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An integrated approach to predict genetic risk for Mosquito-Borne diseases in the local Population of Tehsil Haripur, Khyber Pakhtunkhwa, Pakistan

Rabea Basri[1], Muslim Bin Aqeel[2], Faryal Mehwish Awan[1]✉, Sadiq Noor Khan[1]✉, Ayesha Obaid[1], Rubina Parveen[1], Muhammad Mohsin[3,4], Wajeeha Akhtar[1], Abdal Hussain Shah[1], Tahira Sher Afghan[1], Amir Alam[5], Saira Khan[1] & Anam Naz[6]

Highly variable response shown by individuals against mosquito-borne infections suggests that host genetic factors play an important role in determining mosquito-borne disease onset. Therefore, it is necessary to determine the genetic risk of these diseases in specific populations. The current study aimed to determine the percentage of individuals in the general population carrying mosquito-borne disease susceptibility and protection-related variants. This study initially aggregated mosquito-borne disease susceptibility and protection-related variants from all publically available data and literature. Afterward, the allele frequency was calculated 1009 genetic variants of 366 genes associated with susceptibility and protection to estimate the global prevalence in multiple ethnicities (Middle Eastern, Ashkenazi Jewish, European (Non-Finnish), Latino/Admixed American, South Asian, East Asian, European (Finnish), North Asian, Southeast Asian, African American, and Swedish population). Furthermore, the cumulative allele frequency of all susceptibility and protection-related variants was calculated in diverse ethnic groups and the relationship with mosquito-borne disease-associated morbidity and mortality was examined to determine whether results are consistent with founder effect in these populations. Two prioritized genetic variants of *IL-10* (rs1800871) and *FcγRIIA* (rs1801274) were examined in the Tehsil Haripur population to assess the genetic risks linked to susceptibility and protection against mosquito-borne diseases. The findings of this study revealed overlapping genes most implicated in mosquito-borne disease linked with susceptibility and protection across different ethnic ancestries. In the available sample size, the percentage of TC and TT genotypes in *IL-10* genetic variant (rs1800871) was 12% and 88%, respectively and GA and GG genotypes in *FcγRIIA*(rs1801274) genetic variant were 6% and 94% respectively. Based on statistical analysis, the percentage allele frequency of *IL-10* (rs1800871) variant was 0.2112% and the *FcγRIIA* (rs1801274) variant is 0.1128% in the current study. Additionally, this study reflects that screening of genetic variants associated with susceptibility and protection in a population gives better insights into organizing public health awareness campaigns to control diseases.

Keywords Allele frequency in Pakistan, Genetic factors, Mosquito-Borne diseases, ARMS-PCR

Mosquito-borne diseases (MBDs) cause serious public health concern globally. The primary pathogens transmitted by mosquitoes are chikungunya virus (CHIKV), zika virus (ZIKV), dengue virus (DENV), west

[1]Department of Medical Lab Technology, The University of Haripur (UOH), Haripur, Khyber Pakhtunkhwa, Pakistan. [2]Department of Microbiology, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan. [3]Medical Research Center, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China. [4]Department of Anesthesiology and Perioperative Medicine, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China. [5]The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China. [6]Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore (UOL), Lahore, Pakistan. ✉email: faryal.mehwish@uoh.edu.pk; faryal_mehwish@yahoo.com; snkhan@uoh.edu.pk

nile virus (WNV), yellow fever virus (YFV), rift valley fever virus (RVFV), Japanese encephalitis virus (JEV), *Plasmodium spp* and Wuchereria bancrofti[1]. MBDs have one of the highest burdens in number of cases of morbidity and mortality annually[2–4]. Roughly half of the world's population is at risk of MBDs[5]. The European Centre for Disease Prevention and Control as of October 2023, reported that over 4.2 million cases and over 3,000 dengue-related deaths have been reported from 79 countries/territories globally (https://www.ecdc.europa.eu/en/dengue-monthly). Furthermore, across the Asian population, like Bangladesh, Sri Lanka, Thailand, and Malaysia have experienced a surge in cases of MBDs[6]. Asia has been a hotspot of DENV and CHIKV infections mainly due to its dense human population, unplanned urbanization, and poverty[7]. However, the number of malarial deaths globally remains over 30-fold higher than those from DENV infection, although this ratio is less extreme if one only considers disease burden outside Africa[8,9]. DENV infection has resulted in important socioeconomic burdens placed on different countries around the world, including those in Latin America, especially Brazil[10]. More than a hundred thousand DENV infection cases are diagnosed in India annually, and about half of the country's population carries DENV infection specific antibodies[11]. CHIKV infection, which mainly invades tropical and subtropical regions, is one of the recently emerging pathogens associated with severe morbidity in humans[12]. Since CHIKV infection arrived in Asia, it has become widely established and caused major outbreaks like the one in India with more than 1.4 million cases in 2006[13]. The presence of ZIKV infection antibodies has been reported in Pakistan, indicating active circulation of the virus in the Sindh region. ZIKV infection is a potential cause of seasonal dengue-like illnesses observed in the country[14]. The high urban population density and abundance of competence vectors may result in a high risk of local transmission following the introduction of ZIKV infection through travel in Punjab, Pakistan[15]. In dengue-endemic countries, the recent epidemics of CHIKV infection and WNV disease have created panic among the public and are thought to provoke an outbreak of ZIKV infection in Pakistan[16].

MBDs transmission depends on complex interactions between the environment and the susceptibility, exposure, and adaptive capacity of populations. Variations in climatic factors, including temperature and precipitation which modulate the spatiotemporal distribution of vectors, hosts, and pathogens[17]. The impact of climate change on the incidence, transmission season, duration, and spread of vector-borne diseases characterizes a serious problem[18]. Low- and middle-income countries with higher population density, poor healthcare systems, rapid unplanned urbanization, and global warming-induced changing climatic factors are particularly vulnerable to DENV infection[19] (Fig. 1).

Genome-Wide Association Studies (GWAS) have been conducted across various populations including African, European, Asian European, and Asian populations. The application of GWAS to African populations could offer valuable insights into the genetic pathways underlying resistance to malaria, as well as shed light on the genetic origins of related diseases[20]. Disease prevalence and severity exhibited variations across populations. Many studies provides robust evidence that genetic variation in human populations contributes to susceptibility to infectious diseases[21]. The majority of GWASs have focused on patients of European and Southeast Asian descent, with insufficient representation of studies involving individuals from other ancestral backgrounds[22]. Jian-Wen et al.. conducted a large GWAS on Systemic Lupus Erythematosus (SLE) in a Chinese Han population, analyzing 1,047 cases and 1,205 controls using Illumina Human610-Quad BeadChips. Replication of 78 single nucleotide polymorphisms (SNPs) in two additional cohorts (3,152 cases and 7,050 controls) identified nine new susceptibility loci (*ETS1*, *IKZF1*, *RASGRP3*, *SLC15A4*, *TNIP1*, 7q11.23, 10q11.22, 11q23.3, and 16p11.2) and confirmed seven previously reported risk loci[23]. The *HLA-DR2* (DRB1*1501) and *HLA-DR3* (DRB1*0301) class II genes have been found to consistently associate with SLE in many European populations, with a twofold relative risk conferred by each allele[24]. Similarly, GWAS from European (4,036 cases and 6,959 controls) and Chinese (1,659 cases and 3,398 controls) cohorts studies revealed that these populations share more than half of known SLE-associated genetic loci[25]. Furthermore, a recent study on SLE progression has identified a locus

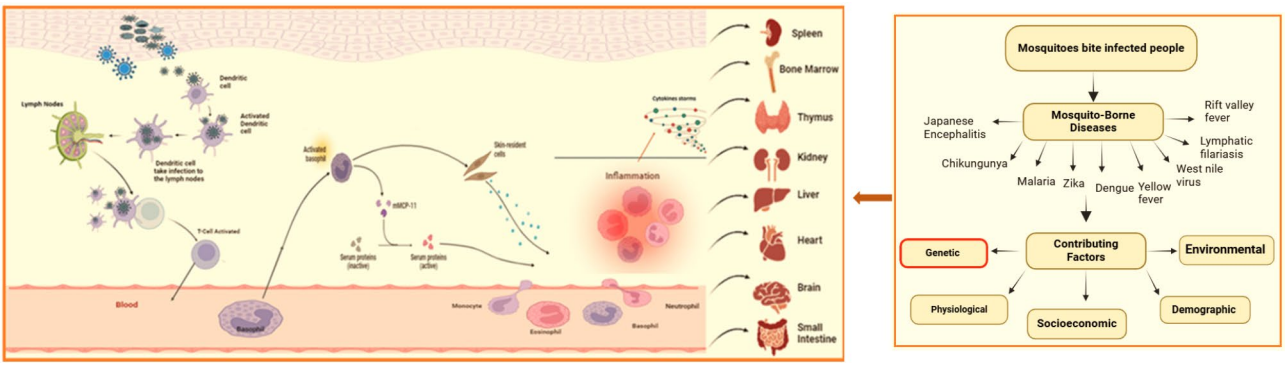

Fig. 1. Transmission of MBDs occurs following a mosquito (*Aedes aegypti* or *Aedes albopictus*, *Culex*, and *Anopheles* mosquitoes) bite. MB infection mostly replicates in the skin, fibroblasts, and a few in liver cells, and disseminates to the liver, muscle, joints, lymphoid tissue (lymph nodes and spleen), small intestine, thymus, one marrow, kidney, and brain. The target cells are indicated for each tissue. Immunogenetic pathways are involved against MBD infections. There is evidence of MBD-specific adaptive immunity (that is, T cell and antibody-mediated responses).

pointing to involvement of the central nervous system in disease outcome as opposed to the enrichment for immunological-related signals observed for disease susceptibility[26].

Khor et al. identified genes that significantly associated with dengue shock syndrome (DSS) including *MHC* and *MICB* in the chromosome 6 and the *PLCE1* on chromosome 10 region[27]. Susceptibility to infection and many other human diseases (including diabetes and ischaemic heart disease) arises from the complex interaction of environmental and host genetic factors[21]. According to Karin et al. the availability of more data is expected to reveal risk variants that are rare in specific populations, shedding light on the shared genetic susceptibility across diverse ancestries[22]. Furthermore, another study highlighted the genetic polymorphisms associated with the immune response to DENV infection. Fang et al. identified several genetic variants modulating host immune responses. This study highlighted the role of specific alleles in determining disease severity[28]. Similarly, Oliveira et al. conducted a meta-analysis on genes associated with dengue fever (DF). The study revealed certain SNPs linked to disease risk across different ethnic groups. This trans-ethnic consistency suggests that shared genetic variants may underlie susceptibility to DENV infection. These studies warrant further investigations to comprehend the genetic architecture of MBDs[29]. Patro et al. demonstrated that polygenic risk scores (derived from GWAS) can predict the risk of dengue hemorrhagic fever (DHF) across multiple ancestries. This emphasizes the need for an integrated approach in predicting disease risk[30]. In addition to DENV, CHIKV and ZIKV infections have also been subjects of GWAS investigations[31]. The concept of genetic susceptibility revolves around whether specific alleles consistently elevate or diminish susceptibility across various regions of the world[32]. Genetic variations play a crucial role in influencing susceptibility to DHF[33]. The DENV genetic heterogeneity (serotype and genotype) adds another dimension to the public health challenge due to the increased risk of disease severity (secondary and tertiary infection)[34]. In certain populations, specific genetic variants are associated with severe complications of DENV infection. Family-based analysis of whole-exome sequencing (WES) provides a high detection rate for prevalent and rare variations[35]. Among neurological conditions, the authors Tan et al. focused on disease survival as well as cognitive or motor decline. In one of the largest studies, researchers have identified three novel loci associated with Parkinson's disease progression[36]. In Crohn's disease, a study has identified four loci for disease progression, indicating distinct genetic contribution from disease susceptibility[37].

This study initially aggregated MBDs susceptibility and protection-related variants from all publicly available information. Further, we propose to develop a framework of methods that can prioritize overlapping and unique genes and their variants to explore new associations with MBDs. The current study expanded our knowledge base regarding the most prevalent genetic variations associated with disease in the local Haripur population. To the best of our knowledge, this study reflects that screening of genetic variants associated with susceptibility and protection in a population gives better insights into organizing public health awareness campaigns to control diseases.

