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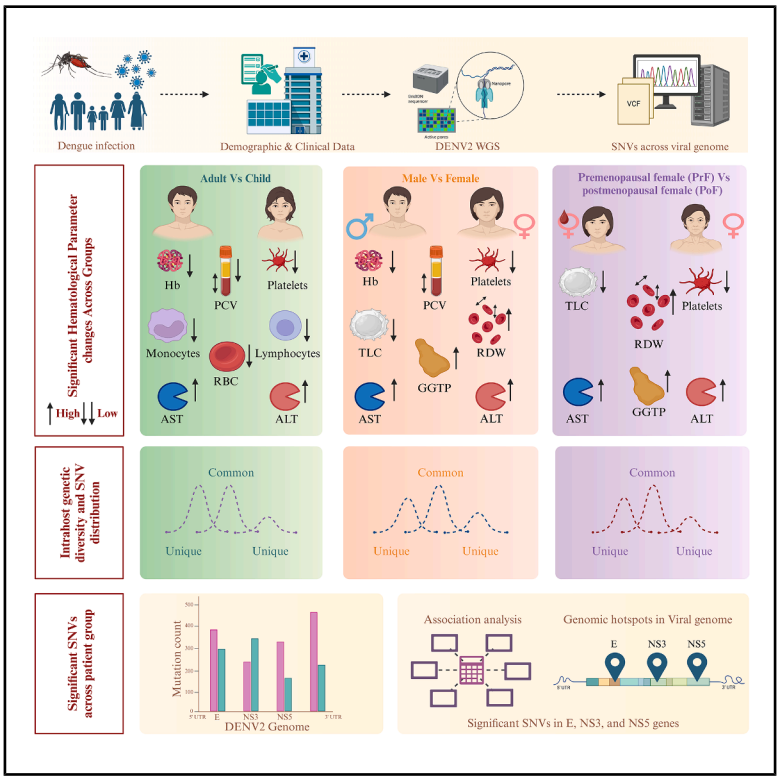
Inter-host diversity associated with age, sex, and menstrual cycle modulates clinical manifestations in DENV-2 patients

Graphical abstract



Highlights

- Young adult males (19–35 years) have a higher prevalence of DENV-2
- Males with increased hepatic injury and females show severe hematological changes
- Premenopausal females harbor more diverse viral populations
- SNV burdens in *E*, *NS3*, and *NS5* genes highlight host-driven DENV-2 viral diversity

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In brief

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Inter-host diversity associated with age, sex, and menstrual cycle modulates clinical manifestations in DENV-2 patients

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SUMMARY

Dengue virus (DENV-2) remains a global threat, yet the influence of age, sex, and menstrual status on its epidemiology and genetic diversity is underexplored. We analyzed 2136 hospitalized DENV-2 patients (ages 0–86) using whole-genome sequencing (WGS) to examine how these host factors shape interhost viral diversity and clinical manifestations. Young adult males (19–35 years) had the highest prevalence with sex-based clinical differences where females exhibited severe hematological changes, while males showed increased hepatic injury. Premenopausal females harbored more diverse viral populations, whereas post-menopausal women experienced pronounced platelet depletion. Dengue virus WGS identified 1100336 single nucleotide variants (SNVs) across 2932 genomic positions, with greater viral diversity in adults and females. Significant SNV burdens were observed in the *E*, *NS3*, and *NS5* genes of the virus. These interconnected findings underscore the profound impact of age, sex, and menstrual status on DENV-2 epidemiology which merits inclusion into the disease pathophysiology.

INTRODUCTION

Dengue, caused by the dengue virus (DENV) and transmitted by *Aedes* mosquitoes, is a widespread and a vector-borne disease of concern, particularly in the tropical and subtropical regions, where it remains a significant public health threat.[1] Climate change, particularly rising temperatures and altered precipitation patterns, enhances the breeding and survival of *Aedes* mosquitoes, the primary vectors of dengue.[2,3] Warmer temperatures accelerate the mosquito life cycle, leading to higher transmission potential. Additionally, elevated temperatures can increase the extrinsic incubation period of the virus within mosquitoes, potentially affecting viral replication rates and transmission efficiency.[4] Furthermore, climate-induced changes in vector distribution have expanded the geographic range of dengue, introducing the virus to new regions and populations.[5,6] This expansion can lead to interactions with diverse host genetic backgrounds, potentially driving viral evolution and influencing virulence. However, direct evidence linking climate change to increased dengue virulence remains limited, and further research is necessary to elucidate these complex interactions. The clinical manifestations of dengue vary widely, ranging from mild flu-like symptoms to severe, life-threatening conditions such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[7] Moreover, the dengue infection is commonly associated with alterations in the hematological parameters/liver dysfunction, with total leukocyte count (TLC) and platelet count reductions being among the most observed and reliable indicators of the disease severity, often leading to immune dysfunction and complications such as vascular leakage, bleeding, and organ failure.[8–11] Liver damage in dengue is thought to be driven by both direct viral cytopathic effects and immune-mediated injury, often indicated by elevated levels of AST (aspartate aminotransferase) and ALT (alanine aminotransferase), which are key biomarkers of liver injury.[12–18]

Besides the comprehensive comparison of clinical parameters, with a focus on potential predictive markers for severe dengue (DSS/DHF), demographic factors such as age and sex can also be considered as risk factors for progression of the disease.[19] Children are often more susceptible to severe plasma leakage and (DSS) due to immature immune systems.[20] Older adults, on the other hand, have a growing proportion of dengue cases, with adults over 50 years experiencing an increased mortality linked to comorbidities such as diabetes

and cardiovascular diseases.[21–25] While previous research has reported that males are often more affected by dengue than females,[26–28] findings vis-a-vis hematological responses vary with some studies reporting higher thrombocytopenia and leukopenia in males,[29] while others note increased leukopenia in females.[30] This fluctuation in blood components holds significance not only in terms of disease progression but also with regard to sex-specific responses that may be influenced by the menstrual cycle in females.[31] Rosin et al.[32] reported that platelet function, including platelet-leukocyte interactions, varies with menstrual cycle phases, particularly peaking at ovulation due to estrogen's effects that may influence susceptibility to thromboembolic and inflammatory conditions. Studies have suggested that platelet reactivity is altered in response to the menstrual cycle.[33] However, this aspect remains largely unexplored in the context of dengue infection, particularly in how it may impact premenopausal (≤50 years) and postmenopausal (>50 years) females differently. This gap in the literature is particularly noteworthy because the menstrual cycle-related changes in blood parameters could potentially modify the severity of dengue infection, adding a layer of complexity that has not been fully investigated.

The genome of DENV is a single-stranded RNA that encodes a polyprotein, which is subsequently cleaved into 10 proteins. These include three structural proteins: C (capsid), prM (Precursor membrane), and E (envelope) which play crucial roles in virus assembly and host cell entry. Seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) are known to be involved in viral replication, immune modulation and RNA synthesis. More specifically, the E (envelope) protein is essential for viral attachment and fusion with the host cell membrane, facilitating entry into target cells. It serves as a primary target for neutralizing antibodies, and SNVs in this region can modulate antigenicity, potentially impacting immune recognition and vaccine efficacy.[34] The prM (pre-membrane) protein, which matures into the M protein, plays a key role in viral assembly and release. It protects the E protein during virion assembly and contributes to antibody-dependent enhancement (ADE), a phenomenon where sub-neutralizing antibodies facilitate viral entry into Fc receptor-bearing cells, potentially exacerbating disease severity.[35] The NS3 protein exhibits dual functionality as a serine protease and RNA helicase. Its protease domain processes the viral polyprotein into functional units required for replication, while the helicase activity unwinds viral RNA, ensuring efficient genome replication. Additionally, NS3 has been implicated in counteracting host immune responses, particularly by interfering with interferon signaling pathways.[36] The NS5 protein, a multifunctional enzyme, plays a pivotal role in viral replication as an RNA-dependent RNA polymerase. It also acts as an interferon antagonist by inhibiting STAT2 phosphorylation, thereby suppressing host antiviral responses and promoting immune evasion.[37] In addition to these, the NS1 protein, a secreted glycoprotein, is involved in immune modulation and endothelial dysfunction. It has been shown to interact with host immune components, contributing to vascular leakage, a hallmark of severe dengue. NS1 is also a major diagnostic marker and a potential target for therapeutic intervention.[38] The significance of these viral proteins underscores the potential impact of genetic variations in shaping disease pathogenesis and host-virus interactions. Studies with low sample numbers have explored the genetic variability and evolution of the DENV genomes,[39–41] highlighting their critical role in shaping disease outcomes and clinical manifestations in dengue patients. Additionally, research has identified potential links between specific genetic variations in the DENV genome and disease severity.[42–44] For example, valine to methionine substitution in the *ancC* position, although will not much alter its hydrophobicity due to the same property of the amino acid being changed, it is associated with the dengue with warning signs and severe dengue. For example, a valine-to-methionine substitution in the ancC protein of DENV-2 has been linked to severe dengue cases.[45] Experimental studies by Prestwood et al. demonstrated that asparagine-to-aspartic acid and lysine-to-glutamic acid substitutions in the E protein of DENV-2 led to severe disease in mice.[46] However, the impact of host factors (age, sex, and menstrual status) on interhost viral diversity remains underexplored, offering more possibilities for the investigation. Therefore, the present study aims to investigate age- and sex-based differences in shaping the interhost genetic diversity of DENV-2 vis-a-vis interhost clinical manifestations of dengue, with a particular focus on premenopausal vs. postmenopausal females, during the 2023 dengue outbreak in National Capital Region of Delhi (DELHI-NCR), India. By integrating clinical and genomic insights, this research seeks to enhance understanding of host-virus interactions, particularly in age- and sex-specific immune responses and viral adaptation, to improve dengue management strategies. Given the large population base of India, approx. 1.4 billion people with 50% being females, study insights from this would have wider implications at the population level.

RESULTS

Higher DENV-2 prevalence in young adult males compared to females (19–35 Years)

Demographic parameters such as age and sex could play a pivotal role in shaping the epidemiology of dengue, influencing susceptibility, clinical severity, immune response, and social determinants of health. Understanding these differences is crucial for improving disease management and reducing the dengue burden in an endemic region. For this, the patients with dengue infection were identified using the NS1 antigen (NS1-Ag) test and confirmed by serotype-specific qRT-PCR. Additionally, successful whole genome sequencing of DENV-2 from RNA isolated directly from clinical serum samples validated the diagnosis in all 2136 patients (Figure 1A). The study cohort of 2136 dengue patients represent all age groups (ranging from 0 to 86 years) and both sexes (male and female). Based on the demographic data, the following study sub-groups were defined: 1) The first subgroup comprised 1844 adult patients (age >12 years) with further stratification of male (n = 1040) and female (n = 804) patients; 2) A subgroup of children (age ≤12 years) (n = 292) with boys (n = 178) and girls (n = 114); 3) In addition, to examine how intricate hormonal changes in premenopausal and postmenopausal females may impact dengue epidemiology, a subset of female patients (n = 804) was specifically selected. This subgroup was further divided into two arms: premenopausal females (n = 676) and postmenopausal females (n = 128)

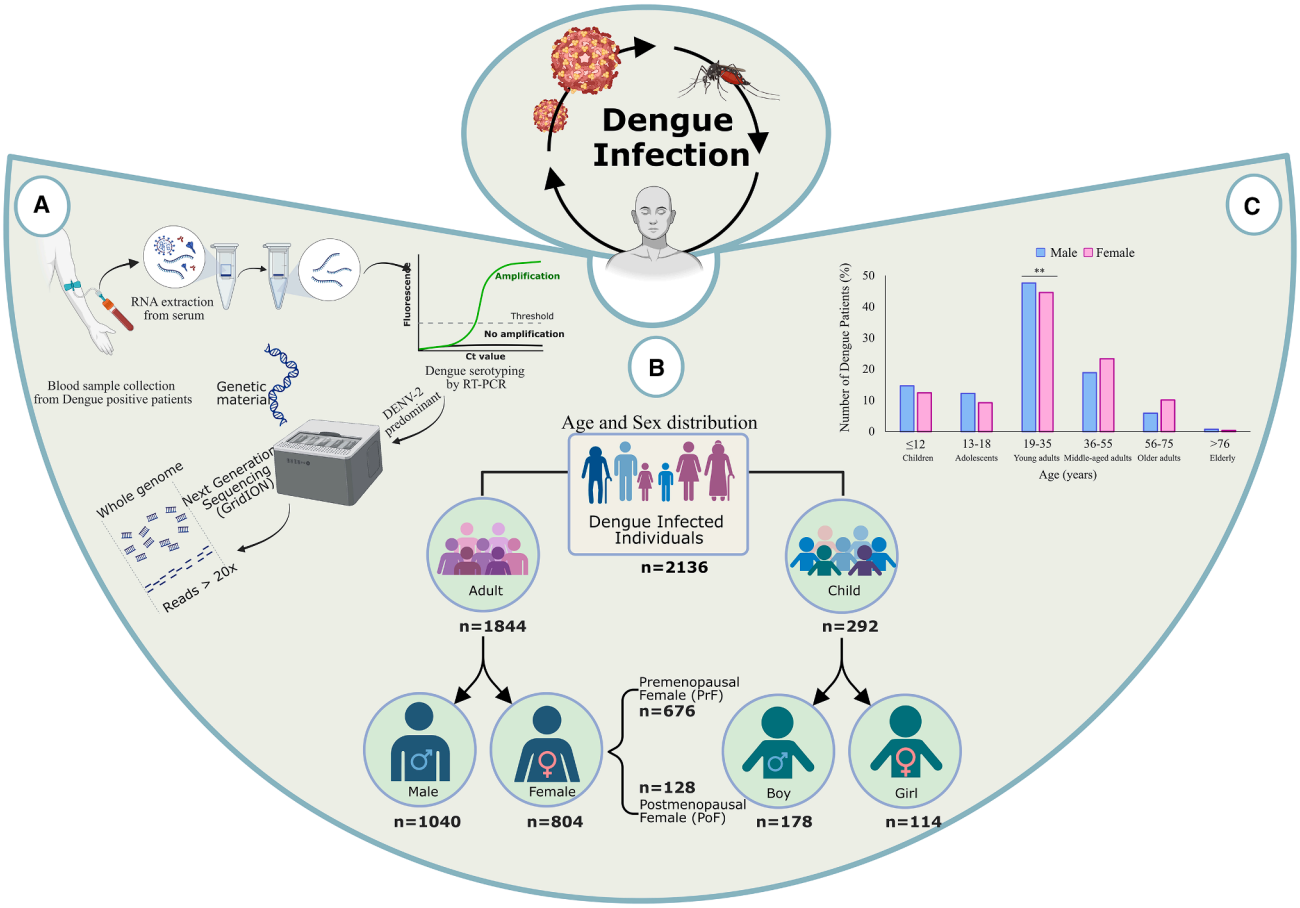


Figure 1. Study design and methodology for investigating DENV infections in patients

(A) Experimental workflow including NS1-Ag-positive serum sample collection, RNA isolation, and serotype detection, followed by whole genome sequencing and data interpretation.

(B) Study overview including 2136 DENV-2 positive patients with further classification based on age and sex to investigate the possible association of the deviations in the clinical parameters with age and sex. Of total 2136 dengue patients, adult = 1844, child = 292. Of adults (1844), male = 1040, female = 804. Of females (804), premenopausal female = 676, postmenopausal female = 128. Of child = 292; boy = 178, girl = 114.

