Risk Factors for Dengue Hemorrhagic Fever

RE Blanton, ML Barreto, MG Teixeira

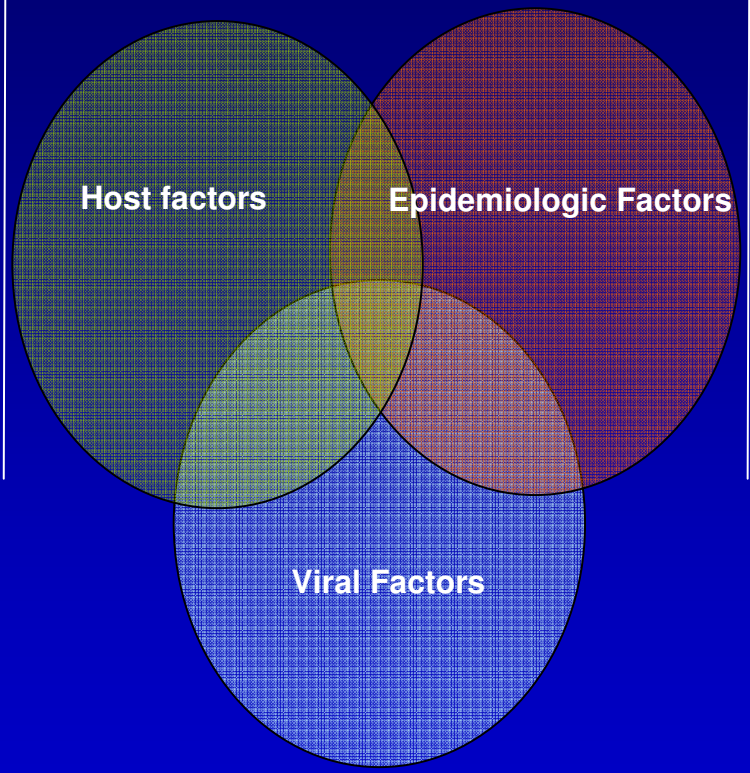
1-Center for Global Health and Diseases, CWRU, Cleveland, OH
2-Institute for Collective Health, Federal University of Bahia, Brazil

Antibody-dependent Enhancement

- Infection produces neutralizing antibodies to the infecting serotype, and non-neutralzing 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 dilema)

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

- Serotype
- Strain virulence

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

- Thisyakorn U, Nimmannitya S. Clin. ID 1993 Feb;16:295-7
  - 100 DHF children
  - 125 Other infections
  - 184 Healthy
- Nguyen TH et al. Am J Trop Med Hyg. 2005 Apr;72:370-4
  - 182 infants with primary DHF
  - 63 DSS
  - 533 Healthy
- Kalayanarooj S, Nimmannitya S.SE Asian J Trop Med Pub Health. 2005 Mar;36:378-84
  - 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

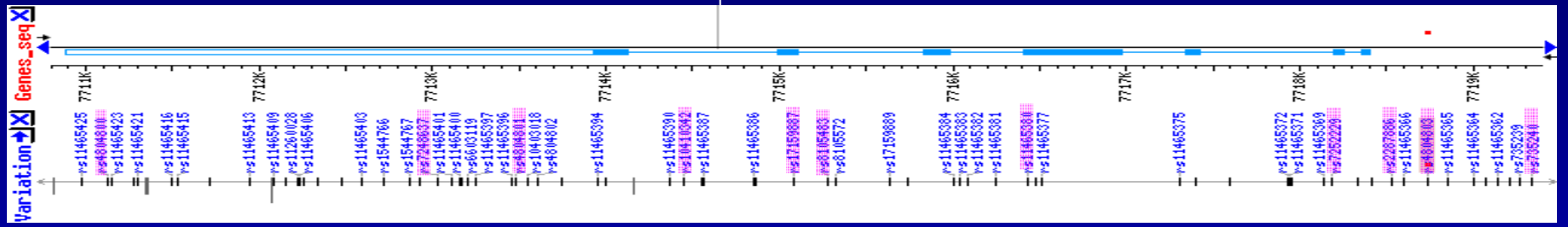
- Fortaleza: 115 cases and 1148 controls from 2003-2005
- Frequency of sickle cell anemia, autoimmue disease, hepato/renal failure, epilepsy too low to assess
- HTN & Diabetes not associated
- Atopy/Asthma associated
- Atopy/Asthma not associated if not taking steriods