Materials and methods

In the current study, we employed a two-step approach to calculate allele frequencies of genetic variants related to susceptibility and protection among MBDs. Firstly, we used a computational pipeline to retrieved genetic variants, process, and calculated allele frequencies of these variants from publicly available whole genome datasets, capturing a comprehensive global perspective. Secondly, we validated our findings by performing Amplification refractory mutation system PCR (ARMS-PCR) on prioritized genetic variants to determine allele frequencies in a local population. ARMS-PCR, also known as allele-specific amplification allows to identify gene barcodes with single-nucleotide resolution and has become a widely used method in clinical practice[38]. ARMS-PCR is more sensitive than Sanger sequencing with its detection limit of about 1%. It is a very simple method for detecting any known mutations by using sequence-specific PCR primers that allow amplification only when the target al.lele is present within the sample[39]. It is based on the principle that PCR amplification is inefficient or completely refractory if there is a mismatch between the 3' terminal nucleotide of a primer and its template sequence. This technique employs two primer pairs to amplify two alleles, one wild-type allele and one mutant allele in a single PCR reaction[40]. This dual approach ensured that our results for global, Asian, and South Asian populations were consistent and applicable at a local level, providing g robust validation for our computational analysis.

Data retrieval and processing

To understand the genetic spectrum of MBDs, a comprehensive search was performed to identify all previously reported disease related genetic variants associated with MBDs. Data was retrieved from Google Scholar, PubMed, Science Direct and Scopus databases using the following combinations of search terms: genetic polymorphism, genetic susceptibility, genetic predisposition, genetic determinants, susceptibility, protection, severity, single nucleotide polymorphism, mutations, variants, variations, mutants, MBDs, genetic susceptibility and protection against dengue, chikungunya, rift valley fever, zika, west nile virus, yellow fever, lymphatic filariasis and malaria infections/diseases (Fig. 2).

Calculation of allele frequency from available whole genomes

Following the screening of shared and divergent genetic variants, the subsequent step involved calculating the allele frequency of each variant present in lymphatic filariasis (LF), JEV, malaria, DENV, ZIKV, CHIKV, RVF, WNV and YF infections. While GWAS has advanced our understanding of the genetic architecture of MBDs, post-GWAS functional genomic analyses are required to prioritize genes that modulate disease susceptibility and nominate candidate genes for further functional validation through ARMS-PCR.

To accomplish this, we utilized various population-specific databases known for their ability to estimate allele frequencies across diverse populations and datasets. We analyzed the available whole genome from

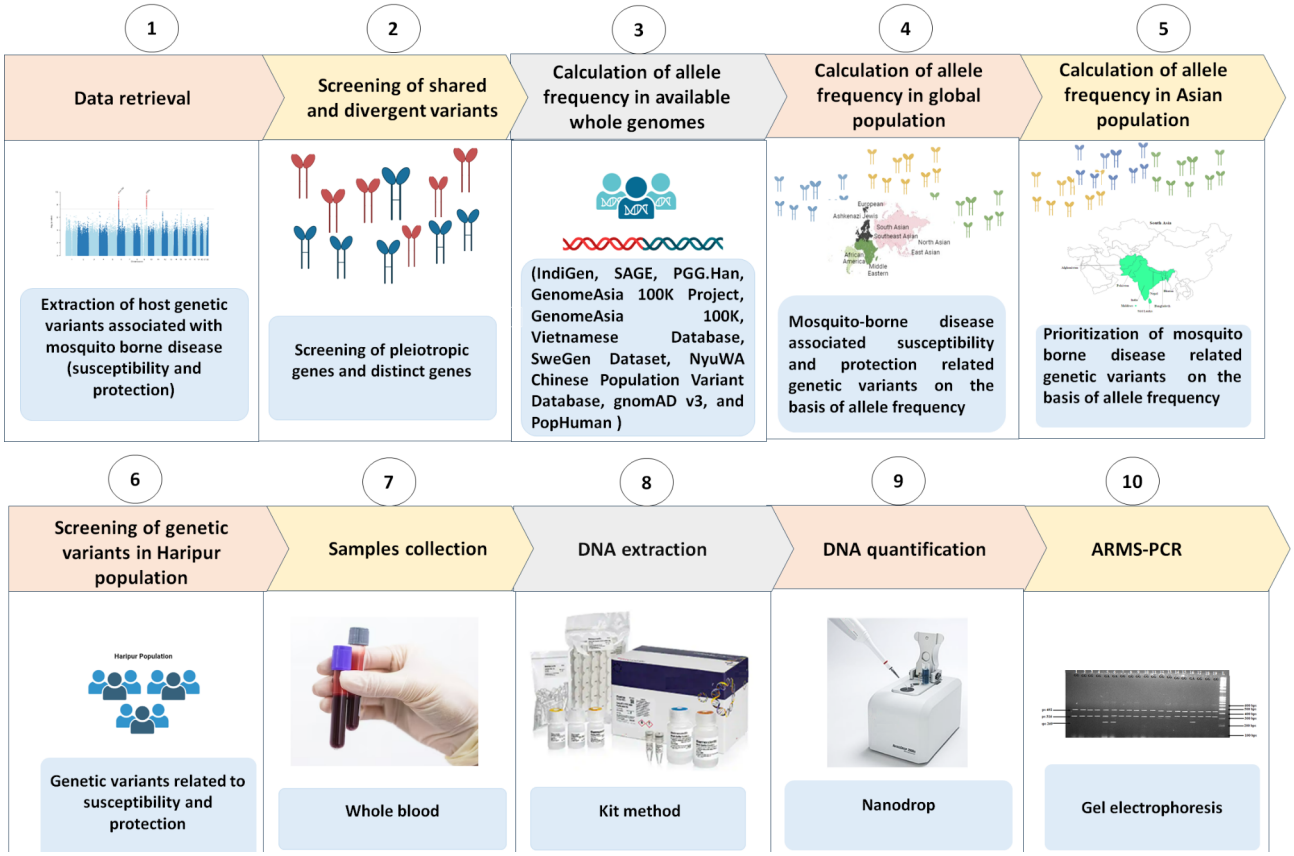

Fig. 2. Schematic representation of prioritization pipeline. Screening and identification of genetic variants related to susceptibility and protection associated with MBDs.

various publicly available next-generation sequencing databases that aggregates exome and genome sequencing information from a wide variety of large-scale projects and stratify allele counts by ethnicity, including Genome Asia 100k (http://www.genomeasia100k.com), IndiGen (https://clingen.igib.res.in/indigen), SAGE (South Asian Genome), PGG.Han[41], GenomeAsia 100 K Project[42](https://browser.genomeasia100k.org/), Vietnamese Genetic Variation Database (https://genomes.vn/), SweGen Dataset[43], NyuWA Chinese Population Variant Database (http://bigdata.ibp.ac.cn/NyuWa_variants/searchact.php#search-denote)[44], gnomAD v3 (Genome Aggregation Database), and PopHuman (http://pophuman.uab.cat). These freely accessible databases provide large-scale genetic datasets from diverse populations to estimate allele frequencies associated with specific genes and their variants. By utilizing multiple resources, we aimed to ensure comprehensive coverage of various populations and regions. This allele frequency data contributes to our understanding of the genetic landscape of these diseases, helping to identify population-specific variations and potential correlations between genetic variants and disease susceptibility or protection.

Screening of prioritized genetic variants in the local population

To confirm and corroborate the initial analysis, preliminary validation of the two prioritized genetic variants was performed using ARMS-PCR. The sample size comprised fifty participants, all residents of Tehsil Haripur, Khyber Pakhtunkhwa (KPK), Pakistan. Preliminary screening of the *IL-10* (rs1800871) and the *FcγRIIA* (rs1801274) genetic variants were performed. Ethical approval for this study was obtained from the Research Ethics/Bioethics Committee of the Directorate of Advanced Studies & Research Board (ASRB) at the University of Haripur, KPK, Pakistan. The study was conducted in accordance with ASRB's relevant guidelines and regulations. Informed consent was obtained from all study participants. This analytical observational study was conducted from December 2022 to August 2023.

Inclusion and exclusion criteria

A random sampling technique was used to collect samples. Individuals aged ≥ 18 years, regardless of gender, with a permanent address of Tehsil Haripur were included in this study. Individuals who had been diagnosed with a genetic disease and those under 18 years of age were excluded. Venous whole blood was collected in EDTA tubes using a sterile disposable syringe. 05 mL whole blood was collected and transported at 04 °C, after which the sample was stored at 4 °C for further analysis.

Primer	F/R	Sequence (5'→3')	Length	Tm °C	GC %	Optimized Tm °C	Product size
FcγRIIA rs1801274	Forward inner	ACAACAGCCTGACTACCTATTACCTT	26	60.7	42	63.7	316 bps
	Reverse inner	CATATTTGTGTCTTTCAGAATGGC	26	60.7	42		269 bps
	Forward outer	GGAACATCCCAGAAATTCTCACA	23	60.1	43		492 bps
	Reverse outer	GAACATCCCAGAAAATTCCACG	22	59.4	45		
IL-10 rs1800871	Forward inner	GTACCCTTGTACAGGTGATGTCAC	24	61.8	50	62.5	380 bps
	Reverse inner	GCAAACTGAGGCACAGAGCTA	21	61.1	52		272 bps
	Forward outer	GGCTCCCCTTACCTTCTACAC	21	63	57		607 bps
	Reserve outer	CAGAGACTGGCTTCCTACAGTACA	24	62.3	50		

Table 1. Sequence information of ARMS-PCR primers used for amplifying *FcγRIIA* (rs1801274) and *IL-10* (rs1800896) gene variants, along with their respective product sizes.

Disease	Total genes	Total genetic variants	Susceptibility related genes/variants	Protection related genes/variants
DENV Disease	21	49	13/22	8/27
Malaria	117	362	94/336	23/26
WNV Disease	99	388	95/388	4/0
CHIKV Disease	24	53	16/45	8/8
LF Disease	21	37	21/37	
JEV Disease	17	16	18/13	4/3
ZIKV	26	32	25/32	1/0
YF	22	2	10/0	12/2
RVF	19	70	17/58	3/12
Total	**366**	**1009**	**309/931**	**63/78**

Table 2. Representation of pleiotropic genes related to susceptibility and protection identified against MBDs.

DNA extraction and quantification

Genomic DNA was isolated from 200 µL of blood using the Gene JET Genomic DNA Purification Catalog no: K0721, (JETTM Thermofisher Genomic DNA Purification and Isolation kit). Genomic DNA was quantified using a nanodrop (OPTIZEN NanoDrop) spectrophotometer. All samples were stored at 4 °C until analysis. High-quality intact genomic DNA with an optical density ratio of 260/280 ~ 1.8 and 260/230 > 1.5 were used for further analysis.

ARMS-PCR amplification

We designed ARMS-PCR primers through Primer1 software (http://primer1.soton.ac.uk/primer1.html), and A-Plasmid Editor software[45]. Furthermore, UCSC Genome Browser (genome.ucsc.edu) was used to check the specificity and sensitivity of designed primers by running in silico PCR (Table 1).

PCR amplifications were performed in 20µL reactions containing 10 µL 2X PCR Taq Master Mix (amaR OnePCR, Cat. No. SM213-0250), 1 µL genomic DNA (1 ng/µL) template, 2 µL of 3.3 µM of tetra primers (0.5 µL for each primer), and 7 µL nuclease-Free water. The PCR regimen was as follows: initial denaturation at 95 °C for 5 min, followed by 33 cycles for 30 s at 94 °C for 01 min, 01 mint at 62–64 °C, 01 min at 72 °C, and then a final extension for 5 min at 72 °C, and finally the PCR products were maintained at 4 °C in the end. PCR products were resolved on a 2.5% agarose gel with 1× Tris–acetate-ethylenediaminetetraacetic acid (TAE) buffer, and then subjected to electrophoresis at 110 V for 30 min. The agarose gel was stained with 0.5 µg/mL ethidium bromide. Bands were visualized under UV light by Automatic Gel Imaging and Analysis System (LABTRON) and the molecular weight of the PCR products was determined by comparing them with the100 base pairs (bps) DNA marker (Cat.no FNB-500304, Fine Biotech Life Sciences).

