Research paper

Genetic risk for dengue hemorrhagic fever and dengue fever in multiple ancestries

Guillaume Pare⁴, Binod Neupane⁵, Sasha Eskandarian⁶, Eva Harris⁷, Scott Halstead⁸, Lionel Gresh⁹, Guillermina Kuan⁵, Angel Balmaseda¹⁰, Luis Villar¹, Elsa Rojas¹, Jorge E. Osorio¹, Dang Duc Anh¹, Aruna Dharshan De Silva¹¹, Sunil Premawansa¹², Gayani Premawansa¹², Ananda Wijewickrama¹², Ivette Lorenzana¹³, Leda Parham¹, Cynthia Rodriguez¹⁴, Ildefonso Fernandez-Salas¹⁵, Rosa Sanchez-Casas¹⁶, Esteban E. Diaz-Gonzalez¹⁷, Khin Saw Aye¹⁸, Win Lai May¹⁹, Min Thein¹⁹, Filemon Bucardo¹⁹, Yaoska Reyes¹, Patricia Blandon¹, Kenji Hirayama¹, Lan Weiss¹,r, Pardeep Singh¹, Jennifer Newton¹, Mark Loeb¹,b,c,*, for the Dengue Population Genetics Program

⁴ Department of Pathology and Molecular Medicine, McMaster University, Ontario L8N 3Z5, Canada
⁵ Department of Health Research, Methods, Evidence, and Impact, Canada
⁶ Institute for Infectious Diseases Research, McMaster University Hamilton, Canada
⁷ Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, CA, United States
⁸ Department of Preventive Medicine and Biometrics, Uniformed University of the Health Sciences, Bethesda, MD, United States
⁹ Sustainable Sciences Institute, Managua, Nicaragua
¹⁰ Health Center Socrates Flores Vivas, Ministry of Health, Managua, Nicaragua
¹¹ Laboratory Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua
¹² Clinical Epidemiology Unit, Universidad Industrial de Santander, Bucaramanga, Colombia
¹³ Centro de Atención y Diagnóstico de Enfermedades Infecciosas, Bucaramanga, Colombia
¹⁴ University of Wisconsin, Wisconsin, United States
¹⁵ National Institute of Hygiene and Epidemiology, Hanoi, Vietnam
¹⁶ Genetech Research Institute, Sri Lanka
¹⁷ Department of Zoology and Environmental Sciences, University of Colombo, Sri Lanka
¹⁸ Department of National Autonomous University of Honduras, Tegucigalpa, Honduras
¹⁹ Universidad Autonoma de Nuevo Leon, Mexico
²⁰ Medical Research, Ministry of Health, Myanmar
²¹ The Faculty of Medical Sciences at the National Autonomous University of Leon, Nicaragua
²² Department of Immunogenetics, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan
²³ Department of Immunology and Microbiology, Pasteur Institute, Ho Chi Minh City, Vietnam

ARTICLE INFO

Article History:
Received 8 August 2019
Revised 28 October 2019
Accepted 26 November 2019
Available online xxx

Keywords:
Dengue
Genetics
Risk
GWAS
Ancestry

ABSTRACT

Background: Genetic risk factors for dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) and dengue fever (DF) are limited, in particular there are sparse data on genetic risk across diverse populations.

Methods: We conducted a genome-wide association study (GWAS) in a derivation and validation sample of 7,460 participants of Latin American, South Asian, and South East Asian ancestries. We then developed a weighted polygenic risk score (PRS) for each participant in each of the validation cohorts of the three ancestries to predict the risk of DHF/DSS compared to DF, DHF/DSS compared to controls, and, DF compared to controls.