(C) Age- and sex-wise prevalence of DENV-2 infection in the study cohort. The highest prevalence was observed in the 19–35-year age group, with a higher proportion of male patients in most age groups, except for the 36–55 and 56–75-year age groups. Data are represented as median +/− SEM. The significance was calculated using the Mann-Whitney U-test ($p < 0.05$). This figure was created using premium licensed version of Biorender.com.[48]

(Figure 1B). Overall, the stratified cohort design enables a detailed investigation into the influence of sex and age on dengue epidemiology. Blood parameters, including platelet count and leukocyte and hemoglobin levels, are vital in understanding how hormonal fluctuations influence disease susceptibility, immune response, and clinical outcomes in female patients, particularly during menstrual cycle.[47] Therefore, examining the premenopausal and postmenopausal female groups can provide insights into the impact of these variations on disease progression. Furthermore, to examine age- and sex-associated risks in dengue, we analyzed the age- and sex-specific patterns of DENV-2 infection in our dataset, where the highest prevalence of DENV-2 infection was found in the 19–35-year age group, followed by the 36–55-year group. Male patients were more affected than female patients across most age groups, except for the 35–55 and 56-75-year age groups, with a statistically significant difference in the 19–35 age group ($p < 0.05$) (Figure 1C).

Age and sex display differential spectrum of clinical manifestations across dengue patients

To investigate the differences in clinical manifestations of dengue, we conducted a comparative analysis of hematological and liver function parameters across age and sex subgroups.

Adult vs. child

The comparative analysis of hematological parameters revealed distinct patterns between adult and pediatric (child) dengue patients. In adults, 45.75% and 51.75% had low platelet counts and total leukocyte count (TLC), respectively, while only 25.42% and 25.70% of pediatric patients exhibited these abnormalities. A Mann-Whitney U test revealed that the differences in the proportions of adults and children with low platelet counts and TLC

were statistically significant (Tables 1 and S1; Figures 2A and 2C). While not statistically significant, pediatric patients showed higher rates of low mean corpuscular volume (MCV: 63.52%) and mean corpuscular hemoglobin (MCH: 56.84%) compared to the adults (MCV: 18.18%, MCH: 20.63%), while red blood cell (RBC) count depletion was more prominent in the adult patients (47.17%). These findings suggest that adult dengue patients are more prone to thrombocytopenia, leukopenia, and RBC depletion, whereas pediatric patients exhibit more reductions in MCV and MCH levels during dengue infection.

Furthermore, it is crucial to highlight that the liver is the most vulnerable organ prone to damage during a dengue infection. The extent of liver dysfunction or potential damage is typically assessed by measuring liver enzyme levels in the blood of dengue patients.[12] In our study, pediatric patients showed a significantly higher prevalence of elevated liver enzyme levels, including aspartate transaminase (AST: 80.6%), alanine transaminase (ALT: 39.19%), and alkaline phosphatase (97.96%), compared to the adult patients (62.58%; 49.06%; 19.25%), respectively, although the absolute levels of AST and ALT were significantly higher in adults than in children (Table 2, Figures 2B and 2D). This indicates potential age-related differences in liver impairment during dengue infection, with children experiencing a higher proportion of liver enzyme alterations, while adults demonstrate more pronounced hepatic injury.

Male vs. female

Following the analysis of differences in clinical manifestations between the adult and child patients, we also examined the sex-wise variations in the hematological profiles, i.e., across male and female dengue patients. A higher proportion of females had low hemoglobin (57.95%) and packed cell volume (PCV: 44.54%) compared to males (hemoglobin: 16.39%, PCV: 19.56%). Both sexes showed reduced TLC, with more males (49.27%) affected by low platelet counts than females (41.30%). Interestingly, a higher percentage of female patients (61.43%) showed increased red cell distribution width (RDW) compared to male patients (41.49%) (Figures 2E and 2G). Females exhibited a greater susceptibility to reductions in hemoglobin, PCV, RBC, and TLC, potentially suggesting that hormonal or physiological factors may exacerbate the impact of dengue infection in this demographic subgroup.

A significantly higher proportion of female patients exhibited increased liver enzymes, including (AST: 78.71%), (ALT: 58.63%), and gamma-glutamyl transpeptidase (GGTP: 43.54%) compared to the male patients (AST: 63.32%; ALT: 54.14%; GGTP: 39.26%) (Figure 2F). However, the median of these enzymes was significantly lower in females than in males (Figure 2H). This discrepancy may reflect sex-related differences in enzyme activity and metabolic responses, with females showing more subtle liver dysfunction and males exhibiting more severe hepatic injury.[49–51]

Premenopausal female (PrF) vs. postmenopausal female (PoF)

We further explored the clinical differences between the premenopausal female (PrF) and postmenopausal female (PoF) dengue patients to uncover potential hormonal or physiological influences on disease manifestations. Across PrF and PoF patients, significant differences were observed in the TLC and platelet counts, with 54.26% of PoF patients having low platelet counts compared to 38.89% of PrF. Conversely, a higher proportion of PrF (59.43%) exhibited a drop in TLC compared to PoF, 50.54% of whom demonstrated TLC below the normal range. Similarly, a greater proportion of PrF patients demonstrated a decrease in the MCH (30.43%) and MCV (26.47%) compared to the PoF patients, indicating possible differences in the erythropoiesis and RBC morphology (Figures 2I and 2K). On the other hand, PoF patients showed a higher prevalence of increased RDW (66.67%) compared to the PrF patients (60.45%) (Figure 2J). Interestingly, despite this, the absolute levels of RDW were significantly higher in PrF patients compared to the PoF (Figure 2L), suggesting that while variability was more prevalent in PoF, the magnitude of the changes was greater in PrF patients.

Moreover, the comparative profiling of liver enzyme levels in the blood of dengue patients revealed that a higher proportion of PoF patients exhibited increased AST (86.89%), ALT (68.25%), alkaline phosphatase (34.69%), and GGTP (53.06%) levels, compared to the PrF (76.24%, 55.81%, 18.24%, and 40.36%, respectively); however, the difference was observed to be non-significant (Table S2). These findings suggest that age and hormonal status influence hematological and hepatic responses in dengue, emphasizing the importance of appropriate clinical monitoring in the PrF and PoF patients.

Boy vs. girl

Comparative profiling of hematological and liver parameters between boys and girls revealed similar proportions of patients exhibiting reductions in hemoglobin, PCV, TLC, and platelet count during dengue virus infection. This uniformity suggests that sex does not significantly influence the hematological response to dengue infection in children. Unlike adults, where sex-specific hormonal or physiological factors may exacerbate or mitigate disease manifestations, the relatively equal hormonal baseline in prepubescent boys and girls could account for this similarity (Figure S1).

Age, sex, and menstrual cycle status distinctly shape the interhost genetic diversity of DENV-2

The genetic diversity of DENV is pivotal in determining a virus population's adaptability and fitness, influencing clinical manifestations and disease outcomes in dengue patients.[52–54] Therefore, to assess the influence of host-associated factors such as age, sex, and menstrual cycle status on the interhost genetic diversity of the virus, we conducted variant calling analysis on 2136 DENV-2 genomes with average genome coverage of 88.1% (Mode: 94.2%) ranging from 50.12% to 98.8%. The study identified single nucleotide variants (SNVs) at 2932 genomic positions and a total of 1100336 SNVs, demonstrating the extent of genetic diversity acquired by the DENV-2 population in human hosts across a diverse range of age, sex, and menstrual cycle statuses. We further categorized the SNVs according to the age, sex, and menstrual cycle status of the patients, as outlined in the study design. The SNVs count across each phenotype vis-a-vis the DENV-2 genome coverage is tabulated in the Table S3. Additionally, the phylogenetic analysis was done categorically for the groups and the result was depicted in the Figures S2–S5. Interestingly, no distinct clustering was observed in the phenotype subgroups. Moving forward, in the adult vs. child group, 53.4% (n = 1566) SNVs were exclusively found in the DENV-2 genomes

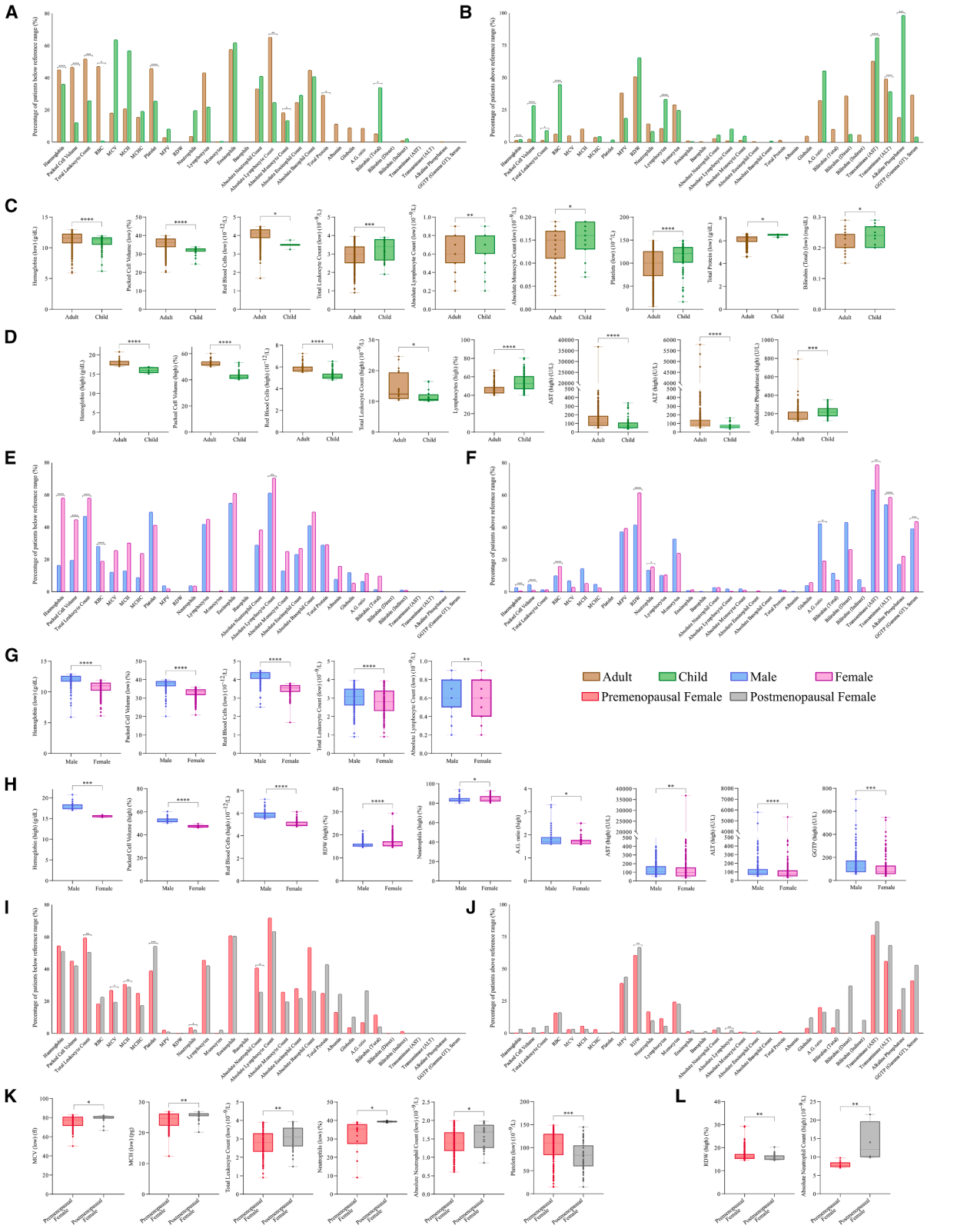
Table 1. Clinical manifestations of dengue patients below the reference range (low) across age and sex subgroupings (Mann Whitney U-test)

Clinical parameters	Adult	Child	Sig	Male	Female	Sig	Boy	Girl	Sig	PrF	PoF	Sig
Hemoglobin	11.6 (5.9–12.94)	11.3 (6.2–11.94)	****	12.1 (5.9–12.9)	11 (6.1–11.95)	****	11.2 (6.2–11.9)	11.6 (8.5–11.94)	ns	11 (6.1–11.95)	10.8 (7.4–11.9)	ns
Packed Cell, Volume	36.2 (20.1–39.9)	32.45 (24.5–33.8)	****	38.15 (20.1–39.9)	33.5 (20.8–35.9)	****	33.8 (24.5–35.8)	33.5 (29.2–35.9)	ns	33.6 (20.8–35.9)	33.4 (25.5–35.8)	ns
Total Leukocyte Count (TLC)	3 (0.9–3.95)	3.4 (1.9–3.9)	***	3.1 (0.9–3.95)	2.8 (0.9–3.93)	****	3.7 (1.8–4.9)	3.7 (1.8–4.9)	ns	2.8 (0.9–3.9)	3.1 (1.5–3.93)	**
RBC	4.12 (1.68–4.49)	3.5	*	4.29 (2.51–4.49)	3.55 (1.68–3.79)	****	3.495 (3.24–3.75)	NA	NA	3.555 (1.68–3.79)	3.55 (2.82–3.79)	ns
MCV	78.6 (50.3–82.9)	78.55 (50.4–82.9)	ns	79.4 (57.4–82.9)	78.15 (50.3–82.9)	ns	78.7 (50.4–82.8)	78.5 (59.5–82.9)	ns	77.4 (50.3–82.9)	80.1 (66.7–82.5)	*
MCH	25.3 (12.4–26.9)	24.9 (12.6–26.9)	ns	25.4 (17.8–26.9)	25 (12.4–26.9)	ns	25.3 (12.6–26.9)	24.7 (18.1–26.8)	ns	24.75 (12.4–26.9)	26 (20.2–26.9)	**
MCHC	30.9 (24.6–31.4)	30.85 (25.1–31.4)	ns	31 (27.4–31.4)	30.9 (24.6–31.4)	ns	30.7 (25.1–31.4)	30.95 (27.8–31.4)	ns	30.9 (24.6–31.4)	31 (28–31.4)	ns
Platelet	100 (6–149)	121 (16–149)	****	100 (6–149)	100 (15–149)	ns	123.5 (16–147)	121 (40–149)	ns	110.5 (15–149)	84 (15–145)	***
MPV	7.485 (6.3–7.79)	7.365 (6.7–7.7)	ns	7.5 (6.3–7.79)	7.4 (6.7–7.7)	ns	7.33 (6.7–7.7)	7.4 (6.8–7.7)	ns	7.43 (6.7–7.7)	7.4	NA
RDW	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Neutrophils	35 (9–39.9)	31.25 (8.1–39.9)	ns	34.6 (17–39.9)	35.5 (9–39.9)	ns	30.8 (12–39.9)	33.9 (8.1–39.4)	ns	35.4 (9–39.1)	39.35 (38.8–39.9)	*
Lymphocytes	12.8 (1–19.9)	13 (3–19.2)	ns	12.6 (1–19.9)	13 (2.7–19.9)	ns	13.35 (3–18.9)	13 (5.1–19.2)	ns	12.75 (2.7–19.9)	15 (3.3–19)	ns
Monocytes	1.25 (0–1.7)	1.5	NA	1.7	1	NA	NA	1.5	NA	NA	0.75 (0–1.5)	NA
Eosinophils	0.2 (0–0.9)	0.1 (0–0.9)	ns	0.2 (0–0.9)	0.2 (0–0.9)	ns	0.1 (0–0.8)	0.2 (0–0.9)	ns	0.1 (0–0.9)	0.2 (0–0.9)	ns
Basophils	2.55 (2.1–3.4)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Absolute Neutrophil Count	1.48 (0.48–1.99)	1.41 (0.37–1.98)	ns	1.53 (0.48–1.99)	1.42 (0.6–1.99)	ns	1.3 (0.53–1.96)	1.46 (0.37–4)	ns	1.395 (0.6–1.99)	1.685 (0.85–1.98)	*
Absolute Lymphocyte Count	0.6 (0.2–0.9)	0.8 (0.2–0.9)	**	0.6 (0.2–0.9)	0.6 (0.2–0.9)	**	0.8 (0.2–0.9)	0.6 (0.3–0.9)	ns	0.6 (0.2–0.9)	0.6 (0.2–0.9)	ns
Absolute Monocyte Count	0.15 (0.03–0.19)	0.16 (0.07–0.19)	*	0.14 (0.04–0.19)	0.15 (0.03–0.19)	ns	0.17 (0.1–0.19)	0.14 (0.07–0.19)	ns	0.15 (0.04–0.19)	0.14 (0.03–0.19)	ns
Absolute Eosinophil Count	0.01	0.01	ns	0.01	0.01	ns	0.01	0.01	ns	0.01	0.01	ns