Data analysis

The Hardy-Weinberg equation ($p2 + 2pq + q2 = 1$)[46] was used to calculate the expected genotype frequencies for heterozygous (2pq) and homozygous (p2) genotypes, using the minor and total allele counts, where p is equal to the cumulative minor allele frequency (cMAF) of causally related variants and q is (1 – p).

Results

Identification of genetic variants showing pleiotropy for MBDs

309 genes and 931 genetic variants associated with susceptibility and 63 genes with 78 genetic variants related to protection against nine MBDs including DENV, CHIKV, ZIKV, RVF, WNV, YF, JEV, malaria and LF infections were identified through a literature search. Moreover, the analysis revealed a total of 1009 genetic variants of 366 genes associated with both susceptibility and protection against MBDs as shown in Table 2 (Fig. 3).

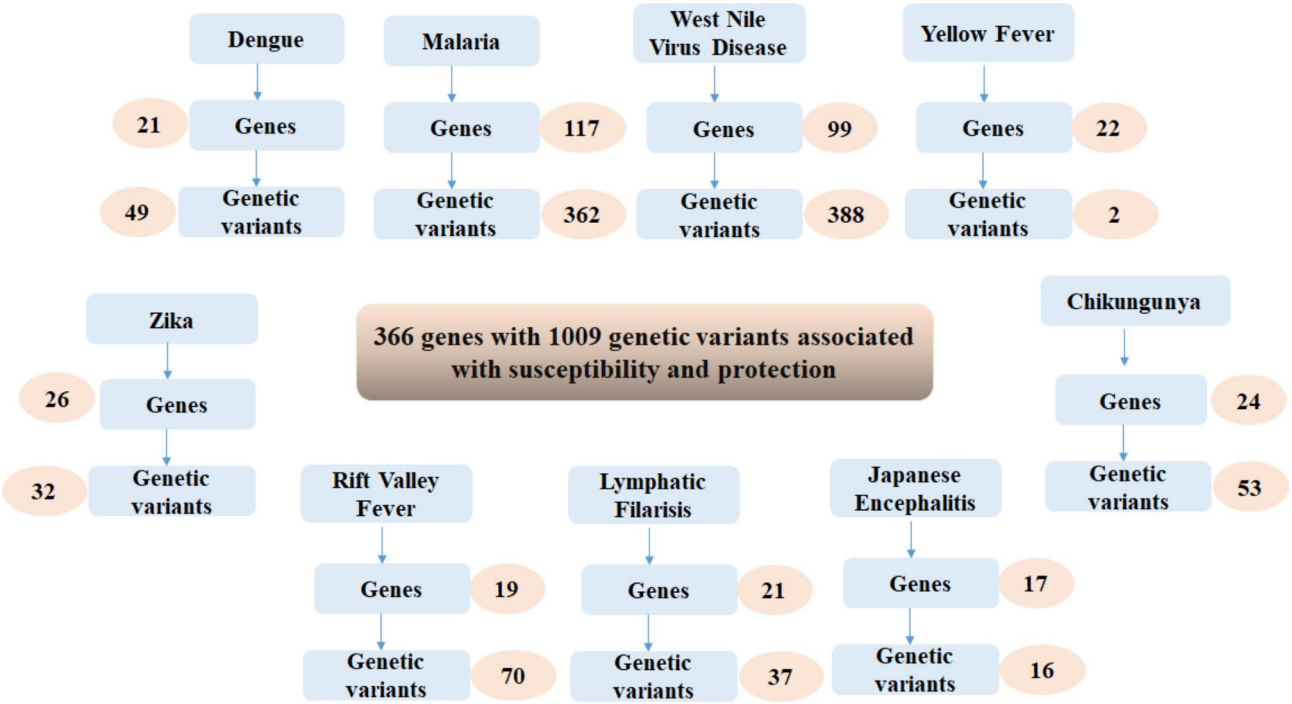

Fig. 3. Representation of pleiotropic genes and their variants related to susceptibility and protection among dengue, malaria, CHIKV, RVF, WNV, YF, JEV and LF diseases.

Identification of pleiotropic genes related to susceptibility and protection in MBDs

Across various search platforms, a collective analysis has identified 21 genes with 49 variants linked with susceptibility and protection against DENV infection (Fig. 3), (Supplementary table S2) in which 27 variants of 08 genes were associated with protection and 22 variants of 13 genes were associated with DENV disease susceptibility as shown in Table 2. A total of 117 genes and 362 variants linked with susceptibility and protection against malaria including 94 genes and 336 variants related to susceptibility and 23 genes with 26 variants linked with protection against malaria as shown in Fig. 3, Table 2 and Supplementary table S5. In WNV disease a total of 99 genes and 388 variants related to susceptibility and protection were obtained, in which 95 genes and 388 variants were associated with susceptibility and 04 genes were linked with protection against WNV disease as shown in Fig. 3, Table 2 and Supplementary table S7. Among ZIKV disease a total of 26 genes and 32 variants were associated with susceptibility and protection against ZIKV infection including 25 genes and 32 variants related to susceptibility and 01 genes linked with protection against ZIKV disease as shown in Fig. 3, Table 2 and Supplementary table S9. In RVF a total of 19 genes and 70 variants were associated with susceptibility and protection, in which 16 genes and 58 variants linked to susceptibility and 03 genes, and 12 variants associated with protection against RVF as shown in Fig. 3, Table 2 and Supplementary table S6. LF infection related studies revealed that 21 genes and 37 variants were associated with disease susceptibility as shown in Fig. 3, Table 2 and Supplementary table S4. Furthermore, JEV disease related studies showed 17 genes, and 16 variants associated with susceptibility and protection including 13 variants of 14 genes were linked with susceptibility and 03 variants of 04 genes related to protection against JEV infection as shown in Fig. 3, Table 2 and Supplementary table S3. In CHIKV disease a total of 24 genes and 53 variants were associated with susceptibility and protection including 16 genes and 45 variants linked with susceptibility and 08 genes, and 08 variants related to protection against disease as shown in Fig. 3, Table 2 and Supplementary table S1. A total of 29 genes and 02 variants were identified against YF related to disease susceptibility and protection in which 12 genes and 02 variants were linked with the protection of the disease and 17 genes were related to susceptibility to the YF disease as shown in Fig. 3, Table 2 and Supplementary table S8.

Identification of common genes (susceptibility and protection) among MBD and their overlap

A total of 67 overlapping genes and variants related to susceptibility and protection among MBDs were identified as common among two or more diseases (Table 3) and (Fig. 4). *IL-1β* gene was common in malaria and JEV diseases. *TNFα* gene overlapping across malaria, DENV, ZIKV, CHIKV, JEV, and WNV diseases. Similarly, *FcγRIIA/CD32* gene overlaps in malaria and DENV diseases. Moreover, the *G6PD* gene was shared between malaria, DENV, and ZIKV infections, whereas the *HLA* gene was common to malaria, ZIKV, and WNV diseases. The *TLR4* gene overlaps across malaria, DENV, and LF diseases. Similarly, the *TLR9* gene was shared among malaria, DENV, JEV and WNV diseases. The *TIRAP* gene was present in both malaria and WNV diseases (Table 3) and (Fig. 4).

Genes	MBDs						
IL-1β	Malaria	JEV					
FcγRIIA/CD32	Malaria	DENV					
IL-8	Malaria	DENV					
RNF2	ZIKV	RVF					
MIR605	DENV	ZIKV					
LIF	DENV	ZIKV					
NOS3	DENV	ZIKV					
JAK1	DENV	WNV					
CD2	ZIKV	WNV					
STAT1	WNV	YF					
CTLA4	WNV	LF					
CXCL8	DENV	ZIKV					
CLEC5A	Malaria	DENV					
MICB	DENV	ZIKV					
IL-4R	Malaria	DENV					
IL-17	Malaria	DENV					
IL-8	Malaria	DENV					
HLA-DRB1	CHIKV	WNV					
HLA-DQA1	CHIKV	WNV					
TLR2	DENV	LF					
DC-SIGN	DENV	CHIKV					
CXCR3	DENV	WNV					
CD40LG	DENV	WNV					
RNO3	ZIKV	RVF					
IRF7	WNV	YF					
TIRAP	DENV	WNV					
VDR	DENV	WNV					
CCR2	ZIKV	JEV					
IL-4	Malaria	ZIKV					
IL-10RA	Malaria	LF					
ADAR	Malaria	YF					
IDO1	DENV	YF					
MDA5	CHIKV	YF					
NOS2	Malaria	DENV	ZIKV				
CCR5	ZIKV	WNV	JEV				
CXCL10	DENV	ZIKV	WNV				
OAS2	DENV	CHIKV	WNV				
OAS3	DENV	CHIKV	WNV				
G6PD	Malaria	DENV	ZIKV				
HLA	Malaria	ZIKV	WNV				
TLR4	Malaria	DENV	LF				
TIRAP	Malaria	WNV					
IRF1	Malaria	WNV	YF				
IL-12B	Malaria	ZIKV	JEV				
ICAM1	Malaria	DENV	JEV				
MBL2	DENV	WNV	LF				
OAS1	DENV	CHIKV	WNV				
IFITM3	DENV	WNV	RVF	YF			
C6	DENV	ZIKV	JEV	YF			
IL-6	DENV	ZIKV	CHIKV	RVF			
IFN-γ	Malaria	DENV	WNV	JEV			
MyD88	Malaria	CHIKV	WNV	RVF			
TLR9	Malaria	DENV	WNV	JEV			
CCL2	DENV	ZIKV	WNV	JEV			
CD209	DENV	CHIKV	WNV	JEV	ZIKV		
TLR8	DENV	CHIKV	WNV	RVF	JEV		
Continued							

Genes	MBDs						
TNFα	Malaria	ZIKV	CHIKV	WNV	JEV	DENV	
IL-10	DENV	Malaria	ZIKV	WNV	LF	JEV	
TLR3	DENV	ZIKV	CHIKV	WNV	JEV	RVF	
TLR7	DENV	ZIKV	CHIKV	WNV	JEV	RVF	
TNF	Malaria	DENV	CHIKV	WNV	LF	JEV	ZIKV

Table 3. Overlapping genes related to MBDs susceptibility and protection.

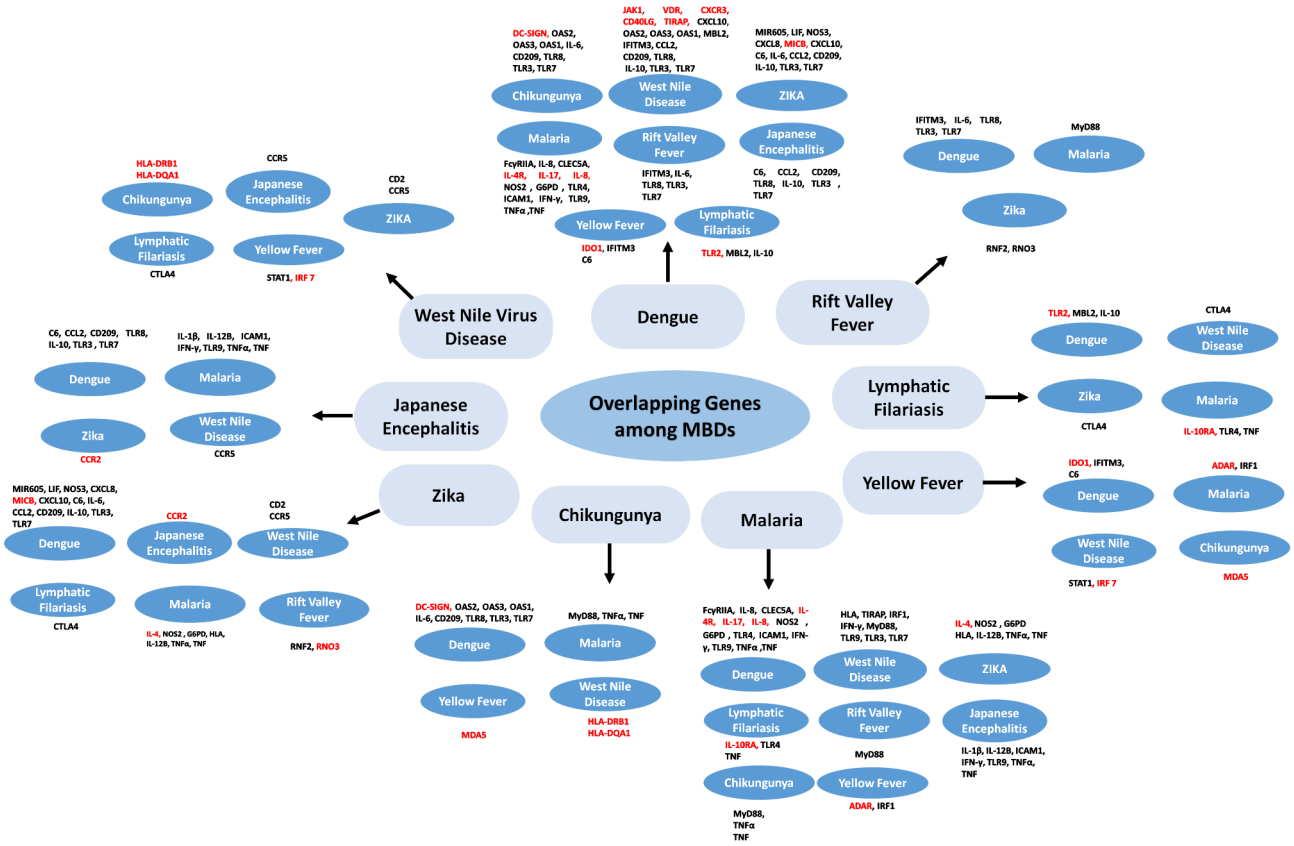

Fig. 4. Overlapping genes associated with disease susceptibility and protection against MBDs. Genes depicted in red are those shared among only two MBDs. Genes depicted in black are those shared by more than two MBDs (DENV, malaria, ZIKV, RVF, YF, CHIKV, LF, JEV, and WNV diseases).

