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Research Article

Gene Expression Profiling and Functional Annotation of Dengue Virus

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Abstract

Background: Detailed examinations of the Gene expression profiles and functional profiling is pre-eminent in characterizing the pathogenesis of dengue virus to promulgate the symptomatic dengue hemorrhagic fever. This paves the way to generate the predicted functions of gene targets in normal versus diseased data sample sets.

Methods: Here, we have investigated the gene expression profiling for dengue hemorrhagic fever, dengue fever, and normal human dataset. For generating DEGs establish, FunCoup2.0 has been used for every one of the systems all through the work and Cytoscape has been used for system perception. For a large portion of our coding and estimations, MATLAB has been utilized.

Results: Our investigation is a prescient plan dependent based on gene expression datasets related to dengue virus where the studies conclusively suggest that pathways and networks of expression and functional profiles contribute towards the prognosis of the disease.

Keywords: Dengue Virus, Pathogenesis, gene expression, innate immunity, dengue hemorrhagic fever, vascular fragility, comparative genomics.

Introduction

Dengue is a viral infection caused by four types of viruses (DENV-1, DENV-2, DENV-3, and DENV-4). These viruses are transmitted through the bite of infected *A. aegypti* and *A. albopictus* female mosquitoes that feed both indoors and outdoors during the daytime (from dawn to dusk) and are emerging or re-emerging pathogens. There are four independent serotypes of dengue virus that infect humans and leads to the diseases ranging from acute self-limiting febrile illness to life-threatening dengue hemorrhagic fever and the dengue shock syndrome. More than 2 billion people in tropical and subtropical regions are at risk of dengue virus infection, thus statistically aligning dengue to be eventually leading to 50 to100 million human infections and 24,000 deaths each year. So far there is no vaccine or antiviral therapy available for prevention and diagnostic (Eong Ooi E, 2011; T, 2012; Firth & Lipkin, 2013; DH, 2013). Risk of Dengue exists at the global level and mainly in tropical and subtropical areas of Central America, South America, Africa, Asia, and Oceania (http://www.infectionlandscapes.org/2011/01/dengue-part-2-mosquito-and-its-ecology.html)(Chaturvedi, Nagar & Shrivastava, 2006; Eong Ooi E, 2011).

Dengue virus is among the leading infectious disease which is potential risk among the developing countries. Being the most critically acclaimed infectious potential of Dengue, it demands deeper insights of molecular cues relating to the genetic aberrations as well as the gene expression modulations associated with the disease. Due to its nature of the devastating impact, it is of potential impact to understand alterations at multiple levels such as gene expression, genetic aberrations, etc. Ongoing work on the dengue virus focuses from multiple perspectives. In a previous study it has been shown that host response to DENV infection can now be presented in two distinct phases by using unique transcriptional markers where DHF signatures have been identified during day 1–3 may have applications in developing early Interepidemic evolution of

the circulating DENV might be responsible for increased severity of disease, suggesting that the circulating DENV might have become more virulent through passage in hosts during the epidemic stage(R, G & D, 1993; Cordeiro et al., 2007; Martina, Koraka & Osterhaus, 2009; Morrison et al., 2010) molecular diagnostics for DHF(Cordeiro et al., 2007; Ubol et al., 2008; Sun et al., 2013; Sim & Hibberd, 2016a) and have shown the global gene expression patterns(Sun et al., 2013). It has also been shown that some of the gene expression signatures displayed accuracy more than 95% which indicates that gene expression profiling with these signatures may prove as an essential step for DHF prognosis at the earlier stages during infection(Nascimento et al., 2009). There is a number of previous works where the gene expression profiling has been studied in DF and DHF and presented a promising perspective. Continuing this type of working strategy, here, we have mainly shown the gene expression profiling and its potential impact on the signaling pathways in a simplified way. For this purpose, we have not only analyzed the DEGs and inferred pathways but also examined the networks of DEGs. Based on this study, we conclude that there are clear sets of genes and their assumed functions for DF and DHF which also correlates with the previous research.

