Genetic Analysis of Dengue Fever - A Comprehensive Investigation of Associated Genes and their Functional Pathways

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Abstract

Dengue fever represents a significant global health challenge with complex pathophysiological mechanisms. This study aimed to elucidate the genetic underpinnings of Dengue fever through comprehensive analysis of associated genes. The top 30 genes related to Dengue fever were retrieved from the DisGeNET database and analyzed using multiple bioinformatics tools including GO terms (Biological Process, Cellular Component, Molecular Function), Wiki Pathways and Jensen databases. R version 4.4.2 was employed for data analysis and visualization. Analysis revealed significant enrichment of genes involved in cytokine production regulation, particularly interleukin-6 and inflammatory response pathways. Key biological processes included STAT protein phosphorylation regulation (OR=66.37, p<0.001) and nitric oxide biosynthesis (OR=133.42, p<0.001). Cellular localization identified extracellular and immune-related compartments as primary sites, while molecular function analysis highlighted cytokine activity (OR=42.35, p<0.001).

Pathway analysis demonstrated significant associations with folate metabolism (OR=168.74, p<0.001) and cytokine inflammatory responses (OR=319.58, p<0.001). Tissue analysis revealed predominant expression in immune-related tissues, particularly the peritoneal cavity (OR=950.52, p<0.001). This study elucidates the complex genetic landscape underlying Dengue fever pathogenesis, highlighting the central role of inflammatory cytokine regulation, particularly IL-6, IFNG, TNF and IL-1B in disease manifestation. These findings provide potential targets for therapeutic intervention and diagnostic biomarker development for improved management of Dengue fever.

Keywords: Dengue fever, Gene ontology, Biological pathways, Cytokine regulation, Inflammatory response.

Introduction

Dengue fever represents one of the most significant mosquito-borne viral diseases globally, affecting millions of individuals annually with increasing geographical spread due to climate change and globalization[2]. The causative agent, Dengue virus (DENV), belongs to the Flaviviridae family in four distinct serotypes (DENV-1 to DENV-4), each capable of causing the full spectrum of disease manifestations[9]. The clinical presentation of Dengue ranges from asymptomatic infection to severe forms including Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS) which are associated with significant morbidity and mortality[27].

The pathogenesis of Dengue fever involves complex interactions between viral factors and host immune responses, with genetic determinants playing crucial roles in disease susceptibility and progression[35]. Previous studies have identified various genetic factors that influence the clinical outcome of Dengue infection including polymorphisms in cytokine genes, human leukocyte antigen (HLA) system and genes involved in innate immunity[37]. However, a comprehensive understanding of the genetic landscape underlying Dengue pathophysiology remains incomplete.

The host immune response to DENV infection is characterized by a complex interplay of innate and adaptive immune mechanisms[30]. Upon infection, viral recognition by pattern recognition receptors triggers a cascade of inflammatory responses, including the production of pro-inflammatory cytokines and interferons[19]. This initial response aims to control viral replication but can also contribute to immunopathology when dysregulated, particularly in secondary infections with heterologous serotypes[33].

The phenomenon of antibody-dependent enhancement (ADE) further complicates the immune response in Dengue where non-neutralizing antibodies from previous infections can enhance viral entry into host cells, particularly monocytes and macrophages, leading to increased viral replication and heightened inflammatory responses[10]. This mechanism partially explains the increased risk of severe disease in secondary infections and represents a significant challenge for vaccine development[13]. Cytokine dysregulation has been consistently observed in severe Dengue cases with elevated levels of pro-inflammatory cytokines including TNF-α, IL-6 and IL-1β contributing to vascular leakage, coagulopathy and shock[15]. The "cytokine storm" phenomenon is characteristic of severe Dengue manifestations and correlates with disease severity[7]. Additionally, alterations in endothelial function, coagulation pathways and complement activation contribute to the complex pathophysiology of dengue[17].

Genetic factors influencing these pathways have been the subject of numerous investigations, with genome-wide association studies (GWAS) and candidate gene approaches identifying various susceptibility loci[6]. Polymorphisms in genes encoding cytokines, chemokines and their receptors, as well as genes involved in viral recognition and immune regulation, have been associated with dengue severity[7]. However, the functional implications of these genetic variations and their interactions remain incompletely understood.

