Human genetic variation and malaria: not only a matter of resistance/susceptibility
Entomological Inoculation Rates in sub-Saharan Africa

Malaria selective pressure

Morbidity

The global distribution of clinical episodes of *Plasmodium falciparum* malaria

Robert W. Snow, Carlos A. Guerra, Abdisalan M. Noor, Hla Y. Myint & Simon I. Hay

*NATURE* VOL 434 | 10 MARCH 2005 |

- 2.2 billion people at risk
- 515 (range 300–660) million episodes of clinical P. falciparum malaria

Africa 70%
South East Asia 25%
1-3 million people die from malaria each year, most of whom are children.
Geographic diffusion of malaria and hemoglobinopathies

Shaded areas > 500 m a.s.l.
Haldane formulated ....

“The corpuscles of the anaemic heterozygote (for thalassaemia) are smaller than normal and more resistant to hypotonic solutions. It is at least conceivable that they are also more resistant to attacks by the sporozoa which cause malaria”

- Haldane, J.B.S., *Disease and evolution*. 1948, Pallanza. (*La Ricerca Scientifica* **1949**)

J.B.S. Haldane

Malaria Hypothesis
.... and Allison demonstrated

A.C. Allison


The challenge of human genetics in malaria

Why only 2% of clinical malaria cases evolve into severe forms of the disease?

Greenwood B et al. Parasitology Today. 1991
The road map

- Descriptive genetic epidemiology
- Malaria protective genes
- Mechanism/s

New control tools?

Not only academic exercises
**MalariaGEN**

MalariaGEN’s goal is to identify specific genes that are critical for protective immunity against malaria.

The billion genotype study of severe malaria (1,000,000 SNPs in 10,000 cases and 10,000 ethnically matched controls).

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Wellcome Trust Sanger Institute - Wellcome Trust Centre for Human Genetics, University of Oxford
From hospital-based disease association studies.

VS

The classic approach
From hospital-based disease association studies.

Haemoglobin C protects against clinical *Plasmodium falciparum* malaria


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Haemoglobin C

(G→A  SNS)

(β6 Glu→Lys)

The classic approach
From hospital-based disease association studies.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>Relative and (absolute) genotype frequencies</th>
<th>Relative and (absolute) allele frequencies</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>AC</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>3513</td>
<td>0.6641</td>
<td>0.2172</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2333)</td>
<td>(763)</td>
</tr>
<tr>
<td>Severe malaria</td>
<td>359</td>
<td>0.8078</td>
<td>0.1755</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(290)</td>
<td>(63)</td>
</tr>
<tr>
<td>Non-complicated malaria</td>
<td>476</td>
<td>0.8004</td>
<td>0.1555</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(381)</td>
<td>(74)</td>
</tr>
<tr>
<td>Malaria patients (total)</td>
<td>835</td>
<td>0.8036</td>
<td>0.1627</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(671)</td>
<td>(157)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comparisons</th>
<th></th>
<th>Odds ratio (95% confidence interval) and P values *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects vs. Malaria patients</td>
<td>2.07</td>
<td>(1.71-2.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.58-0.87)</td>
</tr>
<tr>
<td>Severe malaria vs. non-severe malaria</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Reduction in risk of clinical malaria:
AC heterozygotes: 29%
CC homozygotes: 93%

Reduction in risk of clinical malaria:
AS heterozygotes: 73%
SS homozygotes: lethal
Haemoglobin S and haemoglobin C: ‘quick but costly’ versus ‘slow but gratis’ genetic adaptations to *Plasmodium falciparum* malaria

David Modiano¹,², Germana Bancone¹,², Bianca Maria Ciminelli², Florenza Pompei², Isa Blot³, Jacques Simporé⁴ and Guido Modirano²,⁵

*Human Molecular Genetics. 2008*

Models of geographic diffusion of haemoglobin S (left) and haemoglobin C (right)
Abnormal display of PfEMP-1 on erythrocytes carrying haemoglobin C may protect against malaria

Rick M. Fairhurst¹, Dror I. Baruch¹, Nathaniel J. Brittain¹, Graciela R. Oster⁴, John S. Wallach¹, Holly L. Hoang⁴, Karen Hayton¹, Aldiouma Guindo⁵, Morris O. Makobongo⁵, Owen M. Schwartz⁷, Anatole Tounkara⁸, E. Wellems⁶

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uninfected red blood cell

typical knobs on an infected normal red blood cell

abnormal knobs on an infected cell containing haemoglobin C
... again, from descriptive epidemiology...

