

Review

Host genetic susceptibility to severe dengue infection

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Abstract: Epidemiological evidence indicates that host genetic factors are relevant and predispose DHF/DSS development. Here, we review the host genetic studies concerning human leucocyte antigens, antibody receptors, immune/inflammatory mediators, attachment molecules, cytokines and other factors exerting an immunoregulatory effect as well as the current genome-wide association studies. We also discuss some viewpoints on future challenges related to the design of safe and effective prevention and treatment options.

Key words: dengue fever, dengue hemorrhagic fever, dengue shock syndrome, genetic susceptibility, HLA

1. INTRODUCTION

Increasing evidence for the major role of host genetics has accumulated in research on inter-individual variation in susceptibility to infectious diseases, particularly since a HLA allele was found to be susceptible to leprosy (1980) from early case-control studies of unrelated individuals with disease and unaffected controls. Host genetic studies on infectious diseases have identified a number of novel gene associations that implied the molecular pathways involved in disease pathogenesis [1].

Over the last decade since the completion of the human genome project, host genetic studies have robustly progressed using single nucleotide polymorphism (SNP) analysis, DNA sequencing, DNA microarrays, and cytogenetic methods. Recently, the technological development of high throughput genotyping has provided a powerful tool to examine the genetic basis of disease through Genome-Wide Association Studies (GWASs). This approach has considerably increased the number of known genes associated with major diseases [2].

Dengue infections (DI) are a serious cause of morbidity and mortality in tropical and subtropical areas of the world including Southeast and South Asia, Central and South America, and the Caribbean [3]. The disease is caused by dengue virus (DV), a *flavivirus* transmitted to humans mainly by infected *Aedes aegypti* mosquitoes [4]. Infection by any of the 4 serotypes of DV, DV-1, -2, -3, and -4, may result in a wide clinical spectrum, ranging from asymptomatic to fever (DF), haemorrhagic fever (DHF) and shock syndrome (DSS), the life threatening complications being

characterized mainly by plasma leakage. Recently, WHO suggested a new classification which separates severe dengue patients from those with non-severe dengue [5]. To be consistent with the publications reviewed, we use WHO 1997 criteria in this paper.

The postulated factors exerting an influence on manifestations can be divided into viral and host factors.

Viral factors: Higher blood viral load was detected in DHF patients in comparison to DF patients [6]. DV-2 had a larger pleural effusion index than the other virus serotypes in Thailand [6] and clearly related to the severe clinical forms in a study in Vietnam [7]. Imported to America, this genotype is thought to be the cause of the appearance of DHF in this region [8], providing additional proof for the higher virulence of the Asian DV-2 genotype.

Host factors: Plasma leakage happens often occurs at day 4–6 of fever when the viremia has already declined [9], revealing the role of host immunopathological mechanisms in disease severity. Both dengue-virus-infected monocytes with Antibody-Dependent Enhancement (ADE) phenomenon [10], and activated specific T lymphocytes are responsible for the rapidly increased levels of cytokines in DHF [11, 12]. These cytokines, especially TNF- α , IFN- γ , and chemical mediators, including IL-1 and IL-6 from mast cell [13], play a key role in inducing important clinical manifestations of DHF, that is, plasma leakage and shock. However, how the virus-host interaction causes the clinical outcome remains an important question. Epidemiological evidence indicates that host genetic factors are relevant and predispose the DHF/DSS development.

In this review, we summarize the findings from host

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genetic polymorphism studies concerning human leucocyte antigens, antibody receptors, immune/inflammatory mediators, attachment molecule, cytokines and other factors exerting immunoregulatory effects, as well as the current status of genome-wide association studies. The accumulated evidences, integrated properly, will provide important insights into the pathogenesis of severe dengue and shed light on new prevention and treatment options.

