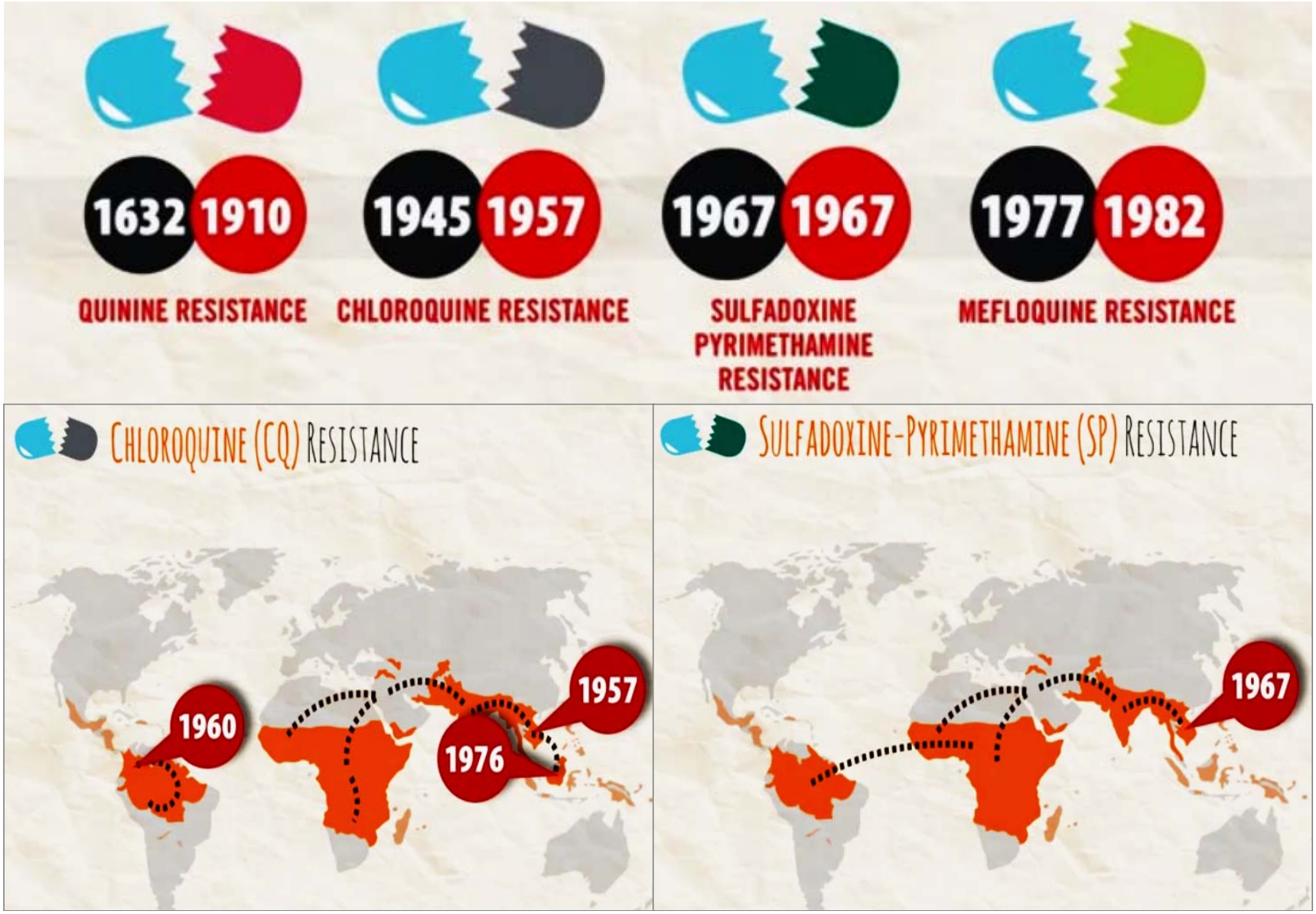
From GWAS to Genomic Surveillance

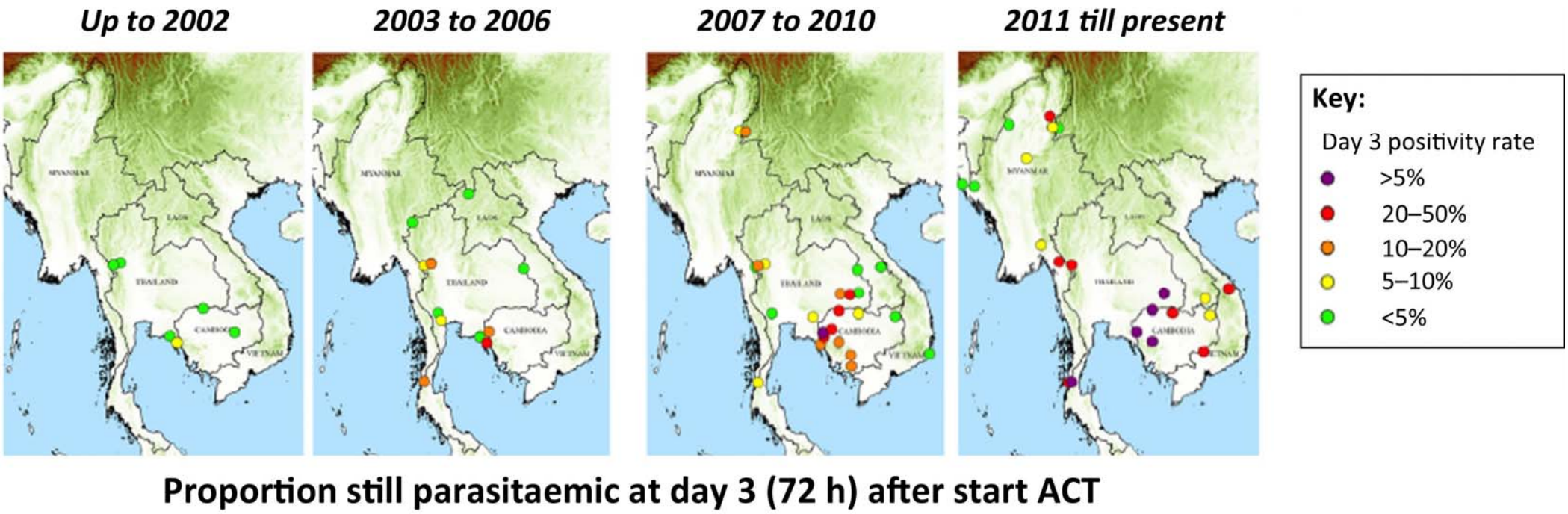
Supporting malaria elimination with intelligence on drug resistance

