Genetic architecture and evolution of emerging artemisinin resistance in *Plasmodium falciparum*
Thanks and credits to....

All Team 112!

All Community Project Investigators
In the previous episodes...
Drug Resistance History

Visit the MalariaGEN website for the complete animation
And today...artemisinin

Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study

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BACKGROUND
Artemisinin-based combination therapy (ACT) has revolutionized the treatment of Plasmodium falciparum malaria in many parts of the world. However, concerns have emerged regarding the efficacy of ACT in the western border regions of Thailand, where the efficacy of ACT has been reported to be declining.

METHODS
The study was a longitudinal surveillance study conducted at the Thai-Myanmar border from 2004 to 2009. Plasma parasitemia was measured at baseline and every 2 weeks during follow-up. The efficacy of ACT was assessed using the World Health Organization's treatment efficacy criteria.

RESULTS
The treatment failure rate was significantly higher in the western border region compared to other regions of Thailand. The failure rate increased from 0% in 2004 to 5% in 2009.

CONCLUSIONS
The emergence of artemisinin-resistant malaria highlights the need for continued surveillance and the development of alternative strategies to control malaria in the western border region of Thailand.
What about artemisinin resistance?

- “Delayed parasite clearance observed after treatment with an artesunate monotherapy, or after treatment with an artemisinin-based combination therapy (ACT)” [WHO]

- Declining efficiency observed in Southeast Asia
  - From 2.5h to >5h
  - Complete treatment failure observed in western Cambodia due to resistance to partner drug

- Urgent priority for global health
  - Hard to measure clinical phenotype
  - Genetic marker would enable large-scale surveillance
  - Hopefully marker leads to causal mutations
The *kelch13* gene

- A molecular marker of artemisinin resistance has been identified in vitro [Ariey et al. Dec 2013].
- Different mutations in the *kelch13* propeller domain were shown to be associated with delayed parasite clearance.

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**ARTICLE**

da:10.1038/nature12876

A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria

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Open questions

- How many genes are involved?
- Are all parasites equally likely to acquire a resistance-causing mutation?
- What is the geographical distribution of the mutations that cause resistance and of the genetic predisposing factors?
- Is it spreading due to migration of resistant parasites, or does it have multiple origins in different locations?
TRAC/NIH GWAS

- **1,612 clinical samples**
  - Full genome sequence
  - 1,063 with phenotypes

- **15 locations** (+2 in Africa)
  - Cambodia, Vietnam, Laos, Thailand, Myanmar and Bangladesh

High genetic and geographical resolution

Map Source: [http://www.map.ox.ac.uk/](http://www.map.ox.ac.uk/)
18K SNPs with MAF>0.01
- Resistance phenotype expressed as parasite clearance half-life
  - number of hours taken for artemisinin to reduce parasite density by half during the steady-state clearance phase of the treatment
- Linear mixed model (Fast-LMM) to account for population structure

**kelch13 C580Y** p=10^{-26}

At least 7 distinct loci with p<10^{-7}

Miotto, Amato et al., under review
Extreme allelic heterogeneity

Miotto, Amato et al., under review
Observations

- C580Y mutations in *kelch13* has a p-value of 1E-26
  - Why the other mutations are not there or why is this mutation there?

- Other loci have significant p-values

- At least 20 non-synonymous mutations in the propeller domain of *kelch13* have a phenotypic effect
  - How does this compare to the rest of the world?
  - How does this compare to the rest of the genome?
  - Are the mutations in *kelch13* all born equal?
Emergence vs spreading
Resistance is emerging

Miotto, Amato et al., under review
Resistance is emerging
Compelling evidences of different origins

Miotto, Amato et al., under review
Haplotype homozygosity
Predisposing genetic background
3,500 samples
Different topologies

Africa

Cambodia
(E)SEA has low endemicity
Population structure: Principal Component Analysis

Miotto et al., Nat Gen 2013
"wild type" recombinants?

PCA in SE Asia

"outlier" clusters

Miotto et al., Nat Gen 2013
Evidence for multiple founder effects

Even MAF spectrum

Long haplotypes and low haplotype diversity
# Surface-associated interspersed genes

<table>
<thead>
<tr>
<th>Region</th>
<th>Samples</th>
<th>Haplotypes (Hs)</th>
<th>Unique Hs</th>
<th>Hs shared by &lt; 5</th>
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**Wild-type**  
**Founder 1**  
**Founder 2**

![Clonal expansion diagrams]

*Extremely rapid clonal expansion*
Subpopulations are ART-R
“Founder drift”
7 founder populations strongly associated with artemisinin resistance

- Each artemisinin-resistant founder populations strongly associated with a specific \textit{kelch13} resistance allele
- But the non-\textit{kelch13} significant SNPs (\textit{background mutations}) are all in there!

\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Population & WT & Y493H & R539T & S534T & P553L & C580Y & Het & Total \\
\hline
VN-C & 69 & 1 & 2 & 4 & 8 & 16 & 4 & 76 \\
KH-C & 122 & 2 & 1 & 3 & 8 & 16 & 2 & 124 \\
VN-F01 & 1 & 20 & 4 & 49 & 3 & 16 & 1 & 21 \\
VN-F04 & 1 & 15 & 32 & 3 & 15 & 1 & 15 \\
WKH-F01 & 2 & 15 & 8 & 2 & 8 & 1 & 9 \\
WKH-F02 & 1 & 15 & 8 & 1 & 9 & 1 & 9 \\
WKH-F03 & 1 & 15 & 8 & 1 & 9 & 1 & 9 \\
NKH-F01 & 1 & 15 & 8 & 1 & 9 & 1 & 9 \\
NKH-F02 & 1 & 15 & 8 & 1 & 9 & 1 & 9 \\
NKH-F03 & 1 & 15 & 8 & 1 & 9 & 1 & 9 \\
NKH-F04 & 1 & 15 & 8 & 1 & 9 & 1 & 9 \\
\hline
Total & 195 & 16 & 15 & 20 & 6 & 89 & 16 & 357 \\
\hline
\end{tabular}

Miotto, Amato et al., under review
**kelch13** and the background alleles have similar geographical distributions.
The genetic background is extremely differentiated even on a short geographic distance.

Miotto, Amato et al., under review
Contextualizing \textit{kelch13} within the genome and across countries
### Geographical distribution of the samples

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<thead>
<tr>
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<th>Sample counts</th>
<th>Country</th>
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Density of the variants in AFR and SEA
Distribution of the mutations within *kelch13*

![Graph showing distribution of mutations](image)

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<th>Category</th>
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Excess of frequent NS mutations in SEA
N/S vs conservation