2. HOST GENETIC FACTORS INVOLVED IN THE CLINICAL COURSE OF DENGUE VIRUS INFECTION

2.1 The major histocompatibility complex (MHC)

a. Human leucocyte antigen (HLA) –class I and class II sub regions

HLA is encoded by the major histocompatibility complex (MHC), located on the chromosome 6 in humans. The genes encoding HLA class I (HLA-A, -B, -C) and class II (HLA-DR, DQ and DP) are the most polymorphic in the human genome [14]. Both class I and II molecules are involved in displaying peptide antigen to host T lymphocytes [15], being the essential recognition elements of acquired and innate immune responses to viruses [16]. However, the interaction between antigenic epitopes and the host immune system varies with the HLA allele involved [17]. Polymorphisms in HLA alleles may correlate with differential T cell profiles that lead to variable anti-viral responses [18]. The host HLA allele profile influenced the reactivity of DV-specific T cells [19] and may be responsible for the immunopathology of DV infection [20]. This is why HLA is the most extensively studied allele regarding the association with severity of DV infection.

Both HLA classes I and II genes were intensively analyzed (Table 1A, 1B). As in many genetic studies, the case-control study of HLA association to dengue infection often compares HLA allele frequencies in DF, DHF/DSS patients (each form or combined) with those in healthy controls of the same ethnicity, or by level of disease severity (DHF/DSS vs. DF or controls). A new trend consistent with 2009 WHO criteria can be seen in studies that separate the most severe form, DSS and DHF/DF [7, 21, 22, 23], or even asymptomatic cases [22, 24].

With certain HLA alleles, DV serotype could affect the clinical outcome, especially DV-2 in Southeast Asia (SEA), as in the case of B*52 allele associated with secondary DF only in DV-2 in the Thai population [25], or DRB1*0901 which particularly protects the development to DSS from DHF in the Kinh ethnic with DV-2 infection [7].

As shown in Table 1, many HLA class I alleles have been shown to be associated with severe dengue in secondary DI, suggesting the importance of the existing primed

memory HLA class I-restricted cross-reactive T cell. This is not always the case, however, as in A*24 association with primary infections in a Vietnamese study [7]. Meanwhile, HLA class II, especially DRB1 alleles, more likely exerted a protective effect on DI [7, 26, 27] and disease severity [26]. A better understanding of this protection mechanism may lead to novel preventive and immuno-therapeutic approaches, including vaccines.

b. HLA-class III sub-region

Located in the central or class III sub-region of MHC region [28, 29], tumor necrosis factor (TNF), an important vasoactive immuno-modulators produced by activated monocytes, is known to be up-regulated in DHF infections [30]. As shown in Table 1C, polymorphism in the promoter region of the TNF- α gene, -308A allele was identified as a risk for the development of DHF in South American patients [23, 31] but not in Southeast Asian patients, despite the much larger sample size of the latter [32, 33]. This SNP was reported to be important in diabetes mellitus, asthma, and allergic rhinitis [34], most being associated with DHF [35]. Another SNP in TNF- α promoter gene, TNF- α -238A, was found to have no significant association in Vietnamese [32] but to be significantly increased in secondary DHF Thai patients when compared with healthy controls [33]. The study in Thailand has also identified an extended HLA class I, II, III (TNF, LTA) haplotype in secondary DHF patients, correlating with in vivo intracellular cytokine production in the acute viraemic phase of infection [33].

c. Integrated comprehension of the MHC associations

Regarding disease severity association, some differences were observed in HLA allele frequency when comparing DHF/DSS and DF, including A*0203, B*52 [25] and DRB1*04 [26] as protective factors; while B*51 [25], DQB1*0302 [35] and TNF- α - LTA haplotype [33] were noted as susceptible factors in Thai and Mexican studies. These findings suggest that DF and DHF arise from distinct immune response processes.

It has been thirty years since the first report on HLA association to dengue infection [36]. Many associations have been cited in the interim, but none has been replicated except for susceptible HLA- A*24 alleles in Vietnamese [7, 32]. Moreover, the latter finding was reproduced in two different study sites in Vietnam. A study in Malaysia also found a DHF risk trend (2 fold) of A*24 in a Chinese group [37]. Further subtyping, A*2402/03/10 with histidine at codon 70, elicited a stronger significant association in the Vietnamese but not in the Malaysian population, although the latter involved a much smaller sample size. The HLA class II associations in the Kinh population, however, were not consistent with the above findings [7, 32].