Methods

In this work, we have gathered gene expression datasets of ordinary, dengue fever, and dengue hemorrhagic fever from Gene Expression Omnibus (GEO)(Nascimento et al., 2009). Dengue datasets have been collected and processed from necessary steps to analysis and interpretation. These gene expression profiling datasets were generated from the Affymetrix Human Genome U133 Plus 2.0 Array. In short, the essential steps involved in the entire study are raw file processing, intensity calculation, and normalization. For normalization (Ideker et al., 2000; Quackenbush, 2002; Simon, 2008), GCRMA(Salomonis et al., 2007; Bild et al., 2009; Reimers, 2010; Girke, 2011; Chen et al., 2014), RMA and EB are the most commonly used approaches. Here, we have used EB for raw intensity normalization. After normalization, we proceed for our goal which is to understand the gene expression patterns (Lapointe et al., 2004; Subramanian & Tamayo, 2005) and its inferred functions(Subramanian & Tamayo, 2005; Mi et al., 2016).

For differential gene expression prediction and statistical analysis, MATLAB functions (e.g., matter) have been used. For pathway analysis, we used the Panther database (Mi et al., 2016) and have our own code designed to the pathway and network analysis.

For generating DEGs network, FunCoup2.0(Alexeyenko & Sonnhammer, 2009a) has been used for all the networks throughout the work and Cytoscape has been used for network visualization. For

most of our coding and calculations, MATLAB has been used. FunCoup predicts four different classes of functional coupling or associations such as protein complexes, protein- protein physical interactions, metabolic, and signaling pathways.(Alexeyenko & Sonnhammer, 2009b).

Results

In this study, we have used the publicly available array expression profiling dataset from GEO (Gene Expression Omnibus) which contains dengue hemorrhagic fever (DHF (9 patients), dengue fever (DF (9 patients), and control sample (ND (8 uninfected with dengue). Here, our primary goal was to study the role of NS5 in the dengue-infected human immune system and associated pathways.

Gene expression profiling during early acute febrile stage of dengue infection can predict the disease outcome:

For the selected dataset, the DEGs (differentially expressed genes) and their inferred pathways have been identified in different combinations (DF versus ND, DHF versus ND, and DF versus DHF).

Dengue virus drives potential change in a large number of genes expression and leads to dengue and dengue hemorrhagic fever:

Based on our analysis we observe that gene expression pattern shows significant diversity in terms of expression level. After infection with dengue virus, a large number of genes display the alterations in the expression level. From ND to DF and DHF, the gene expression change increases and mainly in DHF, we see massive impact of dengue infection on the infected genes (Figure 1B), and similarly, the number of inferred pathways are also very high in case of DHF patients (Figure 1C). The number of shared genes between all the samples are only 10, the shared pathways are 15, DF-DHF specific genes were four and inferred pathways only one, DF-ND particular genes and pathways are 19 and 11, DHF—ND specific genes and pathways are very high (823 DEGs and 50 pathways) compared to the others (Figure 1B and 1C). The shared DEGs and pathways are comparative high between DHF versus ND and DF versus ND.

Top-ranked genes infer the most critical signaling pathways: Here, we have selected top 30 genes (up and down-regulated) and analyzed the inferred pathways which leads to the critical pathways such as T-cell activation, Angiogenesis, EGFR signaling, G-protein signaling, and Wnt signaling (in DHF versus ND), Ubiquitin proteasome signaling, nicotine degradation, Wnt signaling, circadian clock, cell cycle, and p53 pathway (in DF versus ND), and Apoptosis, TLR, IGF, p53, blood coagulation, and metabolic pathways (Figure 2a and 2b and Table S1).