(*Continued on next page*)

Table 1. *Continued*

Clinical parameters	Adult	Child	Sig	Male	Female	Sig	Boy	Girl	Sig	PrF	PoF	Sig
Absolute Basophil Count	0.01	0.01	ns	0.01	0.01	ns	0.01	0.01	ns	0.01	0.01	ns
Total Protein	6.18 (4.6–6.58)	6.52 (6.3–6.52)	*	6.2 (5.09–6.58)	6.1 (4.6–6.55)	ns	6.435 (6.2–6.52)	6.57	NA	6.115 (4.6–6.54)	6.06 (4.99–6.55)	ns
Albumin	3.25 (2.4–3.4)	NA	NA	3.3 (2.8–3.4)	3.2 (2.4–3.4)	ns	NA	NA	NA	3.2 (2.8–3.4)	3.15 (2.4–3.4)	ns
Globulin	2.1 (1.4–2.2)	2.15	ns	2.1 (1.4–2.2)	2 (1.8–2.2)	ns	2.2 (2.1–2.2)	NA	NA	2 (1.8–2.2)	2.1 (2–2.2)	ns
A.G.	1.1 (0.7–1.1)	NA	NA	1.1 (0.8–1.1)	1 (0.7–1.1)	ns	NA	NA	NA	1 (0.7–1.1)	1.1 (0.8–1.1)	ns
Bilirubin (Total)	0.2 (0.15–0.29)	0.24 (0.2–0.29)	*	0.24 (0.19–0.26)	0.2 (0.15–0.29)	ns	0.21 (0.2–0.29)	0.24 (0.2–0.29)	ns	0.2 (0.15–0.29)	0.205 (0.2–0.21)	ns
Bilirubin (Direct)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Bilirubin (Indirect)	0.05 (0.01–0.09)	0.01	NA	0.05 (0.03–0.09)	0.035	ns	0.01	NA	NA	0.035 (0.01–0.06)	NA	NA
Transaminase (AST)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Transaminase (ALT)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Alkaline Phosphatase	13.4	NA	NA	13.4	NA	NA	NA	NA	NA	NA	NA	NA
GGTP (Gamma GT), Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA



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of adult patients, while only 4.5% (n = 131) SNVs were unique to the DENV-2 genomes of child patients (Figure 3A). This differential acquisition of SNVs highlights the potential role of age-related selection pressures on viral evolution. Additionally, 42.1% (n = 1235) SNVs were commonly present in the genomes of both adult and child patients, suggesting the development of genomic variation under similar host selection pressures. To further explore the gross interhost genetic diversity, we calculated the Shannon entropy for both adult and child patient groups. Notably, greater interhost genetic diversity was observed in the DENV-2 genomes from the adult patients compared to those from the child patients (Figure 3B), underscoring the potential impact of host-specific factors on viral diversity.

Furthermore, in the adult group of patients, 29.8% (n = 836) SNVs were uniquely found in DENV-2 genomes from male patients, while 17.7% (n = 495) SNVs were exclusive to the genomes from female patients. Additionally, 52.5% (n = 1470) SNVs were commonly present in both male and female patients (Figure 3C). This underscores the importance of sex as a significant factor in shaping viral genetic variation and highlights that while male and female hosts may exert distinct selective pressures on the virus, they also share common selection pressures that may drive the acquisition of genetic variation. Interestingly, the genetic diversity of female patients was observed to be slightly higher than that of male patients (Figure 3D).

Similarly, in the pediatric group of patients, 26.6% (n = 263) SNVs were unique to boy patients, and 10.2% (n = 140) SNVs to girl patients, with 63.2% shared between them (Figure 3E). This suggests that in the child group, sex differences may not play a significant role in influencing interhost genetic diversity. Instead, child patients may exhibit a higher proportion of shared SNVs, which collectively shape genetic variation and exert selective pressure on viral evolution. Notably, genetic diversity was observed more in boys compared to girls (Figure 3F).

Lastly, in the premenopausal female (PrF) and postmenopausal female (PoF), 45.4% (n = 893) of SNVs were unique to PrF, while only 4% (n = 79) of the SNVs were unique to PoF. They also share 50.6% (n = 994) of SNVs (Figure 3G). Additionally, we have observed that the PrF exhibited higher genetic diversity compared to PoF (Figure 3H). This suggests that the hormonal and physiological characteristics of female patients may significantly influence the interhost genetic diversity of the dengue virus. Conclusively, the genetic diversity and unique SNV patterns indicate host factors driving the heterogeneity in interhost evolution and immune response.[30,55]

Significant SNV burden observed in *E*, *NS3*, and *NS5* regions of DENV-2 genomes across age and sex groupings

With the differential genetic diversity observed vis-à-vis age/sex groupings, we further expanded our exploration of DENV-2 genomic and genetic features across the different phenotypes. In the adult vs. child comparison, the frequency of the SNV spectrum was comparatively higher in *NS3, NS4A*, and *NS4B* regions of the genome, with children exhibiting a comparatively higher frequency of SNVs in the *C* gene (Figure 4A). A chi-square test was applied to assess the significance of SNV burden across genes, considering their presence or absence in adult and child groups. The analysis revealed a significant SNV burden in all genes except *ancC* (Figure 4B), highlighting substantial genomic variability between these age groups.

In line with this comparison, the premenopausal female (PrF) vs. postmenopausal female (PoF) group also showed higher frequencies of the SNV spectrum in *NS2A*, *NS3*, and *NS4B*, whereas PoF showed higher frequencies in *C*, *ancC*, *prM*, and *M* (Figure 4G). SNV burden was observed in all the genes except *5′UTR*, *ancC*, and *M* (Figure 4H). In contrast, the male vs. female cohort exhibited a similar frequency of SNV spectrum throughout the genome (Figure 4C), and SNV burden was observed in *E*, *NS3*, *NS4B*, and *NS5* regions (Figure 4D). Similarly, in the boy vs. girl, frequencies of the SNV spectrum are higher in *E*, *NS1*, and *NS3* in boys, whereas the girls showed elevated frequencies in the *C* and *M* genes (Figure 4E). Further, SNV burden was exhibited in *E*, *NS1*, *NS3*, *NS4A*, and *NS5* (Figure 4F). Physiological features such as age and sex can significantly influence the SNV burden observed in different regions of the DENV-2 genome. Variations in host immune responses, which are often age- and sex-dependent, may lead to differential selection pressures on the virus, affecting its genetic diversity and evolution during infection.[52]

Age- and sex-specific patterns of significant SNVs in key DENV-2 genes reveal differential selection pressures and viral adaptation

To investigate the involvement of SNVs across different demographic subgroups, we focused on significant genes identified through the gene burden analysis (*E*, *NS3*, and *NS5*). Fisher's exact test and the phi correlation coefficient were used to determine significant SNVs and their associations with specific age and sex associated groups, revealing distinct SNV patterns.

A total of 142 significant SNVs were identified in the *E*, *NS3*, and *NS5* genes, with 82 SNVs associated with the adult and 60

Figure 2. Differential clinical manifestations of dengue infection across age, sex, and menstrual cycle status

(A and B) Bar graph representing percentage of patients (A) below and (B) above the reference range for each clinical parameter in the adult vs. child subgroup.
(C and D) Boxplots showing significant deviations for selected clinical features between adult and child for (C) low (below) and (D) high (above) reference range.
(E and F) Bar graph representing percentage of patients (E) below and (F) above the reference range for each clinical parameter in the male vs. female subgroup.
(G and H) Boxplots showing significant deviations for selected clinical features between male and female for (G) low (below) and (H) high (above) reference range.
(I and J) Bar graph representing percentage of patients (I) below and (J) above the reference range for each clinical parameter in the premenopausal female vs. postmenopausal female subgroup.
(K and L) Boxplots showing significant deviations for selected clinical features between premenopausal female and postmenopausal female groups for the (K) low (below) and (L) high (above) reference range. Data are represented as median +/− SEM. Significance was calculated using Mann Whitney U-test and is denoted as *, where * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$, and **** indicates $p \leq 0.0001$.

Table 2. Clinical manifestations of dengue patients above the reference range (high) across age and sex subgroupings (Mann Whitney U-test)

Clinical parameters	Adult	Child	Sig	Male	Female	Sig	Boy	Girl	Sig	PrF	PoF	Sig
Hemoglobin	17.52 (17.02–20.78)	16 (15.1–16.9)	****	17.52 (17.02–20.78)	15.44 (15.31–15.9)	***	16 (15.5–16.9)	15.9 (15.1–16.7)	ns	15.9	15.38 (15.31–15.5)	NA
Packed Cell, Volume	51.9 (50.1–60.2)	42 (40.2–53.3)	****	51.9 (50.1–60.2)	47.5 (46.1–49.6)	****	47.15 (46.1–53.3)	50.6 (46.5–52)	ns	47.1 (46.5–47.7)	47.65 (46.1–49.6)	ns
Total Leukocyte Count (TLC)	12.4 (10.4–24.6)	10.65 (10.08–16.5)	*	12.4 (10.4–23.6)	12.75 (10.8–24.6)	ns	16.3 (13.8–16.5)	NA	NA	11.6 (10.8–12)	13.7 (10.8–24.6)	ns
RBC	5.73 (5.51–7.24)	5.085 (4.81–6.52)	****	5.78 (5.51–7.24)	4.99 (4.81–6.14)	****	5.09 (4.81–6.52)	5.07 (4.81–6.32)	ns	4.99 (4.81–6.14)	5 (4.84–5.6)	ns
MCV	104.8 (101.1–124)	NA	NA	104.7 (101.1–119)	105.2 (101.4–124)	ns	NA	NA	NA	105.65 (101.6–124)	104 (101.4–104.3)	ns
MCH	33.6 (32.1–40.1)	NA	NA	33.55 (32.1–39.8)	33.8 (32.1–40.1)	ns	NA	NA	NA	33.65 (32.1–40.1)	34.1 (32.3–34.7)	ns
MCHC	35 (34.6–36.5)	35.4 (34.6–36)	ns	35 (34.6–36.5)	34.8 (34.6–35.7)	ns	35.3 (34.6–35.7)	35.6 (34.6–36)	ns	34.8 (34.6–35.7)	NA	NA
Platelet	551	435.5 (412–652)	NA	NA	551	NA	441	430 (412–652)	NA	NA	551	NA
MPV	12.5 (11.27–17.8)	12.4 (11.3–16.1)	ns	12.4 (11.29–17.8)	12.5 (11.27–16.8)	ns	12.1 (11.3–14.1)	12.4 (11.3–16.1)	ns	12.6 (11.27–16.8)	12.2 (11.3–15.4)	ns
RDW	15.6 (14.6–29.4)	15.6 (14.6–24.5)	ns	15.4 (14.6–21.7)	15.9 (14.6–29.4)	****	15.5 (14.6–24.5)	15.75 (14.6–20.6)	ns	15.9 (14.6–29.4)	15.4 (14.6–20.3)	**
Neutrophils	83.25 (80.1–94)	82.4 (80.2–89.4)	ns	83 (80.1–94)	84 (80.3–92.7)	*	82.8 (80.2–88)	82 (81.5–89.4)	ns	83.95 (80.3–92.7)	86.2 (80.4–87.5)	ns
Lymphocytes	45 (40.1–67.2)	52.5 (40.1–80.3)	****	45 (40.1–67.2)	45.35 (40.3–64.4)	ns	51 (40.1–79)	53.45 (42–80.3)	ns	45.4 (40.3–64.4)	45 (42.7–51.1)	ns
Monocytes	12 (10.1–25.7)	12.05 (10.1–19.5)	ns	12 (10.1–25.7)	12.1 (10.1–21)	ns	12 (10.1–19.5)	12.5 (10.4–18)	ns	12 (10.1–20)	12.4 (10.3–21)	ns
Eosinophils	7.85 (6.4–18.5)	6.8	NA	8.55 (6.4–18.5)	7.85 (6.7–16.1)	ns	6.8	NA	NA	8.35 (6.7–16.1)	7.45 (6.9–8)	ns
Basophils	NA	NA	NA	2.3 (2.1–2.8)	3.4	NA	NA	NA	NA	NA	3.4	NA
Absolute Neutrophil Count	8.11 (7.06–22.18)	7.89 (7.39–11.34)	ns	8.11 (7.06–22.18)	8.075 (7.07–21.53)	ns	7.965 (7.39–11.34)	7.66 (7.65–7.75)	ns	7.63 (7.07–9.74)	12.08 (9.86–21.53)	**
Absolute Lymphocyte Count	3.3 (3.1–6.9)	3.7 (3.1–9.2)	ns	3.3 (3.1–6.9)	3.7	ns	3.45 (3.1–9.2)	3.8 (3.2–6.9)	ns	NA	3.7 (3.6–3.8)	NA
Absolute Monocyte Count	1.2 (1.03–2.33)	1.31 (1.01–1.93)	ns	1.23 (1.08–1.66)	1.165 (1.03–2.33)	ns	1.31 (1.01–1.93)	NA	NA	1.16 (1.03–1.31)	2.33	NA

(*Continued on next page*)

