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Deciphering Dengue: A Bioinformatic Quest for TLR7/8-Recognized RNA Signals across Serotypes

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Abstract: The Dengue Virus (DENV), comprising four primary serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), poses a significant global health challenge, particularly in tropical regions such as Southeast Asia. Each serotype contains a single-stranded RNA (ssRNA) genome that is recognized by Toll-like receptor proteins (TLRs) within host immune systems, specifically TLR7 and TLR8, which play a critical role in detecting viral ssRNA and triggering immune responses and inflammation. This study aims to identify and compare ssRNA motifs across the DENV serotypes that bind to TLR7 and TLR8, employing bioinformatics tools called SequenceSearcher. Findings indicate that DENV-2 contains the highest number of fully bound TLR7 motifs, potentially eliciting a stronger immune response, while DENV-3 exhibits a higher prevalence of moderately bound motifs, which may facilitate viral replication and immune evasion. Additionally, results suggest that TLR8 may recognize DENV serotypes more efficiently than TLR7, with DENV-2 being the most actively engaged. These insights into TLR-mediated recognition of DENV could inform vaccine development and treatment strategies to reduce the burden of dengue on global health.

Key Words: Dengue Virus; Toll-like receptors; ssRNA motif

1. INTRODUCTION

1.1 Background of the Study

The Dengue Virus (DENV), which comes from the family Flaviviridae and genus Flavivirus, has four serotypes namely DENV-1, DENV-2, DENV-3, and DENV-4 (Murugesan & Manoharan, 2020). These viruses are known to be the cause of severe illnesses like the Dengue Fever (DF) and the Dengue Shock Fever (DHF). Dengue virus infection, which is a mosquito-borne disease, is found in tropical regions like Southeast Asia and Africa (Kok et al., 2023). According to the World Health Organization (2024), there was an estimate of 7.6 million dengue cases as of April 2024 wherein the significant rise was very evident in the American region. Some factors that contribute to the rise of dengue cases in other countries include the adverse changes of the serotypes in a country, the migration of carriers or people infected with the virus, and climate change which could possibly lead to transmission of the virus. Dengue's incident rate in the Philippines was found to be the highest in children ages 5-14 years old, resulting in an 80% dengue-related deaths among young people of ages 20 years old or less. (Undurraga et al., 2017). In 2019, the Philippines declared an epidemic due to the

rapid increase of dengue fever cases wherein there are 5,000 new cases reported every week (Dyer, 2019).

RNA viruses can either have single stranded RNA (ssRNA) or double stranded RNA (dsRNA) as their genetic material. For DENV, all the four mentioned serotypes contain the (+) ssRNA as their genetic material which are directly translated into viral proteins by the host compared to (-) ssRNA (Anusha, et al, 2019). In animal hosts, the innate immune response is activated by Toll-like receptor proteins (TLRs). TLRs help in the innate immune system by acting as a biomarker that detects infections. Inflammation or proinflammation in one's body system is a response to infection and the TLRs create this symptom when it detects a virus. In the study of Moreno-Eutimio, et al. (2020), it was found that using bioinformatics tools, TLRs were able to distinguish which SARS-CoV genome contained more ssRNA that could have the potential to lead to acute lung injury and death. Similarly, the subfamilies TLR7/8 are receptors that recognize non-methylated viral ssRNA, making the genetic material of DENV-1, DENV-2, DENV-3, and DENV-4 recognizable (Posadas-Mondragón, et al., 2020). Bioinformatics tools RefSeq and Sequence Searcher were used. Ref Seq database uses data from NCBI Genbank and curates the information to make data results non redundant and carefully curated for specific research. This database allows the paper to obtain data for DENV serotypes' genomic sequences that are accurate and standard across submitted data in Genbank (O'Leary, et al., 2016). Meanwhile, sequence searcher provides distinct advantages over other available tools due to its adaptability as both an independent program and a component of more advanced bioinformatics platforms. Unlike many sequence analysis tools, it supports the simultaneous search of multiple sequences using regular expressions or fuzzy patterns, allowing greater flexibility in motif identification. Furthermore, its integration into software such as the Viral Genome Organizer and Base-By-Base enhances result visualization by enabling genome annotation compatibility (Moreno-Eutimio et al., 2020). These capabilities make SeqS particularly suitable for this study, as it enables efficient and precise identification of ssRNA motifs associated with Toll-like receptor (TLR) 7 and 8 recognition in Dengue virus genomes.