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

CD209



- 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 ($p=1.4 \times 10^{-7}$, OR 5.8)

Sabkuntabhai et al. Nat Genet. 2005

Study Design-Salvador (2004)

- 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 Markers (AIMs) (e.g. Duffy)

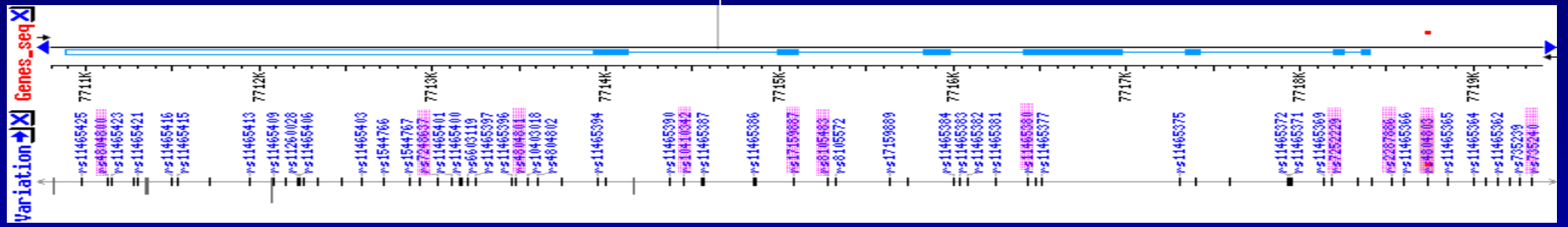
Association of African Ancestry and DHF

- χ^2 – ancestry, income
- Logistic regression
- African ancestry and lower income are independently protective

Variables	DHF vs DF		
	p	OR	CI
Sex	0.94	0.98	0.50 - 1.90
Age	0.18	0.99	0.96 - 1.01
%African Ancestry	0.02	0.13	0.02 - 0.69
Income Index	0.01	9.71	1.70 - 55.62

CD209 (DC-SIGN1) & DHF

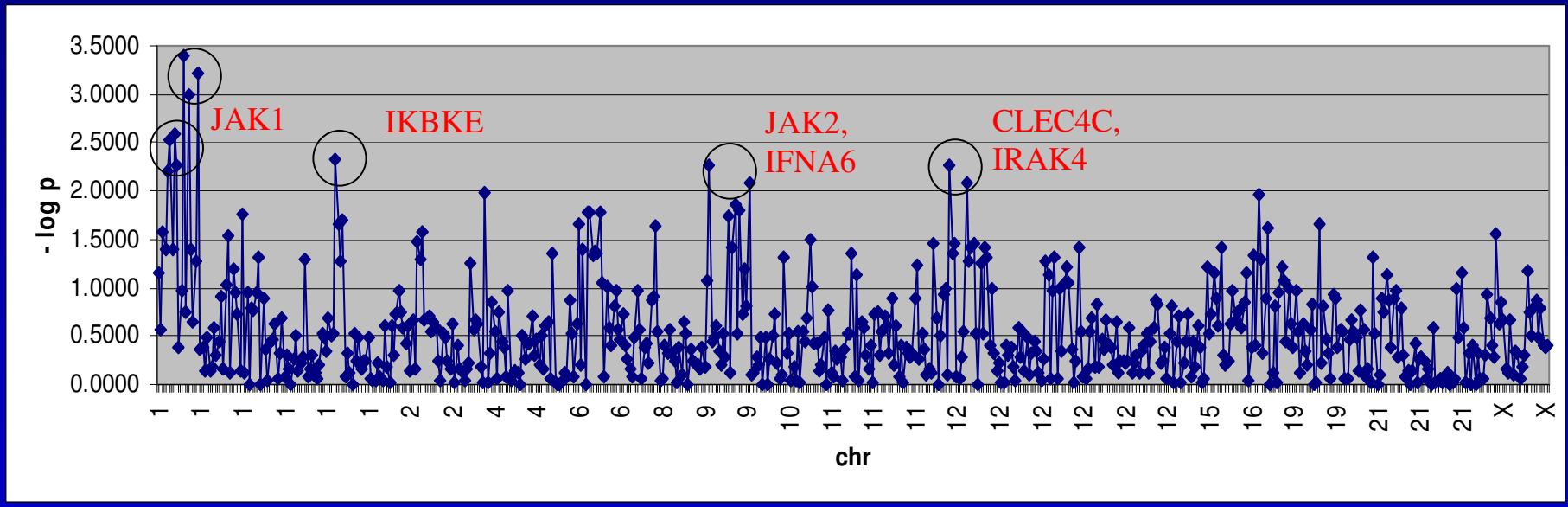
CD209



- rs4804803 (DCSIGN1 -336) for DHF/DF ($p=1.4 \times 10^{-7}$, OR 5.8)
- No signal in our Brazilian population