Olivo Miotto

Joint International Tropical Medicine Meeting **Bangkok, 6 December 2017**


1632 1910
QUININE RESISTANCE
1945 1957
CHLOROQUINE RESISTANCE
1967 1967
SULFADOXINE PYRIMETHAMINE RESISTANCE
1977 1982
MEFLOQUINE RESISTANCE
CHLOROQUINE (CQ) RESISTANCE
1960
1957
1976
SULFADOXINE-PYRIMETHAMINE (SP) RESISTANCE
1967

Today: artemisinin resistance



Dondorp *et al.,* Trends in Parasitology 2017


عربي 中文 English Français
World Health Organization
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Malaria
Eliminating malaria from the Greater Mekong subregion
24 February 2015
Share
WHO is working on developing a regional malaria elimination strategy for the Greater Mekong subregion (GMS). The strategy is being drafted under the aegis of the Regional Hub for the Emergency Response to Artemisinin Resistance in an inclusive and transparent manner.
The process follows the conclusion by the Malaria Policy Advisory Committee (MPAC) in September 2014 that P. falciparum elimination in the GMS is technically feasible and should be the recommended public health response to address the challenge of growing parasite resistance to artemisinin and partner drugs.
In parallel, the 9th East Asia Summit in Myanmar (November 2014) agreed to the goal of an Asia
Related links
More informatic
hub for the Gre
subregion
ERAR regional
11-12 February
ANT
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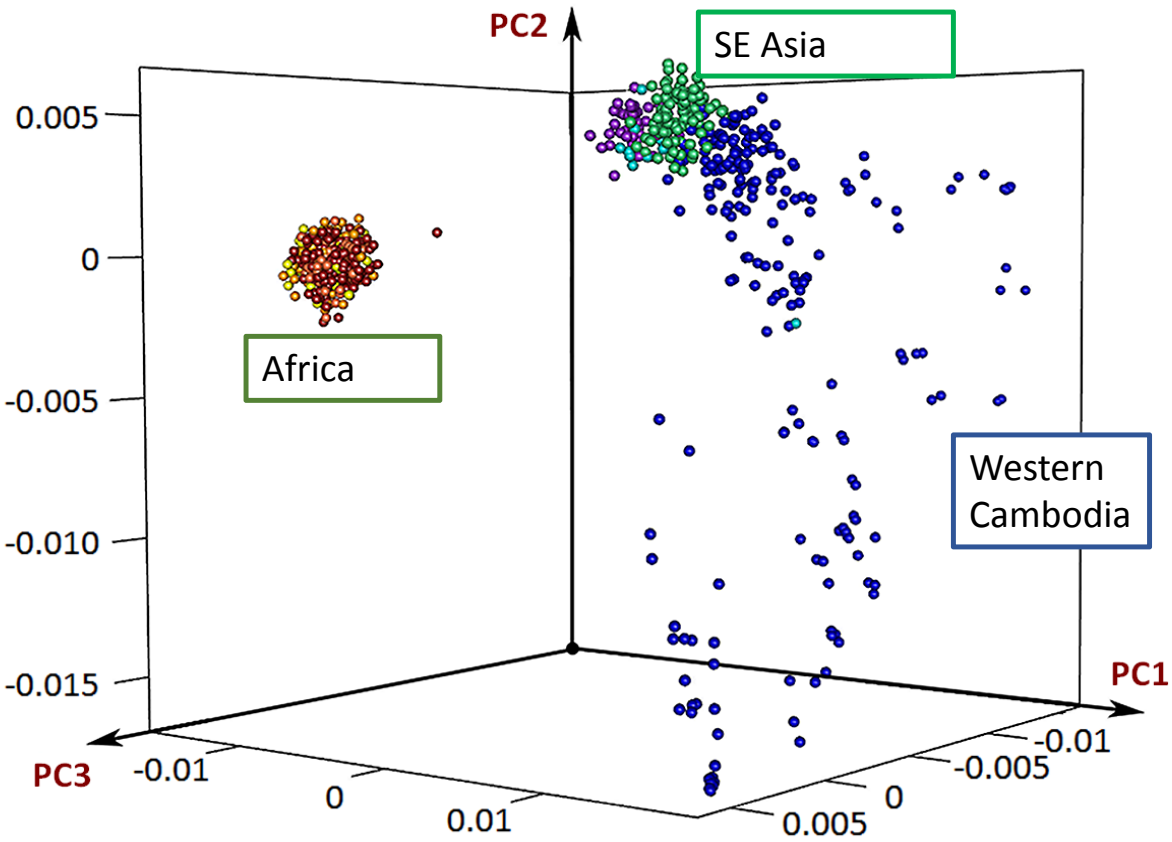
BHUTAN
Lhasa
Jaipur
Lucknow
Ahmadābād
IND
Pune
Bangalore
Laccadive Sea
Male
PALAU