Table 1. Association of classical class I and II HLA polymorphism and dengue

A. Cases vs. healthy controls										
HLA Allele	Infection	Serotype	Case (n)	Control	OR	95% CI	P value	Pc value	Population	Reference
Susceptible										
A*0203	2nd	DV-1	DF (49)	140	2.65	1.14–46.12	0.02	ns	Thai	25
A*0203	2nd	DV-3	DF (26)	140	3.4	1.61–12.03	<0.0005	0.012	Thai	25
A*0203	2nd	all	DF (106)	140	3.09	1.59–6.02	<0.0005	0.012	Thai	25
B*52	2nd	DV-2	DF (17)	140	24.91	4.61–150.14	<0.0001	<0.0001	Thai	25
B*52	2nd	–	DF (106)	140	5.83	1.48–26.80	0.0067	0.027	Thai	25
DQB1*01	–	–	DF (23)	34	3.12	0.09–10.9	0.04	ns	Mexican	35
DQB1*0202	–	–	DF (23)	34	7.00	1.11–73.8	0.012	ns	Mexican	35
DQ1	–	–	DF (64)	201	2.4	2.4	<0.01	0.021	Brazilian	55
A*24, codon 70 histidine	–	–	DHF (59)	200	2.02	1.01–3.95	0.038	–	Vietnamese, VL 04-05	7
A*24, codon 70 histidine	–	–	DHF (117)	250	1.75	1.02–2.98	0.033	–	Vietnamese, HCMC	7
A*0207	2nd	DV-1	DHF/DSS (32)	140	2.73	1.03–7.18	0.043	ns	Thai	25
A*0207	2nd	DV-2	DHF/DSS (36)	140	2.64	1.03–6.70	0.041	ns	Thai	25
A*0207	2nd	DV-1, DV-2	DHF/DSS (103)	140	2.68	1.26–5.72	0.0084	ns	Thai	25
A*0207	2nd	All	DHF/DSS (103)	140	2.35	1.19–4.68	0.012	ns	Thai	25
A*03	–	–	DHF/DSS (51)	95	5.23	1.19–23.02	0.015	ns	Malay, Chinese, Indian	37
B*13	–	–	DHF/DSS (19)	95	–	–	0.049	ns	Malay	37
B*51	2nd	All	DHF/DSS (103)	140	4.11	1.44–12.28	0.0052	0.021	Thai	25
B*51	2nd	DV-1	DHF/DSS (32)	140	4.14	1.0–16.85	0.049	ns	Thai	25
B*53	–	–	DHF/DSS (51)	95	–	–	0.042	ns	Malay, Chinese, Indian	37
A*24	–	–	DHF/DSS (309)	251	1.54	1.05–2.25	0.021	–	Vietnamese	32
A*02	2nd	–	DSS (41)	138	–	–	0.047	–	Thai	36
A*24, codon 70 histidine	–	–	DSS (152)	250	1.89	1.16–3.09	0.008	–	Vietnamese, HCMC	7
A*24, codon 70 histidine	–	–	DSS (170)	200	1.7	1.04–2.79	0.03	–	Vietnamese, VL 02-03	7
A*24, codon 70 histidine	–	–	DSS (96)	200	2.09	1.18–3.70	0.0075	–	Vietnamese, VL 04-05	7
B blank	2nd	–	DSS (41)	138	–	–	<0.02	–	Thai	36
A*31	–	DV-2	DF, DHF/DSS (120)	189	7.6	2.3–27.7	<0.0001	0.0002	Cuban	27
B*15	–	DV-2	DF, DHF/DSS (120)	189	4.46	1.96–10.29	<0.0001	0.0002	Cuban	27
B*51	2nd	DV-3	DF, DHF/DSS (51)	140	4.16	1.22–14.45	0.018	ns	Thai	25
Resistant										
DRB1*11	–	–	DF (47)	34	0.09	0–0.64	0.003	0.03	Mexican	26
DQB1*0302	–	–	DF (23)	34	0.23	0.06–0.84	0.011	ns	Mexican	35
DRB1*0901	1st	–	DHF (59)	200	0.22	0.02–0.95	0.027	0.3	Vietnamese, VL 04-05	7
A*33	–	–	DHF/DSS (309)	251	0.56	0.34–0.93	0.014	–	Vietnamese	32
B*18	–	–	DHF/DSS (51)	95	–	–	0.017	ns	Malay, (Chinese, Indian)	37
B*13	2nd	–	DSS (41)	138	–	–	–	–	Thai	36
DRB1*0901	–	–	DSS (170)	200	0.56	0.34–0.93	0.018	0.2	Vietnamese, VL 02-03	7
DRB1*0901	–	–	DSS (96)	200	0.37	0.18–0.72	0.0018	0.02	Vietnamese, VL 04-05	7
B*35	–	–	DF, DHF/DSS (39)	34	0.12	0.037–0.39	<0.0001	0.01	Mexican	35
DRB1*04	2nd	DV-2	DF, DHF/DSS (77)	189	0.19	0.05–0.63	0.001	0.01	Cuban	27
DRB1*07	–	–	DF, DHF/DSS (120)	189	0.25	0.11–0.55	0.0001	0.0004	Cuban	27
B. DHF/DSS vs. DF										
HLA Allele	Infection	Serotype	DHF/DSS	DF	OR	95% CI	P value	Pc value	Population	Reference
Susceptible										
B*48	2nd	–	132	169	–	–	<0.001	<0.035	Thai	33
B*51	2nd	–	103	106	3.07	1.07–9.22	0.036	ns	Thai	25
DQB1*0302	–	–	16	23	5.02	1.05–25.34	0.018	ns	Mexican	35
Resistant										
A*0203	2nd	–	103	106	0.41	0.2–0.82	0.01	ns	Thai	25
B*52	2nd	–	103	106	0.08	0.00–0.59	0.049	0.02	Thai	25
DRB1*04	–	–	34	47	0.31	0.11–0.85	0.011	ns	Mexican	26
C. Class III										
HLA Allele	Infection	Serotype	Cases	Controls	OR	95% CI	P value	Pc value	Population	Reference
TNF- α (-308 -238 +488) GAG	2nd	–	DHF/DSS (132)	NC (143)	2.38	1.01–5.7	0.03	ns	Thai	33
TNF- α -308 A	–	DV-1/DV-2	DSS (43)	NC (99)	3.51	1.77–7.00	0.00006	0.0001	Cuban	23
TNF- α -308AG	–	DV-1/DV-2	DSS (43)	NC (99)	4.07	1.45–11.43	0.005	0.013	Cuban	23
TNF- α (-308/-238/+488) GGA	1st	–	DHF/DSS (10)	DF (59)	12.2 *	1.73–86.1**	0.019	ns	Thai	33
TNF- α (-308/-238/+488) GAG	2nd	–	DHF/DSS (132)	DF (169)	4.13	1.59–11.17	< 0.001	0.022	Thai	33
TNF-1,4 LTA-1,3 & TNF-1,4 LTA-3,3	2nd	–	DHF/DSS (129)	DF (163)	–	–	<0.001	0.014	Thai	33
TNF-238A & LTA (+249/+365/+720) AGC	–	–	DHF (129)	DF (163)	4	1.4–11.7	0.003	0.034	Thai	33
TNF- α (-308 -238 +488) GGA	1st	–	DF (59)	NC (143)	0.24	0.04–1.15	0.046	ns	Thai	33
TNF- α -308 GG	–	DV-1/DV-2	DSS (43)	NC (99)	0.35	0.16–0.75	0.007	0.001	Cuban	23

