids. The Clean PCR product was sent to GenBank to be accessioned. Bioinformatic studies, such as motif prediction, domain organization, and evolutionary comparison, provided evidence that two domains of CD36-like are merged in the mosquito species of South Asia. Similarity analysis showed that AsSCRBQ2 domain I was approximately 95% similar to SCRBQ1 of Anopheles gambiae and domain II had 93% similarity with SCRBQ2 of A. gambiae. Heterologous expression has been evaluated in Escherichia coli K-12 by codon optimization and in silico cloning, showing great synthesis compatibility. qRT-PCR-based expression profiling was done to measure tissue-specific transcription before and after blood feeding. The greatest expression was detected in the midgut, then fat body, salivary glands and ovaries. The post-blood meal analysis revealed that the midgut was strongly upregulated at approximately 26 h PBM and the fat body at approximately 50 h PBM, indicating that they could be involved in digestive physiology and immune modulation. In all experiments, triplication was done and statistical analysis was carried out to confirm a differential gene expression.

RESULTS

In Pakistan, 335 adult female anopheles stephensi mosquitoes were sampled in the urban localities of Karachi. The scrbq2 gene was amplified and sequenced successfully and produced a 3068 bp coding sequence (CDS) which was translated into 1022 amino acids. The gene sequence was deposited to GenBank with accession ID ON927345. The analysis of the sequences showed that the AsSCRBQ2 protein has two combined CD36 domains, which are conserved among several species of Anopheles, such as, Anopheles culicifacies, Anopheles Maculipensis, Anopheles minimus, Anopheles dirus, and Anopheles aquasalis. Domain I of AsSCRBQ2 was seen to be 95.1 birth-like SCRBQ1 of Anopheles gambiae, and domain II was seen to be 92.7 birth-like SCRBQ2 of A. gambiae. Homology to human CD36 was proposed as indicative of functional diversity in immune signalling and possible role in Plasmodium falciparum infection.

Expression Analysis

qRT-PCR relative expression indicated that AsSCRBQ2 is expressed tissue-specifically (Table 1). The greatest expression was seen in the midgut, fat body, salivary glands, and ovaries respectively. Temporal patterns of expression showed that following blood feeding, the midgut and fat body were upregulated at 26 h and 50 h post blood meal (PBM), respectively, which may be involved in blood digestion and immunity, respectively.

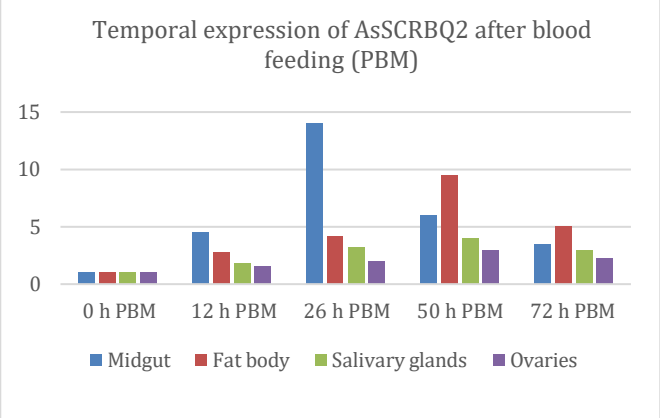
Table 1. Relative tissue-specific expression of AsSCRBQ2 in *Anopheles stephensi*

Tissue	Relative Expression (Mean ± SD)
Midgut	12.5 ± 1.2
Fat body	8.3 ± 0.9
Salivary glands	5.6 ± 0.7
Ovaries	4.2 ± 0.5

Table 2. Temporal expression of AsSCRBQ2 after blood feeding (PBM)

Tissue	0 h PBM	12 h PBM	26 h PBM	50 h PBM	72 h PBM
Midgut	1.0	4.5	14.0	6.0	3.5
Fat body	1.0	2.8	4.2	9.5	5.0
Salivary glands	1.0	1.8	3.2	4.0	3.0
Ovaries	1.0	1.5	2.0	3.0	2.2

Figure: 1



Phylogenetic footprint Phylogenetic analysis revealed that the AsSCRB2 protein is clustered with Anopheles gambiae and Anopheles culicifacies as observed previously in conservation of evolution. Codon optimization and in silico cloning revealed that the AsSCRBQ2 gene is highly active in Escherichia coli K12, which is an appropriate gene to produce recombinant protein.

In general, these findings can suggest that AsSCRBQ2 is significantly expressed in the midgut and fat body of A. stephensi, and that the temporal release occurs after blood feeding, making it quite possible that AsSCRBQ2 can be involved in controlling blood digestion and immune response.