Recent advances in bioinformatics and systems biology approaches have enabled more comprehensive analyses of disease-associated genes and their functional interconnections[1]. Gene ontology (GO) analysis, pathway enrichment and network-based approaches provide valuable insights into the biological processes, cellular components and molecular functions relevant to disease pathogenesis[12]. These methodologies facilitate the identification of key pathways and potential therapeutic targets by placing individual genes in their functional context[25].

In this study, we employed a systematic approach to analyze the top 30 genes associated with dengue fever, as identified through the DisGeNET database, which aggregates gene-disease associations from various sources including curated databases, GWAS catalogs and literature mining[21]. Our analysis encompassed multiple dimensions of gene function including biological processes, cellular localization, molecular activities and pathway involvement, to provide a comprehensive view of the genetic landscape underlying Dengue pathophysiology.

By integrating data from GO annotations, WikiPathways and various specialized databases including those focused on transcription factors, microRNA targets, metabolites, tissue expression, cellular compartmentalization and disease associations, we aimed to elucidate the complex interrelationships among dengue-associated genes[33]. This multi-layered approach allows for a more nuanced understanding of disease mechanisms beyond individual gene effects, potentially identifying novel targets for therapeutic intervention and biomarker development[4].

Objectives

1. To identify and characterize the key biological processes, cellular components and molecular functions associated with genes implicated in Dengue fever pathogenesis.
2. To elucidate the significant pathways and regulatory networks involving Dengue fever-associated genes using integrated bioinformatics analysis.
3. To determine the tissue-specific expression patterns and subcellular localization of dengue-related genes to understand their contextual roles in disease manifestation.
4. To identify potential therapeutic targets and biomarkers for improved diagnosis, prognosis and treatment of dengue fever based on comprehensive genetic analysis.

Material and Methods

Data Acquisition and Gene Selection: The investigation began with the retrieval of genes associated with dengue fever from the DisGeNET database (version 7.0), a comprehensive platform that integrates gene-disease associations from various sources including curated databases, GWAS catalogs, animal models and text mining of scientific literature[20]. The database was queried using the term "Dengue_fever" to extract all relevant gene associations. Based on association scores that consider the number and type of sources supporting each gene-disease relationship, the top 30 genes with the strongest evidence for dengue fever association were selected for further analysis.

Bioinformatics Resources and Tools: To comprehensively analyze the functional characteristics of the selected genes, multiple bioinformatics resources were employed. These included gene ontology (GO) databases for biological processes, cellular components and molecular functions (2023 versions); WikiPathways (2024 Human); ClinVar (2019); Cancer Cell Line Encyclopedia; ChEA (2022); TargetScan microRNA (2017); DrugMatrix; HMDB Metabolites; and Jensen databases for tissues, cellular compartments and diseases[14]. These diverse resources allowed for a multi-dimensional analysis of gene functions, interactions and regulatory relationships.

Gene Ontology Analysis: GO analysis was conducted using the 2023 versions of GO biological process, GO cellular component and GO molecular function databases. This analysis categorized genes based on the biological processes they participate in, their cellular localization and their molecular activities[31]. Enrichment analysis was performed to identify over-represented GO terms within the gene set, calculating odds ratios and combined scores to quantify enrichment significance. Terms with high odds ratios and combined scores represented biological features with substantial relevance to dengue fever pathophysiology.

Pathway Analysis: The WikiPathways 2024 human database was utilized to identify biological pathways significantly enriched among the dengue-associated genes[28]. This analysis provided insights into the broader biological systems and networks in which these genes function. Similar to GO analysis, enrichment was quantified using odds ratios and combined scores, with higher values indicating stronger pathway associations with the gene set.

Transcription Factor and Regulatory Analysis: To understand the regulatory mechanisms controlling the expression of dengue-associated genes, the ChEA 2022 database was employed[16]. This analysis identified transcription factors likely to regulate the selected genes based on chromatin immunoprecipitation (ChIP) experiments. Additionally, TargetScan microRNA 2017 was used to predict microRNA-mediated regulation of the gene set, providing insights into post-transcriptional regulatory mechanisms.

Tissue and Cellular Compartment Analysis: Jensen TISSUES and Jensen COMPARTMENTS databases were utilized to determine the tissue expression patterns and subcellular localization of the dengue-associated genes[24]. This analysis helped contextualize gene functions within specific anatomical and cellular environments, potentially identifying key sites of disease activity and therapeutic intervention.

Metabolite Association Analysis: The HMDB Metabolites database was employed to identify small molecules and metabolites associated with the dengue fever genes[36]. This analysis provided insights into the biochemical processes potentially altered in dengue infection and identified metabolites that might serve as biomarkers or therapeutic targets.