**Different response to *Plasmodium falciparum* malaria in West African sympatric ethnic groups**
(Burkina Faso/Fulani/Mossi/Rimaibé)

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*Istituto di Parasitologia, World Health Organization Collaborating Centre for Malaria Epidemiology, Università di Roma “La Sapienza,” 00185 Rome, Italy; ²Direzione Generale per la Cooperazione allo Sviluppo, Italian Ministry of Foreign Affairs, 00194 Rome, Italy; ³Centre National de Lutte Contre le Paludisme, Ministère de la Santé, Ouagadougou, Burkina Faso; and ⁴Dipartimento di Biologia Molecolare, Cellulare e Animale, Università di Camerino, 62032 Camerino (MC), Italy

Further approaches
The lower susceptibility to *Plasmodium falciparum* malaria of Fulani of Burkina Faso (West Africa) is associated with low frequencies of classic malaria-resistance genes

Among Fulani the proportion of individuals not having any of these protective alleles was more than 3-fold greater than in the Mossi-Rimaibe group (56.8% vs 16.7%; P < 0.001)

Among Mossi 83.3% of the population carries at least one classic malaria-resistance gene (HbS, HbC, alpha-thal, G6PDA-, HLA BW-53)
Negative epistasis between the malaria-protective effects of alpha-thalassemia and the sickle cell trait

Thomas N Williams, et al., Nature Genetics (2005)
.... to protective mechanisms

Functional deficit of T regulatory cells in Fulani, an ethnic group with low susceptibility to Plasmodium falciparum malaria

Maria G. Torcia*, Veronica Santarasci², Lorenzo Cosmi³, AnnMaria Clemente³, Laura Maggi³, Valentina D. Mangano³, Federica Verra³, Germana Bancone³, Isabella Nebia³, Bienvienu Sodjomom Simma³, Francesco Liotta³, Francesca Frosali³, Roberta Angeli³, Carlo Severini³, Anna R. Sannella³, Paolo Bonini³, Maria Lucibello³, Enrico Maggi³, Enrico Garaci³, Mario Coluzzi³, Federico Cozzolino³, Francesco Annunziato³, Sergio Romagnani³, and David Modiano³

Lower expression of genes determinant for Treg activity (TGFβ, TGFβRs, CTLA4, FOXP3) in Fulani compared to both Mossi and Europeans

646–651 | PNAS | January 15, 2008
Besides protection ...
...is there any effect of human genetic variation on *Plasmodium* transmission from the vertebrate host to the vector?
To be infected, mosquitoes should ingest gametocytes
Does human genetic variation affect the production of gametocytes???
Do HbC and HbS affect *P. falciparum* gametocytogenesis *in vivo*?

• **Cross-sectional malarialogical surveys**

Gametocyte rates according to β globin genotypes

(AA, AS, AC, CC)
Cross-sectional survey, Mossi Plateau, Burkina Faso

M-H OR 4.75
95% c.i. 1.89-11.98
P = 0.001
Are these gametocytes infectious?
Transmission experiments

**in vivo**
*(natural conditions)*

**ex vivo**
*(Direct Membrane Feeding Assay)*

Mosquito infection rates according to β globin genotypes
(AA, AS, AC, CC)

*Anopheles* examined: N= 4275
(AA: 892; AS: 1124; AC: 1045; CC: 1214)

*Anopheles* examined: N= 2171
(AA: 573; AS: 438; AC: 586; CC: 574)
Transmission experiments

Mosquitoes presenting oocysts were considered as infected and their oocyst load counted.
**In vivo** *Anopheles* infection after blood meals on human subjects with different β globin genotypes

- AS vs AA: OR 1.57 (1.08-2.30); P=0.018
- AC vs AA: OR 2.31 (1.60-3.34); P<<0.0001
- CC vs AA: OR 2.66 (1.87-3.80); P<<0.0001
Ex vivo: Anopheles infection rate after membrane feeding on blood from human subjects with different β globin genotypes

- AS vs AA: OR 4.46 (1.89-10.89); P <0.001
- AC vs AA: OR 4.08 (1.77-9.76); P <0.001
- CC vs AA: OR 3.89 (1.68-9.37); P <0.001
First demonstration that human genetic variation may influence the transmission dynamics of an infectious disease.
The level of malaria transmission besides being determined by ecological, and socio-cultural factors, is influenced also by the human genetic background.
Fascinating *Homo sapiens* - *P. falciparum* evolutionary scenario

Through single $\beta$ globin mutations, a mutual host-parasite benefit is produced

- Higher resistance to the disease for the host
- Higher transmissibility for the parasite
Human genetics and infectious diseases

First chapter:

• Resistance/susceptibility

 further possible chapters ....

• Dynamics of transmission of pathogens
• Efficacy of control measures (vaccines, drugs, etc)
• Dynamics of selection of drug resistance
Institutions and Funding

- Dipartimento di Sanità Pubblica e Malattie Infettive, Università “La Sapienza” Rome, Italy
- Centre National de Recherche et Formation sur le Paludisme, Ministère de la Santé, Burkina Faso
- Dipartimento di Biologia, Università "Tor Vergata" Rome, Italy
- Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom
- University of Ouagadougou, Burkina Faso
- Institut de Recherche pour le Développement (IRD) – Unité de Recherche 916, Bobo Dioulasso, Burkina Faso
- Institut de Recherche en Sciences de la Santé (IRSS), Direction Régionale de Bobo-Dioulasso, Burkina Faso