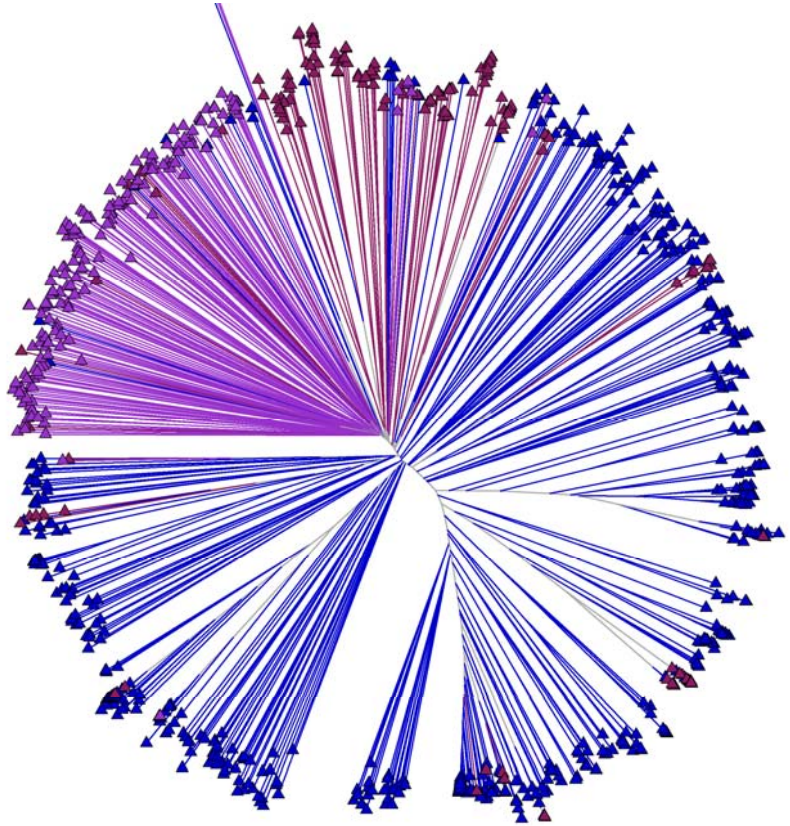
Genomic Epidemiology

- Work from patient blood samples, clinically relevant
- Obtain high-resolution genome-wide data
  - high-throughput whole-genome sequencing technology
  - ~1M high-quality variations across the genome
  - Single-nucleotide polymorphisms (SNPs), insertions/deletions, gene copy numbers
- Large numbers of cases:
  study populations, not individuals

- Multidimensional Analyses
  - Genomic, geographical, temporal, clinical
- Analyze allele frequencies, diversity, similarities between parasites
  - Relate observations to epidemiological models
- MalariaGEN:
  >130 Terabytes of genomic data

Patterns of Drug Resistance in Western Cambodia

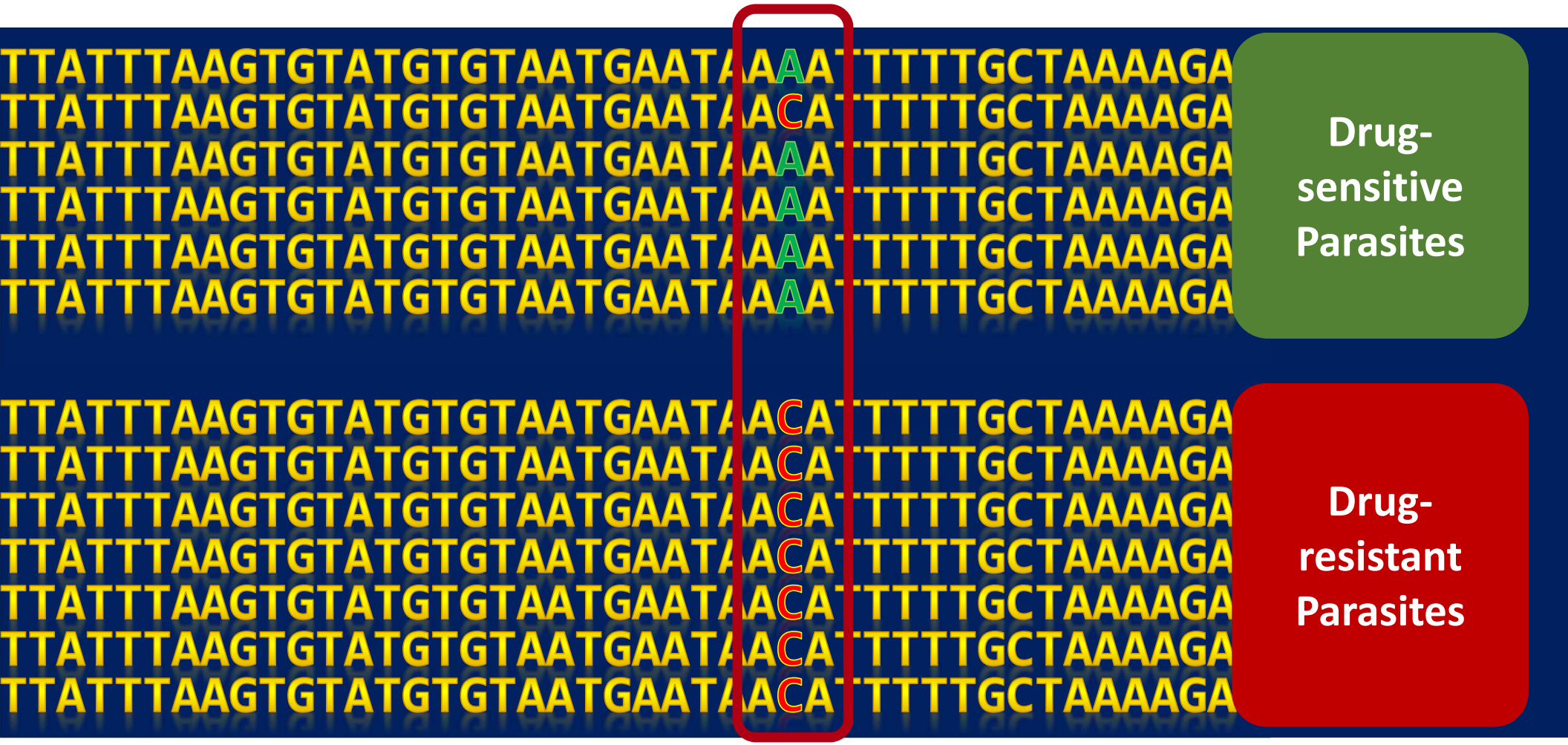




nature genetics

Miotto O et al. (2013) Multiple populations of artemisinin-resistant *Plasmodium falciparum* in Cambodia.