Statistical Analysis and Data Interpretation: For all analyses, statistical significance was assessed using odds ratios and combined scores. The odds ratio represents the strength of association between the gene set and a particular feature (GO term, pathway etc.) while the combined score incorporates both the odds ratio and the p-value to provide a comprehensive measure of significance. Higher combined scores indicate stronger and more statistically significant associations.

Data Visualization and Software: All analyses and visualizations were performed using R version 4.4.2, a powerful statistical computing environment[22]. Various R packages were utilized for data processing, statistical analysis and visualization, including those specifically designed for bioinformatics applications such as enrichment analysis and network visualization. The resulting tables and figures were organized to present the most significant findings from each analysis category.

Data Integration and Interpretation: The results from individual analyses were integrated to construct a comprehensive understanding of dengue fever pathophysiology from a genetic perspective. Overlapping features across different analysis types were identified to highlight processes, pathways and cellular components with the strongest evidence for involvement in dengue fever. This integrated approach allowed for a system-level interpretation of genetic contributions to disease mechanisms, potentially revealing novel insights beyond individual gene effects.

Results

GO_Biological_Process_2023 analysis revealed significant enrichment of genes involved in cytokine regulation, particularly interleukin-6 production (OR=45.98, combined score=825.47) with genes IL10, IL6, IFNG, IL1B, PTPN11 and TNF implicated. The regulation of calcidiol 1-monooxygenase activity showed the highest odds ratio (OR=1109.33, combined score=19201.67), involving IFNG, IL1B and TNF.

Table 1
GO_Biological_Process_2023

Term	Odds. Ratio	Combined. Score	Genes
Regulation of Interleukin-6 Production (GO:0032675)	45.97685	825.4656	IL10;IL6;IFNG;IL1B;PTPN11;TNF
Positive Regulation of Cytokine Production (GO:0001819)	22.91142	409.9476	IL10;CD4;IL6;IFNG;IL1B;PTPN11;TNF;IL2
Positive Regulation of Tyrosine Phosphorylation of STAT Protein (GO:0042531)	79.68	1422.48	IL6;IFNG;TNF;IL2;VEGFA
Regulation Of Membrane Protein Ectodomain Proteolysis (GO:0051043)	180.5701	3166.829	IL10;IFNG;IL1B;TNF
Regulation Of Calcidiol 1-Monooxygenase Activity (GO:0060558)	1109.333	19201.67	IFNG;IL1B;TNF
Positive Regulation of Peptidyl-Tyrosine Phosphorylation (GO:0050731)	40.0121	686.8365	IL6;IFNG;PTPN11;TNF;IL2;VEGFA
Regulation Of Tyrosine Phosphorylation of STAT Protein (GO:0042509)	66.36667	1128.107	IL6;IFNG;TNF;IL2;VEGFA
Regulation Of Inflammatory Response (GO:0050727)	25.78074	431.9913	IL10;IL6;IFNG;CMA1;IL1B;TNF;IL2
Positive Regulation of Nitric Oxide Biosynthetic Process (GO:0045429)	133.4247	2197.289	IFNG;IL1B;HBB;TNF
Positive Regulation of Multicellular Organismal Process (GO:0051240)	18.79683	308.6967	IL10;IL6;IFNG;IL1B;PTPN11;TNF;IL2;VEGFA
Positive Regulation of Nitric Oxide Metabolic Process (GO:1904407)	127.859	2086.053	IFNG;IL1B;HBB;TNF

Positive regulation of tyrosine phosphorylation of STAT protein (OR=79.68, combined score=1422.48) and regulation of membrane protein ectodomain proteolysis (OR=180.57, combined score=3166.83) were also significantly enriched processes as in table 1.

According to table 2, GO_Cellular_Component_2023 analysis identified significant enrichment of genes in the T cell receptor complex (OR=83.84, combined score=663.37) involving CD4 and CD8A and endocytic vesicle lumen (OR=75.00, combined score=578.21) containing HBB and HBA1. Additional enriched components included membrane raft (OR=13.26, combined score=82.15), platelet alpha granule lumen (OR=22.22, combined score=120.57) and polysomal ribosome (OR=25.47, combined score=81.24).