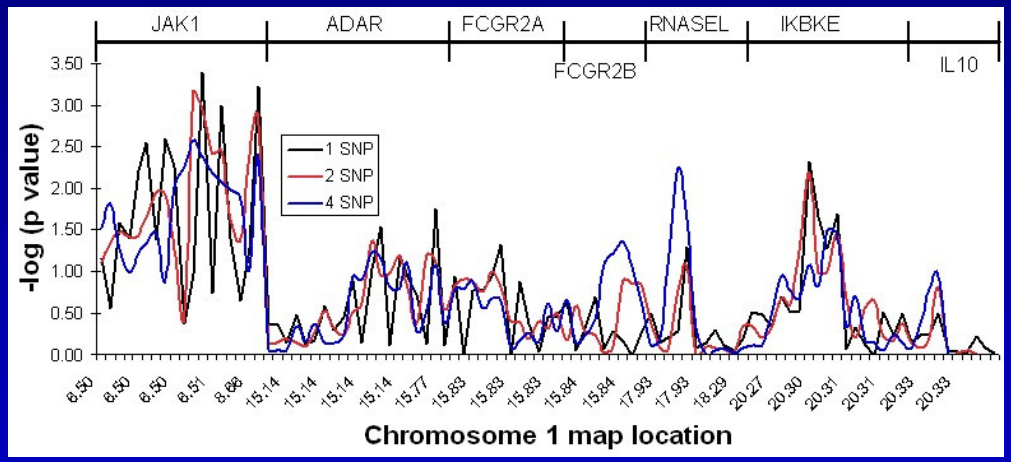
Sabkuntabhai et al. Nat Genet. 2005

Single Locus Analysis: DHF vs DF



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

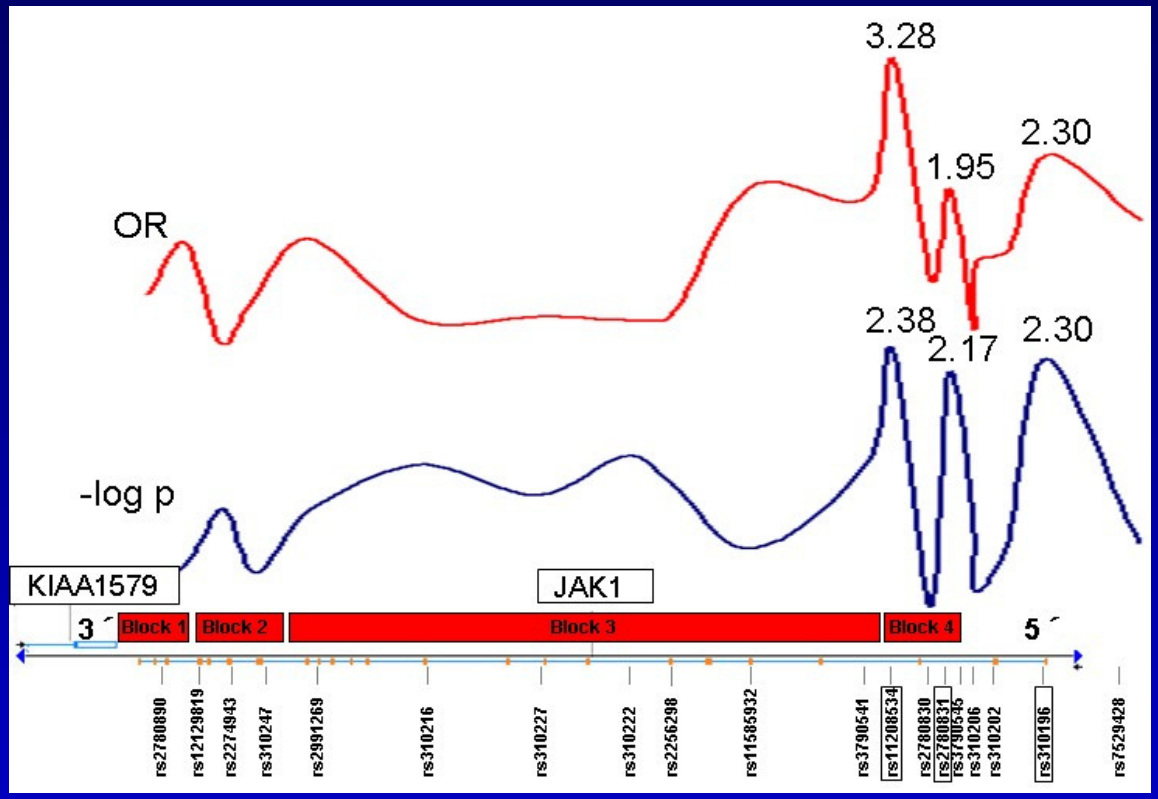
Multilocus Analyses



-log 0.05=1.3

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

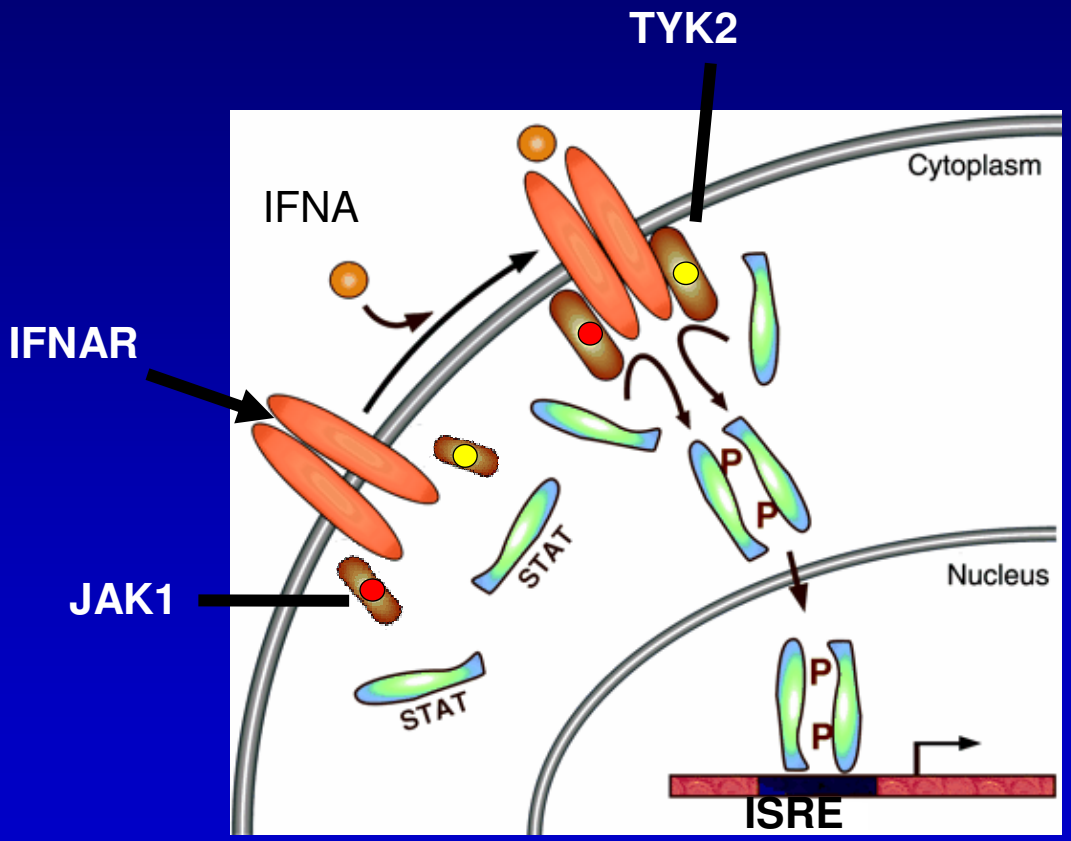
Strength of Association



-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

Acknowledgements

ISC-UFBA

M. Glória Teixeira
Vanesa Morato
Maurício Barreto

Div. Genetic Epidemiology

Katrina Goddard
Antonio Parrado

Funding

NIH R21 AI056263

FIOCRUZ

Dr. Mitermayer G. dos Reis
Luciano K. Silva
Paulo Melo
Eliana Reis

SESAB

Jesuína Costa
Juarez Dias
Maria P Figueiredo

LACEN

Dr. João Rodrigues
Isolina A. Ciuffo