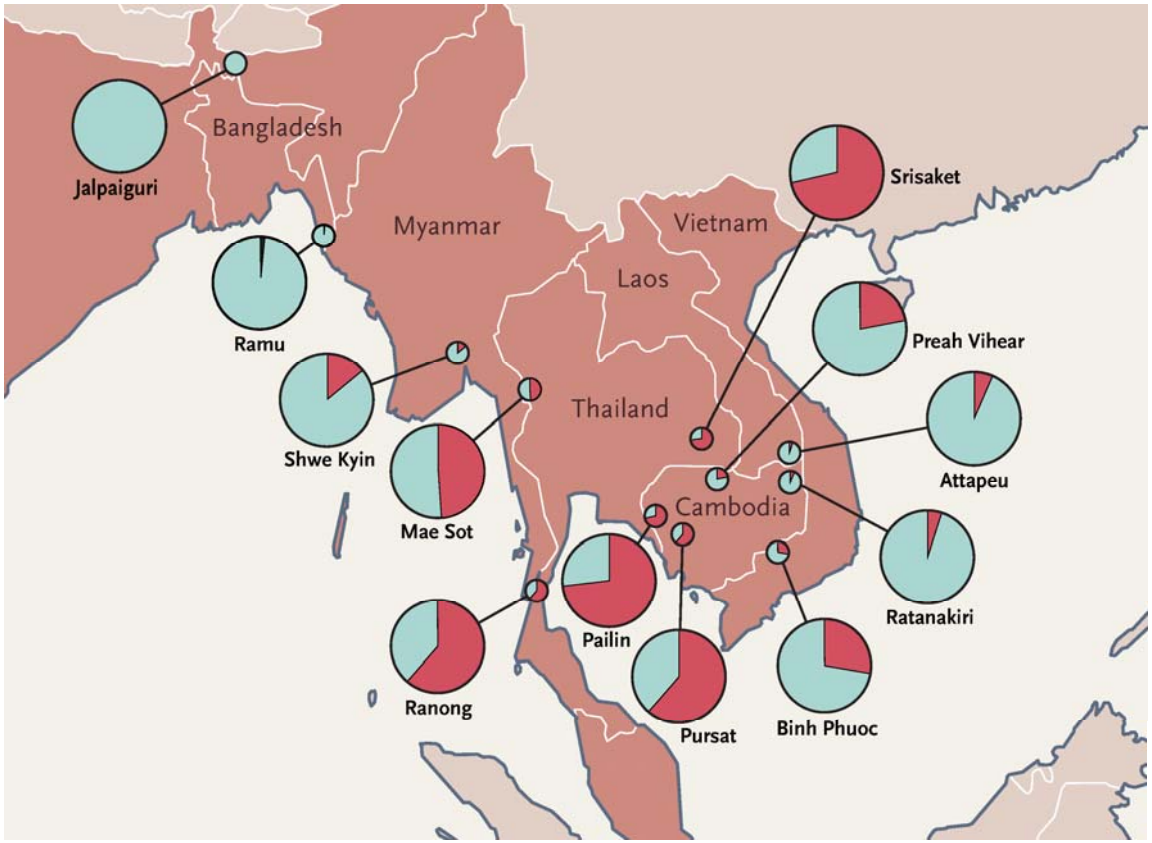
Tackling Drug Resistance - GWAS



TRAC study

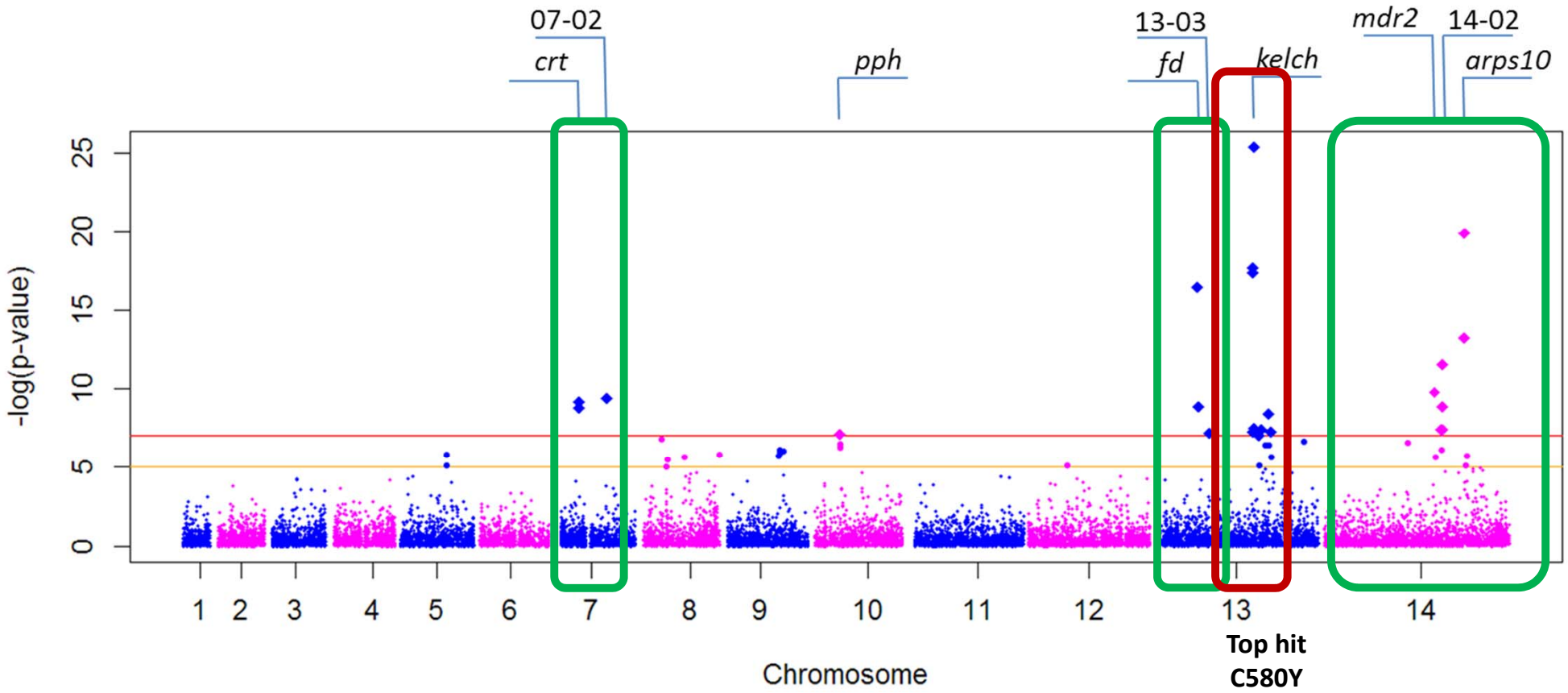
- **1,612 clinical samples**
  - Full genome sequence
  - 1,063 with high-quality clinical phenotypes
- **15 locations in SEA** (+2 in Africa)
  - Cambodia, Vietnam, Laos, Thailand, Myanmar and Bangladesh

High genetic and geographical resolution



Ashley EA et al. (2014) N Engl J Med. 2014 Jul 31;371(5):411-423.