As per table 3, GO_Molecular_Function_2023 analysis highlighted cytokine activity as highly enriched (OR=42.35, combined score=953.64) involving IL10, CXCL10, IL6, IFNG, IL1B, TNF, IL2 and VEGFA. Related functions including receptor ligand activity (OR=27.18, combined score=575.55) and growth factor activity (OR=49.73, combined score=777.69) were also significant. Specialized functions such as deNEDDylase activity (OR=172.12, combined score=842.66) and interleukin-6 receptor binding (OR=137.69, combined score=649.09) showed high specificity.

WikiPathways_2024 analysis revealed strong associations with folate metabolism (OR=168.74, combined score=6785.30) and vitamin B12 metabolism (OR=189.76, combined score=7116.15). The cytokines and inflammatory response pathway showed particularly high enrichment (OR=319.58, combined score=10512.56), as did COVID-19 adverse outcome pathway (OR=554.47, combined score=17209.82), highlighting overlapping mechanisms between viral infections as in table 4.

Table 2
GO_Cellular_Component_2023

Term	Odds.Ratio	Combined.Score	Genes
T Cell Receptor Complex (GO:0042101)	83.83613	663.3672	CD4;CD8A
Endocytic Vesicle Lumen (GO:0071682)	75.00376	578.2103	HBB;HBA1
Intracellular Organelle Lumen (GO:0070013)	5.623529	36.62349	IL6;ALB;HBB;SDC1;HBA1;F2
Membrane Raft (GO:0045121)	13.25569	82.14976	CD4;CD8A;TNF
Platelet Alpha Granule Lumen (GO:0031093)	22.21652	120.5728	ALB;VEGFA
Platelet Alpha Granule (GO:0031091)	16.3243	79.11709	ALB;VEGFA
Endoplasmic Reticulum Lumen (GO:0005788)	7.785291	36.96894	IL6;ALB;F2
Golgi Lumen (GO:0005796)	14.48397	66.95156	SDC1;F2
Endocytic Vesicle (GO:0030139)	7.281296	24.46668	HBB;HBA1
Polysomal Ribosome (GO:0042788)	25.46999	81.2396	NR0B1
Tertiary Granule Lumen (GO:1904724)	13.20822	33.93875	HBB

Table 3
GO_Molecular_Function

Term	Odds. Ratio	Combined. Score	Genes
Cytokine Activity (GO:0005125)	42.35294	953.6364	IL10;CXCL10;IL6;IFNG;IL1B;TNF;IL2;VEGFA
Receptor Ligand Activity (GO:0048018)	27.17972	575.5451	IL10;CXCL10;IL6;IFNG;IL1B;F2;TNF;IL2;VEGFA
Growth Factor Activity (GO:0008083)	49.725	777.693	IL10;IL6;F2;IL2;VEGFA
Growth Factor Receptor Binding (GO:0070851)	43.69011	656.6151	IL10;IL6;IL1B;IL2;VEGFA
Cytokine Receptor Binding (GO:0005126)	41.84211	620.2959	IL10;IL6;IL1B;IL2;VEGFA
MHC Protein Binding (GO:0042287)	44.50446	299.8969	CD4;CD8A
Chemoattractant Activity (GO:0042056)	43.15368	288.2955	CXCL10;VEGFA
Protein Tyrosine Kinase Binding (GO:1990782)	17.11446	84.46633	CD4;PTPN11
deNEDDylase Activity (GO:0019784)	172.1207	842.6623	SENP8
Heme Binding (GO:0020037)	16.71008	81.71977	HBB;HBA1
Interleukin-6 Receptor Binding (GO:0005138)	137.6897	649.092	IL6

Jensen_COMPARTMENTS analysis identified significant association with extracellular ferritin complex (OR=138.06, combined score=6672.24), ferritin complex (OR=73.51, combined score=3350.37) and Fc receptor complex (OR=88.02, combined score=3771.23). Multiple immune-related complexes including NF-kappaB complex, interleukin-23 complex and integrin complexes were also enriched as per table 5.

Jensen_TISSUES analysis showed strongest expression in peritoneal cavity (OR=950.52, combined score=46641.60), bronchoalveolar lavage (OR=150.62, combined score=6937.93) and peritoneal exudate (OR=725.82, combined score=30748.31). Additional significant tissues included mast cells, tendon, synovial tissue and the broader immune system, consistent with dengue's systemic inflammatory effects as in table 6.

ChEA_2022 analysis identified RELA (OR=7.39, combined score=78.91) and RELB (OR=22.60, combined score=222.45) as significant transcriptional regulators of dengue-associated genes, particularly in inflammatory contexts. Additional transcription factors included STAT4, LMO2 and ADNP, suggesting diverse regulatory mechanisms as in table 7.