Immune system and pathways associated with it are significant

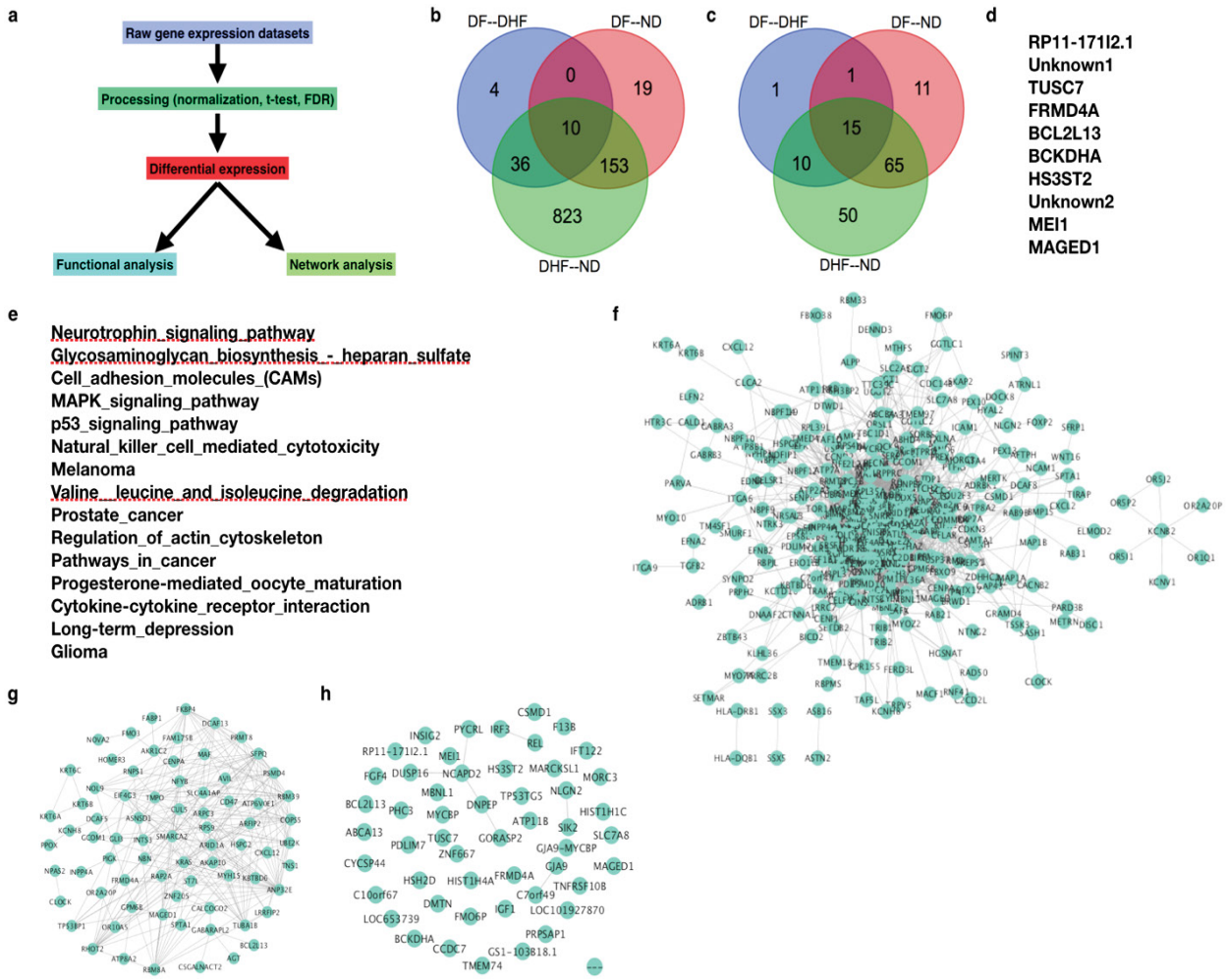

Figure 1: Gene expression profiling in case of dengue infection: (a) Workflow. Venn diagram for (b) DEGs and (c) Inferred pathways. (d) Common DEGs between all the samples (e) Commonly inferred pathways for all the samples. DEG networks (f) DHF versus DN, (g) DF versus DN, and (h) DF versus DHF.

targets for dengue virus: Based on the inferred pathways, we observe that there are a number of paths which either direct part of immune system or potentially control/contribute to healthy immune system function are among the altered pathways list which means that their pathway components (genes) are dominantly present among the DEGs as a result of dengue infection (Table 1A-C and Table 1).