This research focuses on the four DENV serotypes: DENV1, DENV2, DENV3, and DENV4 and ssRNA motifs that are recognized with the TLR7 and TLR8 proteins. The study did not include the newly discovered DENV-5 due to the limited literature available. While the study focuses on DENV, other immunity factors aside from the TLR recognition were not considered. Lastly, the methodological process done was similar to a previous study Moreno-Eutimio et al. (2020) to obtain reproducible results that are aligned with the objectives. Consequently, this study is highly significant in today's society as it deepens the understanding of the Dengue virus through assessing which serotype is more likely to trigger immunity. Viruses with similar genetic material or recognizable genes by TLR 7/8 could be further understood of their capabilities, mutation and pathways that could benefit or harm the environment. The study has the potential to improve healthcare in dengue prone countries through the development of vaccines. Additionally, displaying the importance of Bioinformatics tools can further improve Bioinformatic developments for more computational and biological studies. Analyzing the prevalence of these motifs with the help of the current advancements in technology can further develop antiviral medications and other sustainable solutions to ensure healthy lives and well-being as asserted by Sustainable Development Goal 3. Thus, the study aims to identify the single-stranded RNA (ssRNA) sequences that are read by the toll-like receptors TLR7 and TLR8 across the four DENV serotypes (DENV1, DENV2, DENV3, and DENV4). Through analyzing the role of Toll-like receptor (TLR) proteins, specifically TLR7/8, in recognizing the single-stranded RNA (ssRNA) of different DENV serotypes., the study would provide a thorough understanding on the variability of the motifs found in the different DENV serotypes.

2. METHODOLOGY

2.1 Data Collection

All genomic sequences of Dengue serotypes were obtained from NCBI RefSeq, where they are publicly available. These reference genomes—DENV-1 (NC_001477.1), DENV-2 (NC_001474.2), DENV-3 (NC_001475.2), and DENV-4 (MG272274.1)—served as the basis for the analysis. These sequences were selected based on their high-quality annotation and widespread recognition as reference genomes for each Dengue serotype. Their inclusion ensures methodological reliability and consistency in the identification of ssRNA motifs. This is further supported

by their utilization in studies, such as the U.S. CDC's Genomic Characterization of Circulating Dengue Virus (Centers for Disease Control and Prevention [CDC], 2025), which underscores their significance in Dengue virus research.

2.2 ssRNA Fragment Motif Search

After obtaining the complete genome sequences, the FASTA files were extracted and analyzed using SequenceSearcher to identify ssRNA motifs (Moreno-Eutimio et al., 2020). The ssRNA motif search was particularly necessary for the study, as it enabled the identification of sequence motifs recognized by Toll-like receptors 7 and 8 (TLR7/8), which play a key role in the innate immune response to Dengue virus (Idrees et al., 2023).

2.3 Interpretation and Analysis

After conducting the ssRNA motif search, an analysis presented in a table illustrating the differences among the Dengue Serotypes will be performed. This will include the base pair percentage and the number of oligonucleotide fragments recognized by TLR7, which will be categorized into two groups: Motif fully bound (UUU, UUU_2, UUC, UUC_2, UUC_3) and Motif moderately bound (UUA, UUA_2, UUA_3, UUG, UUG_2). Additionally, it will also include the number of oligonucleotide fragments recognized by TLR8, including UG, UG_2, UG_3, UG_4, and UG_5. The fragments stated above are used These motif sequences are selected because they replicate ssRNA patterns that TLR7 and TLR8 naturally recognize. This approach gives a clearer picture of the immune response and offers clues about each serotype's likelihood of being detected by the host's immune system.

Finally, a comparison between the ssRNA fragment numbers present in each genome recognized by TLR7/8 will be conducted to determine which viral serotypes are most effective at triggering a robust immune response. This comparison will be visually represented using a bar graph. Figure 1 shows an overview of the methodology that will be used to conduct the research described in the paper.