Note: DF: dengue fever, DHF: dengue hemorrhagic fever, DSS: dengue shock syndrome. 1st: Primary infection, 2nd: Secondary infection.

DV: dengue virus (serotype). OR: Odds ratio, 95% CI: 95% confidence intervals, Pc: corrected p, ns: non significant, Author data: *0.08 ; **0.01–0.76

2.2 Non MHC genes

Studies on the association between susceptibility to DI and polymorphic non-HLA gene have increased recently (Table 2).

a. Fcγ receptor II (FcγRII, CD32)

FcγR is a widely distributed receptor for IgG subclasses and can mediate ADE in DI [38]. Homozygotes for the arginine variant at position 131 (R/R131) of the FcγRII gene were shown to be protective against DSS in a Vietnamese population [39], and also against DHF/DSS in a Cuban population with asymptomatic control [22]. This is

the first consistent finding of host genetic analysis in DI between two very different ethnic groups from Southeast Asia and South America.

b. Vitamin D receptor (VDR)

VDR, an immune mediator expressed on monocytes, activated B and T cells. VDR polymorphism (rs731236 T/C) analysis in a Vietnam study has shown that C allele (author named as t allele) at position 352 was resistant to DSS (p trend analysis) [39]. This correlates with a recent report that VDR T allele was a risk factor of type 2 diabetes with insulin secretion capacity [40].