Findings: The risk of DHF/DSS was significantly increased, odds ratio [OR] 1.84 (95%CI 1.47 to 2.31) (195 SNPs), compared to DF, fourth PRS quartile versus first quartile, in the validation cohort. The risk of DHF/DSS compared to controls was increased (OR=3.94; 95% CI 2.84 to 5.45) (278 SNPs), as was the risk of DF compared to controls (OR=1.97; 95%CI 1.63 to 2.39) (251 SNPs). Risk increased in a dose-dependent manner with increase in quartiles of PRS across comparisons. Significant associations persisted for PRS built within ancestries and applied to the same or different ancestries as well as for PRS built for one outcome (DHF/DSS or DF) and applied to the other.

* Corresponding author at: Department of Pathology and Molecular Medicine, McMaster University Hamilton, Ontario L8N 3Z5, Canada.
E-mail address: loebm@mcmaster.ca (M. Loeb).

https://doi.org/10.1016/j.ebiom.2019.11.045
2352-3964/© 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license. (http://creativecommons.org/licenses/by-nc-nd/4.0/)
**1. Introduction**

Infection with dengue virus (DENV), a mosquito-borne flavivirus infection, is of major global public health relevance [1]. Dengue fever (DF), or “break-bone fever”, is characterized by fever, headache, retro-ocular, and joint pain, rash, and, lymphadenopathy [2]. In less than 2% of those infected for a second time, the disease may progress to dengue hemorrhagic fever characterized by thrombocytopenia and vascular leakage and dengue shock syndrome (DSS) (DHF with evidence of systemic hypoperfusion) [3–8]. A secondary heterologous infection, that is infection with a DENV serotype different from that of prior infection, can increase risk of DHF/DSS through antibody-dependent enhancement [9,10]. However, given that only a small proportion of secondary infections result in severe disease, underlying genetic predisposition is likely [11].

The vast majority of genetic studies for dengue have been candidate gene studies, where a number of genetic variants, including single nucleotide polymorphisms and HLA polymorphisms, have been implicated as genetic risk factors [12–19]. Existing data have been conflicting [20]. There has only been one genome-wide association study (GWAS) conducted for dengue, which showed an association between variants in major histocompatibility complex (MHC) class I polypeptide-related sequence B (MICB) and phospholipase C, epsilon 1 (PLCE1) and DSS [21]. However, this study was conducted in a single ancestry population (Vietnamese). Although these variants were replicated when tested alone in another Vietnamese and a Thai population [22,23], less is known about the extent to which genetic variants that predispose for dengue act across ancestries. We report here a GWAS in a sample set with multiple ancestries, which allowed us to test the hypothesis, using polygenic risk scores, that genetic variants across the genome predispose to DHF/DSS and DF.

**2. Methods and methods**

**2.1. Study participants**

The Dengue Population Genetics Program (DPGP) was a genetic epidemiologic study where DNA was obtained from participants of three major ancestries from seven countries: Latin American from Honduras, Mexico, Nicaragua, Colombia; South Asian from Sri Lanka; and Southeast Asian from Vietnam and Myanmar between March 2003 and December 2013. Participants were categorized into one of the following groups: DF, DHF/DSS, and controls (definitions are shown in Table S1 in the Supplementary Appendix). Clinical phenotype data was obtained from community cohort studies and hospitalized patients with DHF/DSS and DF. Infection was confirmed by serology. DENV RNA was demonstrated by RT-PCR. DENV NS1 antigen detection, or, viral isolation [24, 25]. Controls were defined as participants who had evidence of DENV infection but no evidence of DHF/DSS or DF (they consisted of two groups, a group of 513 that seroconverted during the study and 1706 IgG antibody positive participants). They were derived by flow cytometry-based assays or ELISA in cross-sectional samples of participants who had no symptoms of dengue at the time of testing and had no history of hospitalization for dengue, or annual healthy samples by neutralization assay (using plaque reduction neutralization assay or flow cytometry-based assays) for participants in cohort studies [26, 27]. The analysis included participants with primary and secondary DENV infections. A summary of laboratory testing for infection is provided in Table S2 in the Supplementary Appendix.