After analyzing the DEGs and inferred pathways, we have also

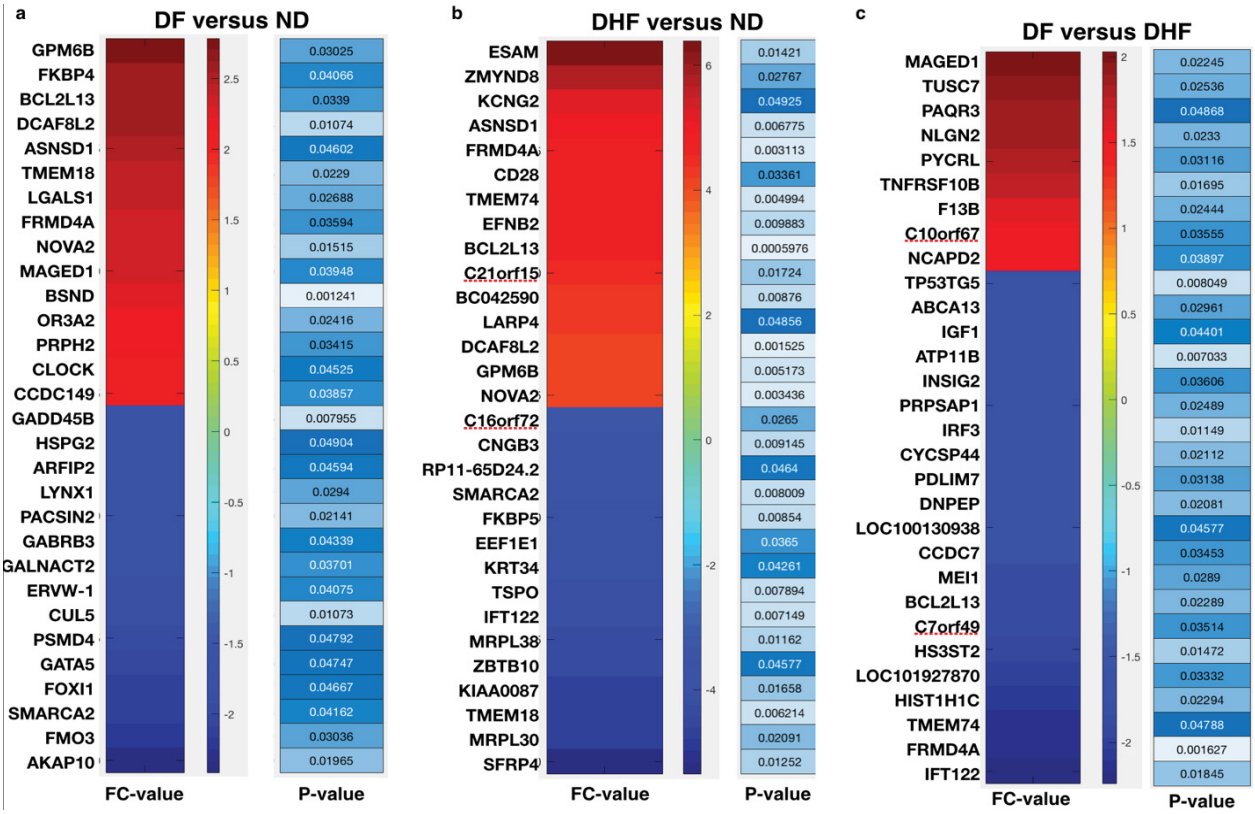

Figure 2: Top 30 DEGs followed by their fold changes and p-values: (a) DF versus ND (b) DHF versus ND, and (c) DF versus DHF.