Table 2. ***Continued***

Clinical parameters	Adult	Child	Sig	Male	Female	Sig	Boy	Girl	Sig	PrF	PoF	Sig
Absolute Eosinophil Count	1.3 (0.56–1.54)	NA	NA	1.54	0.93 (0.56–1.3)	NA	NA	NA	NA	0.56	1.3	NA
Absolute Basophil Count	NA	0.11	NA	NA	NA	NA	0.11	NA	NA	NA	NA	NA
Total Protein	8.48 (8.31–8.85)	NA	NA	8.565 (8.43–8.85)	8.3	ns	NA	NA	NA	8.3	NA	NA
Albumin	5.3	NA	NA	5.3	NA	NA	NA	NA	NA	NA	NA	NA
Globulin	3.8 (3.6–4.3)	NA	NA	3.8 (3.6–4.2)	3.7 (3.6–4.3)	ns	NA	NA	NA	3.95 (3.6–4.3)	3.6 (3.6–4)	ns
A.G.	1.7 (1.6–3.3)	1.8 (1.6–2.2)	ns	1.7 (1.6–3.3)	1.7 (1.6–2.5)	*	1.75 (1.6–2.2)	1.8 (1.6–1.9)	ns	1.65 (1.6–2.5)	1.7 (1.6–2.2)	ns
Bilirubin (Total)	1.455 (1.22–10.59)	NA	NA	1.435 (1.22–10.59)	1.59 (1.22–4.06)	ns	NA	NA	NA	1.6 (1.22–2.1)	1.58 (1.3–4.06)	ns
Bilirubin (Direct)	0.3 (0.21–5.53)	0.24 (0.23–0.3)	ns	0.3 (0.21–5.53)	0.3 (0.21–2.66)	ns	0.24 (0.23–0.3)	NA	NA	NA	0.34 (0.21–2.66)	NA
Bilirubin (Indirect)	1.15 (1.01–5.06)	NA	NA	1.12 (1.01–5.06)	1.23 (1.05–1.47)	ns	NA	NA	NA	1.47	1.2 (1.05–1.4)	NA
Transaminase (AST)	108 (50.03–36826)	70 (35.3–337)	****	107 (50.03–11143)	95 (36–36826)	**	76 (35.3–337)	60 (39–279)	ns	94.5 (36–8805)	97.2 (36.1–36826)	ns
Transaminase (ALT)	88.5 (50.6–5785)	58.2 (35.2–163)	****	89 (50.6–5785)	73 (36–5345)	****	55 (35.2–163)	76 (37–139)	ns	71 (36–2349)	76.2 (36–5345)	ns
Alkaline Phosphatase	154.38 (121–791)	216 (123–351)	***	180.8 (123–791)	143.5 (121–403)	ns	217.6 (123–351)	195 (136–324)	ns	146.83 (121–349)	142 (122–403)	ns
GGTP (Gamma GT), Serum	105 (56–707)	61 (45–77)	ns	111.5 (58–707)	79	***	77	45	NA	78 (39–318)	79 (39–547)	ns

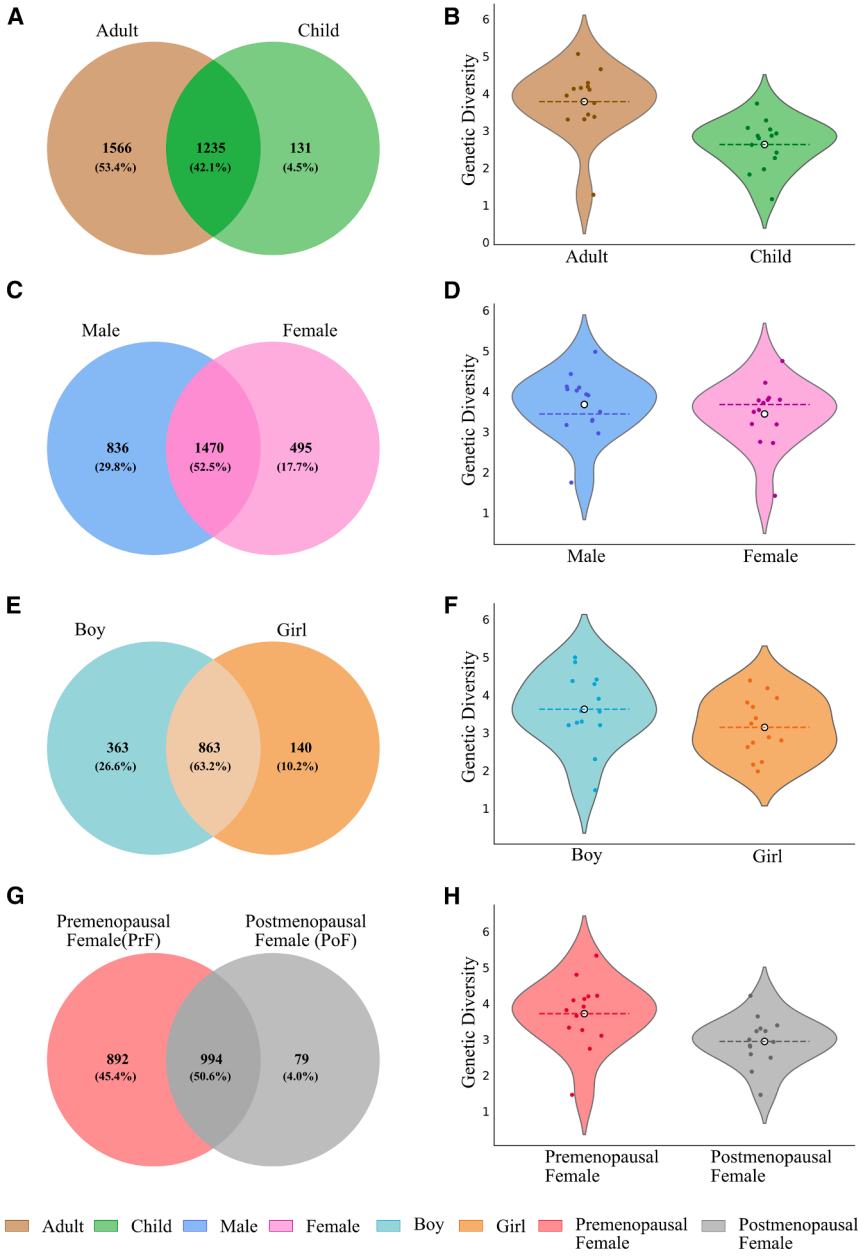

Figure 3. SNV distribution and genetic diversity across age and sex groupings

The Venn diagram depicts the percentage and counts of common and unique SNVs, and the violin plot shows the genetic diversity.

(A) Shared and unique SNVs of adult vs child.

(B) Genetic diversity of adult vs child.

(C) Shared and unique SNVs of male and female.

(D) Genetic diversity of male and female.

(E) Shared and unique SNVs of boy and girl patients.

(F) Genetic diversity of boy and girl.

(G) Shared and unique SNVs of premenopausal female and postmenopausal females.

(H) Genetic diversity of premenopausal female and postmenopausal female.

with the child group. In the *E* gene, of the 35 significant SNVs, 11 of which were associated with the adult and 24 with the child group. Notably, four non-synonymous SNVs were identified—two in adult (A1426G [Ile164Val] and A2105G [Asn309Ser]) and two in child (G1092T [Gln52His] and A2386G [Ile484Val]) (Figure 5A). The number of significant synonymous SNVs associated with the adult and child group in the *E* gene are 9 and 22, respectively. Among 47 significant SNVs in *NS3*, 38 are associated with the adult group and 9 with the child. Three non-synonymous SNVs are found to be associated with the adult group (G5338A [Val273Ile], A5520G [Ile333Met], and A5902G [Ile461Val]) (Figure 5B). The *NS3* gene harbored 35 significant synonymous SNVs in the adult group and 9 in the child group. Further in *NS5*, of the total 60 significant SNVs, 33 are associated with the adult and 27 with the child group (Figure 5C). Five non-synonymous SNVs are observed—three in the child group (GA7820G [Gly85fs], T8308C [Tyr247-His], and T8381C [Ile271Thr]) and two in the adult group (G7613A [Ser15Asn] and A9628G [Ile687Val]). In the *NS5* gene, 31 significant synonymous SNVs were identified in the adult, compared to 24 in the child group.

In the adult group, there are 10 significant SNVs, with 7 associated with male and 3 with female groups, where all 10 are synonymous SNVs (Figure S6). However, all the SNVs except one have less than 1% frequency, suggesting minimal genomic differentiation between males and females. Similarly, in the PrF vs. PoF comparison, we observed 10 significant SNVs, with 8 associated with PrF and 2 with PoF. Both significant SNVs in the PoF are observed in the *NS5* region. All the SNVs found here are synonymous, indicating limited functional impact (Figure 5E). In contrast, a total of 34 significant SNVs are found in the child group, with 33 associated with the boy and only one with the girl group. This group included 17 SNVs in NS3 with all synonymous SNVs and 17 SNVs in *NS5* with four non-synonymous (C8062T [Leu165Phe], G8572A [Val335Ile], C9499A [Gln644Lys], and C9512T [Ala648Val]) and 14 synonymous associated with the boy group (Figure 5D). These findings highlight notable sex-related differences in the genomic variations within the cohort.

Age/sex specific associations for the significant SNV burden genes (*E*, *NS3* and *NS5*)

To explore the relationship between viral SNVs and demographic factors, we performed a logistic regression incorporating age and sex groups. Our findings reveal distinct

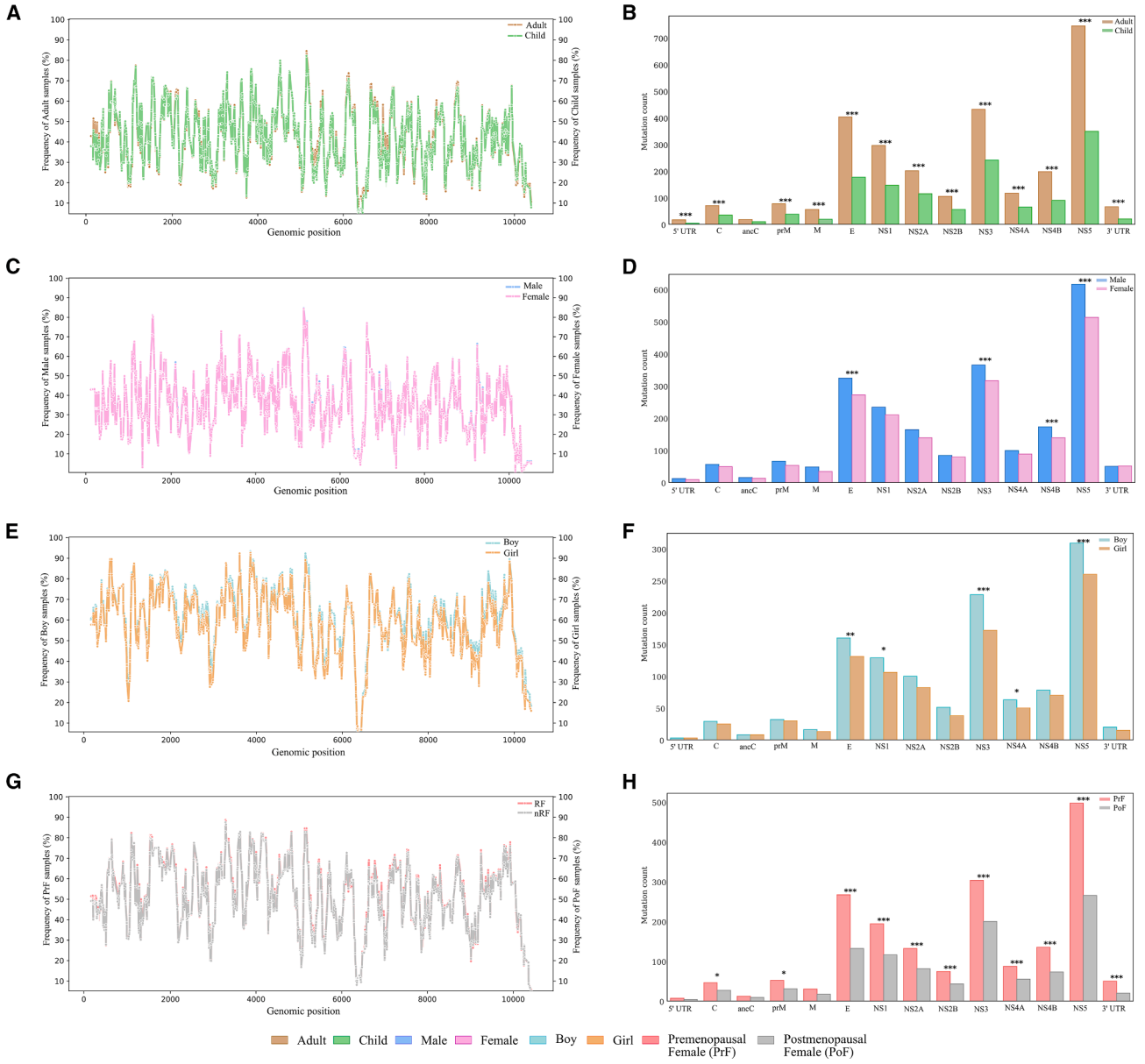

Figure 4. SNV spectrum and gene burden profile across age/sex groupings

The dual line plot represents trends of the SNV spectrum across the DENV-2 genome, and the bar graph depicts the SNV burden in the genes with significant asterisks.

(A) SNV spectrum of adult vs. child.

(B) SNV burden in genes for adult vs child.

(C) SNV spectrum of male vs female.

(D) SNV burden in genes for male vs female.

(E) SNV spectrum of boy vs. girl.

(F) SNV burden in genes for boy vs. girl.

(G) SNV spectrum of premenopausal female vs. postmenopausal female.

(H) SNV burden in genes for premenopausal female vs. postmenopausal female. Data are represented as median +/− SEM. Significance was calculated using the chi-square test and is denoted as *, where * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, *** indicates $p \leq 0.001$, and **** indicates $p \leq 0.0001$.

associations of SNVs in *E*, *NS3*, and *NS5* genes with different phenotype subgroups (Figure 6), suggesting potential age- and sex-specific selection pressures on viral evolution.

In the Male group, *NS3* exhibited a strong positive association (coef = 7.3794, $p = 0.022$), suggesting its significant role in viral adaptation among adult males. Interestingly, in the Boy group,

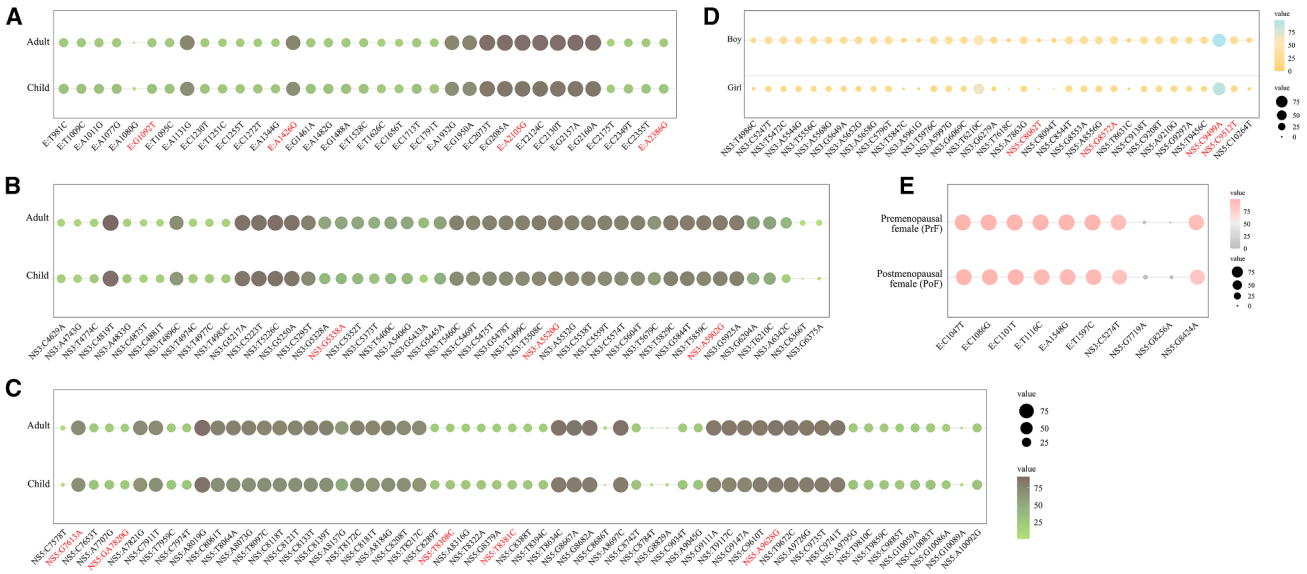

Figure 5. Significant SNVs in *E*, *NS3*, and *NS5* genes associated with specific age/sex

The bubble plot represents the significant SNVs in the respective groups with frequencies as the size of the bubble, and non-synonymous SNVs are represented in red font.