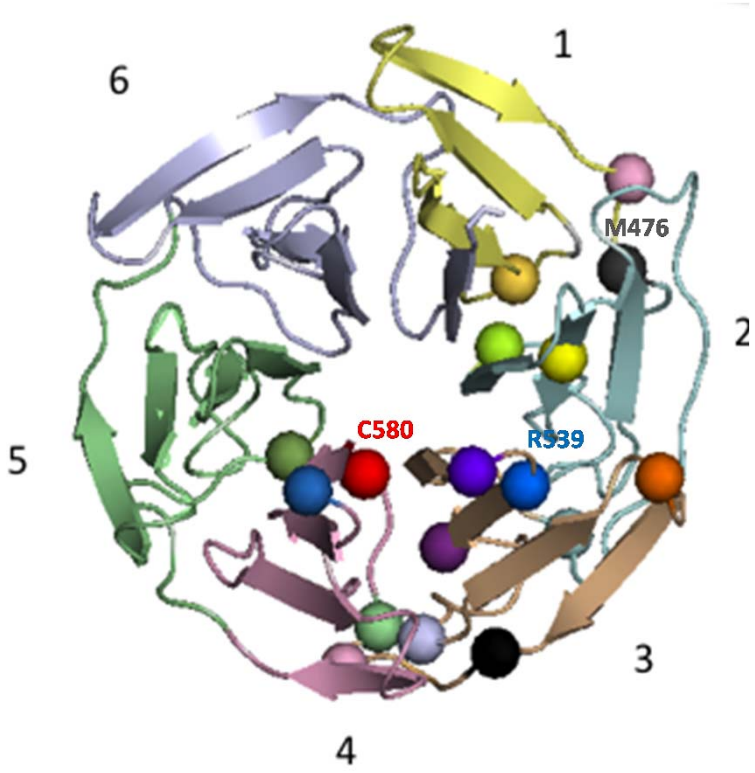
Genome-wide Association Study (GWAS)



Miotto O, Amato R et al. (2015) Genetic architecture of artemisinin-resistant *Plasmodium falciparum*.

nature genetics

Multiple ART-R kelch13 mutations



Each artemisinin-resistant founder populations is strongly associated with a specific *kelch13* mutation

Population	WT	Y493H	R539T	I543T	P553L	C580Y	*Het*	Total
VN-C	69	1			2		4	**76**
KH-C	122						2	**124**
VN-F01				20			1	**21**
VN-F04	1				4		3	**8**
WKH-F01	2					49	3	**54**
WKH-F02	1		15					**16**
WKH-F03						32	2	**34**
WKH-F04		15						**15**
NKH-F02						8	1	**9**
Total	**195**	**16**	**15**	**20**	**6**	**89**	**16**	**357**

Multiple independent emergence events

nature **Ariey, F. et al. *Nature* 505, 50–55 (2014).**

It takes 2 to Tango…

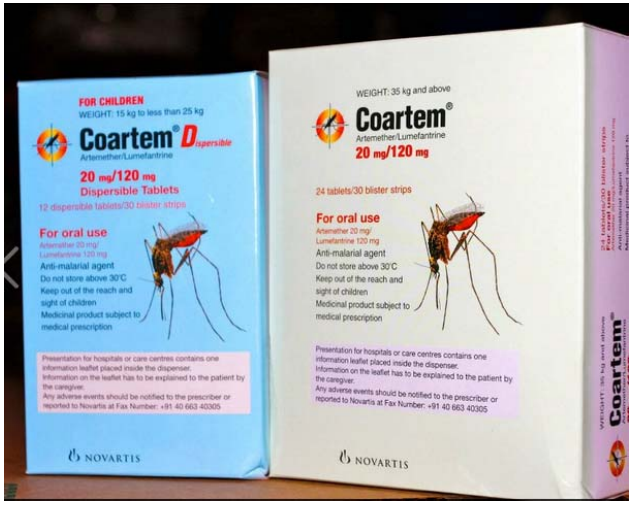
ACT

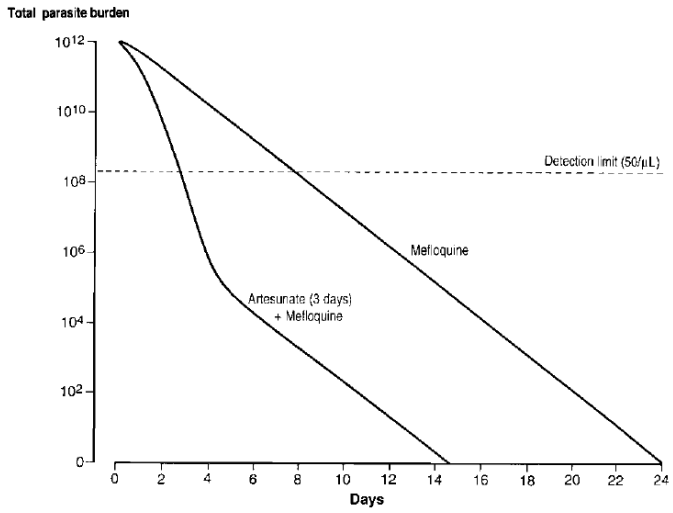
Artemisinin + **Partner Drug**

Fast parasite killer

Kills surviving parasites

Piperaquine
Mefloquine
Lumefantrine





White NJ (1997) Antimicrob Agents Chemother. 41(7):1413-22.