A.	B.	C.
Common DEGs & pathways between DH vs. DHF and DHF vs. ND	**DF-DHF-specific (1 pathway) Complement and coagulation cascades DF-ND- specific (11 pathways)**	**DHF-ND specific (50 pathways)**
IGF1	Renin- angiotensin system Glycosphingolipid biosynthesis-lacto and neolacto series	04350 TGF beta signaling pathway
IFT122		00480 Glutathione metabolism
PDLIM7		00983 Drug metabolism- other enzymes
GJA9-MYCBP		
C10ORF67		05010 Alzheimer's disease
MRAP2	Glycosaminoglycan biosynthesis-chondroitin sulfate	00040 Pentose
IRF3		00020 TCA cycle
TMEM74		00670 One carbon pool by folate
GS1-103B18.1	Glycosylphosphatidy inositol (GPI)- anchor biosynthesis photo transduction	02020 Two component system
NLGN2		00140 Steroid hormone biosynthesis
MORC3	Glycosaminoglycan biosynthesis-keratan sulfate	00460 Cyanoamino acid metabolism
GJA9		00600 Sphingolipid metabolism
PAQR3		03050 Proteasome
C7orf49		04130 SNARE interaction
FGF4	Amino sugar and nucleotide sugar metabolism	00590 Arachidonic and metabolism
SIK2		04940 Type I diabetes mellitus
CYCSP44	Basal cell carcinoma Lysosome	00071 Fatty acid metabolism
CSMD1		04270 Vascular smooth muscle
SLC7A8		04950 Maturity onset diabetes
INSIG2	N-Glycan biosynthesis	05416 Viral myocarditis

ATP11B	Streptomycin biosynthesis	04330 Notch signaling pathway
MBNL1		04630 Jak-STAT signaling pathway
DNPEP		00625 Tetrachloroethene degradation
F13B		04020 Calcium signaling pathway
NCAPD2		00361 Gamma- hexacholrocycolhexane
TNFRSF10B		00591 Linoleic acid metabolism
HSH2D		00230 Purine metabolism
LOC101927870		00830 Retinol metabolism
REL		00363 Bisphenol A degradation
GORASP2		00290 Valine, leucine and isoleucine
MYCBP		04520 Adherence junction
DUSP16		00270 Cysteine and methionine
CCDC7		00770 Pantothenate
PYCRL		00650 Butanoate metabolism
LOC653739		04920 Adipocytokine signaling pathway
ABCA13		05140 Leishmaniasis
Arginine and proline metabolism		04640 Hematopoietic cell linage
mTOR signaling pathway		00260 Glycine, threonine and serine
ABC transport		
Oocyte meiosis		00430 Taurine
Cytosolic DNA-sensing pathway		03020 RNA polymerase
RIG-I-like receptor signaling pathway		
Apoptosis		00620 Pyruvate metabolism
Focal adhesion		00970 Amino acyl tRNA biosynthesis
Cell cycle-yeast		04621 NOD like receptor signaling
Toll-like receptor signaling pathway		03030 DNA replication
		00240 Pyrimidine metabolism
		05414 Dilated cardiomyopathy
		05142 Chagas disease
		05310 Asthma
		05222 Small cell lung cancer
		00120 Primary bile acid biosynthesis 03022 Basal Transcription factor

Table 1: Condition-specific pathways: (A) Common DEGs & pathways between DH vs. DHF and DHF vs. ND (B) DF—DHF-specific (1 pathway) and (C) DF—ND-specific (11 pathways) and DHF—ND-specific (50 pathways).

investigated the genes connectivities within the DEGs networks (Figure 3), and the genes connectivity in the DEG networks for DHF versus DN and the inset figure represents top 50 genes with the highest connectivity, DF versus DN, and DF versus DHF. Here, we observe that the top-ranked genes are associated with the significant pathways which mostly infer the critical disease-associated pathways. Based on this result, we conclude that the DHF versus DN DEGs network contains the genes which have the comparatively very high number of genes altered and since more than 50 genes display the connectivity with 20 or higher number of genes, so it also leads to higher level of alterations.