**2.2. Genotyping**

We conducted the study using a two-stage design. In the first stage, we used the Illumina OmniExpress BeadChip array of 958,178 single nucleotide polymorphisms (SNPs) with genome-wide coverage to identify genetic associations in three separate comparisons: DHF/DSS versus DF, DHF/DSS versus controls, and, DF versus controls using a derivation cohort of 2498 subjects. We included genetic variants that had previously been associated with DHF/DSS or DF (e.g., DC-SIGN, vitamin D receptor, Fc gamma receptor II, TNF alpha, IL-10, HLA-A, HLA-B, TAP 1, Tap 2, CTLA-4, MICB and, PLCE1) in the derivation analyses [11, 21]. In the second stage, SNPs with p-values <0.0001 for at least one of three comparisons in the analysis of the entire derivation cohort were pruned at the linkage disequilibrium cut-off of r^2=0.20 using a 500 kb window. These, along with the genetic variants previously associated DHF/DSS or DF, were genotyped in a validation (replication) cohort of 6879 subjects using the Illumina GoldenGate BeadXpress platform using a total of 1536 SNPs.
2.3. Quality control

In the overall derivation cohort, samples with <95% call rates and sex mismatch were removed, as were the SNPs with <95% call rate, <1% minor allele frequency (MAF) or Hardy-Weinberg p-value < 0.000001 in controls. For the ancestry-specific derivation cohort, we further removed SNPs with MAF <5% because the sample size was limited. In the overall validation cohort, we removed samples with <95% call rates, SNPs with <95% call rate, or SNPs with <1% MAF in order to avoid excluding SNPs that were narrowly < 5%. The quality controls in derivation and validation cohorts were performed using PLINK (version 1.09).

A total of 9377 DNA samples was received for analysis, of which for the derivation cohort 999 were from participants with DHF/DSS, 999 DF, and, 500 were from controls. For the validation cohort, there were 1715 samples from participants with DHF, 2731 DF, and, 2433 controls. Following quality control steps, there were a total of 2248 DNA samples from the derivation phase and 5212 samples from the validation phase with the final dataset consisting of 7460 samples. The characteristics of participants in this final dataset are summarized in Table 1. Of the total of 958,178 SNPs genotyped in the derivation phase, 662,390 met quality control and the MAF criteria, as did 1165 of the 1536 SNPs tagged for the validation phase.

2.4. Association testing and replication

We calculated heritability using Genome Wide Complex Trait Analysis [28]. We used the Grammar-Gamma method to test for the associations of SNPs with DHF/DSS versus DF, DF versus controls, and, DHF/DSS versus controls in the entire derivation cohort (all ancestries), adjusted for age and sex, and population structure was taken into account by the kinship coefficient matrix of the variance component model [29]. The Grammar-Gamma analysis was also performed separately for each derivation sample of Latin American, South Asian, and, Southeast Asian ancestries, where only SNPs with allele frequency ≥ 5% were analyzed and a heritability coefficient (h²) of 0.3 was assumed (rather than estimated from the data) in a sample to ease model convergence as ancestry-specific sample sizes were small. We also checked the data for outliers from ancestries other than Latin American, South Asian, and Southeast Asian. We used a significance level of p < 5 × 10⁻⁵ to account for multiple testing. In the replication cohort, a separate logistic regression model was fitted adjusting for age, sex and ancestry for each SNP for each comparison. In order to exclude the possibility of population stratification, we did the analyses adjusted for principal components.

