Risk Factors for Dengue Hemorrhagic Fever

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Antibody-dependent Enhancement

- Infection produces neutralizing antibodies to the infecting serotype, and non-neutralizing antibodies to the others.
- Non-neutralizing antibodies in a subsequent infection enhance viral entry into macrophages.
- Many infected macrophages are eliminated, releasing substances that reduce platelets, produce vascular permeability and hemorrhagic manifestations of DHF and DSS.
- DHF = 4% of secondary infections (The central dilemma).
Risk Factors for DHF/DSS: An integrated framework

- Secondary infection
- Age
- Nutrition
- Medications
- Chronic diseases
- Race
- Genetics

- History of viral circulation
- Number of susceptibles
- Vector density
- Intensity of viral circulation

Adapted from Gúzman 2002
Host Risk Factors

• Non-neutralizing antibodies
• Age
• Nutrition
• Chronic disease
• African ancestry
• Genetics
  – HLA
  – Dengue receptor CD209
  – The IFNα response pathway (JAK1)
Age

• In Southeast Asia, children are most affected

• In the Americas, all age- groups are affected, but the demographics are changing to mirror those in Asia

• Strong support for the antibody-dependent enhancement hypothesis
Nutrition

  - 100 DHF children
  - 125 Other infections
  - 184 Healthy
  - 182 infants with primary DHF
  - 63 DSS
  - 533 Healthy
  - Over and Undernourished – more complications
  - Undernourished – fewer infections
Chronic Disease

- Hypertension
- Diabetes
- Auto immune disease
- Sickle cell anemia
- Asthma/Atopy

Koury et al., 1988; Cunha et al, 1997
Dengue & Chronic Disease
Maria Aparecida Araújo Figueiredo

- Fortaleza: 115 cases and 1148 controls from 2003-2005
- Frequency of sickle cell anemia, autoimmue disease, hepato/renal failure, epilepsy too low to assess
- HTN & Diabetes not associated
- Atopy/Asthma associated
- Atopy/Asthma not associated if not taking steroids
Dengue & Genetics

- Race-Ethnicity-Ancestry
- HLA
  - Mexico – HLA DR4 protective
  - Cuba – HLA DR1 protective
  - Thailand – HLA-A*0207HLA-B*51 more susceptible
- CD 209
- JAK1
CD209 (DC-SIGN1) & DHF

- DHF, DF and normal blood donors
- All Thai children ≤ 15
- Studied markers only in CD 209
- Replication at 3 sites
- Demonstrated functionality in vitro
- rs4804803 (DCSIGN1 -336) for DHF/DF (p=1.4 X 10^{-7}, OR 5.8)

Sabkuntabhai et al. Nat Genet. 2005

• Population
  – DHF cases identified: 82
  – DHF Cases Collected: 55
  – DF controls: 289
  – Asymptomatic controls: 286

• Demographic survey

• Dengue serology (+) for 90%

• Illumina Microbead Array Genotyping
  – 78% polymorphic
  – Error rates <0.1%
Candidate Gene Categories

• 768 SNP markers
• 71 genes
  – viral sensing/receptors (e.g. CD209, TLRs)
  – control of IFNα induction (e.g. NFKB1, MYD88, EIF2AK2)
  – IFNα production (e.g. IFNA 1-21)
  – IFNα suppression (e.g. DNAJC3)
  – IFNα signaling (e.g. IFNARs, JAKs, STATs, MAPK1)
  – effector molecules (e.g. OASs, RNASEL, MX1, ADAR)
  – effector suppression (e.g. SOCSs)
  – published associations (e.g. IL18, RANTES-CCL5, VDR)
  – Ancestry informative (e.g. Duffy) Markers (AIMs)
Association of African Ancestry and DHF

- $\chi^2$ – ancestry, income
- Logistic regression
- African ancestry and lower income are independently protective

<table>
<thead>
<tr>
<th>Variables</th>
<th>DHF vs DF</th>
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<tbody>
<tr>
<td></td>
<td>p</td>
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<tr>
<td>Sex</td>
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<tr>
<td>Age</td>
<td>0.18</td>
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<tr>
<td>%African Ancestry</td>
<td>0.02</td>
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<tr>
<td>Income Index</td>
<td>0.01</td>
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</tbody>
</table>
• rs4804803 (DCSIGN1 -336) for DHF/DF ($p=1.4 \times 10^{-7}$, OR 5.8)

• No signal in our Brazilian population

Sabkuntabhai et al. Nat Genet. 2005
Single Locus Analysis: DHF vs DF

-log 0.05=1.3
FDR q<0.2 for rs11208534, rs310196, rs2780831
Multilocus Analyses

- Sliding window analysis also shows that JAK1 produces strongest signal of all loci

- \(-\log 0.05=1.3\)
Strength of Association

- log 0.05 = 1.3

- P value and OR localized to 5’ end of the JAK1 gene
JAK1 Function

Katze et al. Nat. Rev. Imm. 2002
Plausibility

**Publications**

- Muñoz-Jordan et al 2003 (PNAS) and (J Virol.) 2005: DENV2 infection and NS4B expression block STAT1 phosphorylation in cell culture

- Simmons et al 2007 (JID): Patterns of host genome-wide gene transcript abundance in the peripheral blood of patients with acute dengue hemorrhagic fever

**Abstracts ASTMH Annual Meeting**

- Hoang, LT et al: Marked attenuation of immune response genes associated with Type 1 interferon induced responses by microarray analysis
Sources of Error and Solutions

- Sample size
- Trait heterogeneity
- Genetic heterogeneity
  - age
- Whole genome association
The Next Step: Repetition, Extension and Functional Validation

- 115 cases and 1148 controls from 2003-2005
- Collect and genotype additional cases from other cities
- Genotype childhood cases
- Resequencing
- Cell culture & in vitro infections
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