Roberto Amato

Rick Fairhurst

- ~300 clinical samples
- 3 provinces of Cambodia in 2011-2013
- Whole-genome sequences (WGS) on Illumina
- *In vivo* and *in vitro* phenotypes

Articles

Genetic markers associated with dihydroartemisinin–piperaquine failure in *Plasmodium falciparum* malaria in Cambodia: a genotype–phenotype association study

Roberto Amato, Pharath Lim, Olivo Miotto, Chanaki Amaratunga, Dalin Dek, Richard D Pearson, Jacob Almagro-Garcia, Aaron T Neal, Sokunthea Sreng, Seila Suon, Eleanor Drury, Dushyanth Jyothi, Jim Stalker, Dominic P Kwiatkowski, Rick M Fairhurst

Summary

Background As the prevalence of artemisinin-resistant *Plasmodium falciparum* malaria increases in the Greater Mekong subregion, emerging resistance to partner drugs in artemisinin combination therapies seriously threatens global efforts to treat and eliminate this disease. Molecular markers that predict failure of artemisinin combination therapy are urgently needed to monitor the spread of partner drug resistance, and to recommend alternative treatments in southeast Asia and beyond.

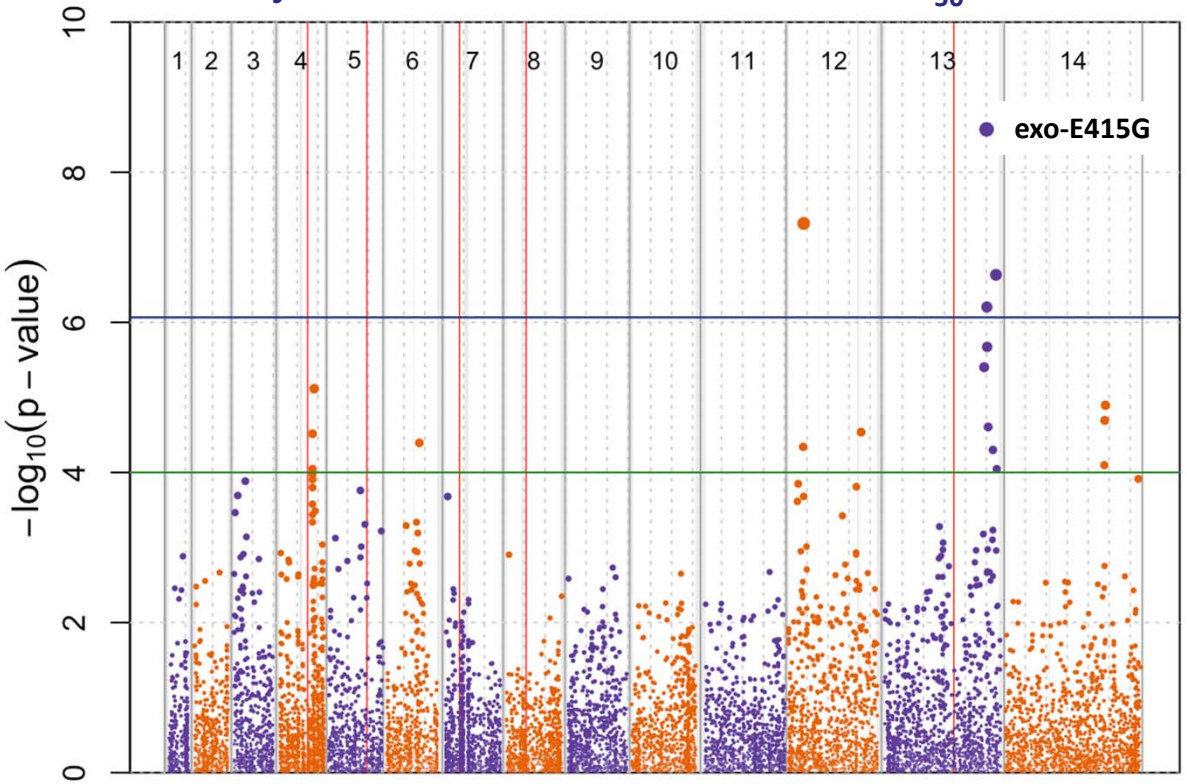
Methods We did a genome-wide association study of 297 *P falciparum* isolates from Cambodia to investigate the relationship of 11 630 exonic single-nucleotide polymorphisms (SNPs) and 43 copy number variations (CNVs) with in-vitro piperaquine 50% inhibitory concentrations (IC_{50}s), and tested whether these genetic variants are markers of treatment failure with dihydroartemisinin–piperaquine. We then did a survival analysis of 133 patients to determine whether candidate molecular markers predicted parasite recrudescence following dihydroartemisinin–piperaquine treatment.

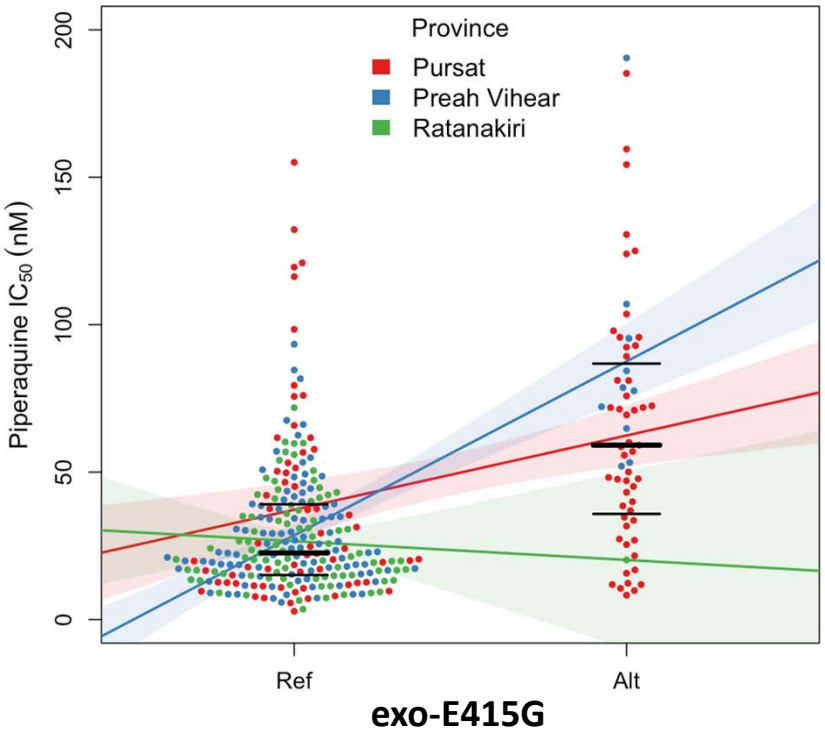
Lancet Infect Dis 2016
Published Online
November 3, 2016
http://dx.doi.org/10.1016/S1473-3099(16)30409-1
See Online/Comment
http://dx.doi.org/10.1016/S1473-3099(16)30414-5
See Online/Articles
http://dx.doi.org/10.1016/S1473-3099(16)30415-7