The *IRF1* gene was shared among malaria, WNV, and YF diseases. The *IL-4* gene overlaps in malaria and ZIKV infections, while the *IL-12B* gene was shared among malaria, ZIKV, and JEV diseases. Additionally, the *ICAM1* gene was found in malaria, DENV, and JEV diseases. The *IFN-γ* gene was common to malaria, DENV, JEV, and WNV diseases. Moreover, the *MyD88* gene was shared across malaria, CHIKV, RVF, and WNV diseases (Table 3) and (Fig. 4). The *IL-8* gene overlaps in malaria and DENV diseases. Similarly, the *IL-10RA* gene was shared between malaria and LF diseases. Additionally, the *ADAR* gene overlaps in malaria and YF, while the *IDO1* gene was shared between DENV and YF diseases. Moreover, the *MDA5* gene overlaps in YF and CHIKV diseases, and the *C6* gene was present in YF, ZIKV, JEV, and DENV diseases. Furthermore, the *RNF2* gene overlaps in ZIKV and RVF, while the *MIR605* gene was shared between DENV and ZIKV infections. Additionally, the *LIF* gene was common to DENV and ZIKV infections, and the *NOS3* gene overlaps in DENV and ZIKV infections. Lastly, the *NOS2* gene was shared among malaria, DENV and ZIKV infections. Similarly, the *CCL2* gene was common in DENV, ZIKV, JEV, and WNV disease. Additionally, the *CXCL8* gene was associated with DENV and ZIKV diseases, while *CXCL10* overlaps in DENV, ZIKV, and WNV diseases. The *CCR2* gene was shared between ZIKV and JEV infections, and *CCR5* was found in ZIKV, WNV and JEV diseases. Moreover, *OAS2* was involved in DENV, CHIKV, and WNV infections, and *OAS3* was found in DENV, CHIKV, and WNV diseases. *IFITM3* was associated with DENV, WNV, RVF, and YF, while *JAK1* shared among DENV and WNV diseases. *CD2* was common in ZIKV and WNV diseases, and *STAT1* overlaps in YF and WNV diseases. *CTLA4* linked with WNV and LF, while *TLR3* was shared among RVF, ZIKV, CHIKV, WNV, JEV, and DENV diseases. Furthermore, *MBL2*

was associated with DENV, WNV, and LF diseases, and *IRF7* was common among YF and WNV infections. *TIRAP* overlaps in DENV and WNV infections, and *VDR* associated with DENV and WNV diseases. Lastly, *OAS1* was common among DENV, CHIKV, and WNV diseases, while *CD209* is implicated in DENV, ZIKV, CHIKV, WNV, and JEV infections (Table 3) and (Fig. 4).

The *TLR8* gene overlaps in DENV, CHIKV, WNV, RVF, and JEV diseases. Similarly, the *CXCR3* gene was shared between DENV and WNV diseases, while the *CD40LG* gene was associated with DENV and WNV diseases. The *RNO3* gene overlaps in RVF and ZIKV infections, and the *IL-6* gene was shared among RFV, ZIKV, CHIKV, and DENV diseases. Moreover, the *TLR7* gene was common in RVF, ZIKV, CHIKV, WNV, JEV, and DENV diseases. The *TLR2* gene overlaps in DENV and LF diseases, while the *DC-SIGN* gene overlaps among DENV and CHIKV infections. Additionally, the *CLEC5A* gene overlaps in malaria and DENV diseases, and the *VDR* gene was shared between DENV and WNV diseases. The *JAK1* gene was common in DENV and WNV diseases, while the MICB gene overlaps in DENV and ZIKV infections. The *IL-4R* gene was shared between malaria and DENV infections, and the *CXCL10* gene overlaps in DENV, ZIKV, and WNV diseases. The *ICAM-1* gene overlaps in malaria, DENV and JEV infections, while the *IL-17* gene was shared among malaria and DENV diseases. Furthermore, the *IL-8* gene was shared between malaria and DENV diseases, and the *HLA-DRB1* and *HLA-DQA1* genes overlap in CHIKV and WNV diseases. Across diseases such as DENV, ZIKV, malaria, CHIKV, WNV, RVF, YF, LF and JEV infections the genes *IL-10*, *TLR7*, *TLR3*, *TNFα*, *TLR8*, *CD209*, *CCL2*, *TLR9*, *MyD88*, *IFN-γ*, *IL-6*, *C6*, and *IFITM3* showed the maximum combination and co-occurrence, suggesting substantial interrelatedness across these diseases at the genetic level as mentioned in Table 3 and Fig. 4.

Identification of unique genes (susceptibility and protection) among MBD showing no overlap

A total of 205 unique genes and their variants related to susceptibility and protection against MBDs (malaria, WNV, ZIKV, RVF, DENV and CHIKV diseases) were identified as shown in Table 4 and Fig. 5. In malaria a total of 80 distinct genes and their variants related to susceptibility and protection were obtained including *IL-13*, *RTN3*, *HBB*, *ATP2B4*, *FREM3*, *USP38*, *GYPE A*, *ABO*, *MARVELD3*, *HBA1*, *HBA2*, *HbC*, *HbE*, *GYPB*, *TLR5*, *TNFPD*, *LTA*, *BAT2*, *SLC22A4*, *SLC22A5*, *LOC441108*, *IL-5*, *RAD50*, *KIF3A*, *SEPT8*, *ANKRD43*, *IL-12 A*, *IL-12RB1*, *IL-4*, *IL4 VNTR*, *IL-1 A*, *CD36*, *CD37*, *CD31*, *PECAM-1*, *CR1*, *CD14*, *IL-17RC*, *TRIM*, *DARC*, *GBP7*, *IL17RD*, *TLR1*, *CTL4*, *IL20RA*, *CFTR*, *NOD1*, *TRIM5*, *IL-22*, *SPTB*, *ADCY9*, *IL-4R*, *ADORA2B*, *EMR1*, *GNAS*, *DERL3*, *IL-18*, *FPN*, *HLA-F*, *TPI1*, *ADRB2*, *BF*, *SCO1*, *HbS*, *FcγRIIB*, *FcγRIIC*, *FcγRIIIA*, *NF-kB1*, *FcγR*, *RNASE 3*, *ABCB1*, *ADORA2A*, *GRK5*, *STAT6*, *TNFRSF18*, *FOXO3A*, *CD40L*, *IRF1*, *IL17RE*, and *ABO GTG*. A total of 64 genes and their variants related to susceptibility and protection related to WNV disease were found, including *ANPEP*, *SCN1A*, *RFC1*, *HLA-DRB1*, *HLA-DQA1*, *HLA-DPA1*, *DQA1*01:02*, *CD58*, *FcγRIIA*, *RNASEL*, *EIF2AK2*, *IFIH1*, *PRKRA*, *CD28*, *ICOS*, *PDCD1*, *CD80*, *CD86*, *PTX3*, *NFKB1*, *CASP3*, *GZMA*, *IFNGR1*, *IRF5*, *INDO*, *TRAM1*, *JAK2*, *PDCD1L1*, *PDCD1L12*, *IFNB1*, *IFNA*, *TRAF1*, *PRF1*, *IFIT2*, *IFIT1*, *IFITM2*, *IFITM1*, *CD81*, *TRAF6*, *CREB3L1*, *RARRES3*, *FADD*, *PRKRIR*, *IL-10RA*, *CLEC4C*, *IRAK4*, *STAT2*, *TBK1*, *OASL*, *ISGF3G*, *GZMB*, *IFI27*, *SOCS1*, *CCL3*, *SOCS3*, *TICAM1*, *TNFSF14*, *CLEC4M*, *IRF3*, *CD40*, *IFNGR2*, *MX1*, *ICOSLG*, and *SAT1* as shown in Table 4 and Fig. 5.

In ZIKV disease a total of 32 genes and their variants associated with susceptibility and protection were distinctly identified including *DISP3*, *IL-12RB2*, *SPG7*, *RYR1*, *CAPN3*, Fig. 4, *COL2A1*, *CRB1*, *FGFR3*, *FLNB*, *SHH*, *FBN2*, *TNXB*, *KMT2A*, *CHD4*, *COL4A2*, *ZNF423*, *DNM2*, *LMNB2*, *IFNAR*, *TP53*, *LILRB1*, *LILRB2*, *HLAG*, *HLA-G*, *MDM2*, *VEGFA*, *PTGS2*, *TREM1*, *IFNR1*, *CXCR1* and *TFRC* genes. A total of 12 genes and their variants related to susceptibility and protection were uniquely found in RVF including *RVFS-1*, *RVFS-2*, *RVFS-3*, *RNO9*, *CHF*, *DDX58*, *MVS*, *TRIF*, *RNO3*, *IFITM3*, *ALDH2*, and *IL28B*. In DENV disease, a total of 09 genes along with their variants related to susceptibility and protection were distinctly identified, including *CXCL11*, *TAP1*, *PLEC1*, *IL-6R*, *GYPA*, *CXCR3*, *IFNL1*, *RXRA*, and *BAK1*. Additionally, a total of 06 genes and variants related to susceptibility and protection were uniquely found in CHIKV disease, including *OAS*, *CRP*, *COMP*, *IL-2R*, *IL-1RN*, and *MIF* as shown in Table 4 and Fig. 5.

Calculation of allele frequency of the overlapping of genes and their genetic variants in population-specific databases

Aiming to assess allele frequency of worldwide genetic variants related to susceptibility and protection among DENV, ZIKV, CHIKV, LF, JEV, malaria, RVF, YF and WNV diseases. Overall, 366 genes with 1009 genetic variants associated with susceptibility and protection genes were screened. These variants were stratified according to the ethnical group of the individuals carrying population sequencing data from IndiGen, SAGE, PGG.Han, GenomeAsia 100 K Project, GenomeAsia 100 K, Vietnamese Genetic Variation Database, SweGen Dataset, NyuWA Chinese Population Variant Database, PopHuman, and gnomAD database (Africans, East Asians, South Asians, Latinos, Europeans (non-Finnish, and Finnish), based on the information provided in the gnomAD database (version 3.1; all data are based on GRCh37/hg19) and bioinformatics algorithm to calculate allele frequency of these variants among multiple ethnics group as shown in Fig. 5.