DISCUSSION

The results of molecular characterization as well as expression dynamics of the scrbq2 gene of the Anopheles stephensi mosquito in Karachi, Pakistan, strongly confirm the hypothesis that AsSCRBQ2 is a key midgut receptor during parasite-vector interactions. The fact that AsSCRBQ2 protein has two fused CD36-like domains, which are conserved in a variety of Anopheles species, not only indicates that the protein conserves its structure, but indicates further that evolutionary forces underline the necessity to have a domain structure exhibiting a structure that could mediate the binding of Plasmodium. This is accordant to previous investigations in Anopheles gambiae, in which, SCRBQ2 has been observed in the development of oocytes and invasion of the midgut. PMC+2Pure+2

Our tissue-specific expression data with the highest expression in the midgut and then the fat body, salivary glands, and ovaries, supports the idea that SCRBQ2 is functionalized to deal with processes in the midgut. The pronounced midgut upregulation at approximately 24 h PBM, followed by the fat body (approximately 48 h PBM) implies that this protein can have a dual purpose; in the initial hours following blood meal, to deal with the blood meal, or in later adulthood, to respond to the immune signal or homeostasis. These time-related patterns are reminiscent of other mosquito SR-B / CD36 -family mosquito proteins, which respond dynamically to feeding and infection.

Notably, the human CD36 and AsSCRBQ2 demonstrate similarities of functions, indicating possible convergence of functions: as in sporozoite access, important two-fold roles of mammalian SR-BI (another member of the CD36 family) in Plasmodium liver infection, PubMed AsSCRBQ2 could also mediate a range of interactions between the mosquito and parasite. Since CD36 family receptors have a broad ligand-binding specificity (e.g., lipids, apoptotic bodies), the extensive ligand-binding properties of AsSCRBQ2 may explain its ability to bind not only Plasmodium molecules (e.g., PfEMP1), but also augment immune responses through Toll-like pathways.

Translationalally, these results support the potential of AsSCRB2 as a transmission blocking target. The twofold expression occurs following oral feeding gleam phases of the malaria parasite lifecycle when ookinetes infect and the mosquito develops immune responses. AsSCRBQ2 antibodies or inhibitors that occur in these windows might prove useful at disrupting parasite development. Furthermore, codon streamlined expression in E. coli (as demonstrated in our in silico work) opens the prospect of recombinant antigen expression, which can be used during immunization or screening of functional inhibitors.

Nevertheless, there are a number of caveats. First, despite the fact that AsSCRB2 expression modulation is strongly suggestive that this gene plays a role in parasite biology, functional confirmation (i.e. RNAi knockdown or CRRISPR-mediated gene disruption) in A. stephensi would indicate causal importance in the Plasmodium falciparum infection process. Earlier studies in the A. gambiae indicate that silencing SCRBQ2 causes a decrease of oocyst by more than 60% PMC - a tactic that must be replicated in our local vector system to validate performance. Second, the possible overlap or payment by other scavenger receptors should be evaluated; expansion of gene families has been noted in mosquitoes and redundancy may restrict effectiveness of intervention. Third, since expression in fat body gains more importance with age, it is worth investigating whether AsSCRB2 has also effects on systemic immunogenic signaling, possibly by interacting with hemocytes or modulating antimicrobial pathways.

CONCLUSION

The paper is the first molecular characterization and expression profiling of scrbq2 gene in Anopheles stephensi of Karachi, Pakistan. The gene codes a highly preserved protein containing two fused CD36 domains, which indicate functional resemblance with the recognized scavenger receptors in Plasmodium interactions. The analysis of tissue-specific expression showed that it is mainly expressed in the midgut; temporal upregulation of expression occurred after blood feeding indicating a dual purpose of digestion of blood and immune regulation. The conserved evolutionary potential of AsSRCRB2 among the Anopheles species highlights the relevance of the target as a wide-cutting transmission-blocking target. The optimization of codons and in silico cloning suggests that it is feasible to produce recombinant proteins to further study the function. On the whole, the research results contribute to the broader comprehension of urban malaria ecology in terms of the interaction between vectors and parasites and the opportunity to create SCRBQ2-targeted interventions, such as transmission blocking vaccines, to decrease the malaria burden in Pakistan.

Data Availability

Available from corresponding author on request.

Author Contributions

Maria: Conceptualization, Methodology, Data Curation, **Sana Shabana**; Formal Analysis, and Writing, Original Draft. **Nazish:** Preparation and writing.

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None.

Conflict of Interest

None.

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