HMDB_Metabolites analysis revealed associations with simvastatin (OR=146.15, combined score=2454.34), atorvastatin (OR=95.02, combined score=773.48) and butyric acid (OR=68.83, combined score=282.99). These associations suggest potential metabolic pathways affected in dengue fever and possible therapeutic approaches as in table 8.

Discussion

The comprehensive analysis of genes associated with Dengue fever provides significant insights into the molecular mechanisms underpinning disease pathogenesis. The results highlight the central role of inflammatory processes, particularly cytokine regulation, in dengue fever pathophysiology, consistent with the hallmark "cytokine storm" observed in severe cases[32].

The significant enrichment of genes involved in interleukin-6 (IL-6) production regulation (Table 1) underscores the pivotal role of this pleiotropic cytokine in dengue pathogenesis. IL-6 serves as a critical mediator of the acute phase response and fever during infection and elevated IL-6 levels have been consistently associated with severe dengue manifestations[3]. The involvement of multiple genes (IL10, IL6, IFNG, IL1B, PTPN11, TNF) in this process suggests a complex regulatory network controlling IL-6 production, potentially offering multiple intervention points for therapeutic development.

Similarly, the striking enrichment of genes involved in STAT protein phosphorylation (OR=79.68) highlights the importance of JAK-STAT signaling pathways in Dengue infection.

Table 4
WikiPathways 2024

Term	Odds. Ratio	Combined Score	Genes
Folate Metabolism WP176	168.7373	6785.301	CRP;IL6;IFNG;IL1B;ALB;HBB;HBA1;F2;TNF;IL2
Vitamin B12 Metabolism WP1533	189.7619	7116.149	CRP;IL6;IFNG;IL1B;ALB;HBB;HBA1;F2;TNF
Selenium Micronutrient Network WP15	110.7217	3661.652	CRP;IL6;IFNG;IL1B;ALB;HBB;HBA1;F2;TNF
Cytokines And Inflammatory Response WP530	319.5812	10512.56	IL10;IL6;CD4;IFNG;IL1B;TNF;IL2
COVID 19 Adverse Outcome Pathway WP4891	554.4722	17209.82	IL10;CXCL10;IL6;IL1B;TNF;IL2
Network Map Of SARS CoV 2 Signaling WP5115	46.99957	1440.15	CRP;IL10;CXCL10;CD4;IL6;IFNG;CD8A;IL1B;ALB;HBB;TNF
Immune Infiltration In Pancreatic Cancer WP5285	189.6277	5642.53	IL10;IL6;IFNG;IL1B;TNF;IL2;VEGFA
Post COVID Neuroinflammation WP5485	293.4265	8228.323	IL10;IL6;IFNG;IL1B;TNF;IL2
Prostaglandin Signaling WP5088	184.6574	4737.775	CXCL10;IL6;IFNG;IL1B;TNF;VEGFA
Overview Of Proinflammatory And Profibrotic Mediators WP5095	59.65139	1497.601	IL10;CXCL10;IL6;IFNG;IL1B;TNF;IL2;VEGFA
T Cell Antigen Receptor TCR Pathway During Staph A. Infection WP3863	88.90179	1926.541	IL10;CD4;IFNG;CD8A;TNF;IL2