2.5. Polygenic risk score analysis

To test for the possibility of polygenic risk for DENV infection outcome (DHF/DSS versus DF, DHF/DSS versus control, and DF versus control), we developed a weighted polygenic risk score (PRS) for each participant in each of the validation cohorts of three ancestries (Latin American, South Asian, Southeast Asian) separately. For each participant, the PRS was the sum of the product of log-odds ratios of a set of SNPs passing a p-value threshold in a derivation cohort and the number of effect alleles at the corresponding SNPs (see Methods section in the Supplementary Appendix). Polygenic risk scores were constructed using all three ancestries (the primary PRS analysis) as well as single ancestries for sensitivity analyses. We also conducted the analysis using five principal components as covariates. If the allele at a SNP for a participant was missing, the number of effect alleles was imputed using the average number of effect alleles in all participants within the same ancestry with the same outcome. Nine different p-value thresholds ranging from 0.1 to 0.00001 were used for selecting SNPs to compute PRSs. All SNPs had a p-value < 0.001 for at least one analysis but could have had larger p-values for other comparisons.

2.6. Overall effect

To test the association of PRS with each of three comparisons (DHF/DSS versus DF, DHF/DSS versus controls and DF versus controls), first a logistic regression model was fitted by comparing the second to fourth quartile PRS to the first (referent), adjusting for age and sex in each validation ancestry. Based on plots of the p-value threshold and effect size for selecting SNPs for the PRS, which remained similar from a range of 0.1 to 0.001, we selected a p-value of 0.01 for the PRS (Figure S1 in the Supplementary Appendix). To assess the association of PRS in the overall validation cohort (overall effect), the results (the log-odds ratios and corresponding standard errors) from all three ancestries were then pooled using the fixed-effect model of meta-analysis. We also conducted a similar set of analyses by demonstrating the effect using a standardized PRS (i.e., PRS divided by its standard deviation, SD) adjusting for age and sex in each validation ancestry.

2.7. Secondary analyses

We conducted secondary analyses using derivation and validation cohorts of different ancestries. We examined 1) the effect of a PRS derived from all three ancestries and applied to a validation cohort of a single ancestry, 2) the effect of a PRS from the derivation cohort of a single ancestry applied to the validation cohort of the same ancestry, 3) the association of PRS from one derivation ancestry applied to a validation cohort of two different ancestries, 4) the effect of PRS derived from one single ancestry applied to a validation cohort of all ancestries, 5) a PRS from the derivation cohort of either DHF/DSS versus control or DF versus control then applied to the validation cohort of the other syndrome comparison. We examined these using a standardized PRS with the OR expressed per change in SD or by comparing quartiles.

The Grammar-Gamma analyses were performed using the R package GenABEL (version 1.8–0). Statistical Gene score analyses were performed using R software, version 3.2.4.