Table 2. Non-HLA polymorphism and dengue

A. Cases vs. healthy controls										
Factors	Infect	Serotype	Case	Control	OR	95% CI	p	p _c	Population	Reference
Susceptible										
IL-10 (-1082/-819/-592) ACC/ATA	–	DV-1/DV-2	DSS (43)	NC (99)	2.54	1.12–5.73	0.02	0.03	Cuban	23
TNFα-308A & INFγ 874T	–	DV-1/DV-2	DSS (43)	NC (99)	3,968	1.48–10.62	0.004	0.009	Cuban	23
TNFα-308A & IL-10 (-1082/-819/-592) ACC/ATA	–	DV-1/DV-2	DSS (43)	NC (99)	10,057	2.03–49.72	0.001	0.003	Cuban	23
TNFα-308A & IL-10 (-1082/-819/-592) ACC or ATA	–	DV-1/DV-2	DSS (43)	NC (99)	3,924	1.78–8.64	0.0004	0.001	Cuban	23
IFN-γ & IL10 (-1082/-819/-592) ACC/ATA	–	DV-1/DV-2	DSS (43)	NC (99)	17,306	2.05–145.73	0.001	0.002	Cuban	23
TNFα-308A & TGF1 codon 25C	–	DV-1/DV-2	DSS (43)	NC (99)	5,333	1.50–18.89	0.005	0.013	Cuban	23
TNFα-308A & INFγ + 874T & IL10 (-1082/-819/-592) ATA	–	DV-1/DV-2	DSS (43)	NC (99)	9,162	1.81–46.31	0.002	0.006	Cuban	23
DC-SIGN1-336G	–	–	DHF/DSS (454)	NC (696)	0.204	–	2×10 ⁻⁶	–	Thai (3 cohorts)	41
TAP1 333 Val & HPA 1b	–	–	DHF/DSS (107)	NC (100)	–	–	<0.05	–	South Indian	42
TNF-308 AA or AG & IL-10-1082 AA	–	–	DHF (25)	NC (46)	19.47	1–378.2	0.013	ns	Venezuelan	31
TAP1 333 Ile/Val	–	–	DHF (75)	NC (100)	2.7	1.46–5.01	0.005	–	South Indian	42
FcγRIIa H/H131	–	DV-4	DF (68)	SI (42)	4,425	1.10–20.52	0.016	–	Cuban	22
HPA1 1a/1b	–	–	DSS (32)	DHF (75)	4.75	–	0.003	–	South Indian	42
FcγRIIa H/H131	–	DV-4	DSS (29)	SI (42)	10.56	2.33–54.64	0.00018	–	Cuban	22
TNF-308 AA or AG & IL-10-1082 AA	–	–	DHF (25)	DF (41)	17.4	0.89–338	0.017	ns	Venezuelan	31
Resistant										
Vitamin D receptor 352 C	–	–	DSS (352)	NC (251)	–	–	0.033	–	Vietnamese	32
TGFβ1 codon25 G allele	–	DV-1/DV-2	DSS (43)	NC (99)	0.38	0.21–0.69	–	0.002	Cuban	23
TGFβ1 codon25 GG	–	DV-1/DV-2	DSS (43)	NC (99)	0.34	0.15–0.76	–	0.01	Cuban	23
TNFα-308G & INFγ + 874A & TGFβ1 codon25 GG	–	DV-1/DV-2	DSS (43)	NC (99)	0.291	0.09–0.89	0.025	0.044	Cuban	23
B. DHF/DSS vs. DF										
Factors	Infect	Serotype	Case	Control	OR	95% CI	p	p _c	Population	Reference
Susceptible										
TAP1 333 Ile/Val	–	–	DHF (75)	DF (91)	2.58	–	0.007	–	South Indian	42
HPA 1a/1a	–	–	DHF (75)	DF (91)	1.93	–	0.006	–	South Indian	42
HPA 2a/2b	–	–	DHF (75)	DF (91)	2.8	–	0.007	–	South Indian	42
TGFβ1-509 CC	–	DV-2	DHF (100)	DF (150)	1.94	1.04–3.61	0.034	–	Taiwanese	49
CTLA-4 +49 G & TGFβ1-509 CC	–	–	DHF (100)	DF (150)	2.10	1.07–4.09	0.028	–	Taiwanese	49
JAK1 rs11208534 (TT)	–	–	DHF (50)	DF/ SI	5.19	2.13–12.66	<0.05	–	Brazilian	24
Resistant										
DC-SIGN1-336G	–	–	DHF/DSS (454)	DF (152)	5.84	2.77–12.31	1.4×10 ⁻⁷	–	Thai (3 cohorts)	41
TNF-308 GG & IL10-1082 AA	–	–	DHF (25)	DF (41)	0.275	0.094–0.805	0.023	ns	Venezuelan	31
JAK1 rs310196 (TT)	–	–	DHF (50)	DF/ SI	0.3	0.15–0.57	<0.05	–	Brazilian	24