Allele frequency in the Latino/Admixed American population

In the Latino/Admix American population, we calculated the allele frequency of variants related to susceptibility and protection. The analysis found that the *IL-6* (rs1800795), *OAS2* (rs15895), *MICB* (rs3132468), *OAS3* (rs2285932), and *TLR7* (rs179010) variants showed 0.8085%, 0.8271%, 0.8203%, 0.8326% and 0.8511% allele frequency respectively. Among these variants the *TLR7* gene (rs179010) variants showed the highest allele distribution about (0.8511%), among the Latino/Admixed American population, while almost absent among other ethnic groups in the world like in South Asia, America, Oceania, Africa, West Eurasia, Southeast Asia, Northeast Asia) based on the data recorded in gnomAD. Furthermore, the *G6PD* gene (rs1050828) variant

Malaria	WNV disease	ZIKV disease	RVF	DENV disease	CHIKV disease
IL-13	*ANPEP*	*DISP3*	*RVFS-1*	*CXCL11*	*OAS*
RTN3	*SCN1A*	*IL-12RB2*	*RVFS-2*	*TAP1*	*CRP*
HBB	*RFC1*	*SPG7*	*RVFS-3*	*PLEC1*	*COMP*
ATP2B4	*HLA-DRB1*	*RYR1*	*RNO9*	*IL-6R*	*IL-2R*
FREM3	*HLA-DQA1*	*CAPN3*	*CHF*	*GYPA*	*IL-1RN*
USP38	*HLA-DPA1*	Figure 4	*DDX58*	*CXCR3*	*MIF*
GYPE, B, A	*ABO-ve*	*COL2A1*	*MVS*	*IFNL1*	
ABO	*ABO D-ve*	*CRB1*	*TRIF*	*RXRA*	
MARVELD3	*DQA1*01:02*	*FGFR3*	*RNO3 LEW*	*BAK1*	
HBA1, HBA2	*CD58*	*FLNB*	*IFITM3*		
HbC	*FcγRIIA*	*SHH*	*ALDH2*		
HbE	*RNASEL*	*FBN2*	*IL28B*		
GYPB	*EIF2AK2*	*TNXB*			
TLR5	*IFIH1*	*KMT2A*			
TNFPD	*PRKRA*	*CHD4*			
LTA	*CD28*	*COL4A2*			
BAT2	*ICOS*	*ZNF423*			
SLC22A4	*PDCD1*	*DNM2*			
SLC22A5	*CD80*	*LMNB2*			
LOC441108	*CD86*	*IFNAR*			
IL-5	*PTX3*	*TP53*			
RAD50	*NFKB1*	*LILRB1*			
KIF3A	*CASP3*	*LILRB2*			
SEPT8 GENE	*GZMA*	*HLAG*			
ANKRD43	*IFNGR1*	*HLA-G*			
IL-12 A	*IRF5*	*MDM2*			
IL -12RB1	*INDO*	*VEGFA*			
IL4 – 33	*TRAM1*	*PTGS2*			
IL4 VNTR	*JAK2*	*TREM1*			
IL-1 A	*PDCD1L1*	*IFNR1*			
CD36	*PDCD1L12*	*CXCR1*			
CD37	*IFNB1*	*TFRC*			
CD31	*IFNA*				
PECAM-1	*TRAF1*				
CR1	*PRF1*				
CD14	*IFIT2*				
IL-17RC	*IFIT1*				
TRIM	*IFITM2*				
DARC	*IFITM1*				
GBP7	*CD81*				
IL17RD	*TRAF6*				
TLR1	*CREB3L1*				
CTL4	*RARRES3*				
IL20RA	*FADD*				
CFTR	*PRKRIR*				
NOD1	*IL-10RA*				
TRIM5	*CLEC4C*				
IL-22	*IRAK4*				
SPTB	*STAT2*				
ADCY9	*TBK1*				
IL-4R	*OASL*				
ADORA2B	*ISGF3G*				
EMR1	*GZMB*				
GNAS	*IFI27*				
DERL3	*SOCS1*				
IL-18	*CCL3*				
Continued					

Malaria	WNV disease	ZIKV disease	RVF	DENV disease	CHIKV disease
FPN	*SOCS3*				
HLA-F	*TICAM1*				
TPI1	*TNFSF14*				
ADRB2	*CLEC4M*				
BF	*IRF3*				
SCO1	*CD40*				
HbS	*IFNGR2*				
FcγRIIB	*MX1*				
FcγRIIC	*ICOSLG*				
FcγRIIIA	*SAT1*				
NF-k B1					
FcγR					
RNASE 3					
ABCB1					
ADORA2A					
GRK5					
STAT6					
TNFRSF18					
FOXO3A					
CD40L					
IRF1					
IL17RE					
ABO GTG					

Table 4. Representation of distinct genes related to susceptibility and protection against MBDs.

showed the least allele frequency about 0.004137% in the Latino/Admix American population as mentioned in Fig. 5.

Allele frequency in the African/African American population

In the African/African American population the analysis revealed that *IL-6* (rs1800795), *OAS2* (rs15895), *OAS3* (rs2285932), *CXCR3* (rs2280964), *TLR7* (rs179010) and *MICB* (rs3132468) variants showed 0.927%, 0.9414%, 0.9481%, 0.83755%, 0.842%, and 0.8093% allele frequency respectively. Among these variants, the *OAS3* variant (rs2285932) was highly prevalent in the African/African American population, with an allele frequency of 0.9481%, while this variant was absent in the South Asian population. Furthermore, in the Latino/Admix American population, the *TLR*4 gene variant (rs4986791) exhibited a lower allele frequency (0.01448%) as mentioned in Fig. 5.

Allele frequency in the Ashkenazi jewish population

In the Ashkenazi Jewish population, the variants of *MICB* (rs3132468), *TLR3* (rs6552950), *IL-10* (rs1800872), and *TLR8* (rs5741883) gene have allele frequencies of 0.8093%, 0.8576%, and 0.7138%, respectively. Notably, *TLR3* (rs6552950) showed a significantly high prevalence among Ashkenazi Jewish population about 0.8576%, while it was absent in the South Asian population. Furthermore, the *G6PD* gene variant (rs1050828) exhibited no allele frequency (0%) in the Ashkenazi Jewish population as mentioned in Fig. 5.

Allele frequency in the European (Finnish) population

In the European (Finnish) population, the allele frequencies of genetic variants including *OAS3* (rs2285932), *OAS2* (rs15895), *TLR3* (rs6552950), *OAS1* (rs10774671), *TLR8* (rs5741883), and *FcγRIIA* (rs10774671) were observed at 0.7999%, 0.7825%, 0.7597%, 0.7366%, 0.7374%, and 0.7366%, respectively. Among these variants, the *OAS3* variant (rs2285932) demonstrates a notable prevalence at 0.7999% in this population. Furthermore, it was observed that (0%) allele frequencies for both variants including *CCL2* (rs1024611) and *G6PD* (rs1050828) within the European (Finnish) population as mentioned in Fig. 5.

Allele frequency in the European (non-Finnish) population

In the European (non-Finnish) population, the allele frequencies of the *IL-10* (rs1800872), *TLR3* (rs6552950), *TLR8* (rs5741883), and *MICB* (rs3132468) variants were reported at 0.7646%, 0.7546%, 0.733%, and 0.743%, respectively. Notably, the *IL-10* gene variant (rs1800872) demonstrates a significant prevalence with an allele frequency of 0.7999%. Additionally, the *G6PD* (rs1050828) variant exhibited the lowest allele frequency of about 0.0001946%, marking it as among the least frequent variants observed within this ethnic group as mentioned in Fig. 5.

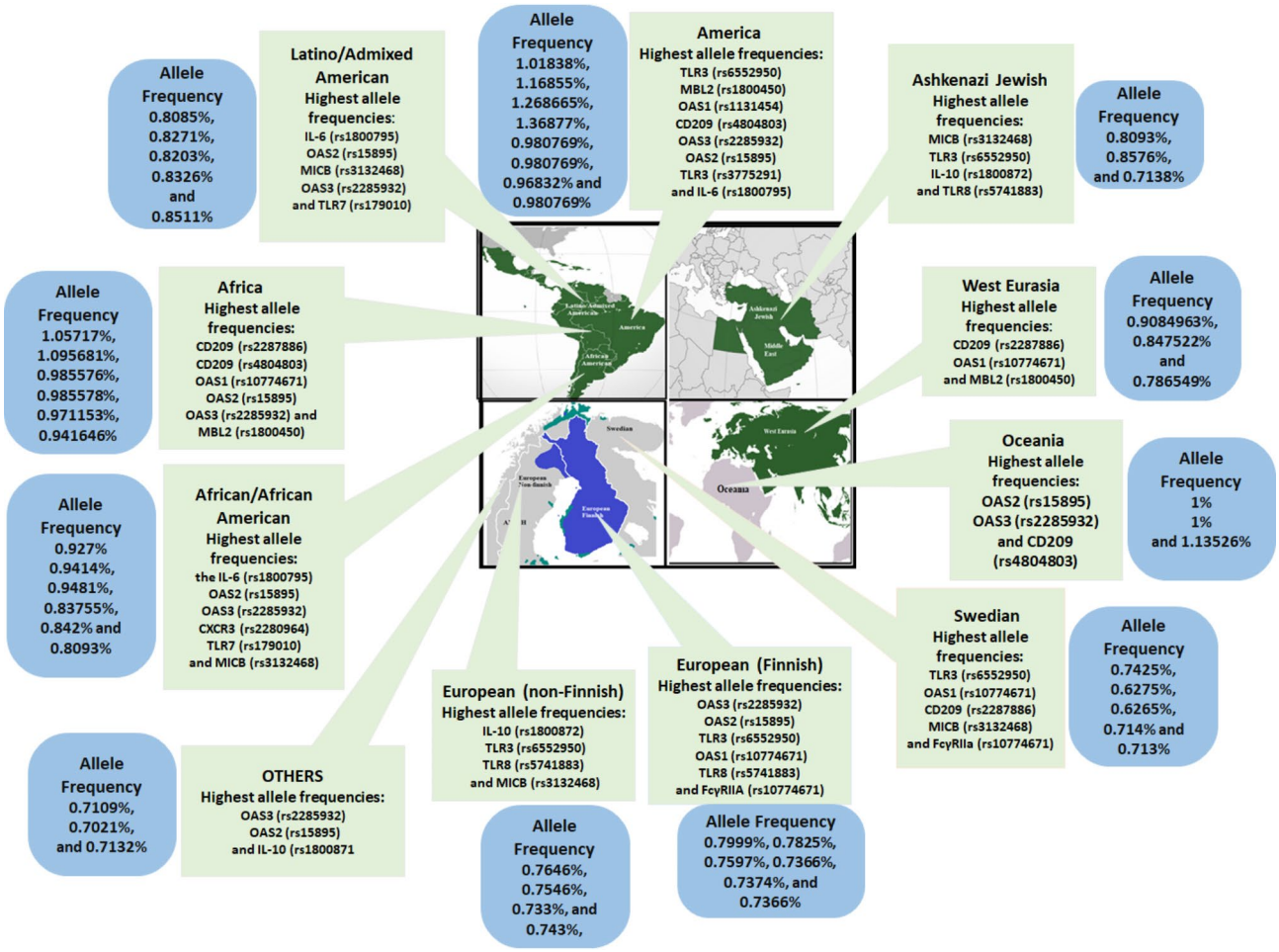

Fig. 5. Worldwide allele frequency distribution of susceptibility and protection-related genetic variants associated with MBDs and the genetic variants exhibiting the highest allele frequencies across multiple ethnic groups.

Allele frequency in OTHERS population

In the context of the gnomAD (Genome Aggregation Database), "OTHER population" refers to individuals whose ancestry or ethnicity does not fall clearly into the predefined major ancestry groups. These groups typically include populations like African, Latino, East Asian, South Asian, and European[47]. In the population designated as OTHERS, the analysis indicates allele frequencies for the *OAS3* (rs2285932), *OAS2* (rs15895), and *IL-10* (rs1800871) gene variants of 0.7109%, 0.7021%, and 0.7132%, respectively. Notably, the *OAS3* variant (rs2285932) was more prevalent in this population, exhibiting an allele frequency of 0.7109%. Additionally, within the OTHERS population, the *G6PD* (rs1050828) variant showed a minimal allele frequency of 0.003005% as mentioned in Fig. 5.

Allele frequency in the American population

In American population the observed genetic diversity showed that *TLR3* (rs6552950), *MBL2* (rs1800450), *OAS1* (rs1131454), *CD209* (rs4804803), *OAS3* (rs2285932), *OAS2* (rs15895), *TLR3* (rs3775291) and *IL-6* (rs1800795) gene variants revealed 1.01838%, 1.16855%, 1.268665%, 1.36877%, 0.980769%, 0.980769%, 0.96832% and 0.980769% allele frequency respectively. Among these variants, the *CD209* (rs4804803), was highly prevalent in the American population, with an allele frequency of 0.7109%. Furthermore, in the American population, the *MyD88* (rs6853) variant exhibited no allele frequency about (0%) as mentioned in Fig. 5.