(A) Significant SNVs from E gene of adult vs. child.

(B) Significant SNVs from NS3 gene of adult vs. child.

(C) Significant SNVs from NS5 gene of adult vs. child.

(D) All significant SNVs from boy vs. girl.

(E) All significant SNVs from PrF vs. PoF. Data are represented as median +/− SEM.

NS3 was negatively associated (coef = −10.8069, p = 0.039), indicating a differential effect compared to the males, possibly reflecting age-dependent immune pressures or differential host-virus interactions in children. The *E* and *NS5* genes displayed group-dependent variability. The *E* gene demonstrated a strong positive effect in the Girl (coef = 21.3622, p = 0.065) and Boy (coef = 16.9, p = 0.08) groups, though the p-values were marginally above the significance threshold. This suggests that immune escape SNVs in the *E* gene are more prominent in children than in adults, highlighting a potential age-specific selection pressure on the viral entry mechanisms. Meanwhile, *NS5* SNVs were preferentially associated with females, with a positive effect in PoF (coef = 28.8001, p = 0.073) and PrF (coef = 4.19, p = 0.1). This suggests that SNVs in *NS5*, which is critical for viral replication and immune modulation, may be more frequently observed in the female patients, possibly reflecting sex-specific differences in the immune responses and disease progression. These results collectively indicate that host demographic factors may influence the selection and fixation of viral SNVs, contributing to dengue virus evolution in a population-specific manner.

DISCUSSION

Dengue virus (DENV) infection has become an escalating global health concern, marked by its intricate interplay between host demographic factors, hematological parameters, and genetic variability.[56,57] The increasing rates of single nucleotide variations (SNVs) in the DENV genome highlight the virus's remarkable ability to adapt to host immune pressures and environmental changes, further emphasizing the urgent need for robust clinical and community genomic surveillance systems.[54,57,58] These efforts are crucial not only for unraveling the evolutionary dynamics of DENV but also for understanding the clinical implications of its genetic diversity. This study delves into these aspects, specifically investigating the influence of age, sex, and menstrual cycle status on dengue severity. By examining the clinical manifestations and genetic diversity of the virus during the 2023 dengue outbreak in Delhi, India, the research provides valuable insights into how host demographics intersect with viral genetic variability to shape disease outcomes.

In this study, we observed the prevalent DENV-2 infection among the early adult age group (19–35 years), followed by the 36-55-year age group. This finding is in line with several previous studies where dengue infections are highest among younger adult groups.[9,22,59–61] Younger individuals are more exposed to mosquito bites due to occupational, educational, and recreational activities in urban settings like Delhi, where high *Aedes aegypti* densities and poor waste management facilitate dengue transmission.[62] Also, among the age group of 19–35, male patients were observed to be predominantly and significantly infected with DENV compared to the females. Several studies have reported a higher prevalence of dengue fever among males compared to females, as observed in research from Malaysia, western Uttar Pradesh, and other parts of India.[63–65] This trend may be partly attributed to societal and cultural norms, with males often engaging in more outdoor activities, increasing their exposure to mosquito bites. Interestingly, we observed an

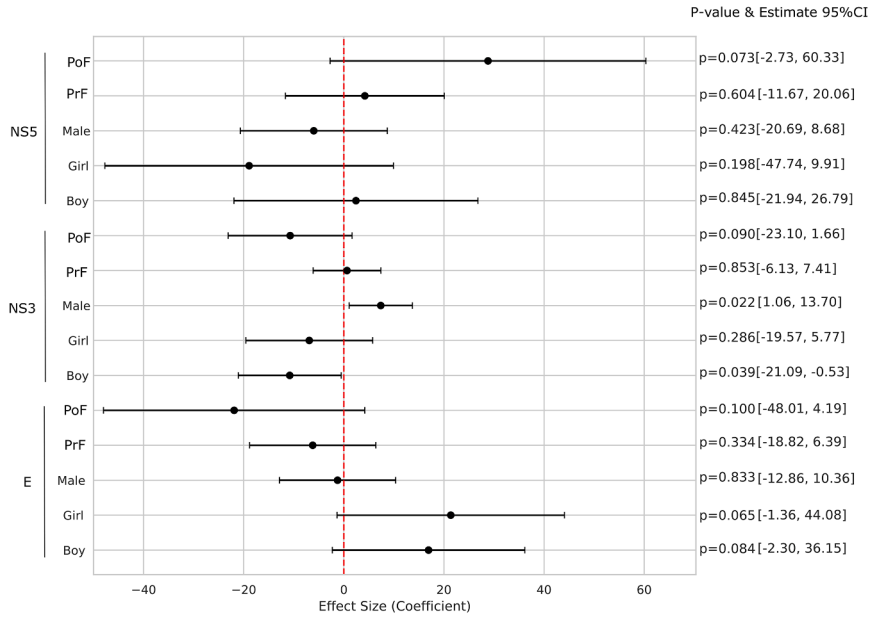

Figure 6. Multivariate analysis depicting the association of demographics (age and sex) with SNVs across significant genes

Forest plot showing the effect size (odds ratio) in the x axis and their dependent variable *E*, *NS3* and *NS5* for the respective demographic groups in the y axis, with their *p*-value and confidence interval on the parallel y axis.

increase in the proportion of the female population over the age of 36 years with DENV infection. This higher prevalence could be associated with a combination of immunological, physiological, and social factors within the female.

The dengue infection is understood through the perspective of hematological and liver function parameters, which are suggestive indicators of disease severity.[66] The reduction within the total leukocyte count (TLC) and thrombocytes (platelets) is often considered an early indicator to assess disease severity in dengue infection.[67] Our analysis identifies adults as being more prone to thrombocytopenia and leukopenia when compared to pediatric patients, suggesting impairment of the immune response thus contributing to the worsening of the disease.[14,29,68] Moreover, the liver function disparities, with children showing higher prevalence but adults exhibiting greater severity of enzyme abnormalities (AST and ALT), underscore the age-associated variations in dengue's hepatic manifestations, likely influenced by the differences in metabolic activity and liver resilience.[69] Sex-specific differences in dengue's impact further reflect the influence of biological and physiological factors. Female patients demonstrated greater susceptibility to reductions in hemoglobin, PCV, and RBC counts, as well as a higher prevalence of elevated RDW, suggesting increased erythropoietic stress. This heightened vulnerability could be linked to menstrual blood loss, which lowers hemoglobin levels and RBC counts, amplifying the hematological impact of dengue.[70] Hormonal factors such as estrogen may modulate immune responses and erythropoiesis, potentially exacerbating the hematological impact of dengue in females.[71] Male patients were found to exhibit elevated levels of liver enzymes, including AST, ALT, and GGTP, pointing to sex-related differences in enzyme activity, liver size, and metabolic rates, indicating possible hepatic injury in male patients.[72]

The comparison between premenopausal female (PrF) and postmenopausal female (PoF) patients highlighted significant differences in hematological and hepatic profiles, likely influenced by hormonal changes associated with aging and menopause. The PoF patients were more likely to exhibit thrombocytopenia and elevated RDW, indicative of impaired hematopoietic recovery and heightened erythropoietic stress. Conversely, PrF patients showed greater reductions in TLC, MCH, and MCV, suggesting a distinct pattern of immune and erythropoietic response in this group.[70] Hepatic parameters also varied, with PoF patients exhibiting a higher prevalence of elevated liver enzymes, including AST, ALT, and GGTP, which could reflect age-related liver vulnerability and the absence of protective hormonal effects seen in younger females.[73] These findings underscore the complex interplay of age, hormonal status, and immune responses in shaping the clinical manifestations of dengue in women. Interestingly, the lack of significant sex differences among pediatric patients suggests that hormonal and physiological differences, which become more pronounced after puberty, play a minimal role in shaping dengue's clinical impact in children. This uniformity aligns with previous studies indicating similar immune and hematological responses between boys and girls in prepubertal stages, where extrinsic factors such as environmental exposure may be more influential.[74]

It is important to note that the difference in viral genetic diversity could have significant implications for disease progression in dengue patients.[75,76] Patients with diverse viral populations might experience more varied clinical manifestations, such as wide-spectrum changes in hematological parameters as well as deviations in the level of enzymes related to liver functions.[77] Therefore, we investigated the impact of the physiological characteristics of the human host in shaping the interhost genetic diversity of the DENV-2 population sequenced directly from the clinical serum samples of the dengue patients. Our findings underscore the role of host-associated factors, such as age, sex, and menstrual cycle status, in shaping the interhost diversity in the viral genome and influencing its overall fitness. By analyzing the 2136 DENV-2 genomes, we observed significant differences in genetic diversity between different patient groups, suggesting that host factors exert selective pressures that contribute to the evolution of the virus within the host.

One of the most striking observations in our study was the differential acquisition of SNVs in the viral genome between adult and child patients. A majority of SNVs were unique to adult patients, while a smaller proportion were exclusive to child patients.

This suggests that age-related factors, such as immune system maturity, may influence the viral genome's evolution.[56] The greater diversity of SNVs in adult patients, as evidenced by the higher Shannon entropy, supports the idea that older individuals experience more complex host-virus interactions, allowing for a broader spectrum of viral variants to emerge. In contrast, the lower diversity in child patients might reflect a less diversified immune response, potentially leading to a more homogeneous viral population[52].

Sex is another host factor that has appeared to influence DENV genetic diversity. A higher proportion of SNV genomes were unique in the DENV-2 sequenced from the male patients (29.8%) as compared to the female patients, suggesting that male and female patients exert different selective pressures on the virus. One possible explanation could be the differences in immune responses between males and females, which have been well-documented in viral infections.[30] Females generally exhibit stronger innate and adaptive immune responses, which could lead to a more robust selection of viral variants and result in slightly higher interhost genetic diversity in females[78] as observed in our study. Interestingly, in child patients, sex differences in viral genetic diversity were less pronounced. Although there was still a higher diversity of SNVs in boys (26.6%) compared to the girls (10.2%), the proportion of shared variants was substantially higher in children than in adults. This could indicate that other factors, such as the immature immune system in both male and female children, lead to less sex-based variation in the viral response. Alternatively, it might reflect a more uniform selection pressure in children, where other factors, such as genetics or environmental factors, play a greater role than sex in shaping viral evolution.[79,80]

The menstrual status of female patients emerged as another important factor influencing viral genetic diversity. Our analysis revealed that premenopausal female patients exhibited significantly higher genetic diversity compared to the postmenopausal age group females. Approximately 45.4% of SNVs were unique to premenopausal females, suggesting that hormonal and physiological changes associated with the menstrual cycle might play a role in modulating immune responses and, consequently, viral evolution. The shared SNVs between premenopausal and postmenopausal females (50.6%) further suggest that some common selection pressures might influence both groups, but the menstrual cycle status likely contributes to a distinct viral evolution pattern as previously reported in the case of SARS-CoV-2.[55]

Within the virus, the intra-host genetic diversity is mostly driven by the SNVs. In our study, the SNV burden among all the inter-groups was significant for the genes *E*, *NS3*, and *NS5*. The E protein plays a crucial role in the virus's ability to infect host cells, through mediating viral entry by interacting with host cell receptors, allowing the virus to attach to and fuse with the host cell membrane.[81] Variations in the E protein, through SNVs, can affect its structure and antigenicity, which may influence viral infectivity and can affect how the immune system responds to the virus, influencing disease progression.[41] The protease function of NS3 protein is critical for cleaving the DENV polyprotein into its functional components, necessary for viral replication.[82] NS3 also plays a role in immune evasion, particularly by inhibiting the host's innate immune response. It achieves this by interfering with the interferon signaling pathways, thereby suppressing the host's antiviral defenses.[83] SNVs in NS3 may enhance viral replication and immune suppression, contributing to persistent infection.[84] The NS5 protein possesses an RNA-dependent RNA polymerase (RdRp) domain, responsible for synthesizing the viral RNA genome during replication.[85] Beyond its role in viral replication, NS5 plays a significant role in immune evasion by inhibiting type I interferon signaling, which is essential for antiviral immune responses.[86] Meanwhile, NS5 SNVs can alter viral replication efficiency and the virus's ability to avoid immune detection, leading to variable clinical outcomes in patients with underlying immune dysfunction.[87,88] By examining the diversity in these proteins, we can gain insights into how host factors, such as age, sex, and menstrual status, may influence viral evolution, ultimately shaping disease outcomes in different populations. Interestingly, the SNV burden acquired more significant SNVs associated with child within the *E* gene, whereas the adult group exhibited more significant SNVs linked with the *NS3* gene. In children, increased SNVs within the *E* gene may indicate adaptations that allow the virus to evade the developing immune system, potentially leading to more severe manifestations of dengue. This aligns with previous findings where children are observed at risk for severe dengue due to their unique immune profiles.[89,90] Conversely, adults exhibiting increased SNVs in the *NS3* gene may help the viral evolution under different selective pressures. The *NS3* protein is crucial for viral replication and immune evasion, and SNVs here could enhance viral fitness in adults.[56] Similarly, in the premenopausal females, the SNVs are prevalent within the *E* gene, while postmenopausal females exhibit increased significant SNVs associated with the *NS5* region. Unfortunately, there are no studies highlighting the differences in hormones and physiology that shape the interhost genetic diversity in DENV. This study could emphasize the critical interplay between host factors and viral genetic diversity, underscoring the need for further studies to elucidate the role of hormonal and immune variations in shaping interhost viral evolution.

Finally, the findings from the multivariate analysis, underscore the importance of demographic stratifications when interpreting viral genetic diversity. While *NS3* SNVs emerge as strong independent predictors in specific groups, *E* and *NS5* SNVs exhibit population-dependent variability, suggesting that viral adaptation is not uniform across the age and sex groups. The differential patterns of SNV burden reinforce the idea that dengue virus dynamics is shaped by complex host-virus interactions that vary across the demographic groups. This highlights the need for demographic considerations in viral pathogenesis studies, ultimately leading to a more precise understanding of dengue disease progression and immune modulation. This may help to uncover the molecular mechanisms that underlie varying clinical manifestations of dengue, providing a more comprehensive understanding of the disease.

Limitations of the study

This study provides important insights into the plausible influence of host demographic factors on dengue infection and viral genetic diversity in one of the dengue epidemic regions of the world; however, a few limitations must be acknowledged which will improve

future studies on dengue. The relatively small sample size within certain subgroups (albeit large sample size overall), particularly postmenopausal females and pediatric patients, may limit the statistical power of our findings. Higher sample numbers for those in future studies would be useful. Additionally, the analysis of hematological and hepatic parameters was based on admission-time clinical reports, lacking longitudinal data to capture disease progression. While we observed significant differences in viral genetic diversity across the patient subgroups, the functional impact of these SNVs on viral fitness and immune evasion remains unexplored. Furthermore, the lack of direct hormonal measurements across the female patients prevents us from definitively linking hormonal fluctuations to immune responses and viral evolution. Lastly, as this study was conducted during a single outbreak in Delhi, India, the future investigations that incorporates the findings based on annual natural biological replicates would enhance our understanding of the interplay between epidemiological factors and clinical manifestations across dengue patients' vis-a-vis genetic diversity of DENV populations.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Rajesh Pandey (rajeshp@igib.in).

Materials availability

This study did not generate new unique reagents and material.