Discussion

Dengue virus is among the leading infectious disease which is potential risk among the developing countries (Seema & Jain, 2005). Due to its nature of the devastating impact, it is of potential implications to understand alterations at multiple levels such as gene expression, genetic aberrations, etc. However, still, it is a great challenge to understand the molecular basis of pathogenesis of the different manifestations of dengue virus infections in humans. In the previous studies, the focus has been towards gene expression profiling (Ubol et al., 2008),(Sim & Hibberd, 2016b) in dengue infection but different from the previous study. The innate immune mechanisms provide the host with the time needed to induce the more slowly developing adaptive immunity maximally. Here, we have mainly presented gene expression profiling and its potential impact on the signaling pathways. For this purpose, we have not only analyzed the DEGs and inferred pathways but also examined the networks of DEGs (Figure 1F—1H).

As we have mentioned that top 30 genes (up and down-regulated) and analyzed the inferred pathways which leads to the critical pathways such as T-cell activation, Angiogenesis, EGFR signaling, G-protein signaling, and Wnt signaling (in DHF versus ND), Ubiquitin proteasome signaling, nicotine degradation, Wnt signaling, circadian clock, cell cycle, and p53 pathway (in DF versus ND), and Apoptosis, TLR, IGF, p53, blood coagulation, and metabolic pathways. Based on this we conclude that there are some pathways which are exclusively affected for DHF and DF.

In the past few decades, a large number of research on dengue has resulted in a host of literature, which strongly suggests that the pathogenesis of DHF and DSS involves viral virulence factors and detrimental host responses.(Martina, Koraka & Osterhaus, 2009). In particular, the DHF-explicit top positioned pathways are T-cell initiation which is one of the fundamental pieces of the versatile invulnerable framework. There are other essential pathways in for DF and DHF explicit which will be useful to continue toward the definite and improved precision conclusion. A customized way to deal with the investigation of dengue pathogenesis will illustrate the