Allele frequency in the oceanian population

In the Oceanian population, genetic diversity analysis revealed that allele frequencies of 1% for *OAS2* (rs15895), 1% for *OAS3* (rs2285932), and 1.13526% for *CD209* (rs4804803) gene variants. Notably, both *OAS2* and *OAS3* variants are highly prevalent among Oceanians, with an allele frequency of 0.7109% for each. Moreover, within this population, the *TNF* gene variants (rs1800629), *TLR4* (rs4986791), and *TLR9* (rs5743836) exhibited an absence of allele frequency (0%) as mentioned in Fig. 5.

Allele frequency in the African population

In African population the observed genetic diversity showed that *CD209* (rs2287886), *CD209* (rs4804803), *OAS1* (rs10774671), *OAS2* (rs15895), *OAS3* (rs2285932) and *MBL2* (rs1800450) gene variants exhibited 1.05717%, 1.095681%, 0.985576%, 0.985578%, 0.971153%, 0.941646% allele frequency respectively. Among these variants, the *OAS2* and *OAS3*, both were highly prevalent in the African population, with an allele frequency of 0.7109%. Furthermore, *TLR4* (rs4986791) exhibited the least allele frequency (0.004807%) in the African population compared with other ethnic groups as mentioned in Fig. 5.

Allele frequency in the West eurasian population

In the West Eurasian population, the observed genetic diversity showed that *CD209* (rs2287886), *OAS1* (rs10774671), and *MBL2* (rs1800450) gene variants exhibited 0.9084963%, 0.847522%, and 0.786549% allele frequency respectively. Among these variants, the *OAS2* and *OAS3* were both highly prevalent in the West Eurasian population, with an allele frequency of 0.7109%. Furthermore, *TLR4* (rs4986790) exhibited the least allele frequency (0.070175%) in the West Eurasian population compared with other ethnic groups as mentioned in Fig. 5.

Allele frequency in Sweden's population

In Sweden's population, the observed genetic diversity showed that *TLR3* (rs6552950), *OAS1* (rs10774671), *CD209* (rs2287886), *MICB* (rs3132468) and *FcγRIIA* (rs10774671) gene variants exhibited 0.7425%, 0.6275%, 0.6265%, 0.714% and 0.713% allele frequency respectively. Among these variants, the *TLR3* (rs6552950) was highly prevalent in the Sweden population, with an allele frequency of 0.7425%. Furthermore, *TNF* (rs361525) exhibited the least allele frequency (0.034%) in the Swedish population compared with other ethnic groups as mentioned in Fig. 5.

Allele frequency in the southeast Asian population

In the Southeast Asian population, the observed genetic diversity showed that *IL-6* (rs1800795), *OAS2* (rs15895), *OAS3* (rs2285932), *CTLA4* (rs231775) gene variants exhibited 0.988439%, 0.994219%, 0.91763%, and 0.992020 allele frequency respectively. Among these variants, the CTLA4 (rs231775) was highly p333revalent in the Southeast Asian population, with an allele frequency of 0.992020%. Furthermore, *TLR9* (rs5743836) exhibited the least allele frequency (0.001445%) in the Southeast Asian population compared with other ethnic groups as mentioned in Fig. 6.

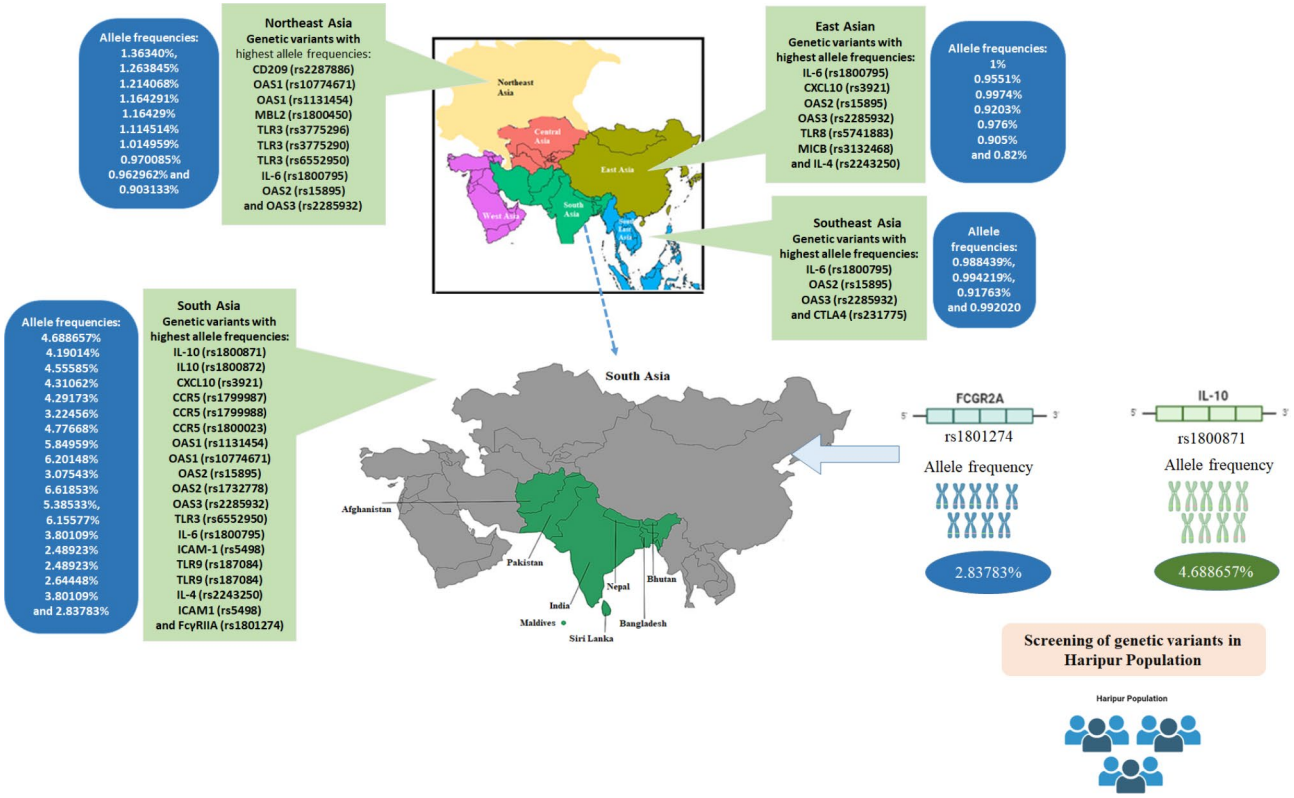

Fig. 6. Genetic variants containing highest allele frequency in the South Asian population.

Allele frequency in the northeast Asian population

In Northeast Asian population the observed genetic diversity showed that *CD209* (rs2287886), *OAS1* (rs10774671), *OAS1* (rs1131454), *MBL2* (rs1800450), *TLR3* (rs3775296), *TLR3* (rs3775290), *TLR3* (rs6552950), *IL-6* (rs1800795), *OAS2* (rs15895) and *OAS3* (rs2285932) variants exhibited 1.36340%, 1.263845%, 1.214068%, 1.164291%, 1.16429%, 1.114514%, 1.014959%, 0.970085%, 0.962962% and 0.903133% allele frequencies respectively. Among these variants, the CD209 (rs2287886) was highly prevalent in the Northeast Asian population, with an allele frequency of 1.36340%, while this variant was absent in the South Asian population. Furthermore, TLR4 (rs4986790) exhibited the least allele frequency (0.004273%) in the Northeast Asian population compared with other ethnic groups as shown in Fig. 6.

Allele frequency in the east Asian population

In the East Asian population, the *IL-6* (rs1800795), *CXCL10* (rs3921), *OAS2* (rs15895), *OAS3* (rs2285932), *TLR8* (rs5741883), *MICB* (rs3132468), and *IL-4* (rs2243250) variants showed 1%, 0.9551%, 0.9974%, 0.9203%, 0.976%, 0.905% and 0.82% allele frequency respectively. Among these variants, the *IL-6* (rs1800795) variant, was highly prevalent in the East Asian population, with an allele frequency of about 1%. Furthermore, in the East Asian population, the *G6PD* (rs1050828) variant exhibited about 0% allele frequency as mentioned in Fig. 6.

Allele frequency in the south Asian population

In the Southeast Asian population, the observed genetic diversity showed that *IL-10* (rs1800871), *IL10* (rs1800872), *CXCL10* (rs3921), *CCR5* (rs1799987), *CCR5* (rs1799988), *CCR5* (rs1800023), *OAS1* (rs1131454), *OAS1* (rs10774671), *OAS2* (rs15895), *OAS2* (rs1732778), *OAS3* (rs2285932), *TLR3* (rs6552950), *IL-6* (rs1800795), *ICAM-1* (rs5498), *TLR9* (rs187084), *TLR9* (rs187084), *IL-4* (rs2243250), *ICAM1* (rs5498) and *FcγRIIA* (rs1801274) genetic variants exhibited 4.688657%, 4.19014%, 4.55585%, 4.31062%, 4.29173%, 3.22456%, 4.77668%, 5.84959%, 6.20148%, 3.07543%, 6.61853%, 5.38533%, 6.15577%, 3.80109%, 2.48923%, 2.48923%, 2.64448%, 3.80109% and 2.83783% allele frequency respectively. Among these variants, the *OAS2* (rs15895) was highly prevalent in the Southeast Asian population, showing the highest allele frequency of 6.20148%. Furthermore, in the South Asian population, the *G6PD* (rs1050828) variant exhibited minimum allele frequency about (0.000367%) mentioned in Fig. 6).

Validation of prioritized genetic variants against MBDs among the Haripur population

Genetic variants prevalent in the South Asian population were examined within the local Haripur population. Two specific variants from *IL-10* (rs1800871) and *FcγRIIA* (rs1801274) mentioned were analyzed on the local population to validate our study findings. In this study, allele and genotype frequencies for *IL-10* (rs1800871) and *FcγRIIA* (rs1801274) were examined in a sample of 50 participants according to the inclusion-exclusion criteria defined in the methodology section. For *IL-10* (rs1800871), the genotype distribution showed that the wild-type (TT) genotype and heterozygous genotype (TC) present in the local Haripur population were 88% and 12% respectively in the available sample size. Similarly, for *FcγRIIA* (rs1801274) variant, the frequencies of wild-type (GG) and heterozygous GA genotypes were 6% and 94%, respectively in the available sample size. Statistical analysis revealed an allele frequency of 0.1128% for the *FcγRIIA* variant and 0.2112% for *IL-10* variant as shown in Table 5 and Fig. 7.

Discussion

The varied genetic architectures across ethnic groups highlight the importance of region-specific studies, as genetic factors may differentially influence disease pathogenesis. South Asia's unique genetic diversity offers both challenges and opportunities for population-based genetic studies[48,49]. Advancements since the Human Genome Project, including SNP analysis, DNA sequencing, and high-throughput genotyping, have enabled large-scale GWAS that have revolutionized our understanding of complex diseases[50–52]. In MBDs research, GWAS have been instrumental in identifying genetic variations in mosquito vectors, human hosts, and pathogens. These studies have been instrumental in helping us to understand more about disease pathogenesis and various associations between parasite and host genetic factors[51,52].

In the current study, we systematically identified candidate genes, and their genetic variants associated with both susceptibility and protection against MBDs, focusing on their distribution across diverse ethnic groups. Our analysis revealed notable differences in allele frequencies, highlighting population-specific genetic resilience. African populations and their descendants exhibited greater protection against severe MBDs compared to Southeast and Northeast Asians. Similarly, individuals of European ancestry and neighboring regions showed genetic protection against DF, whereas Southeast and Northeast Asians were more susceptible[29].