Note: DF: dengue fever, DHF: dengue hemorrhagic fever, DSS: dengue shock syndrome. 1st: Primary infection, 2nd: Secondary infection. DV: dengue virus (serotype). OR: Odds ratio, 95%CI: 95% confidence intervals, Pc: corrected p, ns: non-significant

c. Dendritic Cell-Specific ICAM-3 Grabbing Nonintegrin (DC-SIGN, CD209)

DC-SIGN is an essential attachment molecule for dengue virus, expressed on the surface of DCs. The fact that DC-SIGN variant (rs4804803) protects for the supposition against DF but not DHF/DSS in three cohorts in Thailand [41] provided further evidence that DHF/DSS is caused by a different pathogenic mechanism than DF. This study found a decreased transcription activity of G allele versus A allele of DC-SIGN1-336, suggesting a protective effect of G allele against DF by decreased levels of the viral receptor expression of host cells. The subsequent analysis of this SNP in Brazilian population, however, did not show any association [24].

d. Transporter associated with Antigen Processing (TAP)

TAP is a protein that specializes in delivering cytosolic peptides to class I molecules in the endoplasmic reticulum. TAP gene variants have shown their effect on the outcome of HPV, hepatitis C infections and autoimmune disorders [42]. A TAP1 polymorphism (rs1057141) study in South India showed that a heterozygous at 333 position (Ile/Val) was susceptible to DHF [42].

e. Human Platelet Antigen (HPA)

HPA facilitates the interaction between platelets and endothelial wall components. HPA-1a and 1b are the 33Leu substitution for 33Pro in the b3 component of allb3 complex. The Indian study found that HPA polymorphism associated with the severity of DI, especially heterozygous HPA 1a/1b, increases the risk of developing DSS rather than DHF [42]. These authors proposed that the positive correlation of TAP1 333 and HPA1 in DHF could contribute to the role of TAP in viral peptide selection and cross-reactive immune response against HPA1 antigen.

f. Cytokine polymorphisms and dengue

Cytokine storm after T cell activation has been cited as a cause of plasma leakage [43]. The production of cytokines is modulated by genetic polymorphisms that are associated with susceptibility to disease [31]. Interleukin (IL)-10, IL-6, Transforming Growth Factor b-1 (TGFβ1), TNF-α, and IFN-γ polymorphisms that may play a role in enhancing severe dengue disease [15] were selected for the study.

IL-10 is a major immunomodulation mediator produced by monocyte, DCs, and T, B lymphocytes, and it is an important anti-inflammatory cytokine [44]. IL-10-1082 genotyping did not show any association with dengue in a Venezuelan study [31], but IL-10 (-1082/-819/-592) ACC/ATA haplotype was significantly associated with DHF in a Cuban study (OR = 2.54, Pc = 0.03) [23]. Since IL-10 was involved with TNF-α in the thrombocytopenia and hemorrhagic manifestation in dengue infection, the combination of TNF-α and IL-10 polymorphism is of interest.