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Latin America</th>
<th>South Asia</th>
<th>South East Asia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derivation cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>210</td>
<td>44</td>
<td>151</td>
<td>405</td>
</tr>
<tr>
<td>Female sex,%</td>
<td>61.4</td>
<td>47.7</td>
<td>39.1</td>
<td>51.6</td>
</tr>
<tr>
<td>Mean age (SD), yr</td>
<td>22.1(18.5)</td>
<td>35.4(14.3)</td>
<td>27.0(21.9)</td>
<td>26.0(19.8)</td>
</tr>
<tr>
<td>Cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF</td>
<td>498</td>
<td>119</td>
<td>308</td>
<td>925</td>
</tr>
<tr>
<td>Female sex,%</td>
<td>57.8</td>
<td>35.3</td>
<td>44.8</td>
<td>50.6</td>
</tr>
<tr>
<td>Mean age (SD), yr</td>
<td>21.7(16.9)</td>
<td>29.5(15.4)</td>
<td>12.6(11.0)</td>
<td>19.7(16.0)</td>
</tr>
<tr>
<td>DHF/DSS</td>
<td>457</td>
<td>396</td>
<td>918</td>
<td></td>
</tr>
<tr>
<td>Female sex,%</td>
<td>49.5</td>
<td>50.0</td>
<td>50.9</td>
<td>50.1</td>
</tr>
<tr>
<td>Mean age (SD), yr</td>
<td>22.1(15.7)</td>
<td>17.3(12.9)</td>
<td>11.9(8.0)</td>
<td>18.5(14.6)</td>
</tr>
<tr>
<td>Replication cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1449</td>
<td>127</td>
<td>238</td>
<td>1814</td>
</tr>
<tr>
<td>Female sex,%</td>
<td>60.9</td>
<td>48.0</td>
<td>55.0</td>
<td>59.3</td>
</tr>
<tr>
<td>Mean age (SD), yr</td>
<td>22.1(18.0)</td>
<td>37.0(16.1)</td>
<td>31.5(19.7)</td>
<td>24.4(18.7)</td>
</tr>
<tr>
<td>Cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF</td>
<td>1386</td>
<td>354</td>
<td>361</td>
<td>2101</td>
</tr>
<tr>
<td>Female sex,%</td>
<td>55.8</td>
<td>37.6</td>
<td>51.0</td>
<td>51.9</td>
</tr>
<tr>
<td>Mean age (SD), yr</td>
<td>22.3(18.2)</td>
<td>29.4(12.8)</td>
<td>7.9(8.4)</td>
<td>21.0(17.4)</td>
</tr>
<tr>
<td>DHF/DSS</td>
<td>295</td>
<td>233</td>
<td>769</td>
<td>1297</td>
</tr>
<tr>
<td>Female sex,%</td>
<td>56.9</td>
<td>42.1</td>
<td>48.0</td>
<td>49.0</td>
</tr>
<tr>
<td>Mean age (SD), yr</td>
<td>25.6(16.1)</td>
<td>27.0(12.0)</td>
<td>8.9(7.4)</td>
<td>16.0(13.8)</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation; DF, Dengue fever; DHF, Dengue hemorrhagic fever; DSS, Dengue Shock Syndrome.
2.8. Role of the funding source

The study sponsor played no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; nor in the decision to submit the paper for publication.

2.9. Ethics statement

This study was approved by the McMaster research ethics board and that of all participating institutions. Participants provided informed consent for the analyses done in this study.

3. Results

3.1. Heritability estimates

By testing for chip-wide heritability, that is, the portion of the phenotypic variation that can be explained by genotyped genetic markers [30], overall and by ancestry, we found significant values for DHF/DSS compared to DF for Latin Americans and Southeast Asians (0.18 and 0.21), for DHF/DSS compared to controls for Latin Americans, South Asians, and Southeast Asians (0.35, 0.21, 0.18 respectively), and, for DF compared to controls for Latin Americans and Southeast Asians (0.20 and 0.56 respectively), all p-values <0.05. The establishment of such heritability supported testing for genetic risk of variants.

3.2. Association testing

In the analysis of the entire derivation sample, none of the SNPs reached the significance level of p<5 x 10^-8 for any comparison (Figure S2, Table S3 in the Supplementary Appendix). For the DHF/DSS versus DF comparison, the top associated SNP in the meta-analysis of derivation and replication phases was rs6675033 with p-value of 2.4 x 10^-10, for the fourth-quartile PRS to reference comparison. Similarly, the risk for DF increased compared to controls, OR 1.97 (95% CI 1.63 to 2.39), p-value 2.12 x 10^-12, fourth-quartile PRS compared to reference. The effect size increased in a dose-dependent manner as the PRS increased, with a highly significant effect for third quartile versus referent comparison for all three outcomes (Fig. 1). Although ORs >1 for the PRS in the second quartile compared to the first was observed for all three outcomes, they were only significant for DF versus controls.

Using a standardized PRS, the results were similar (Table 2, Fig. 2). The effect of PRS using a p-value threshold of 0.01 resulted in the following effects: OR 1.31 (1.20 to 1.42) per SD for DHF/DSS versus DF, p-value 1.95 x 10^-11; OR 1.89 (1.69 to 2.11) per SD for DHF/DSS versus control, p-value 1.49 x 10^-13; OR 1.29 (1.21 to 1.38) per SD, DF versus control, p-value 5.80 x 10^-13. Using a different p-value threshold of 0.0005 to derive the PRS resulted in similar effect sizes: OR 1.29 (1.19 to 1.40) for DHF/DSS versus DF, p-value 2.5 x 10^-10; OR 1.76 (1.57 to 1.96) for DHF/DSS versus control, p-value 3.87 x 10^-27; OR 1.22 (1.14 to 1.31), DF versus control, p-value 1.28 x 10^-6. The effect size of all p-value thresholds is shown in Figure S1. Our results did not change when we included principal components as covariates.