Table 5
Jensen_COMPARTMENTS

Term	Odds. Ratio	Combined. Score	Genes
Extracellular ferritin complex	138.0642	6672.236	CRP;IL10;HBB;HBA1;F2;TNF;IL2;CD4;IL6;IFNG;CD8A;IL1B;ALB
Ferritin complex	73.51493	3350.366	CRP;IL10;HBB;GPT;HBA1;F2;TNF;IL2;VEGFA;CD4;IL6;IFNG;CD8A;IL1B;ALB
Fc receptor complex	88.0212	3771.228	CRP;IL10;CMA1;PTPN11;TNF;IL2;CD4;IL6;FCGR2A;IFNG;CD8A;IL1B;ALB
Integrin alpha4-beta1 complex	88.0212	3771.228	IL10;HBA1;TNF;IL2;VEGFA;CXCL10;CD4;IL6;IFNG;CD8A;IL1B;ALB;SDC1
NF-kappaB complex	29.5242	1047.95	CRP;IL10;STAT2;PTPN11;TNF;IL2;VEGFA;CXCL10;CD4;IL6;FCGR2A;IFNG;CD209;CD8A;IL1B;ALB;SDC1
IgG immunoglobulin complex	34.59715	1208.48	CRP;IL10;F2;TNF;IL2;VEGFA;CXCL10;CD4;IL6;FCGR2A;IFNG;CD8A;IL1B;ALB;SDC1
Interleukin-23 complex	38.65837	1340.284	CRP;IL10;STAT2;PTPN11;TNF;IL2;VEGFA;CXCL10;CD4;IL6;FCGR2A;IFNG;CD8A;IL1B
Type III intermediate filament	26.80277	911.0763	CRP;IL10;HBA1;F2;TNF;IL2;VEGFA;CXCL10;CD4;IL6;IFNG;CD8A;MPZ;IL1B;ALB;PMP22;SDC1
Interleukin-12 complex	34.78571	1157.877	CRP;IL10;TNF;IL2;VEGFA;CXCL10;CD4;IL6;FCGR2A;IFNG;CD209;CD8A;IL1B;ALB
Thrombospondin complex	42.98361	1372.151	IL10;CD4;IL6;IFNG;CD8A;IL1B;ALB;SDC1;HBA1;F2;TNF;VEGFA
Integrin alpha5-beta1 complex	68.84028	2194.296	CD4;IL6;CD209;CD8A;IL1B;ALB;HBA1;TNF;IL2;VEGFA

Table 6
JensenTISSUES

Term	Odds. Ratio	Combined. Score	Genes
Peritoneal cavity	950.5238	46641.6	IL10;IL6;CD4;CD8A;IL1B;ALB;TNF;IL2;VEGFA
Bronchoalveolar lavage	150.6212	6937.932	CRP;IL10;CXCL10;CD4;IL6;IFNG;CD8A;IL1B;ALB;TNF;IL2;VEGFA
Mast cell	107.5415	4870.937	IL10;CMA1;PTPN11;TNF;IL2;VEGFA;CXCL10;CD4;IL6;IFNG;CD8A;IL1B;ALB
Tendon	133.8114	5987.838	CRP;IL10;CD4;IL6;IFNG;CD8A;IL1B;ALB;HBA1;TNF;IL2;VEGFA
Synovial tissue	157.7988	6805.719	CRP;IL10;CXCL10;CD4;IL6;IFNG;CD8A;IL1B;TNF;IL2;VEGFA
Immune system	36.92788	1568.415	CRP;IL10;CMA1;STAT2;HBB;PTPN11;HBA1;F2;TNF;IL2;VEGFA;CXCL10;CD4;IL6;FCGR2A;IFNG;CD8A;IL1B;ALB;SDC1
Peritoneal exudate	725.8182	30748.31	IL10;IL6;CD4;CD8A;IL1B;ALB;TNF;IL2
Stromal cell	77.64706	2999.351	IL10;CXCL10;CD4;IL6;IFNG;CD8A;IL1B;SDC1;HBA1;TNF;IL2;VEGFA
Sputum	203.3469	7738.582	CRP;IL10;CD4;IL6;IFNG;CD8A;IL1B;ALB;TNF
Neonate	129.1753	4878.093	CRP;IL10;IL6;IFNG;CD8A;IL1B;ALB;F2;TNF;IL2
Marrow	112.9659	4125.557	CD4;IL6;CD8A;IL1B;ALB;HBB;SDC1;TNF;IL2;VEGFA

This pathway represents a central mechanism for cytokine signal transduction and has been implicated in both antiviral defense and immunopathology in viral infections[11]. The dual role of JAK-STAT signaling in promoting both protective and harmful immune responses presents a complex therapeutic challenge, necessitating carefully timed interventions to balance viral clearance and immunopathology prevention. The exceptionally high odds ratio for regulation of calcidiol 1-monooxygenase activity (OR=1109.33) is particularly intriguing. This enzyme catalyzes the conversion of 25-hydroxyvitamin D to the active form 1,25-dihydroxyvitamin D, suggesting a potential link between vitamin D metabolism and Dengue pathogenesis[26].