TNF-α-308 AA or AG and IL-10-1082 AA genotype (High TNF/low IL-10 phenotype) was more frequent in DHF patients as compared with controls (OR = 19.47) or DF (OR = 17.4); by contrast, the combination of TNF-α-308 GG and IL-10-1082 AA genotype (Low TNF/low IL-10 phenotype) was less frequent in DHF than in DF (OR = 0.275) [31]. Many combinations of TNF-α, IFN-γ, TGFβ1 polymorphisms and IL-10 haplotype were found to be associated with DHF as compared with controls by Perez 2010, as shown in Table 2.

Transforming Growth Factor β-1 (TGFβ1) is a multi-functional cytokine. Chen RF *et al.* (2009) showed that individual carried TGFβ1-509 CC genotype was about twice more likely to have DHF than DF in Taiwanese. Moreover, its combination with CTLA-4 +49 G allele increased the risk of DHF and had higher DV-2 virus load than in patients with CTLA-4 +49 G allele - TGFβ1-509 T allele (p = 0.013). This finding indicated a relationship among immune gene, viral load and disease severity. Conversely, Perez *et al.* (2010) analyzed TGFβ1 at codon 25 and noted the protective effect of G allele and GG genotype against DHF.

2.3 Genome-wide study

The results of candidate gene studies show that the sequencing data of the human genome and the HapMap project have identified millions of SNPs facilitating the implementation of GWASs.

Illumina microbead array technology was used to genotype 728 SNPs in 56 key genes of the Type I TNF response pathway and other well selected genes in a Brazilian study [24]. There were 58 markers with p < 0.05, among which 11 markers are in Janus-Activated Kinase gene (JAK1), representing the largest proportion of significant markers. The most significant SNPs as well as DHF risk SNPs (rs11208534, rs2780831, rs310196) were located toward the 5' end of the JAK1 gene. Linkage disequilibrium (LD) analysis showed that the SNPs rs11208534 and rs2780831 were located in the same linkage block and that rs310196 was an independent marker (D' rs2780831/rs11208534 = 1, rs2780831/rs310196 = 0.924, rs11208534/rs310196 = 0.856). JAK1 is a signaling protein associated with the type IFN receptor, and polymorphism in this gene may provoke the under-expression observed in severe dengue as in the expression profile of DSS patients vs. DF [45].

The technological development of high throughput genotyping has provided a powerful tool to examine the genetic basis of disease through GWAS. The GWAS approach, underway since 2007, has achieved success in identifying genes for many diseases like Crohn's disease,

rheumatoid arthritis, type 1 and type 2 diabetes, prostate cancer macular degeneration [2], but little information on large scale SNP analysis of infectious diseases, including dengue, is available. With the large number of markers typed, the stringent statistical criteria necessary to minimize false positive results may be difficult to establish.

2.4 Transcript analysis

Understanding the pathogenesis of a complicated disease like dengue remains elusive because of the lack of a suitable animal model and the complex immune interactions in infected subjects. Gene expression studies provided an opportunity to observe the simultaneous biological relevance and interaction of genes in the disease [46].

The expression of mRNA transcripts and protein products of selected immune response genes has been measured in the plasma of DF and DHF patients during the acute and convalescent phases of DENV infection. High levels of soluble IL-2 receptor, IL-13, IL-18, IL-10, soluble VCAM-1, soluble CD8, macrophage inhibitory factor and TNF all correlate with disease severity in dengue and indicate a high degree of T cell activation [11, 30, 47, 48, 49].

2.5 Genome expression

The development of genomics technology, microarray and high throughput quantitative PCR have revolutionized the way we study gene expression modification on a large scale and look for each change that could be important in the pathogenic process. Information from this high-

throughput screening of whole transcriptomes requires careful confirmation at the phenotype level.