Wellcome Trust Sanger Institute, Hinxton, UK

Identifying the molecular markers: SNP GWAS

One major locus associated with elevated IC_{50}s

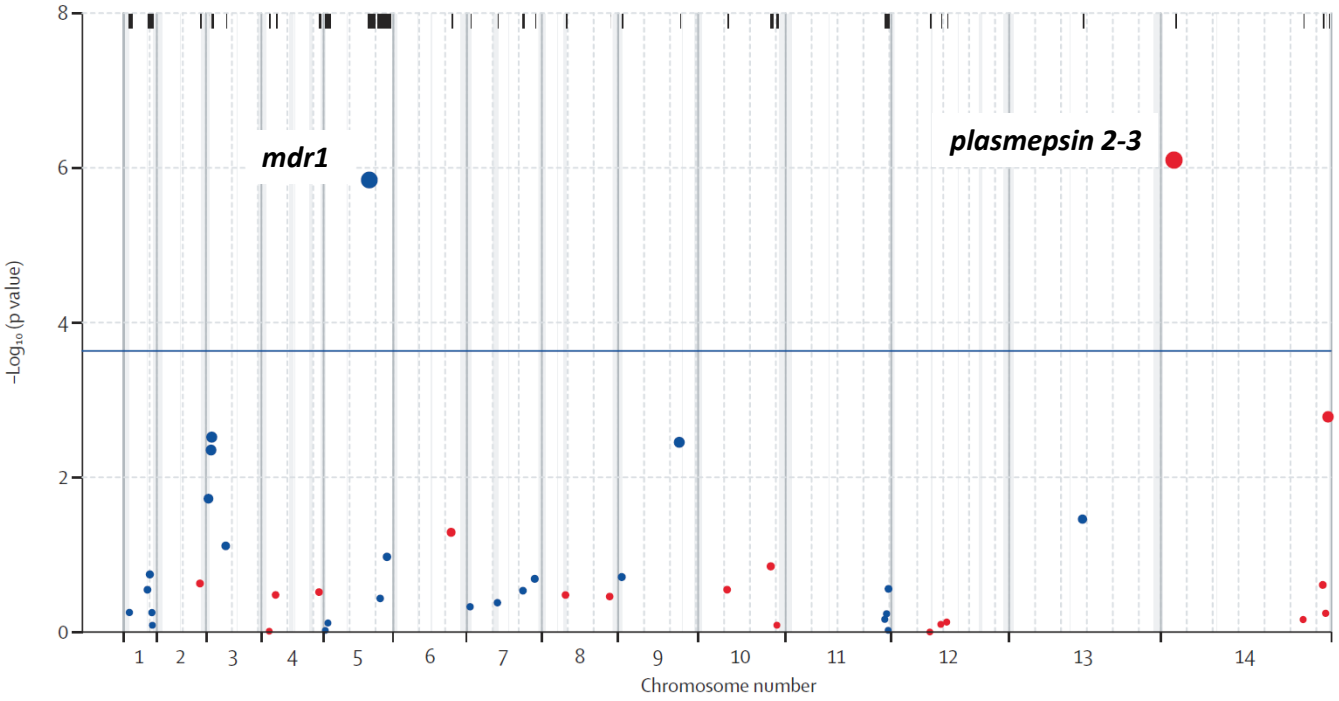


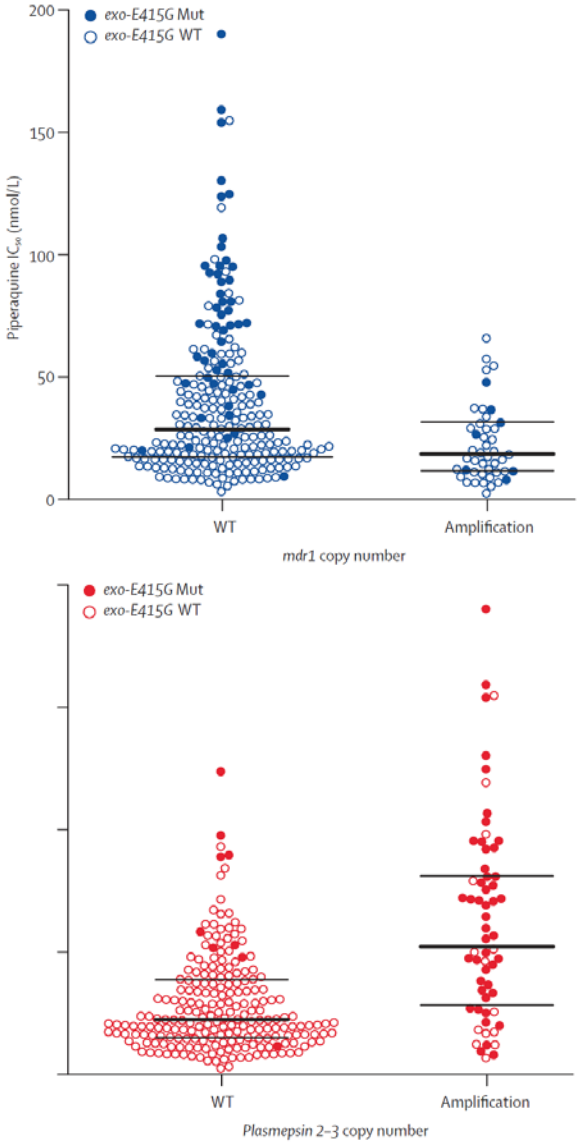


Amato et al. (2016) Lancet ID

Identifying the molecular markers: copy-number GWAS

Two CNVs associated with elevated IC_{50}s