3.4. Secondary analyses

For each of the three DENV infection outcome comparisons (DHF/DSS versus DF, DHF/DSS versus control, DF versus control), the results of our five secondary analyses were similar in direction and

---

Fig. 1. Association of polygenic risk scores with dengue virus infection outcomes. Notations/abbreviations: N1 and N0, total number of cases and comparisons in the validation cohort; P, p-value; OR, odds ratio; CI, confidence interval. "Both derivation and validation cohorts were entire samples of all three ancestries (Latin American, South Asian and Southeast Asian). For each outcome comparison, the estimate of odds ratio of per standard deviation increase in polygenic risk score in the overall validation cohort was obtained by pooling the corresponding log-OR estimates across three ancestries using a fixed-effect meta-analysis model."
magnitude to our overall effects (Tables S4-S9, Figures S3-S6). When we derived the PRS from the overall derivation cohort for DF versus control and applied it to a validation cohort for DHF versus control, the effect size was similar to our previous estimates, OR 1.60 (95% CI 1.45 to 1.77), p-value $2 \times 10^{-19}$ (Table S9). Conversely, when the PRS was derived from DHF/DSS versus control and applied to a validation cohort of DF versus control, the effect was similar, OR 1.37 (95% CI 1.28 to 1.46), p-value $8.45 \times 10^{-19}$.

**4. Discussion**

We found a strong genetic risk, derived from PRS from a multi-ancestry population, when DHF/DSS was compared to DF, DHF/DSS compared to controls, and DF compared to controls. Moreover, this polygenic risk had a dose effect, with the risk increasing with an increasing number of risk alleles in the PRS. Sensitivity analyses demonstrated that PRS derived from single ancestries and applied to a