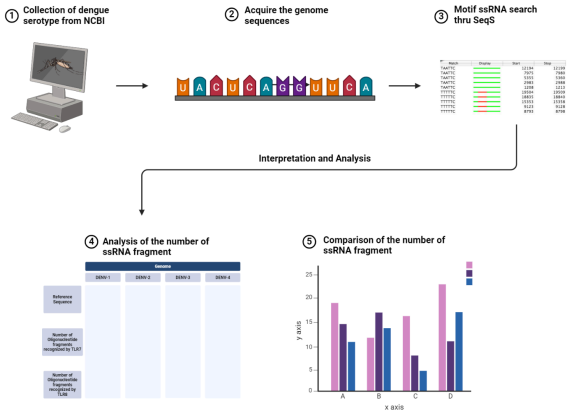

Fig.1. Systematic overview of the methodology

3. RESULTS AND DISCUSSION

Results for motifs fully bound to TLR7 showed that the DENV-2 has the highest number of UUU ssRNA fragments, which is only 5.38% higher than the DENV-1 or the second highest number. Similarly, DENV-2 had the highest $(UUU)_2$ motifs that is 50% higher than DENV-1. UUC and UUC_2 fragments were also found to be the highest among the DENV-2 serotypes which is then followed by DENV-3, DENV-4, and DENV-1. However, motif moderately bound UUA fragments were the highest among both DENV-1 and DENV-3 serotypes and followed closely by DENV-2. Similarly, DENV-3 had the highest UUG serotypes as well. Based on the results shown for TLR7, it can be inferred that the DENV-2 serotype has the highest probability of interacting with TLR7/8 for motifs that are fully bound while DENV-3 for moderately bound.

According to Greulich et al. (2019), UUG fragments exhibit binding with the TLR8 gene with the UU part being inactive. Therefore, the UG fragment was used to investigate across the DENV serotypes for TLR8 in which results presented that DENV-2 had the highest number.

Table 1. Analysis of the number of ssRNA sequences from DENV serotypes recognized by TLR7/8

Genome	DENV-1	DENV-2	DENV-3	DENV-4
Reference Sequence:	NC_001477.1	NC_001474.2	NC_001475.2	MG272274.1
U% (bp)	21.42	21.05	21.30	21.38
A% (bp)	31.91	33.13	31.99	30.95
C% (bp)	20.87	20.52	20.72	21.28
G% (bp)	25.80	25.30	25.99	26.39
Total Nucleotides (bp)	10,735	10,723	10,707	10,652
Number of oligonucleotide fragments recognized by TLR7				
Motif fully bound				
	DENV-1	DENV-2	DENV-3	DENV-4
UUU	260	274	253	237
(UUU)2	12	18	10	5
UUC	346	395	363	357
(UUC)2	11	20	17	12
(UUC)3	2	0	0	0
Motif moderately bound				
UUA	101	100	101	92
(UUA)2	2	0	3	1
(UUA)3	0	0	0	0
UUG	273	271	290	246
(UUG)2	5	6	6	7
(UUG)3	1	0	0	1
Number of oligonucleotide fragments recognized by TLR8				
UG	901	931	926	881
(UG)2	72	71	74	65
(UG)3	5	6	7	6
(UG)4	0	0	0	0
(UG)5	0	0	0	0
*UUG	273	271	290	246

The figures 2, 3, and 4 depict graphs comparing the number of ssRNA fragments from DENV that are recognized by TLR7/8.

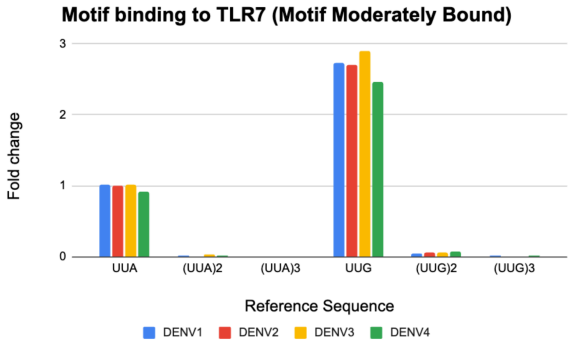

Fig.2. Motif binding to TLR7 (Motif Moderately Bound)

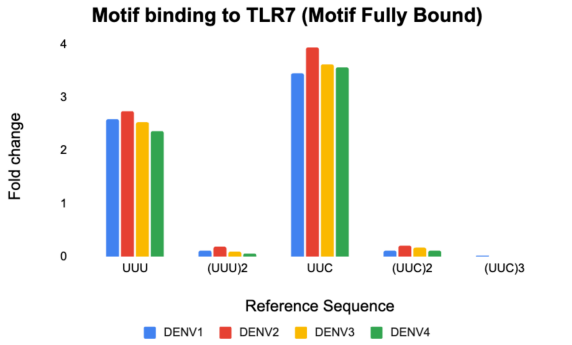

Fig.3. Motif binding to TLR7 (Motif Fully Bound)