Host gene expression was deciphered in cells infected with DENV in vitro [50, 51] and subsequently in patients [45, 51, 52, 53, 54] (Table 3). These studies mainly tried to distinguish the DSS from DF or DHF grades I/II patients (uncomplicated dengue) by comparing whole blood transcription profiles of the acute stage with those of the convalescent stage, and the healthy controls. Despite the limited sample number, the studies revealed many upregulated genes, and the host response pathways during dengue infection were elucidated by the functional studies that ensured. Gene expression profile in the early samples suggested that the innate immune response, endoplasmic reticulum (ER) stress response, cellular mitotic activity, B cell activation, innate immune response, apoptosis and oxygen transport, and ubiquitin proteasome dominated in DV- infection [45, 51]. The most important findings were the less abundant transcriptions among DSS-gene signature than in those with less-severe dengue at the time of cardiovascular decompensation of both innate and acquired immune responses including T, NK cell response [52, 53], IFN-stimulated genes (ISGs) [45], and type I IFN pathway [9, 45, 51].

Considering that early host responses may reflect components of the disease pathogenesis, Tolfvenstam *et al.* preferred to focus on disease timing rather than disease severity by comparing dengue infection vs. non-dengue infection groups. They observed a strong activation of the

Table 3. Genome expression of dengue infection

Population	Subjects	Sampling time	Genes (abundant) expression associated with DI	Gene expression associated with DSS	Reference
1 Vietnamese adults	6 DSS: 8 non-DSS: 4 control (2nd)	- Admission - A month later	The ER stress response, cellular mitotic activity, B cell activation, innate immune response, apoptosis & oxygen transport.	Increased B cell activation. Decreased ISGs, IFN-regulated, immune response.	45
2 Singapore adults	10 DF: 10 non-dengue	- before day3 - day 7 - 3-4 weeks later	NF- κ B (IP-10), type I IFN (I-TAC) and ubiquitin proteasome.	NA	51
3 Thai children	1 DSS: 3 DHF : 5 DF	- Admission - A month later	Metabolic and signal transduction, IFN-inducible and IFN-induced genes.	Decreased both innate & acquired immune responses: cytokine/chemokine signaling molecule production, T and B cell activation, & killer cell activation.	52
4 Vietnamese (adult?)	9 DSS: 9 non-DSS	- Day 4 - A month later	Oxidative metabolism, interferon signaling, protein ubiquitination, apoptosis, and cytokines.	Decreased apoptotic and type I IFN pathways.	9
5 Cambodian children	19 DSS: 13 DHF: 16 DF	-	-	Decreased T, NK lymphocyte responses, increased anti-inflammatory, repair/remodeling transcripts, innate immunity, inflammation and lipid metabolism.	53
6 Singapore adults	31 DI: 26 non- den	- before day3 - day 4-7 - 3-4 weeks later	Innate immune response: IFN-signaling, pathogen recognition, and complement activation, biosynthesis, metabolism. Decreased T cell associated pathways.	NA	54

Note: DF: dengue fever, DHF: dengue hemorrhagic fever, DSS: dengue shock syndrome, DI: dengue infection, 2nd: Secondary infection, ER: endoplasmic reticulum, ISGs: IFN-stimulated genes, NA: not applicable

innate immune response (chemokines CCL2 (MCP-1), CCL8 (MCP-2), CXCL10, (IP-10) and CCL3 (MIP-1 α)) in the early dengue fever phase (before 72 h of fever), while the adaptive immune response, biosynthesis and metabolism dominated in the defervescence phase (day 4–7 of fever).

Again, the expression profiles are so variable that it may be difficult to reach a consensus and may even cause controversy. Some authors have highlighted the importance of timing in the course of dengue disease and suggested that the change in DSS transcription profile may occur earlier than clinical manifestations [9]. Devignot *et al.* concluded that a shift from DSS to uncomplicated transcriptional profile may occur within a very short time by showing that DSS samples at three days after shock exhibited a profile very close to uncomplicated dengue [53].

3. CONCLUSION

Taken together, these studies reveal that associations between host genetics, DV, and clinical outcome are complex. The question of how many genes contributes to dengue susceptibility, how they interact to cause severe manifestations, and the extent to which the pathogenesis mechanism might be genetically predicted remains unknown. It is still a challenge to identify appropriate epitopes for vaccines, and further exploration is needed to identify specific medicine candidates.

Many essential issues will exert an impact on future study design, including uniform case definition, adequate sample size, quality control in typing technique, and statistical analysis method with locus-wise correction of *p* value. The associations should also be verified subsequently by well-designed functional studies.

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