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Total</th>
<th>Cases</th>
<th>Comparators</th>
<th>Threshold</th>
<th>#SNPs</th>
<th>OR [95%CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF vs. Ctrl</td>
<td>3915</td>
<td>2101</td>
<td>1814</td>
<td>0.1</td>
<td>20</td>
<td>1.33 [1.24, 1.43]</td>
<td>$2.20 \times 10^{-16}$</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>332</td>
<td></td>
<td></td>
<td></td>
<td>1.33 [1.25, 1.43]</td>
<td>$1.50 \times 10^{-16}$</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>251</td>
<td></td>
<td></td>
<td></td>
<td>1.29 [1.21, 1.38]</td>
<td>$1.99 \times 10^{-13}$</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>221</td>
<td></td>
<td></td>
<td></td>
<td>1.27 [1.19, 1.36]</td>
<td>$5.17 \times 10^{-12}$</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>179</td>
<td></td>
<td></td>
<td></td>
<td>1.24 [1.16, 1.32]</td>
<td>$8.11 \times 10^{-10}$</td>
</tr>
<tr>
<td></td>
<td>0.0005</td>
<td>155</td>
<td></td>
<td></td>
<td></td>
<td>1.22 [1.14, 1.31]</td>
<td>$6.71 \times 10^{-9}$</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td>1.13 [1.05, 1.20]</td>
<td>$4.63 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>0.00005</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>1.00 [0.93, 1.07]</td>
<td>$9.29 \times 10^{-1}$</td>
</tr>
<tr>
<td>DHF/DSS vs Ctrl</td>
<td>3111</td>
<td>1297</td>
<td>1814</td>
<td>0.1</td>
<td>434</td>
<td>1.67 [1.68, 2.09]</td>
<td>$1.52 \times 10^{-28}$</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>374</td>
<td></td>
<td></td>
<td></td>
<td>1.87 [1.67, 2.09]</td>
<td>$2.31 \times 10^{-28}$</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>278</td>
<td></td>
<td></td>
<td></td>
<td>1.89 [1.69, 2.11]</td>
<td>$3.09 \times 10^{-29}$</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>256</td>
<td></td>
<td></td>
<td></td>
<td>1.88 [1.68, 2.10]</td>
<td>$4.52 \times 10^{-29}$</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>199</td>
<td></td>
<td></td>
<td></td>
<td>1.75 [1.56, 1.95]</td>
<td>$2.98 \times 23$</td>
</tr>
<tr>
<td></td>
<td>0.0005</td>
<td>176</td>
<td></td>
<td></td>
<td></td>
<td>1.76 [1.57, 1.96]</td>
<td>$9.71 \times 24$</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td>1.43 [1.29, 1.59]</td>
<td>$9.11 \times 12$</td>
</tr>
<tr>
<td></td>
<td>0.00005</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td>1.21 [1.10, 1.34]</td>
<td>$1.30 \times 4$</td>
</tr>
<tr>
<td></td>
<td>0.00001</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1.09 [0.97, 1.23]</td>
<td>$1.47 \times 1$</td>
</tr>
<tr>
<td>DHF/DSS vs DF</td>
<td>3398</td>
<td>1297</td>
<td>2101</td>
<td>0.1</td>
<td>317</td>
<td>1.35 [1.24, 1.46]</td>
<td>$5.63 \times 13$</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>265</td>
<td></td>
<td></td>
<td></td>
<td>1.34 [1.23, 1.45]</td>
<td>$3.07 \times 12$</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>195</td>
<td></td>
<td></td>
<td></td>
<td>1.31 [1.20, 1.42]</td>
<td>$1.16 \times 10$</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>183</td>
<td></td>
<td></td>
<td></td>
<td>1.29 [1.19, 1.40]</td>
<td>$6.78 \times 10$</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>167</td>
<td></td>
<td></td>
<td></td>
<td>1.29 [1.19, 1.40]</td>
<td>$7.62 \times 10$</td>
</tr>
<tr>
<td></td>
<td>0.0005</td>
<td>136</td>
<td></td>
<td></td>
<td></td>
<td>1.29 [1.19, 1.40]</td>
<td>$8.65 \times 10$</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td>1.08 [1.00, 1.17]</td>
<td>$4.76 \times 2$</td>
</tr>
<tr>
<td></td>
<td>0.00005</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td>1.05 [0.97, 1.14]</td>
<td>$2.25 \times 1$</td>
</tr>
<tr>
<td></td>
<td>0.00001</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>1.07 [0.99, 1.16]</td>
<td>$1.10 \times 1$</td>
</tr>
</tbody>
</table>

**Abbreviations:** DF, dengue fever; DHF/DSS, dengue hemorrhagic fever; OR, odds ratio; CI, confidence interval.

* Both derivation and validation cohorts were the same. Figures are from replication cohort.

**Table 2**

Pooled association of PRS derived from overall derivation cohort in overall validation cohort.

Fig. 2. Forest plot of the associations of polygenic risk scores* quartiles. Notations/abbreviations: N1 and N0, total number of cases and comparisons in overall validation cohort; P, p-value; OR, odds ratio; CI, confidence interval. * Both derivation and validation cohorts were entire samples of all three ancestries of Latin American, South Asian, and Southeast Asian validation cohorts were meta-analyzed using fixed-effect model.
global validation cohort remained significant predictors of risk, as did multi-ancestry PRS when applied to single ancestries, and single ancestries PRS when applied to the same ancestry in the validation cohort. We also found that PRS derived from one syndrome predicted risk in the validation cohort of another syndrome.