Table 7
ChEA_2022

Term	Odds. Ratio	Combined. Score	Genes
RELA 24523406 ChIP-Seq FIBROSARCOMA Human	7.387476	78.91253	CRP;IL10;CXCL10;IL6;IFNG;CD209;IL1B;TNF;IL2
RELB 30642670 ChIP-Seq CTB1 Human Placenta Inflammation	22.60399	222.4534	IL10;IL1B;TNF;VEGFA
LMO2 26923725 Chip-Seq HEMANGIOBLAST Mouse	4.838239	34.27527	IL10;IVNS1ABP;IFNG;IL1B;PMP22;SDC1;GPT;VEGFA
STAT4 19710469 ChIP-ChIP TH1 Mouse	4.639814	25.96471	IL10;CXCL10;IL6;IFNG;STAT2;TNF
ADNP 35650610 ChIP-Seq mESC Mouse Stem Autism	6.613351	35.53287	IVNS1ABP;CD8A;SDC1;VEGFA
EBF1 22473956 ChIP-Seq LYMPHODE Mouse	3.752946	19.26744	CRP;IL10;IVNS1ABP;MPZ;IL1B;F2;IL2
PU.1 20513432 ChIP-Seq MACROPHAGES Mouse	3.742141	19.15795	IL10;IVNS1ABP;MPZ;STAT2;PMP22;GPT;VEGFA
RXR 22158963 ChIP-Seq LIVER Mouse	3.644857	18.18173	CRP;IVNS1ABP;CXCL10;CD4;STAT2;SDC1;GPT
NFI 21473784 ChIP-Seq ESCs Mouse	3.637174	18.10537	IL10;IVNS1ABP;IL6;CMA1;MPZ;PMP22;IL2
RAC3 21632823 ChIP-Seq H3396 Human	3.611777	17.85375	CRP;CXCL10;CD4;FCGR2A;HBA2;HBA1;VEGFA
LXR 22158963 ChIP-Seq LIVER Mouse	3.564415	17.38771	IVNS1ABP;CD4;MPZ;ALB;SDC1;GPT;VEGFA

Table 8
HMDB_Metabolites

Term	Odds.Ratio	Combined.Score	Genes
Simvastatin (HMDB05007)	146.1465	2454.34	IL6;IFNG;F2;TNF
Atorvastatin (HMDB05006)	95.02381	773.4757	CRP;TNF
Butyric acid (HMDB00039)	68.82759	282.9949	TNF
Phosphorylcholine (HMDB01565)	62.5674	251.8564	CRP
Oxygen (HMDB01377)	9.69863	37.62567	HBB;HBA1
L-Alanine (HMDB00161)	43.00431	158.2847	GPT
$C_{34}H_{34}N_4O_4$.Fe (HMDB03178)	8.47006	30.76854	HBB;HBA1
1,4-Naphthalenedione, 2-methyl- (HMDB01892)	36.20871	127.4662	F2
Bilirubin (HMDB00054)	29.90555	99.91101	ALB
Oxoglutaric acid (HMDB00208)	18.57689	53.71402	GPT
Famotidine (HMDB01919)	15.97995	43.93137	GPT

Recent studies have implicated vitamin D in modulating immune responses to various infections including viral diseases, through its effects on both innate and adaptive immunity[18]. This finding warrants further investigation into the potential protective or pathogenic roles of vitamin D in Dengue fever.

The cellular component analysis (Table 2) revealed significant enrichment in T cell receptor complex and immune-related compartments, emphasizing the central role of T cell-mediated immunity in Dengue pathophysiology. The involvement of CD4 and CD8A genes aligns with extensive literature documenting the contributions of both CD4+ and CD8+ T cells to both protection and immunopathology in Dengue[34]. The enrichment in endocytic vesicle components further supports the importance of viral entry and processing mechanisms in disease pathogenesis.

The molecular function analysis (Table 3) highlighted cytokine activity as the predominant function of Dengue-associated genes, with the involvement of multiple cytokines (IL10, CXCL10, IL6, IFNG, IL1B, TNF, IL2, VEGFA) reflecting the complex inflammatory milieu characteristic of Dengue infection. The high enrichment of growth factor activities suggests potential impacts on tissue repair and vascular function. The specific enrichment of interleukin-6 receptor binding further emphasizes the central role of IL-6 signaling in disease mechanisms. Pathway analysis through WikiPathways (Table 4) revealed unexpected associations with folate and vitamin B12 metabolism (ORs of 168.74 and

189.76 respectively). While not traditionally linked to Dengue pathogenesis, these metabolic pathways support rapid cell proliferation and DNA synthesis, processes critical for both immune cell activation and viral replication[5]. Additionally, folate deficiency has been associated with altered cytokine profiles and impaired immune responses, potentially influencing disease susceptibility and severity.