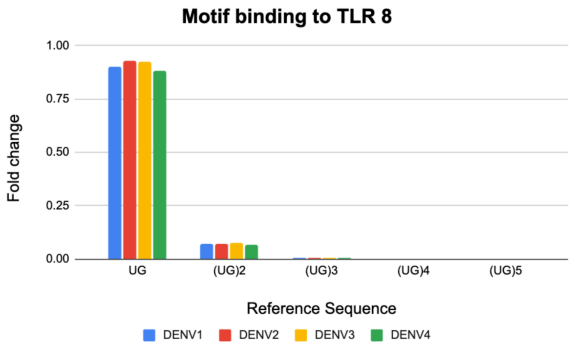

Fig.4. Motif binding to TLR8

Based on Figure 2, the highest amount of detected ssRNA nucleotides moderately bound to TLR7 was DENV3 (400) which makes it the most detected virus with ssRNA. The moderately bound motifs can be related with the cases of asymptomatic dengue infections which are known to reach 40-80% that are classified as only "mild" infections (Santis et al., 2023). Thus, asymptomatic infections produce low amounts of inflammatory cytokines that signal a lower immune response. Given that the motifs are only moderately bound, this suggests that the virus in the DENV-3 serotype does not trigger the full immune response of the host which could make the virus adapt to the host's environment. They can replicate faster and spread without the recognition of the host's immune system. With this, the DENV pathogens can escape the host's immune response resulting in rapid growth and immune evasion. This is consistent with previous studies as it was reported that DENV-3 cases are silent or asymptomatic compared to other serotypes (Yung et al., 2015).

Contrastingly, Figure 3 shows that the highest amount of detected ssRNA nucleotides fully bound to TLR7 is DENV-2 (707). Detection of these nucleotides is highly dependent on the ligand binding with the TLR which can activate varying signaling cascades that serves as the host's defense against the pathogens (Salwa Refat El-Zayat et al., 2019). Binding between the TLR7 and DENV-2 fully bound motifs suggest a stronger immune response by the host due to the stronger interaction between the ssRNA and TLR. This allows the host to have a better potential to fight back against the virus but also increase the inflammatory symptom on the host leading to acute lung injury (Moreno, 2020; Salvi, 2021). Additionally, a study by Sakinah et al. (2018) concluded that the histopathological lung damage which is repeatedly infected by DENV2 resulted in a pulmonary complication. This injury was caused by the presence of the inflammatory cells infiltration as a sign of the body's immune response. Specifically, with the rapid response of the receptors to the viral genetic materials, the increase of cytokines create a cytokine storm where overly increased amounts of anti- and pro-inflammatory symptoms arise and cause severe dengue infections which could result in the dengue fever and dengue fever shock (Bhatt et al., 2024). Thus, the DENV2 serotype can cause a stronger inflammatory response upon virus infection. The total amount of fully bound motifs is 2,592 while moderately bound is 1,506 which can be seen in Table 1. This suggests that the virus has a 'rapid' infection rather than a slow and replicative type. The higher number of fully bound motifs can be an implication of how DENV can establish itself quickly in the host which explains why Dengue outbreaks are usually severe yet quick. According to Smith (2023), Dengue fever is a self limited disease which explains the fast duration of the infection that can occur within just two weeks.

Consequently, there are more TLR8 fully bound motifs compared to the TLR7 fully bound motifs which could suggest that hosts that have TLR8 may detect the presence of the DENV more efficiently than TLR7, especially the DENV-2 serotype. However, targeting the TLR8 in antiviral therapies for DENV can decrease the viral load yet cause an increase in inflammatory damage (Patel et al., 2014). Considering that not all mammals contain both TLR7/8, this can result in a lower immune response compared to the mammals that contain both TLR 7 and 8. Some losses of these receptors could be due to the interactions between viruses in previous species lineages that have evolved and changed their immune system (Neves, 2022).

4. CONCLUSIONS

Overall, the study suggests that TLR7 fully bound motifs and TLR8 are more recognized among DENV2, indicating a stronger host immunity. However, the increase in immunity also has a probability of bringing inflammatory symptoms like acute lung injury. These fully bound motifs also had a higher number compared to the moderately bound motifs, indicating that Dengue cases are quick yet highly virulent. Meanwhile, TLR7 moderately bound motifs are mostly

prominent among DENV3. The moderately bound motifs do not suggest full immunity against the pathogen resulting in a decrease in immune response and eventually higher occurrence of asymptomatic cases in the DENV3 serotype. Consequently, not all mammals have TLR7 and TLR8 receptors that can also result in lower immunity.

The researchers propose future studies focusing on other factors that can influence the immune responses among the DENV serotypes. Although the study implied that DENV2 and DENV3 were most recognizable among TLR7 and TLR8, it is still crucial to investigate the mechanisms of the other serotypes to ensure an extensive immunity against the virus. With this, it is recommended to further explore the possible mechanisms that elucidates the underlying concepts of drugs, vaccines or other forms of medical interventions that can regulate the generation of these serotypes among humans. Furthermore, it is also proposed to look into the influence of both TLR7 and TLR8 among other prominent diseases caused by viruses such as HIV or Mpox. Lastly, the researchers also recommend looking at the recognition of the TLRs on the last serotype which is DENV-5.

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