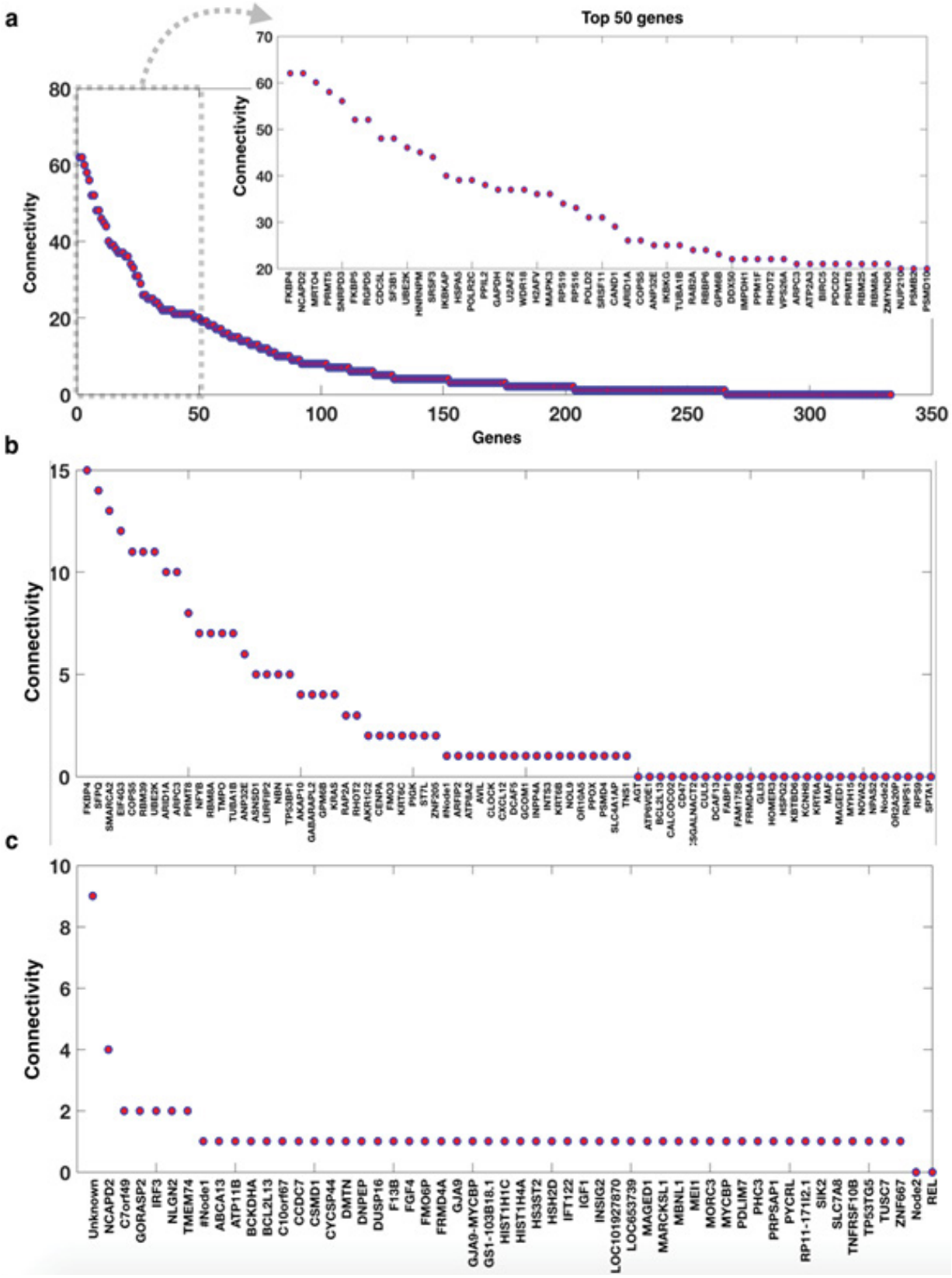

Figure 3: Genes connectivity in the DEG networks (A) DHF versus DN and the inset figure represents top 50 genes with the highest connectivity, (B) DF versus DN, and (C) DF versus DHF.

premise of particular hazard for the advancement of DHF and DSS as distinguishing the hereditary and natural bases for contrasts in danger for improvement of a severe ailment. In this way, on the off chance that we look from future and significant points of view, it is of potential noteworthiness and promising for biomarker advancement and improved demonstrative methodology.

Conclusion

Dengue is one of the noteworthy dangerous ailments which impacts about 2.5 billion people who live in dengue-endemic regions. The genome of dengue diseases is single-stranded positive RNA. In this endeavor, we expected to inspect the pathways initiated by the dengue ailment by separating quality articulation data. Our outcome prompts the end that on account of dengue disease potential focuses for dengue infection are either connected with the insusceptible framework or the pathways related to it and their parts. Ramifications of these discoveries give robotic bits of knowledge into the natural insusceptible framework in balancing dengue infection transmission and its pathogenesis.

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Conflicts of interest

There is no conflict.