The extraordinarily high enrichment of cytokines and inflammatory response pathway (OR=319.58) aligns with the established inflammatory nature of dengue pathogenesis. Interestingly, the COVID-19 adverse outcome pathway showed the highest combined score (17209.82), highlighting significant overlap between the pathogenic mechanisms of these viral infections, particularly regarding inflammatory cascades and cytokine dysregulation. This finding suggests potential value in investigating therapeutic approaches effective in COVID-19 for application in dengue fever.

The subcellular localization analysis (Table 5) identified significant associations with various immune-related complexes including NF-kappaB complex, interleukin complexes and Fc receptor complex. The enrichment in NF-kappaB complex (OR=29.52) is particularly relevant given its central role in regulating inflammatory responses and its activation during Dengue virus infection. This transcription factor complex controls the expression of numerous cytokines implicated in dengue pathogenesis and represents a potential therapeutic target for modulating excessive inflammation.

Tissue expression analysis (Table 6) revealed remarkable enrichment in peritoneal cavity (OR=950.52) and peritoneal exudate (OR=725.82), suggesting significant involvement of peritoneal immunity in dengue pathophysiology, an aspect not extensively explored in previous literature. The high expression in bronchoalveolar lavage and synovial tissue aligns with the respiratory symptoms and arthralgia frequently observed in dengue patients. The broad expression across multiple immune-related tissues reflects the systemic nature of dengue infection and its widespread immunological impacts.

Transcription factor analysis (Table 7) identified RELA and RELB, components of the NF-κB family, as significant regulators of dengue-associated genes, particularly in inflammatory contexts. The enrichment of STAT4, involved in IL-12 signaling and T helper 1 (Th1) cell differentiation, suggests a skewing toward Th1 responses in dengue. This finding aligns with observations of elevated Th1 cytokines in acute dengue infection, although the balance between Th1 and Th2 responses likely influences disease outcomes. The metabolite associations (Table 8) revealed intriguing connections with statins (simvastatin and atorvastatin), suggesting potential roles for cholesterol metabolism in Dengue pathogenesis or therapeutic opportunities. Statins possess pleiotropic effects beyond cholesterol lowering including anti-inflammatory and immunomodulatory properties that could potentially attenuate the excessive inflammation in severe dengue. The association with butyric acid, a short-chain fatty acid with anti-inflammatory properties, further supports the relevance of immunomodulatory approaches in dengue management. Overall, this comprehensive genetic analysis illuminates the complex interplay of inflammatory pathways, immune cell activation, signaling cascades and metabolic processes in dengue pathophysiology. The consistent involvement of key cytokines (IL-6, TNF, IFN-γ, IL-1β) across multiple analyses reinforces their central roles in disease mechanisms and their potential as therapeutic targets. The identification of novel associations with metabolic pathways and specific cellular compartments opens new avenues for research and intervention development.

Conclusion

This comprehensive analysis of Dengue fever-associated genes provides significant insights into the molecular underpinnings of disease pathophysiology. Our findings highlight the central role of inflammatory pathways, particularly those involving IL-6, TNF, IFN-γ and IL-1β, in dengue fever manifestation. The significant enrichment of genes involved in cytokine production regulation, STAT protein phosphorylation and immune cell activation reflects the complex immunological landscape characteristic of dengue infection.

The unexpected associations with metabolic pathways including folate and vitamin B12 metabolism, suggest broader systemic impacts of Dengue virus infection than previously appreciated. Similarly, the strong tissue-specific expression patterns, particularly in peritoneal cavity and immune-related tissues, provide context for understanding the systemic manifestations of dengue fever. Our analysis identifies several potential therapeutic targets including cytokine signaling pathways, transcription factors such as NF-κB and metabolic modulators including statins. These findings align with emerging approaches to manage cytokine storm syndromes in viral infections through targeted immune modulation rather than immune suppression.

The significant overlap observed between dengue fever and COVID-19 pathways underscores common inflammatory mechanisms in severe viral infections and suggests potential for therapeutic approaches. Future research should focus on validating these genetic associations through functional studies and exploring their translational potential for biomarker development and therapeutic intervention. Longitudinal studies examining gene expression patterns throughout disease progression would further elucidate the temporal dynamics of these pathways and would identify optimal timing for interventions.

In conclusion, this study provides a comprehensive genetic framework for understanding dengue fever pathophysiology, highlighting key inflammatory mediators, cellular pathways and potential therapeutic targets that may

contribute to improved management of this significant global health challenge.

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