Moreover, MBD-related genetic variants were aggregated from publicly available datasets and then subjected to broader analysis to prioritize overlapping and unique genes using a structured framework. This approach facilitated the categorization of gene-variants (overlapping and unique) among MBDs. Also, it helped in identification of their associations across MBDs. After prioritization based on overlapping genes and their variants among MBDs, we calculated allele frequencies of these variants across multiple global populations, including Middle Eastern, Ashkenazi Jewish, European (Non-Finnish and Finnish), Latino/Admixed American, South Asian, East Asian, North Asian, Southeast Asian, African American, and Swedish populations. By examining allele frequencies in this diverse set of populations, we captured a comprehensive global perspective on genetic susceptibility and protection against MBDs.

Our results revealed that *TLR7* (rs179010) variant have highest allele frequency of about 0.8511% in Latino/Admixed American population and which reflects that this population linked with disease susceptibility

Participant	Age	Gender	IL-10		FcγRIIA	
			Wild Type Allele	Mutant Allele	Wild Type Allele	Mutant Allele
1	19	Female	TT		GG	
2	22	Female	TT	TC	GG	
3	19	Male	TT		GG	
4	20	Female	TT		GG	
5	20	Male	TT		GG	GA
6	21	Male	TT	TC	GG	
7	18	Male	TT	TC	GG	
8	20	Female	TT	TC	GG	
9	20	Male	TT		GG	
10	20	Female	TT		GG	
11	20	Male	TT		GG	
12	21	Male	TT		GG	
13	25	Male	TT		GG	
14	26	Male	TT	TC	GG	GA
15	23	Male	TT	TC	GG	GA
16	27	Male	TT		GG	
17	27	Male	TT		GG	
18	20	Female	TT		GG	
19	22	Male	TT		GG	
20	23	Female	TT		GG	
21	19	Female	TT		GG	
22	19	Female	TT		GG	
23	20	Female	TT		GG	
24	21	Female	TT		GG	
25	19	Female	TT		GG	
26	20	Female	TT		GG	
27	19	Male	TT		GG	
28	22	Male	TT		GG	
29	21	Male	TT		GG	
30	68	Male	TT		GG	
31	42	Male	TT		GG	
32	38	Female	TT		GG	
33	45	Female	TT		GG	
34	42	Female	TT		GG	
35	23	Female	TT		GG	
36	22	Female	TT		GG	
37	22	Female	TT		GG	
38	21	Female	TT		GG	
39	23	Female	TT		GG	
40	28	Male	TT		GG	
41	35	Female	TT		GG	
42	32	Female	TT		GG	
43	64	Male	TT		GG	
44	70	Female	TT		GG	
45	27	Female	TT		GG	
46	67	Female	TT		GG	
47	38	Male	TT		GG	
48	55	Male	TT		GG	
49	25	Male	TT		GG	
50	45	Male	TT		GG	

Table 5. The allele frequency of the two prioritized genetic variants in the Haripur population.

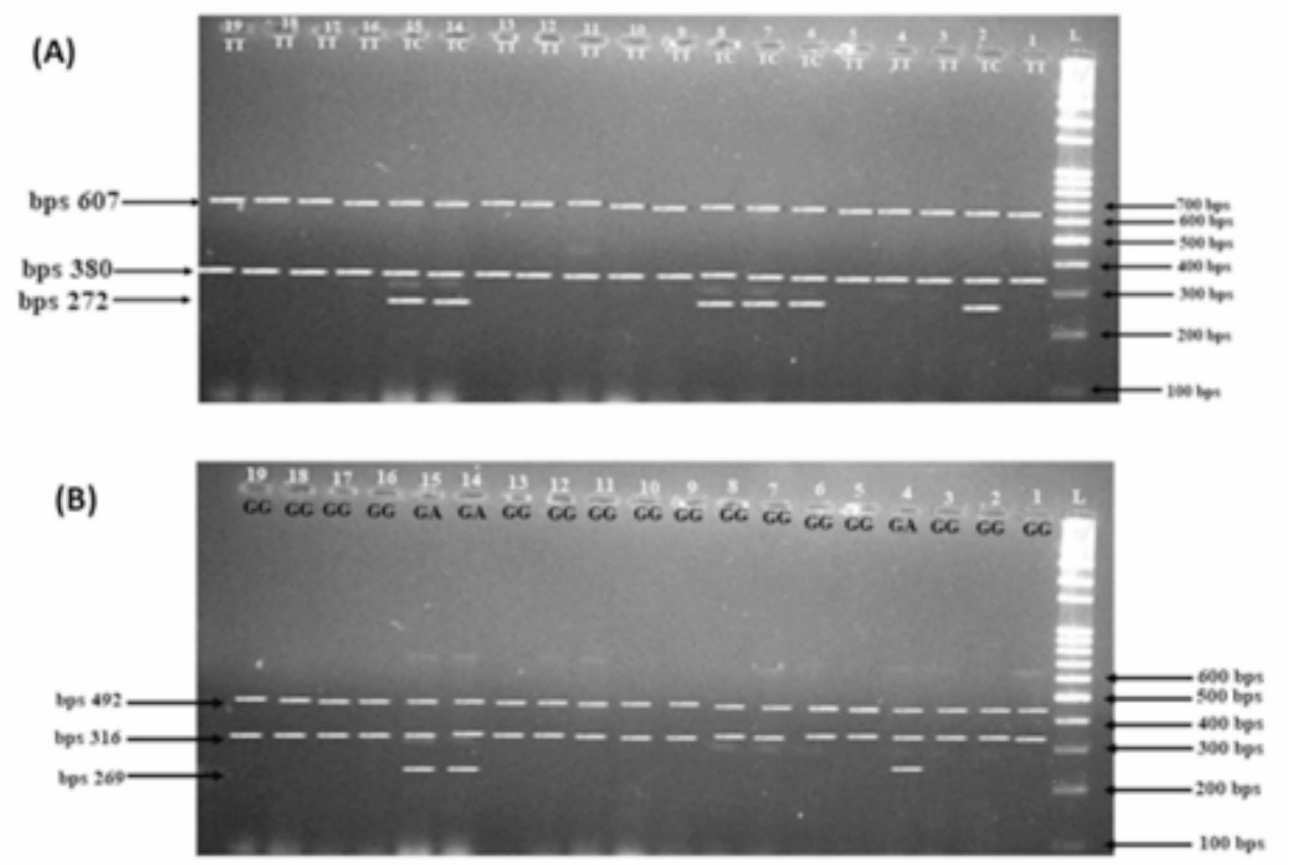

Fig. 7. (**A**) ARMS-PCR using tetra primers to study genetic variations in the *FcγRIIA* and *IL-10* gene. In *IL-10* (rs1800871) the outer primers (OF and OR) amplified a 607 bp product. The IF primer generated a wild allele with an amplicon size of 380 bp while the IR primer generated a mutated allele with an amplicon size of 272 bp. (**B**) The outer primers (OF and OR) for *FcγRIIA* (rs1801274) amplified a 492 bp product. The IF primer generated a wild allele with an amplicon size of 316 bp while the IR primer generated a mutated allele with an amplicon size of 269 bp. Representative PCR products obtained by ARMS are interpreted from an agarose gel. Lane (L) presents the DNA ladder that is 100 base pairs long.

against CHIKV, DENV, JEV, RVF and WNV diseases as shown in Supplementary tables S1, S2, S3, S6 and S7. *OAS3* (rs2285932) variant exhibited highest allele frequency of about of 0.9481% in African/African American population, which reflect that this population is susceptible to CHIKV and WNV diseases as mentioned in Supplementary tables S2 and S7.

In the Ashkenazi Jewish population, the *TLR3* genetic variant (rs6552950) exhibited the highest allele frequency of about 0.8576% among all studied variants. This observation suggests a potential genetic predisposition in these populations, correlating with increased susceptibility to diseases including CHIKV, DENV, JEV, RVF, WNV, and ZIKV infections as mentioned in Supplementary tables S1, S2, S3, S6, S7 and S9. The *OAS3* genetic variant (rs2285932) demonstrates the highest allele frequency of about 0.7999% in the European (Finnish) population. The highest allele frequency of this variant showed that this population might be susceptibility to CHIKV, DENV, and WNV diseases, emphasizing population-specific genetic predispositions that influence responses to viral infections as shown in Supplementary tables S1, S2 and S7.

Furthermore, our results revealed that the *IL-10* (rs1800872) variant showed highest allele frequency in the European (non-Finnish) population and its associations with susceptibility to various infectious diseases. With an allele frequency of 0.7999%, it was one of the most prevalent variants in this population. This polymorphism allele frequency revealed that this population increased susceptibility to DENV, JEV, WNV, malaria and ZIKV infections as mentioned in Supplementary tables S2, S3, S7, S5 and S9. The *OAS3* (rs2285932) variant demonstrates the highest allele frequency within the European (Finnish) population and OTHERS population. This observation suggests a potential genetic predisposition in these populations, correlating with increased susceptibility to diseases such as CHIKV, DENV, and WNV diseases. The heightened frequency of this variant may indicate a population-specific genetic factor influencing disease outcomes or responses to viral infections as shown in Supplementary tables S1, S2 and S7.

The *CD209* (rs4804803) variant was highly prevalent in the American population, with an allele frequency of 0.7109%. This variant has been strongly associated with increased susceptibility to various viral infections, including CHIKV, DENV, JEV, WNV, and ZIKV diseases as mentioned in Supplementary tables S1, S2, S3, S7

and S9. Its high prevalence might play a significant role in shaping immune responses and disease outcomes within this population. In the Oceanian, African and West Eurasian population, the *OAS2* variant (rs15895) and *OAS3* (rs2285932) variant exhibited the highest allele frequencies of about 0.7109%. Among these, the *OAS3* (rs2285932) variant showed a stronger association with susceptibility to diseases such as CHIKV, DENV and WNV diseases as shown in Supplementary tables S1, S2 and S7. On the other hand, the *OAS2* variant (rs15895) was linked to disease susceptibility against DENV, CHIKV, and WNV diseases as well, emphasizing the potential role of these genetic markers in influencing the immune response and vulnerability to these infections as mentioned in Supplementary tables S2, S1 and S7.

Sweden's population revealed that *TLR3* (rs6552950) variant have highest allele frequency among all variants which showed that this population is susceptible to DENV, CHIKV, JEV, RVF, WNV and ZIKV infections as mentioned in Supplementary tables S2, S1, S3, S6, S7 and S9. The *CTLA4* (rs231775) variant was highly prevalent in the Southeast Asian population, with an allele frequency of 0.992020%. This variant allele frequency revealed that this population was more susceptible to various MBDs, including JEV and WNV diseases as shown in Supplementary tables S3 and S7. In Northeast Asian population the *CD209* (rs2287886) was highly prevalent, with an allele frequency of 1.36340% showed that this population was more susceptible against CHIKV, DENV, JEV, ZIKV and WNV diseases as shown in Supplementary tables S1, S2, S3, S9 and S7 as well their potential role of this genetic variant in influencing the immune response and vulnerability to these infections. The *IL-6* (rs1800795) variant revealed an allele frequency of about 1% in the East Asian population. This variant provides protection against infections caused by several viruses, including DENV, ZIKV, RVFV, and CHIKV infections as shown in Supplementary tables S2, S9, S6 and S1.

In South Asian population, the *OAS2* (rs15895) variant exhibited the highest allele frequencies of about 0.7109%. This variant allele frequency revealed that this population might be more susceptible to CHIKV, DENV, and WNV diseases as mentioned in Supplementary tables S1, S2 and S7 as well, emphasizing the potential role of this genetic variant in influencing the immune response and vulnerability to these infections. Furthermore, we explored the correlation of observed allele frequencies with available data on morbidity and mortality rates of MBDs in different populations. The presence of *MIC-B* and *PLCE1* allelic variants in in African ancestry are linked with the severity of DENV infection in individuals[53,54]. Another study showed the involvement of *TNF*, *IFNG* and *IL-10* genetic variation with increased susceptibility to DHF in the Brazilian population[55]. In Malaysian population genetic variants of *HLA* may increase the risk of DHF in DENV infected patients[33]. Mark Loeb et al. identified genetic variants of 3 genes (*RFC1*, *SCN1A*, *ANPEP*) that potentially implicated in susceptibility to WNV disease neurological complications in United States and Canadian populations[56]. Our findings suggest that the observed patterns align with the founder effect, where population-specific genetic adaptations may influence disease outcomes[57].