References

1. Eong Ooi E (2011) Dengue and dengue hemorrhagic fever. Tropical Infectious Diseases New York: Saunders Elsevier, USA.
2. Jong E, Stevens TT (2012) Arboviruses of medical importance. Netter's Infectious Diseases. New York: Saunders Elsevier, USA.
3. Firth C, Lipkin WI (2013) The genomics of emerging pathogens. Annual Review of Genomics and Human Genetics 14: 281-300.
4. DH L (2013) Dengue and dengue hemorrhagic fever. Hunter's Tropical Medicine and Emerging Infectious Diseases. New York: Saunders Elsevier, USA.
5. Chaturvedi UC, Nagar R, Shrivastava R (2006) Dengue and dengue haemorrhagic fever: implications of host genetics. FEMS Immunology & Medical Microbiology 47: 155-166.
6. RS, GB, DS (1993) A primary dengue 2 epidemic with spontaneous haemorrhagic manifestations. Lancet (London, England) 342: 560-561.
7. Cordeiro MT, Silva AM, Brito CAA, Nascimento EJM, Magalhães MCF (2007) Characterization of a dengue patient cohort in Recife, Brazil. The American Journal of Tropical Medicine and Hygiene 77: 1128-1134.
8. Martina BEE, Koraka P, Osterhaus ADME (2009) Dengue virus pathogenesis: An integrated view. Clinical Microbiology Reviews 22: 564-581.
9. Morrison AC, Minnick SL, Rocha C, Forshey BM, Stoddard ST (2010) Epidemiology of dengue virus in Iquitos, Peru 1999 to 2005: Interepidemic and epidemic patterns of transmission. PLOS Neglected Tropical Diseases 4: e670.
10. Ubol S, Masrinoul P, Chaijaruwanich J, Kalayanarooj S, Charoensirisuthikul T (2008) Differences in global gene expression in peripheral blood mononuclear cells indicate a significant role of the innate responses in progression of dengue fever but not dengue hemorrhagic fever. Journal of Infectious Diseases 197: 1459-1467.
11. Sim S, Hibberd ML (2016). Genomic approaches for understanding dengue: Insights from the virus, vector, and host. Genome Biology 1-15.
12. Sun P, García J, Comach G, Vahey MT, Wang Z (2013) Sequential waves of gene expression in patients with clinically defined dengue illnesses reveal subtle disease phases and predict disease severity. PLoS Neglected Tropical Diseases 7: 2298.
13. Nascimento EJM, Braga-Neto U, Calzavara-Silva CE, Gomes ALV, Abath FGC (2009) Gene expression profiling during early acute febrile stage of dengue infection can predict the disease outcome. Plos One 4: 7892.
14. Ideker T, Thorsson V, Siegel AF, Hood LE (2000) Testing for differentially-expressed genes by maximum-likelihood analysis of microarray data. Journal of Computational Biology 7: 805-817.
15. Quackenbush J (2002) Microarray data normalization and transformation. Nature Genetics 32: 496-501.
16. Salomonis N, Hanspers K, Zambon AC, Vranizan K, Lawlor SC (2007) GenMAPP 2: New features and resources for pathway analysis. BMC Bioinformatics 8: 217.
17. Bild AH, Parker JS, Gustafson AM, Acharya CR, Hoadley KA (2009) An integration of complementary strategies for gene-expression analysis to reveal novel therapeutic opportunities for breast cancer. Breast Cancer Research 11: 55.
18. Reimers M (2010) Making informed choices about microarray data analysis. PLOS Computational Biology 6: 1000786.
19. Girke T (2011) Microarray analysis. 1: 42.
20. Chen KH, Wang KJ, Tsai ML, Wang KM, Adrian AM (2014) Gene selection for cancer identification: a decision tree model empowered by particle swarm optimization algorithm. BMC Bioinformatics 15: 1-10.
21. Lapointe J, Li C, Higgins JP, Van de Rijn M, Bair E (2004) Gene expression profiling identifies clinically relevant subtypes of prostate cancer. Proceedings of the National Academy of Sciences of the United States of America 101: 811-816.
22. Subramanian A, Tamayo P (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles 2: 1.
23. Mi H, Poudel S, Muruganujan A, Casagrande JT, Thomas PD (2016) PANTHER version 10: Expanded protein families and functions, and analysis tools. Nucleic Acids Research 44: 336-442.
24. Alexeyenko A, Sonnhammer ELL (2009) Global networks of functional coupling in eukaryotes from comprehensive data integration. Genome Research 19: 1107-1116.
25. Alexeyenko A, Sonnhammer ELL (2009) Global networks of functional coupling in eukaryotes from comprehensive data integration. Genome Research 19: 1107-1116.
26. Seema, Jain SK (2005) Molecular mechanism of pathogenesis of dengue virus: Entry and fusion with target cell. Indian Journal of Clinical Biochemistry 20: 92-103.
27. Sim S, Hibberd ML (2016) Genomic approaches for understanding dengue: insights from the virus, vector and host. Genome Biology pp. 1-15.