Data and code availability

- DENV-2 genomic data has been uploaded to GISAID and is publicly available as of the date of publication. Accession numbers are listed in the key resources table. All the data reported in this paper will be shared by the lead contact upon request.
- This paper does not report the original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Methodology: K.K., M.I., and V.R.; formal analysis: K.K., V.R., and P.M.; investigation: K.K., M.I., V.R., R.M., and S.H.; data curation: K.K. and V.R.; visualization: K.K., M.I., and V.R.; writing – original draft: K.K., M.I., V.R., and R.M.; writing – review and editing, funding acquisition, conceptualization, and supervision: R.P.; resources: R.P., T.P., B.T., and S.B.

DECLARATION OF INTERESTS

All the authors affirm that there is no conflict of interest while conducting the study. We also confirm that the funding body did not have any role in planning, execution and inferences drawn from the study.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Sample collection and patient demographics
- METHOD DETAILS
  - RNA isolation and DENV serotyping
  - Library preparation and whole genome sequencing of DENV-2
  - Analysis of sequencing reads
  - Variant calling and groupwise SNVs analysis
  - Phylogenetic analysis for the age/sex subgroups
  - Genetic diversity in age/sex
  - Integrative analysis of SNVs across the DENV-2 genome and age/sex
  - Multivariate analysis for age/sex groups and gene-specific SNV burden
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

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REFERENCES

1. Islam, M.T., Quispe, C., Herrera-Bravo, J., Sarkar, C., Sharma, R., Garg, N., Fredes, L.I., Martorell, M., Alshehri, M.M., Sharifi-Rad, J., et al. (2021). Production, Transmission, Pathogenesis, and Control of Dengue Virus: A Literature-Based Undivided Perspective. BioMed Res. Int. *2021*, 4224816. https://doi.org/10.1155/2021/4224816.
2. Kolimenakis, A., Heinz, S., Wilson, M.L., Winkler, V., Yakob, L., Michaelakis, A., Papachristos, D., Richardson, C., and Horstick, O. (2021). The role of urbanisation in the spread of Aedes mosquitoes and the diseases they transmit-A systematic review. PLoS Negl. Trop. Dis. *15*, e0009631. https://doi.org/10.1371/journal.pntd.0009631.
3. Liu, Z., Zhang, Q., Li, L., He, J., Guo, J., Wang, Z., Huang, Y., Xi, Z., Yuan, F., Li, Y., and Li, T. (2023). The effect of temperature on dengue virus transmission by Aedes mosquitoes. Front. Cell. Infect. Microbiol. *13*, 1242173. https://doi.org/10.3389/fcimb.2023.1242173.
4. Childs, M.L., Lyberger, K., Harris, M., Burke, M., and Mordecai, E.A. (2025). Climate warming is expanding dengue burden in the Americas and Asia. Preprint at medRxiv. https://doi.org/10.1101/2024.01.08.24301015.
5. Ryan, S.J., Carlson, C.J., Mordecai, E.A., and Johnson, L.R. (2019). Global expansion and redistribution of Aedes-borne virus transmission risk with climate change. PLoS Negl. Trop. Dis. *13*, e0007213. https://doi.org/10.1371/journal.pntd.0007213.
6. Hussain, S.S.A., and Dhiman, R.C. (2022). Distribution expansion of dengue vectors and climate change in india. GeoHealth *6*, e2021GH000477. https://doi.org/10.1029/2021GH000477.
7. Chaturvedi, U.C., Agarwal, R., Elbishbishi, E.A., and Mustafa, A.S. (2000). Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. FEMS Immunol. Med. Microbiol. *28*, 183–188. https://doi.org/10.1111/j.1574-695X.2000.tb01474.x.
8. Kalayanarooj, S. (2011). Clinical manifestations and management of dengue/dhf/dss. Trop. Med. Health *39*, 83–87. https://doi.org/10.2149/tmh.2011-S10.

9. Vijay, J., Anuradha, N., and Anbalagan, V.P. (2022). Clinical presentation and platelet profile of dengue fever: A retrospective study. Cureus *14*, e28626. https://doi.org/10.7759/cureus.28626.

10. Jayanthi, H.K., and Tulasi, S.K. (2016). Correlation study between platelet count, leukocyte count, nonhemorrhagic complications, and duration of hospital stay in dengue fever with thrombocytopenia. J. Family Med. Prim. Care *5*, 120–123. https://doi.org/10.4103/2249-4863.184635.

11. Tejo, A.M., Hamasaki, D.T., Menezes, L.M., and Ho, Y.-L. (2024). Severe dengue in the intensive care unit. J. Intensive Med. *4*, 16–33. https://doi.org/10.1016/j.jointm.2023.07.007.

12. Swamy, A.M., Mahesh, P.Y., and Rajashekar, S.T. (2021). Liver function in dengue and its correlation with disease severity: a retrospective cross-sectional observational study in a tertiary care center in Coastal India. Pan Afr. Med. J. *40*, 261. https://doi.org/10.11604/pamj.2021.40.261.29795.

13. de Azeredo, E.L., Monteiro, R.Q., and de-Oliveira Pinto, L.M. (2015). Thrombocytopenia in Dengue: Interrelationship between Virus and the Imbalance between Coagulation and Fibrinolysis and Inflammatory Mediators. Mediators Inflamm. *2015*, 313842. https://doi.org/10.1155/2015/313842.

14. Das, S., Abreu, C., Harris, M., Shrader, J., and Sarvepalli, S. (2022). Severe Thrombocytopenia Associated with Dengue Fever: An Evidence-Based Approach to Management of Thrombocytopenia. Case Rep. Hematol. *2022*, 3358325. https://doi.org/10.1155/2022/3358325.

15. Wongtrakul, W., Charatcharoenwitthaya, K., Karaketklang, K., and Charatcharoenwitthaya, P. (2024). Incidence of acute liver failure and its associated mortality in patients with dengue infection: A systematic review and meta-analysis. J. Infect. Public Health *17*, 102497. https://doi.org/10.1016/j.jiph.2024.102497.

16. Alam, R., Rukunuzzaman, M., and Nahid, K.L. (2023). Hepatic involvement in childhood dengue infection. Egypt. Liver J. *13*, 45. https://doi.org/10.1186/s43066-023-00281-9.

17. Fernando, S., Wijewickrama, A., Gomes, L., Punchihewa, C.T., Madusanka, S.D.P., Dissanayake, H., Jeewandara, C., Peiris, H., Ogg, G.S., and Malavige, G.N. (2016). Patterns and causes of liver involvement in acute dengue infection. BMC Infect. Dis. *16*, 319. https://doi.org/10.1186/s12879-016-1656-2.

18. Leowattana, W., and Leowattana, T. (2021). Dengue hemorrhagic fever and the liver. World J. Hepatol. *13*, 1968–1976. https://doi.org/10.4254/wjh.v13.i12.1968.

19. Sangkaew, S., Ming, D., Boonyasiri, A., Honeyford, K., Kalayanarooj, S., Yacoub, S., Dorigatti, I., and Holmes, A. (2021). Risk predictors of progression to severe disease during the febrile phase of dengue: a systematic review and meta-analysis. Lancet Infect. Dis. *21*, 1014–1026. https://doi.org/10.1016/S1473-3099(20)30601-0.

20. Thai, K.T.D., Nishiura, H., Hoang, P.L., Tran, N.T.T., Phan, G.T., Le, H.Q., Tran, B.Q., Nguyen, N.V., and de Vries, P.J. (2011). Age-specificity of clinical dengue during primary and secondary infections. PLoS Negl. Trop. Dis. *5*, e1180. https://doi.org/10.1371/journal.pntd.0001180.

21. Junior, J.B.S., Massad, E., Lobao-Neto, A., Kastner, R., Oliver, L., and Gallagher, E. (2022). Epidemiology and costs of dengue in Brazil: a systematic literature review. Int. J. Infect. Dis. *122*, 521–528. https://doi.org/10.1016/j.ijid.2022.06.050.

22. Hossain, M.S., Noman, A.A., Mamun, S.M.A.A., and Mosabbir, A.A. (2023). Twenty-two years of dengue outbreaks in Bangladesh: epidemiology, clinical spectrum, serotypes, and future disease risks. Trop. Med. Health *51*, 37. https://doi.org/10.1186/s41182-023-00528-6.

23. Badawi, A., Velummailum, R., Ryoo, S.G., Senthinathan, A., Yaghoubi, S., Vasileva, D., Ostermeier, E., Plishka, M., Soosaipillai, M., and Arora, P. (2018). Prevalence of chronic comorbidities in dengue fever and West Nile virus: A systematic review and meta-analysis. PLoS One *13*, e0200200. https://doi.org/10.1371/journal.pone.0200200.

24. Kulkarni, A.V., Choudhury, A.K., Premkumar, M., Jain, P., Gupta, E., and Sarin, S.K. (2019). Spectrum, Manifestations and Outcomes of Dengue Infection in Individuals with and without Liver Disease. J. Clin. Transl. Hepatol. *7*, 106–111. https://doi.org/10.14218/JCTH.2018.00047.

25. Lee, I.-K., Hsieh, C.-J., Lee, C.-T., and Liu, J.-W. (2020). Diabetic patients suffering dengue are at risk for development of dengue shock syndrome/severe dengue: Emphasizing the impacts of co-existing comorbidity(ies) and glycemic control on dengue severity. J. Microbiol. Immunol. Infect. *53*, 69–78. https://doi.org/10.1016/j.jmii.2017.12.005.

26. Bhattarai, B.R., Mishra, A., Aryal, S., Chhusyabaga, M., and Bhujel, R. (2023). Association of Hematological and Biochemical Parameters with Serological Markers of Acute Dengue Infection during the 2022 Dengue Outbreak in Nepal. J. Trop. Med. *2023*, 2904422. https://doi.org/10.1155/2023/2904422.

27. Khan, Z. (2024). Gender-based hematological variations in dengue fever: evidence from a tertiary healthcare setting in Peshawar, Pakistan. JPTCP *31*, 2457–2464. https://doi.org/10.53555/jptcp.v31i6.6924.

28. Ang, L.W., Cutter, J., James, L., and Goh, K.T. (2015). Seroepidemiology of dengue virus infection in the adult population in tropical Singapore. Epidemiol. Infect. *143*, 1585–1593. https://doi.org/10.1017/S0950268814002507.

29. Castilho, B.M., Silva, M.T., Freitas, A.R.R., Fulone, I., and Lopes, L.C. (2020). Factors associated with thrombocytopenia in patients with dengue fever: a retrospective cohort study. BMJ Open *10*, e035120. https://doi.org/10.1136/bmjopen-2019-035120.

30. Zeb, F., Haleem, K.S., Almuqbil, M., Rashid, M., Hussain, W., Maqbool, F., Tauseef, I., Jafri, L., Mannasaheb, B.A., Hussain, S.A., et al. (2024). Age, gender, and infectious status-wise assessments of hematological parameters among patients with dengue infection. Heliyon *10*, e34053. https://doi.org/10.1016/j.heliyon.2024.e34053.

31. Berlin, G., Hammar, M., Tapper, L., and Tynngård, N. (2019). Effects of age, gender and menstrual cycle on platelet function assessed by impedance aggregometry. Platelets *30*, 473–479. https://doi.org/10.1080/09537104.2018.1466387.

32. Rosin, C., Brunner, M., Lehr, S., Quehenberger, P., and Panzer, S. (2006). The formation of platelet-leukocyte aggregates varies during the menstrual cycle. Platelets *17*, 61–66. https://doi.org/10.1080/09537100500227021.

33. Korniluk, A., Koper-Lenkiewicz, O.M., Kamińska, J., Kemona, H., and Dymicka-Piekarska, V. (2019). Mean platelet volume (MPV): new perspectives for an old marker in the course and prognosis of inflammatory conditions. Mediators Inflamm. *2019*, 9213074. https://doi.org/10.1155/2019/9213074.

34. Munt, J.E., Henein, S., Adams, C., Young, E., Hou, Y.J., Conrad, H., Zhu, D., Dong, S., Kose, N., Yount, B., et al. (2023). Homotypic antibodies target novel E glycoprotein domains after natural DENV 3 infection/vaccination. Cell Host Microbe *31*, 1850–1865. https://doi.org/10.1016/j.chom.2023.10.004.

35. Dowd, K.A., Sirohi, D., Speer, S.D., VanBlargan, L.A., Chen, R.E., Mukherjee, S., Whitener, B.M., Govero, J., Aleshnick, M., Larman, B., et al. (2023). prM-reactive antibodies reveal a role for partially mature virions in dengue virus pathogenesis. Proc. Natl. Acad. Sci. USA *120*, e2218899120. https://doi.org/10.1073/pnas.2218899120.

36. Yu, C.-Y., Chang, T.-H., Liang, J.-J., Chiang, R.-L., Lee, Y.-L., Liao, C.-L., and Lin, Y.-L. (2012). Dengue virus targets the adaptor protein MITA to subvert host innate immunity. PLoS Pathog. *8*, e1002780. https://doi.org/10.1371/journal.ppat.1002780.

37. Petit, M.J., Kenaston, M.W., Pham, O.H., Nagainis, A.A., Fishburn, A.T., and Shah, P.S. (2021). Nuclear dengue virus NS5 antagonizes expression of PAF1-dependent immune response genes. PLoS Pathog. *17*, e1010100. https://doi.org/10.1371/journal.ppat.1010100.

38. Glasner, D.R., Puerta-Guardo, H., Beatty, P.R., and Harris, E. (2018). The good, the bad, and the shocking: the multiple roles of dengue virus

nonstructural protein 1 in protection and pathogenesis. Annu. Rev. Virol. *5*, 227–253. https://doi.org/10.1146/annurev-virology-101416-041848.