The effect sizes we observed, such as almost a four-fold risk of DHF/DSS compared to controls, are among the highest risk factors reported for dengue [2]. Studies that have tested variants shown to be significant in a GWAS study reported effect sizes that were substantially lower (0.5) [21–23]. Even when examining other risk factors for severe dengue, including age, sex, and, nutritional status, our PRS is the strongest risk factor [31]. The large size of this effect is compatible with a polygenic effect for all three DENV infection outcome comparisons, i.e. DHF/DSS versus DF, DHF/DSS versus controls, DF versus controls. In the overall validation cohort of all three ancestries, the evidence of polygenic risk was stronger for DHF/DSS versus control compared to DF versus control when derived from overall or ancestry-specific GWAS cohorts, suggesting that not only the risk is polygenic but also that the risk of more severe complications increases with higher burden of associated genetic variants.

Our secondary analyses, where we derived the polygenic risk scores from one ancestry population or degree of severity of DENV infection to validate in another ancestry or another severity level, indicate that the association of polygenic risk score is indeed robust. Given the polygenic origin of these traits with large numbers of SNPs with very small effects, it is not surprising that our GWAS findings were not replicated, and this may help explain inconsistencies in the results of previous genetic association studies [11]. Importantly, these secondary analyses demonstrate that the genetic susceptibility we observed is universal, that is, the same alleles increase or reduce susceptibility in diverse regions of the world. This is suggestive of a more general immune mechanism rather than adaptation to DENV-specific strain effects, which would be expected given the large geographical distribution of participants. Moreover, the effect of a similar risk when PRS was derived from one syndrome (DHF/DSS or DF) and applied to the other, suggests a common pathological mechanism.

Strengths of this study are the diverse ancestry of participants and the consistency of the findings. We acknowledge that use of participants that had no symptoms at the time of testing and no history of dengue, including age, sex, and, nutritional status, our PRS is the strongest risk factor [31]. The large size of this effect is compatible with a polygenic effect for all three DENV infection outcome comparisons, i.e. DHF/DSS versus DF, DHF/DSS versus controls, DF versus controls. In the overall validation cohort of all three ancestries, the evidence of polygenic risk was stronger for DHF/DSS versus control compared to DF versus control when derived from overall or ancestry-specific GWAS cohorts, suggesting that not only the risk is polygenic but also that the risk of more severe complications increases with higher burden of associated genetic variants.

In summary, our data provide strong evidence that multiple genetic variants confer clinically important risk for both DHF/DSS and DF. To our knowledge, this is the first report to demonstrate such polygenic risk for an infectious disease. Our data offer insight into how genetic variants predispose to dengue as well as the utility of using PRS in the study of infectious diseases.

Declaration of competing interest
Dr. Pare reports other from Amgen, other from Sanofi, outside the submitted work; the other authors have no conflicts of interest.

Acknowledgments
We thank Laura Childerhouse from McMaster University. We thank Andrea Núñez and William Avilés for overseeing quality and data management of the studies based in Nicaragua, respectively. We thank the study teams of the Nicaraguan Pediatric Dengue Cohort Study and the Pediatric Dengue Hospital-based Study at the Centro de Salud Sócrates Flores Vivás (Nicaragua Ministry of Health), the Hospital Infantil Manuel de Jesús Rivera (Nicaragua Ministry of Health), the Laboratorio Nacional de Virología at the Centro Nacional de Diagnóstico y Referencia (Nicaragua Ministry of Health), and the Sustainable Sciences Institute, as well as the study participants and their families.

Funding sources
Funding for the study was provided by the National Institute of Health (HHSN272201000026C). The funders had no role in study design, data collection, data analysis, interpretation, writing of the report.

Supplementary materials
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2019.11.045.

References


Title: Genetic risk for dengue hemorrhagic fever and dengue fever in multiple ancestries.

Date: 2020-01


Persistent Link: http://hdl.handle.net/11343/296950

License: CC BY-NC-ND