While our study encompassed a broad range of populations, we placed particular emphasis on the South Asian population, given the region's significant burden of emerging infectious diseases and its critical role in global public health. The South Asian region has been a focal point for diseases with pandemic potential, posing substantial public health and economic challenges. In South Asian population we calculated the allele frequency 1009 genetic variants of 366 genes among these variants 19 were revealed the highest allele frequency in this population. The observed genetic diversity in this population revealed that *IL-10* (rs1800871), *IL10* (rs1800872), *CXCL10* (rs3921), *CCR5* (rs1799987), *CCR5* (rs1799988), *CCR5* (rs1800023), *OAS1* (rs1131454), *OAS1* (rs10774671), *OAS2* (rs15895), *OAS2* (rs1732778), *OAS3* (rs2285932), *TLR3* (rs6552950), *IL-6* (rs1800795), *ICAM-1* (rs5498), *TLR9* (rs187084), *TLR9* (rs187084), *IL-4* (rs2243250), *ICAM1* (rs5498) and *FcγRIIA* (rs1801274) genetic variants exhibited 4.688657%, 4.19014%, 4.55585%, 4.31062%, 4.29173%, 3.22456%, 4.77668%, 5.84959%, 6.20148%, 3.07543%, 6.61853%, 5.38533%, 6.15577%, 3.80109%, 2.48923%, 2.48923%, 2.64448%, 3.80109% and 2.83783% allele frequency respectively. These findings suggests that in South Asian region the observed allele frequencies of these variants showed that this population is most susceptible against most of the MBDs including DENV, ZIKV, RFV, WNV, CHIKV, LF, malaria and JEV infections.

In order to validate our study findings, we selected two genes and their variants (related to susceptibility and protection) that were more prevalent in studies related to South Asian populations, underscoring the importance of region-specific genetic insights in understanding and mitigating MBDs risk. *FcγRIIA* and *IL10* genes play an important role in immune responses and pathogenesis of malaria and DENV infection that were more prevalent in Pakistani population. They have been shown to influence individual susceptibility and the overall immune landscape of infections such as DENV, malaria, and ZIKV[58–62]. Immune-modulating genes may function synergistically or antagonistically in populations with unique genetic backgrounds, altering the dynamics of infection spread and severity[62].

ARMS PCR analysis of the genomic samples in local Haripur population were performed for *IL-10* (rs1800871) and *FcγRIIA* (rs1801274) alleles. Based on our findings, the TT genotype of *IL-10* (rs1800871) represented a homozygous normal allele, indicating a typical genomic pattern that confers protection against MBDs. In contrast, the TC genotype was associated with increased susceptibility to MBDs. While for *FcγRIIA* (rs1801274), the GG genotype is related to protection, with the GA genotype was related to susceptibility against MBDs. In our sample of local population, it was shown that the TT genotype of *IL-10* (rs1800871) was predominant (88%) in local population, whereas the TC genotype was less common (12%). Similarly, for *FcγRIIA* (rs1801274), the GG genotype was most prevalent (94%), with the GA genotype being a minority (6%). This pattern suggests that the local population is somewhat protected against MBDs due to minimum allele frequency against MBDs. These patterns are consistent with larger results in European and African region, implying that specific genotypes give either vulnerability or protection, which may differ between groups due to evolutionary and environmental variables.

IL-10 is an anti-inflammatory cytokine that plays an essential role in dampening immune responses during infection to avoid excessive tissue damage[63]. Although its functions in the regulation of inflammatory pathways

are protective, but the immunosuppressive properties of *IL-10* impaired the host's ability to mount effective immune defenses, allowing pathogens perhaps to persist or replicate unchecked[64]. Genetic polymorphism in *IL-10* (rs1800871), have been substantially linked to greater susceptibility to DENV infection. Moreover, in the Mexican population, the *IL-10* (rs18001871) variant indicates the there is an independent relationship between TC and CC alleles with DENV disease susceptibility. This study highlights the relationship between DENV disease and the *IL-10* (rs18001871) variant[65]. This variant affect the expression levels of *IL-10*, leading to an imbalanced immune response that promote virus persistence and exacerbate the severity of the disease[65]. Thus, *IL-10* is a potential biomarker for predicting disease prognosis not only in DENV infection but also in other viral diseases[58]. Furthermore, the rs1800871 polymorphism in *IL-10* gene is associated with clinical malaria in children and IL-10 production[66], and *IL-10* genetic variation with increased susceptibility to DHF in the Brazilian population[55]. A study in Mozambique found higher IL-10 levels in cord blood cells exposed to infected erythrocytes, linked to the presence of the *IL-10* rs1800871 G allele. This association remained significant after adjusting for various confounders, including maternal factors and malaria interventions[66]. The rs1800871 polymorphism has been found to have contradictory associations, with some cases indicating increased risk and others suggesting protection against mosquito-borne infections. Specifically, the GG genotype of rs1800871 was linked with protection compared to CC, indicating a reduced risk of severe complications from MBDs[67]. Another study reported that *IL10* variants associated with increased IL-10 production elevate the risk of clinical malaria in children. Elevated levels of circulating IL-10 have been observed in patients with cerebral, severe, and moderate malaria. Similar findings from pre-clinical models indicate that African children with severe anemia exhibit lower plasma IL-10 levels compared to those with moderate anemia or cerebral malaria, underscoring the role of IL-10 in preventing severe anemia[66,68,69].

FcγRIIA plays a significant role in the recognition and response of the immune system to pathogen-antibody complexes, especially regarding antibody-dependent enhancement. This receptor mediates immune effector functions, including phagocytosis, antibody-dependent cytotoxicity, and the stimulation of inflammatory pathways[70]. The *FcγRIIA* gene variation of rs1801274 has been linked to immune regulation during infections, increasing vulnerability to illnesses against DENV diseases[71]. The H131R polymorphism, have been linked in altering the affinity of the receptor to immune complexes and thus susceptible to a range of vector-borne diseases, such as malaria and DENV infections[72,73].

In the context of malaria, *FcγRIIA* variants have been associated with variable disease severity, depending on the allele; the resultant ADE-infectivity enhancement enhances virus entry into host cells, promoting higher viral replication and inflammation[74]. The *FcγRIIA* gene polymorphism (rs1801274), having R131H, was found to be protective against blood-stage malaria infections[75]. Similarly, the coding mutation is *FcγRIIA*-H131R (rs1801274), which impacts the activation of monocytes through *FcγRIIA*, leading to alterations in IgG recognition and the phagocytosis of IgG parasites[73,75]. Another study from Pakistan revealed that mutation in the *FcγRIIA* gene (rs1801274) affects receptors where immunoglobin G shows binding[71]. The mutated A allele of the *FcγRIIA* gene rs1801274 has been associated with protection against malaria, indicating the significant role of *FcγRIIA* in susceptibility to the disease[61].

FcγRIIA has also been associated, with DENV infection, with an increased risk for the development of severe outcomes of DENV infection including DHF and DSS, when pre-existing antibodies against this virus are present within the host[71,76,77]. In the Vietnamese population genetic variant of *FcγRIIA* R131H (rs1801274) in patients with DSS showed a significant association with recessive protection of the R allele[78]. Another study in Mexican population revealed that genetic polymorphism in the *FcγRIIA* variant rs1801274 was associated with individuals carrying the histidine allele, whether homozygous (GG) or heterozygous (GA), showing protection from symptomatic DENV infection compared to those with the asymptomatic genotype. Additionally, homozygous variants of the *FcγRIIA* gene were less likely to result in severe DENV infection[79]. Individuals with the R allele and R/R genotype are protected from symptomatic DENV infection, while those with the H allele and H/H genotype are associated with both DF and DHF. This highlights rs1801274 as a crucial genetic determinant for severe DENV infection[80]. Another study revealed that genetic susceptibility to DF is associated with *FcγRIIA* gene polymorphism in Pakistani population[81]. Similarly, the *FcγRIIA* rs1801274 gene variant is a crucial factor in susceptibility and protection against CHIKV infection. The *FcγRIIA* gene variation plays a key role in regulating immune responses during CHIKV infection, determining whether an individual is susceptible or resistant to the virus[82]. It has been found that the significant genetic variation rs1801274 within the *FcγRIIA* gene regulates the immune response to CHIKV infection[83]. Furthermore, CHIKV disease susceptibility is connected to the *CD209* gene's rs4804803 GG genotype, *OAS* and *CD209* genes may affect CHIKV infection symptomatology[84].

Our current investigations further strengthen the significance of these *IL-10* polymorphisms in modifying host responses and emphasize their predictive potential for disease prognosis in diverse geographic regions and populations. A better understanding of the genetic basis of immune responses in MBDs is crucial for developing targeted, personalized health therapies. The implementation of more effective public health measures, such as novel vaccinations and antiviral treatments can be designed to alter these immune pathways to enhance disease outcomes.

Implications and contributions

This study lays the groundwork for understanding the genetic architecture of MBDs in South Asian populations, particularly in areas with high illness burden. Identifying protective and susceptibility variations can help drive public health efforts such as targeted screening and population-specific treatments. Furthermore, this study emphasizes the need of incorporating genetic data into disease prevention strategies to increase their efficacy and sustainability. Population genomics could therefore be used to help develop effective new interventions, as well as to support the efficient deployment of existing disease control measures through resistance surveillance. Population genomics will play an important role in future vaccine development efforts by mapping differences in

vaccine target polymorphism profiles across populations and identifying immunogenic protein targets that are not highly polymorphic in any geographical region. Furthermore, incorporating genetic screening into public health policies may allow for more effective risk prediction and mitigation of MBDs, while research into the socioeconomic and cultural obstacles to genetic testing adoption might guarantee that such treatments are more widely applicable and accepted.

Limitations and future research directions

This study is a preliminary effort focused on screening genetic variants associated with susceptibility and protection against MBDs. Due to the small sample size, the analysis was limited to a restricted number of individuals from the local population of Tehsil Haripur, which may not fully represent the genetic diversity of the entire region. Additionally, many other factors which contribute to genetic risk in population like environmental, behavioral, and other epidemiological factors influencing susceptibility to MBDs were not included in this study. Genetic research and screening programs require significant funding, technical expertise, and infrastructure, which are often lacking in regions where MBDs are endemic. This limits the feasibility of large-scale genetic risk assessments in resource-constrained areas. Despite these limitations, this study provides valuable insights and lays the groundwork for future research. It is the first study conducted in this region to explore genetic variants linked to MBDs, addressing an important gap in understanding the genetic basis of disease susceptibility and protection. These findings can serve as a baseline for larger-scale studies and contribute to the development of targeted strategies for disease prevention and control. We believe that this study more accurately predicted the genetic screenings in local population. In addition, this study was a meaningful study that could confirm that public data analysis is helpful toward predicting the variant spectrum of actual patients. To our knowledge, this is the first report to demonstrate such polygenic risk for MBDs. Our data provides valuable insights into how genetic variants influence susceptibility to MBDs. Additionally, it highlights the importance of leveraging population-specific data to inform and organize more effective public health awareness campaigns aimed at controlling these diseases.

Conclusion

Genetic research has played an instrumental role in the discovery of new biological pathways underpinning infectious diseases and the evaluation of new targets for therapeutic development. Public awareness campaigns can play a significant role in mitigating the impact of MBDs in vulnerable populations by increasing knowledge about prevention, early detection, and treatment options. It can be useful in promoting and even accelerating the implementation to enroll subjects for genome projects, whether they are for research or clinical applications, in different countries. The lack of knowledge on the techniques and usage of results from genetic testing leads to misconceptions, unwarranted fear, and rejection of technologies on prejudicial beliefs. Every aspect of education plays a key role since attitudes toward genetic testing are hugely influenced by religious, cultural, and moral views. As diversity in population genetics plays a significant role in viral pathogenesis, drug resistance, viral evolution, and immune escape, further studies must be conducted on worldwide population genetics to expedite the development of population-specific therapeutics to mitigate this worldwide challenge.

Data availability

All data supporting the findings of this study were included within the manuscript, and no external datasets were used. The data was provided in the main manuscript and the supplementary information files.

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Author contributions

Conceptualization, FMA and SNK. RB acquisition, analysis, and interpretation of the data. RB and FMA writing—original draft preparation. RB, MBA, FMA, SNK, AO, MM, WA, RP, AHS, MBA, TSA, SK, AN, writing, review and editing. FMA and SNK supervision, project administration and funding acquisition. All authors contributed to the article and approved the submitted version.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to F.M.A. or S.N.K.