39. Hill, V., Cleemput, S., Fonseca, V., Tegally, H., Brito, A.F., Gifford, R., Tran, V.T., Kien, D.T.H., Huynh, T., Yacoub, S., et al. (2024). A new lineage nomenclature to aid genomic surveillance of dengue virus. Preprint at medRxiv. https://doi.org/10.1101/2024.05.16.24307504.
40. Maduranga, S., Valencia, B.M., Sigera, C., Adikari, T., Weeratunga, P., Fernando, D., Rajapakse, S., Lloyd, A.R., Bull, R.A., and Rodrigo, C. (2023). Genomic surveillance of recent dengue outbreaks in colombo, sri lanka. Viruses *15*, 1408. https://doi.org/10.3390/v15071408.
41. Mushtaq, S., Khan, M.I.U., Khan, M.T., Lodhi, M.S., and Wei, D.Q. (2023). Novel mutations in structural proteins of dengue virus genomes. J. Infect. Public Health *16*, 1971–1981. https://doi.org/10.1016/j.jiph.2023.10.005.
42. Choudhary, M.C., Gupta, E., Sharma, S., Hasnain, N., and Agarwala, P. (2017). Genetic signatures coupled with lineage shift characterise endemic evolution of Dengue virus serotype 2 during 2015 outbreak in Delhi, India. Trop. Med. Int. Health *22*, 871–880. https://doi.org/10.1111/tmi.12898.
43. Makhluf, H., Buck, M.D., King, K., Perry, S.T., Henn, M.R., and Shresta, S. (2013). Tracking the evolution of dengue virus strains D2S10 and D2S20 by 454 pyrosequencing. PLoS One *8*, e54220. https://doi.org/10.1371/journal.pone.0054220.
44. Hapuarachchi, H.C., Chua, R.C.R., Shi, Y., Thein, T.L., Lee, L.K., Lee, K. S., Lye, D.C., Ng, L.C., and Leo, Y.S. (2015). Clinical outcome and genetic differences within a monophyletic Dengue virus type 2 population. PLoS One *10*, e0121696. https://doi.org/10.1371/journal.pone.0121696.
45. Torres, M.C., Martins Karl, A.L., Müller Pereira da Silva, M., Dardenne, L. E., and Bispo de Filippis, A.M. (2021). In Silico Analysis of Dengue Virus Serotype 2 Mutations Detected at the Intrahost Level in Patients with Different Clinical Outcomes. Microbiol. Spectr. *9*, e0025621. https://doi.org/10.1128/Spectrum.00256-21.
46. Prestwood, T.R., Prigozhin, D.M., Sharar, K.L., Zellweger, R.M., and Shresta, S. (2008). A mouse-passaged dengue virus strain with reduced affinity for heparan sulfate causes severe disease in mice by establishing increased systemic viral loads. J. Virol. *82*, 8411–8421. https://doi.org/10.1128/JVI.00611-08.
47. Wulandari, E., and Hapsari, R.A.F. (2024). Innovative hematology analysis using menstrual blood. Med. Devices *17*, 285–293. https://doi.org/10.2147/MDER.S468500.
48. Scientific Image and Illustration Software | BioRender https://www.biorender.com/.
49. Amacher, D.E. (2014). Female gender as a susceptibility factor for drug-induced liver injury. Hum. Exp. Toxicol. *33*, 928–939. https://doi.org/10.1177/0960327113512860.
50. Ikejima, K., Enomoto, N., Iimuro, Y., Ikejima, A., Fang, D., Xu, J., Forman, D.T., Brenner, D.A., and Thurman, R.G. (1998). Estrogen increases sensitivity of hepatic Kupffer cells to endotoxin. Am. J. Physiol. *274*, G669–G676. https://doi.org/10.1152/ajpgi.1998.274.4.G669.
51. Souza, L.J.d., Alves, J.G., Nogueira, R.M.R., Gicovate Neto, C., Bastos, D.A., Siqueira, E.W.d.S., Souto Filho, J.T.D., Cezário, T.d.A., Soares, C. E., and Carneiro, R.d.C. (2004). Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1,585 cases. Braz. J. Infect. Dis. *8*, 156–163.
52. Sim, S., Aw, P.P.K., Wilm, A., Teoh, G., Hue, K.D.T., Nguyen, N.M., Nagarajan, N., Simmons, C.P., and Hibberd, M.L. (2015). Tracking Dengue Virus Intra-host Genetic Diversity during Human-to-Mosquito Transmission. PLoS Negl. Trop. Dis. *9*, e0004052. https://doi.org/10.1371/journal.pntd.0004052.
53. Nonyong, P., Ekalaksananan, T., Phanthanawiboon, S., Overgaard, H.J., Alexander, N., Thaewnongiew, K., Sawaswong, V., Nimsamer, P., Payungporn, S., Phadungsombat, J., et al. (2023). Intrahost Genetic Diversity of Dengue Virus in Human Hosts and Mosquito Vectors under Natural Conditions Which Impact Replicative Fitness In Vitro. Viruses *15*, 982. https://doi.org/10.3390/v15040982.
54. Torres, M.C., Lima de Mendonça, M.C., Damasceno Dos Santos Rodrigues, C., Fonseca, V., Ribeiro, M.S., Brandão, A.P., Venâncio da Cunha, R., Dias, A.I., Santos Vilas Boas, L., Felix, A.C., et al. (2021). Dengue Virus Serotype 2 Intrahost Diversity in Patients with Different Clinical Outcomes. Viruses *13*, 349. https://doi.org/10.3390/v13020349.
55. Liu, P., Cai, J., Tian, H., Li, J., Lu, L., Xu, M., Zhu, X., Zhong, H., Jia, R., Fu, X., et al. (2024). Intra-host genetic diversity of SARS-CoV-2 Omicron variants in children. J. Infect. *88*, 106132. https://doi.org/10.1016/j.jinf.2024.106132.
56. OhAinle, M., Balmaseda, A., Macalalad, A.R., Tellez, Y., Zody, M.C., Saborío, S., Nuñez, A., Lennon, N.J., Birren, B.W., Gordon, A., et al. (2011). Dynamics of dengue disease severity determined by the interplay between viral genetics and serotype-specific immunity. Sci. Transl. Med. *3*, 114ra128. https://doi.org/10.1126/scitranslmed.3003084.
57. Dolan, P.T., Taguwa, S., Rangel, M.A., Acevedo, A., Hagai, T., Andino, R., and Frydman, J. (2021). Principles of dengue virus evolvability derived from genotype-fitness maps in human and mosquito cells. Elife *10*, e61921. https://doi.org/10.7554/eLife.61921.
58. Dang, T.T., Pham, M.H., Bui, H.V., and Van Le, D. (2020). Whole genome sequencing and genetic variations in several dengue virus type 1 strains from unusual dengue epidemic of 2017 in Vietnam. Virol. J. *17*, 7. https://doi.org/10.1186/s12985-020-1280-z.
59. Gupta, S., Shakya, A., Mishra, N., Islahi, S., Singh, S., Nag, S., Dwivedi, A., Shukla, M., Sen, S., Dwivedi, P., et al. (2024). Investigating the Clinico-Demographic Characteristics of Dengue Fever and Its Seroprevalence at a Tertiary Care Hospital in Northern India. Cureus *16*, e57640. https://doi.org/10.7759/cureus.57640.
60. N Sirisena, P.D.N., Mahilkar, S., Sharma, C., Jain, J., and Sunil, S. (2021). Concurrent dengue infections: Epidemiology & clinical implications. Indian J. Med. Res. *154*, 669–679. https://doi.org/10.4103/ijmr.IJMR_1219_18.
61. Annan, E., Treviño, J., Zhao, B., Rodriguez-Morales, A.J., and Haque, U. (2023). Direct and indirect effects of age on dengue severity: The mediating role of secondary infection. PLoS Negl. Trop. Dis. *17*, e0011537. https://doi.org/10.1371/journal.pntd.0011537.
62. Telle, O., Nikolay, B., Kumar, V., Benkimoun, S., Pal, R., Nagpal, B.N., and Paul, R.E. (2021). Social and environmental risk factors for dengue in Delhi city: A retrospective study. PLoS Negl. Trop. Dis. *15*, e0009024. https://doi.org/10.1371/journal.pntd.0009024.
63. Mustafa, Z., Khan, H.M., Azam, M., Sami, H., Ali, S.G., Ahmad, I., Raza, A., and Khan, M.A. (2023). Insight into the seroepidemiology and dynamics of circulating serotypes of dengue virus over a 4 year period in western Uttar Pradesh, India. Access Microbiol. *5*, acmi000567.v4. https://doi.org/10.1099/acmi.0.000567.v4.
64. Kumar, M., Verma, R.K., and Mishra, B. (2020). Prevalence of Dengue Fever in Western Uttar Pradesh, India: A Gender-Based Study. Int. J. Appl. Basic Med. Res. *10*, 8–11. https://doi.org/10.4103/ijabmr.IJABMR_337_18.
65. Rob, M.A., Hossain, M., Sattar, M.A., Ahmed, I.U., Chowdhury, A.F.M.N., Mehedi, H.M.H., Mohammed, N., Maruf Ul Quader, M., Hossain, M.Z., Rahman, M., et al. (2024). Circulating dengue virus serotypes, demographics, and epidemiology in the 2023 dengue outbreak in Chittagong, Bangladesh. Eur. J. Microbiol. Immunol. *14*, 272–279. https://doi.org/10.1556/1886.2024.00069.
66. Haq, F.U., Imran, M., Aslam, Z., Mukhtar, F., Jabeen, K., Chaudhry, M., Rahman, S.U., and Muhammad, N. (2023). Severity of Dengue Viral Infection Based on Clinical and Hematological Parameters among Pakistani Patients. Am. J. Trop. Med. Hyg. *109*, 1284–1289. https://doi.org/10.4269/ajtmh.23-0309.
67. Vogt, M.B., Lahon, A., Arya, R.P., Spencer Clinton, J.L., and Rico-Hesse, R. (2019). Dengue viruses infect human megakaryocytes, with probable clinical consequences. PLoS Negl. Trop. Dis. *13*, e0007837. https://doi.org/10.1371/journal.pntd.0007837.

68. Raadsen, M., Du Toit, J., Langerak, T., van Bussel, B., van Gorp, E., and Goeijenbier, M. (2021). Thrombocytopenia in virus infections. J. Clin. Med. *10*, 877. https://doi.org/10.3390/jcm10040877.

69. Martínez Vega, R., Phumratanaprapin, W., Phonrat, B., Dhitavat, J., Sutherat, M., and Choovichian, V. (2016). Differences in Liver Impairment Between Adults and Children with Dengue Infection. Am. J. Trop. Med. Hyg. *94*, 1073–1079. https://doi.org/10.4269/ajtmh.15-0507.

70. Napolitano, M., Dolce, A., Celenza, G., Grandone, E., Perilli, M.G., Siragusa, S., Carta, G., Orecchioni, A., and Mariani, G. (2014). Iron-dependent erythropoiesis in women with excessive menstrual blood losses and women with normal menses. Ann. Hematol. *93*, 557–563. https://doi.org/10.1007/s00277-013-1901-3.

71. Harding, A.T., and Heaton, N.S. (2022). The Impact of Estrogens and Their Receptors on Immunity and Inflammation during Infection. Cancers (Basel) *14*, 909. https://doi.org/10.3390/cancers14040909.

72. Palmal, S., Chakraborty, S., Ganguly, S., Kundu, S., Dey, J.B., Pramanik, K., and Pattanayak, A.K. (2023). Study of Hepatic Dysfunction Associated with Dengue Epidemiology in a Tertiary Care Hospital, Kolkata. ACS Omega *8*, 6632–6637. https://doi.org/10.1021/acsomega.2c07119.

73. van Beek, J.H.D.A., de Moor, M.H.M., de Geus, E.J.C., Lubke, G.H., Vink, J.M., Willemsen, G., and Boomsma, D.I. (2013). The genetic architecture of liver enzyme levels: GGT, ALT and AST. Behav. Genet. *43*, 329–339. https://doi.org/10.1007/s10519-013-9593-y.

74. Muenchhoff, M., and Goulder, P.J.R. (2014). Sex differences in pediatric infectious diseases. J. Infect. Dis. *209*, S120–S126. https://doi.org/10.1093/infdis/jiu232.

75. Descloux, E., La Fuentez, C., Roca, Y., and De Lamballerie, X. (2014). Clinical significance of intra-host variability of Dengue-1 virus in venous and capillary blood. Clin. Microbiol. Infect. *20*, O167–O175. https://doi.org/10.1111/1469-0691.12368.

76. Zhang, L., Zhao, L., Zhang, Z., Hong, W., Wang, J., Qiu, S., Yang, H., Gan, M., Sun, J., Zhao, J., et al. (2021). Genetic and pathogenicity diversity of dengue virus type 2 strains circulating in Guangdong, China. Biosafety Health *3*, 333–342. https://doi.org/10.1016/j.bsheal.2021.08.001.

77. Bhatt, P., Sabeena, S.P., Varma, M., and Arunkumar, G. (2021). Current understanding of the pathogenesis of dengue virus infection. Curr. Microbiol. *78*, 17–32. https://doi.org/10.1007/s00284-020-02284-w.

78. Vadakedath, S., Kandi, V., Mohapatra, R.K., Pinnelli, V.B.K., Yegurla, R.R., Shahapur, P.R., Godishala, V., Natesan, S., Vora, K.S., Sharun, K., et al. (2021). Immunological aspects and gender bias during respiratory viral infections including novel Coronavirus disease-19 (COVID-19): A scoping review. J. Med. Virol. *93*, 5295–5309. https://doi.org/10.1002/jmv.27081.

79. Parameswaran, P., Wang, C., Trivedi, S.B., Eswarappa, M., Montoya, M., Balmaseda, A., and Harris, E. (2017). Intrahost selection pressures drive rapid dengue virus microevolution in acute human infections. Cell Host Microbe *22*, 400–410. https://doi.org/10.1016/j.chom.2017.08.003.

80. Simon-Lorière, E., and Vignuzzi, M. (2017). Drivers of dengue intrahost evolution. Cell Host Microbe *22*, 260–261. https://doi.org/10.1016/j.chom.2017.08.015.

81. Hu, T., Wu, Z., Wu, S., Chen, S., and Cheng, A. (2021). The key amino acids of E protein involved in early flavivirus infection: viral entry. Virol. J. *18*, 136. https://doi.org/10.1186/s12985-021-01611-2.

82. Nitsche, C., Holloway, S., Schirmeister, T., and Klein, C.D. (2014). Biochemistry and medicinal chemistry of the dengue virus protease. Chem. Rev. *114*, 11348–11381. https://doi.org/10.1021/cr500233q.

83. Latanova, A., Starodubova, E., and Karpov, V. (2022). Flaviviridae nonstructural proteins: the role in molecular mechanisms of triggering inflammation. Viruses *14*, 1808. https://doi.org/10.3390/v14081808.

84. Teramoto, T. (2023). Dengue virus serotypic replacement of NS3 protease or helicase domain causes chimeric viral attenuation but can be recovered by a compensated mutation at helicase domain or NS2B, respectively. J. Virol. *97*, e0085423. https://doi.org/10.1128/jvi.00854-23.

85. Ackermann, M., and Padmanabhan, R. (2001). De novo synthesis of RNA by the dengue virus RNA-dependent RNA polymerase exhibits temperature dependence at the initiation but not elongation phase. J. Biol. Chem. *276*, 39926–39937. https://doi.org/10.1074/jbc.M104248200.

86. Fernandes, P.O., Chagas, M.A., Rocha, W.R., and Moraes, A.H. (2021). Non-structural protein 5 (NS5) as a target for antiviral development against established and emergent flaviviruses. Curr. Opin. Virol. *50*, 30–39. https://doi.org/10.1016/j.coviro.2021.07.001.

87. Gonzalez Lopez Ledesma, M.M., Costa Navarro, G., Pallares, H.M., Paletta, A., De Maio, F., Iglesias, N.G., Gebhard, L., Oviedo Rouco, S., Ojeda, D.S., de Borba, L., et al. (2023). Dengue virus NS5 degrades ERC1 during infection to antagonize NF-kB activation. Proc. Natl. Acad. Sci. USA *120*, e2220005120. https://doi.org/10.1073/pnas.2220005120.

88. Dwivedi, V.D., Tripathi, I.P., Tripathi, R.C., Bharadwaj, S., and Mishra, S.K. (2017). Genomics, proteomics and evolution of dengue virus. Brief. Funct. Genomics *16*, 217–227. https://doi.org/10.1093/bfgp/elw040.

89. Wong, J.M., Adams, L.E., Durbin, A.P., Muñoz-Jordán, J.L., Poehling, K.A., Sánchez-González, L.M., Volkman, H.R., and Paz-Bailey, G. (2022). Dengue: A growing problem with new interventions. Pediatrics *149*, e2021055522. https://doi.org/10.1542/peds.2021-055522.

90. Yung, C.-F., Lee, K.-S., Thein, T.-L., Tan, L.-K., Gan, V.C., Wong, J.G.X., Lye, D.C., Ng, L.-C., and Leo, Y.-S. (2015). Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, singapore. Am. J. Trop. Med. Hyg. *92*, 999–1005. https://doi.org/10.4269/ajtmh.14-0628.

91. Li, H. (2018). Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics *34*, 3094–3100. https://doi.org/10.1093/bioinformatics/bty191.

92. Zheng, Z., Li, S., Su, J., Leung, A.W.-S., Lam, T.-W., and Luo, R. (2022). Symphonizing pileup and full-alignment for deep learning-based long-read variant calling. Nat. Comput. Sci. *2*, 797–803. https://doi.org/10.1038/s43588-022-00387-x.

93. Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X., and Ruden, D.M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) *6*, 80–92. https://doi.org/10.4161/fly.19695.

94. Huddleston, J., Hadfield, J., Sibley, T.R., Lee, J., Fay, K., Ilcisin, M., Harkins, E., Bedford, T., Neher, R.A., and Hodcroft, E.B. (2021). Augur: a bioinformatics toolkit for phylogenetic analyses of human pathogens. J. Open Source Softw. *6*, 2906. https://doi.org/10.21105/joss.02906.

95. Hadfield, J., Megill, C., Bell, S.M., Huddleston, J., Potter, B., Callender, C., Sagulenko, P., Bedford, T., and Neher, R.A. (2018). Nextstrain: real-time tracking of pathogen evolution. Bioinformatics *34*, 4121–4123. https://doi.org/10.1093/bioinformatics/bty407.

96. Letunic, I., and Bork, P. (2024). Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Res. *52*, W78–W82. https://doi.org/10.1093/nar/gkae268.

97. Stubbs, S.C.B., Blacklaws, B.A., Yohan, B., Yudhaputri, F.A., Hayati, R.F., Schwem, B., Salvaña, E.M., Destura, R.V., Lester, J.S., Myint, K.S., et al. (2020). Assessment of a multiplex PCR and Nanopore-based method for dengue virus sequencing in Indonesia. Virol. J. *17*, 24. https://doi.org/10.1186/s12985-020-1294-6.

98. Core Pipeline - artic pipeline https://artic.readthedocs.io/en/latest/minion/.

99. bcftools(1) https://samtools.github.io/bcftools/bcftools.html.

100. Bardou, P., Mariette, J., Escudié, F., Djemiel, C., and Klopp, C. (2014). jvenn: an interactive Venn diagram viewer. BMC Bioinf. *15*, 293. https://doi.org/10.1186/1471-2105-15-293.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Serum RNA extraction	QIAamp Viral RNA Mini Kit (250), Qiagen	Cat. No. 52906
TRUPCR® Dengue Virus Serotyping Kit	3B BlackBio Biotech India Ltd	Cat. No. 3B237
LunaScript RT Supermix	New England Biolabs	Cat. No. E3010L
Q5 High-Fidelity 2X Master Mix	New England Biolabs	Cat. No. M0494L
NEBNext Ultra II End Repair/ dA-Tailing Module	New England Biolabs	Cat. No. E7546L
Native Barcoding Kit 24v14	Oxford Nanopore Technology (ONT)	Cat. No. SQK-NBD114.24
AMPure XP	Beckman Coulter	Cat. No. A63881
Qubit dsDNA HS Assay kit	Symbio (Thermo Fisher Scientific)	Cat. No. Q32854
Deposited data		
DENV-2 Nanopore Sequenced data	GISAID EpiArbo database	GISAID: EPI_SET_250401uc [https://doi.org/10.55876/gis8.250401uc]
Software and algorithms		
Dorado	NA	https://github.com/nanoporetech/dorado; RRID: SCR_025883
minimap2	Li et al.[91]	https://github.com/lh3/minimap2; RRID: SCR_018550
AlignTrim	NA	https://github.com/rln0005/minION_align
Artic Mask	NA	https://github.com/artic-network/fieldbioinformatics/blob/master/artic/mask.py
Clair3	Zeng et al.[92]	https://github.com/HKU-BAL/Clair3; RRID: SCR_026063
bcftools	NA	https://samtools.github.io/bcftools/bcftools.html; RRID: SCR_005227
SnpEff	Cingolani et al.[93]	https://pcingola.github.io/SnpEff/; RRID: SCR_005191
Augur align	Huddleston et al.[94]	https://github.com/nextstrain/augur/blob/master/augur/align.py
Nextstrain	Hadfield et al.[95]	https://nextstrain.org/; RRID: SCR_018223
iTOL	Letunic et al.[96]	https://itol.embl.de/; RRID: SCR_018174
GraphPad Prism 9	NA	https://www.graphpad.com/
R 4.3.2	NA	https://www.r-project.org/; RRID: SCR_001905

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Sample collection and patient demographics

The research was carried out in line with the Helsinki Declaration and received approval from the ethics board of the Council of Scientific & Industrial Research-Institute of Genomics and Integrative Biology in Delhi, India (Reference Number: CSIR-IGIB/IHEC/2023-24/04). All participants provided their written consent before taking part in the study. For participants below 18 years of age, formal written consent was obtained from the parent/legal guardian. The study utilized serum samples collected from symptomatic dengue-suspected patients presenting at Max Super Speciality Hospital, New Delhi, India, between June and November 2023. Serum samples from patients testing positive for NS1 antigen (NS1-Ag) were transported daily to the laboratory at the CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB), New Delhi, under controlled cold storage conditions. Upon arrival, the samples were aseptically transferred into 2 mL tubes, assigned unique barcode identifiers, and stored at −20°C until RNA extraction.

The study cohort comprised 2,136 NS1-Ag-positive dengue patients (age 0–86 years) from the DENV Genomic Surveillance of 2023, Delhi, India, enabling an in-depth investigation of age- and sex-specific differences in dengue epidemiology. Additionally, clinical data, including complete blood count (CBC) and liver function test (LFT) results, were retrieved for a subset of patients from the hospital's electronic health records. Among the 2,136 patients, CBC reports were available for 1,756 individuals, while LFT reports were obtained for 790 patients. Based on the demographic and major confounding factors (age and sex), the study design has been classified into four groups: 1) Adult vs. Child comprised 1844 adult patients (age >12 years); 2) With further stratification of the Adult

group, Male vs. Female male (n = 1040) and female (n = 804) patients; 3) A study group of children as Boy vs. Girl (age $\leq$12 years) (n = 292) with the boy (n = 178) and girl (n = 114); 4) In addition, to examine how intricate hormonal changes in premenopausal and postmenopausal females impact dengue epidemiology, a subset of female patients (n = 804) was specifically selected. This group was further taken as Premenopausal Female (PrF) vs. Postmenopausal Female (PoF) as follows: premenopausal females (n = 676) and postmenopausal females (n = 128).

METHOD DETAILS

RNA isolation and DENV serotyping

The frozen serum samples were thawed on ice prior to RNA extraction. Total RNA was isolated from 150 μL of each serum sample using the QIAamp Viral RNA Mini Kit (Qiagen, Cat. No. 52906) according to the manufacturer's protocol. The extracted RNA was stored at −80°C for further analysis. RNA quality and concentration were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). A portion of the extracted RNA was used for DENV serotyping through qRT-PCR. The serotyping was performed with a DENV serotyping kit (TRUPCR, Cat. No. 3B237), following the provided protocol, which included both positive and negative controls. The serotyping results indicate DENV-2 as the only serotype detected across all 2136 samples.

Library preparation and whole genome sequencing of DENV-2

A total of 2,136 DENV-2-positive samples, identified through serotype-specific RT-PCR, were selected for whole-genome sequencing utilizing Oxford Nanopore Technology (ONT). RNA isolated from these samples was reverse transcribed into complementary DNA (cDNA) using LunaScript RT Supermix (New England Biolabs, Cat. No. E3010L). Amplification of the DENV-2 genome was carried out with a tiling PCR approach, employing 39 overlapping primer pairs, adapted with modifications from the protocol.[97] The primers, synthesized by Integrated DNA Technologies, were validated for specificity and listed in Table S4.

To streamline amplification, the primers were divided into two pools: odd-numbered primers (pool 1) and even-numbered primers (pool 2). Separate PCR reactions were conducted for each pool using Q5 High-Fidelity 2X Master Mix (New England Biolabs, Cat. No. M0494L). After amplification, products from pools 1 and 2 were combined, followed by purification using Ampure XP (AXP) beads (Beckman Coulter, Cat. No. A63881). DNA concentrations of the purified PCR products were quantified using a Qubit 4.0 fluorimeter (Invitrogen, USA). The pooled PCR products were processed for library preparation, beginning with end-repair and A-tailing using the NEBNext Ultra II End Repair/dA-Tailing Module (New England Biolabs, Cat. No. E7546L). Barcoding was performed with the Native Barcoding Kit 24v14 (SQK-NBD114.24), and barcoded libraries were further purified with Ampure XP beads. Concentrations were measured with a Qubit 4.0 fluorimeter before the ligation of native adapters, also using the Native Barcoding Kit 24v14. The final libraries underwent additional purification steps to ensure quality. The prepared libraries were loaded onto R10.4.1 flow cells and sequenced using an ONT GridION platform. High-quality genome reads were generated, enabling downstream analyses to uncover key features of the DENV-2 genome.

Analysis of sequencing reads

The sequencing data from the GridION device were processed on a high-performance computing (HPC) cluster. Initially, raw reads underwent basecalling and demultiplexing using the Dorado basecaller using high accuracy basecaller ('hac basecaller'), ensuring a phred Q-score quality threshold of >9. The resulting high-quality reads were then mapped to the DENV-2 reference genome (NC_001474) with minimap2,[91] an advanced mapping tool designed for long-read sequences. This mapping ensured precise alignment of the sequencing data to the reference genome, enabling accurate downstream analysis. To enhance the quality of the aligned data, primer sequences were trimmed using AlignTrim,[98] which eliminates primers and facilitates cleaner datasets for variant analysis. Furthermore, Artic Mask[98] was applied to identify and exclude low-quality reads and genomic regions, generating 'coverage-mask' files that defined reliable areas for analysis while masking ambiguous or unreliable data.

Variant calling and groupwise SNVs analysis

Variant calling was performed on the primer-depleted high-quality reads using Clair3,[92] a state-of-the-art, deep-learning-based tool optimized for detecting SNVs and small indels in long-read sequencing data. Post-variant calling, the VCF files underwent stringent filtering using BCFtools.[99] Variants with a genotype quality below 3 or a read depth less than 20 were excluded (labeled as 'fail vcf'), while variants meeting the quality thresholds (genotype quality >3, depth >20) were retained in the 'pass vcf' file. For generating the consensus sequence, positions marked as 'fail vcf' or included in the 'coveragemask' were masked, and the SNVs from 'pass vcf' were incorporated into the reference genome using bcftools. Coverage metrics, including depth and breadth, were assessed against the wild-type DENV-2 genome (Table S5). Samples achieving more than 50% genome coverage were merged. Following the merging of multiple VCFs, preprocessing included 'IndelGap' correction and resolution of 'multiallelic' variants using bcftools, ensuring that only a single variant was retained per genomic position. To streamline downstream analysis, we performed variant normalization using 'norm' and 'sort,' which enabled accurate SNVs merging while preserving a consistent positional order from start to end. Further, SNV annotations were carried out using Snpeff.[93] In line with the study design, the group-wise SNVs were called [adult, child, male, female, boy, girl, premenopausal female, and postmenopausal female], and their frequencies were tabulated in Table S6.

Phylogenetic analysis for the age/sex subgroups

The DENV-2 reference sequence (GenBank: NC_001474) was used to perform multiple sequence alignment with our cohort dataset taken in categorically, Adult Vs Child, Male Vs Female, Boy Vs Girl and Premenopausal Female (PrF) Vs Postmenopausal Female (PoF) through Augur align.[94] Prior to phylogenetic analysis, genotypes of all the DENV sequences are identified through nextstrain.[95] All are found to be cosmopolitan genotypes. In total, four phylogenetic trees were built using 'augur tree' using *'iq-tree'* with the '*maximum-likelihood'* algorithm. The tree was visualized using iTOL.[96]

Genetic diversity in age/sex

To compare the intra-group genome differences, we have inquired about common and unique SNVs in each group [adult vs. child, male vs. female, boy vs. girl, and premenopausal female vs. postmenopausal female] using jvenn.[100] To further explore how these intra-grouping differences help in altering the inter-host genetic diversity, Shannon entropy was applied for all the sub-groupings, and statistical significance was assessed using the Wilcoxon rank-sum test, following the methodology outlined by[55] (Table S7). Shannon entropy and Wilcoxon rank-sum test was carried out in scipy.stats library in python.

Integrative analysis of SNVs across the DENV-2 genome and age/sex

Inter-host genetic diversity was observed in viral populations between the intra-subgroups, driven by the accumulation of single nucleotide variations (SNVs). By analyzing genome-wide and gene-specific SNV distributions, it becomes possible to discern how these SNVs influence genetic divergence and impact viral adaptation within subgroups. To investigate gene-wise differences within the previously defined intra-group comparisons, the SNV burden across genes was analyzed. This involved tabulating the presence and absence of SNVs for intra-group comparisons, including adult vs. child, male vs. female, boy vs. girl, and PrF vs. PoF (Table S8). A Chi-square test was then applied to the resulting presence-absence contingency table. Further, *p*-values are adjusted using Bonferroni correction. For genes identified as commonly burdened across all the intra-groups, the importance of SNVs making the burden was determined. To achieve that, the nonparametric Fisher's exact test was conducted to assess the independence of two categorical variables and to compare SNV profiles across groups. Two-sided *p*-values were calculated, with a significant threshold set at 0.05. The direction and strength of the association between SNVs and group classifications were evaluated using the phi coefficient (ϕ). The Chi-square, Bonferroni correction, and Fisher's exact tests were performed using the scipy.stats and stats.model library in Python, while phi correlation analysis was conducted with the psych library in R (Table S9).

Multivariate analysis for age/sex groups and gene-specific SNV burden

To evaluate the contribution of the multiple variants to the SNV burden within specific genes vis-à-vis corresponding their functional relevance to the phenotype subgroups, we analyzed five phenotypes as demographics aligning the theme of our study: Male [$n = 1040$], Boy [$n = 178$], Girl [$n = 114$], Premenopausal Female (PrF) [$n = 676$], and postmenopausal female (PoF) [$n = 128$] as unique groups since these are subset of Adult, Child and Female. The SNV burden was assessed for three key genes: E [$n = 42$], NS3 [$n = 69$], and NS5 [$n = 85$], by normalizing the significant SNVs in each gene against the total SNVs observed in that gene. Normalization and formatting done using pandas and numpy libraries in python. To investigate the association between SNV burden and demographic factors, logistic regression was conducted using the statsmodels library, with genes set as dependent variables. Finally, the likelihood-ratio estimates from the logistic regression model were visualized using forest plots generated with the seaborn library, enabling a clear representation of associations between SNV burden and demographic variables.

QUANTIFICATION AND STATISTICAL ANALYSIS

A Mann-Whitney U-test was performed across age and sex-wise prevalence of DENV-2 infection to determine the significance of deviations in clinical parameters from the normal range (both below and above range) across each grouping. Shannon entropy carried out for determining genetic diversity. Further, Wilcoxon rank-sum test was performed to assess significance for inter-host genetic diversity across different subgroups. Chi-square test was performed to analyze the SNV burden across genes by comparing the presence-absence distribution of SNVs between intra-groups. Additionally, Bonferroni correction was used for the false positive significance. Fisher's exact test to assess the independence of SNVs across intra-groups, while the phi coefficient (ϕ) measured the direction and strength of the association between SNVs and group classifications. GraphPad Prism was used to perform Mann-Whitney U-test and their data visualization. Shannon entropy, Wilcoxon rank-sums test, Chi-square test of independence, Bonferroni correction, Fischer exact test carried out in Python; data handling and formatting carried out using pandas and numpy, statistical calculation using scipy.stats and stats.models. Visualization using matplotlib and seaborn. The phi-correlation coefficient test was carried out in R; under Pshych library. Visualization of Bubble plot was done using SR-plot.