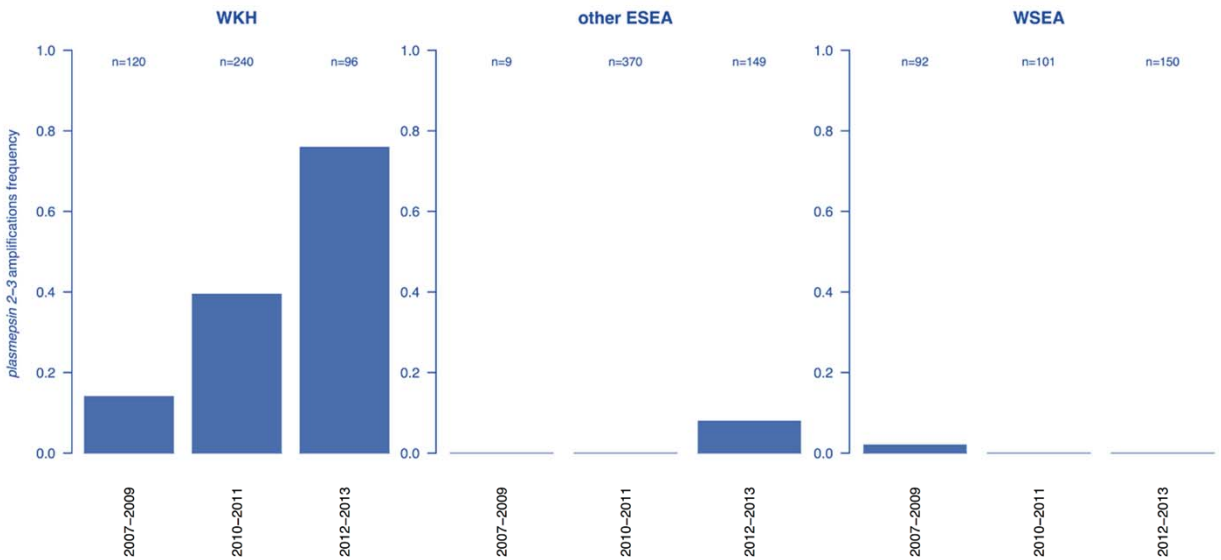




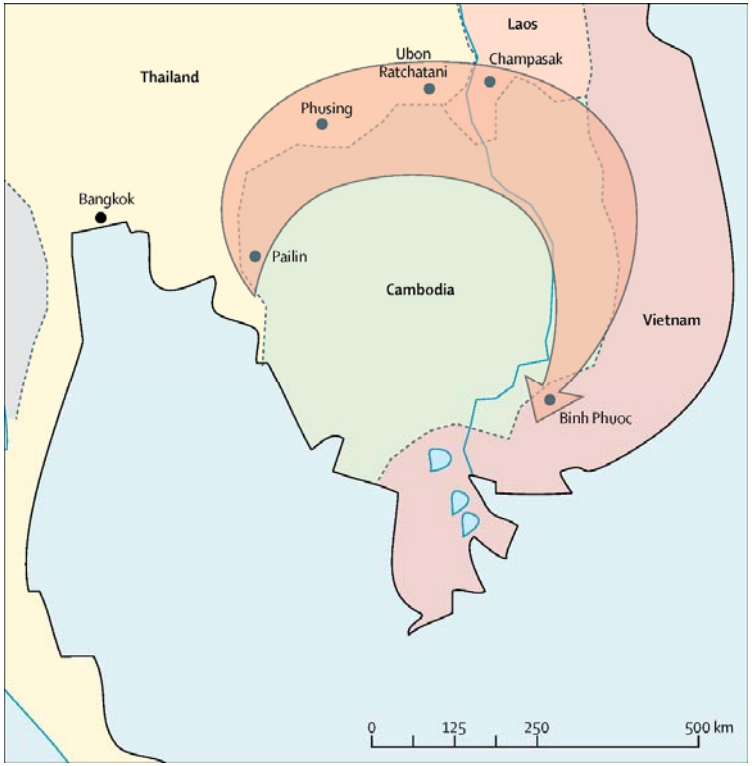
Amato et al. (2016) Lancet ID

DHA-PPQ resistance has spread dangerously

Single major origin of *plasmepsin 2-3* amplifications (PLA1)



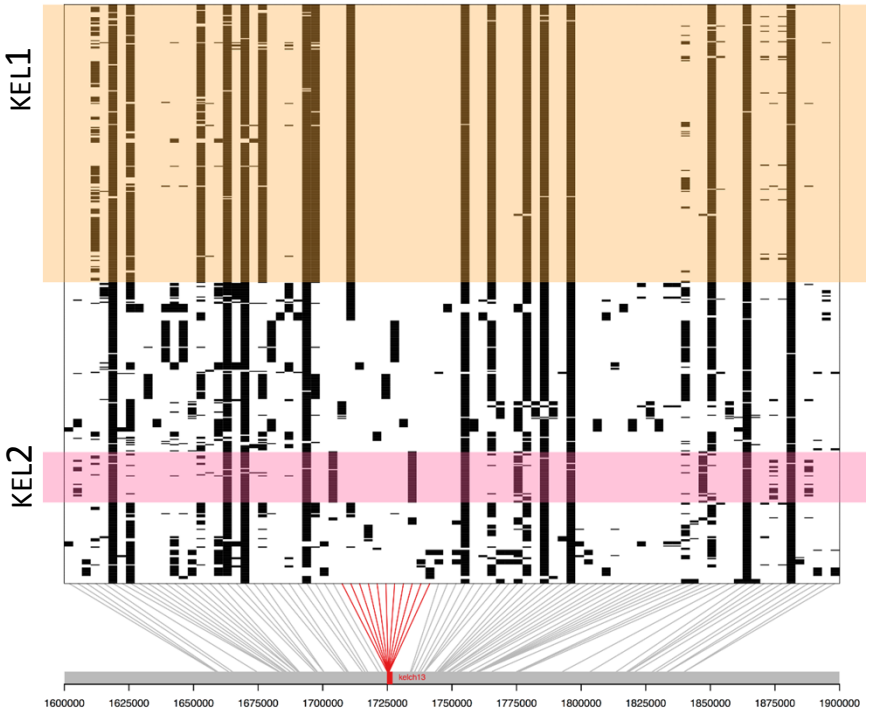
Amato R. *et al.* (2017) Lancet ID (in press)



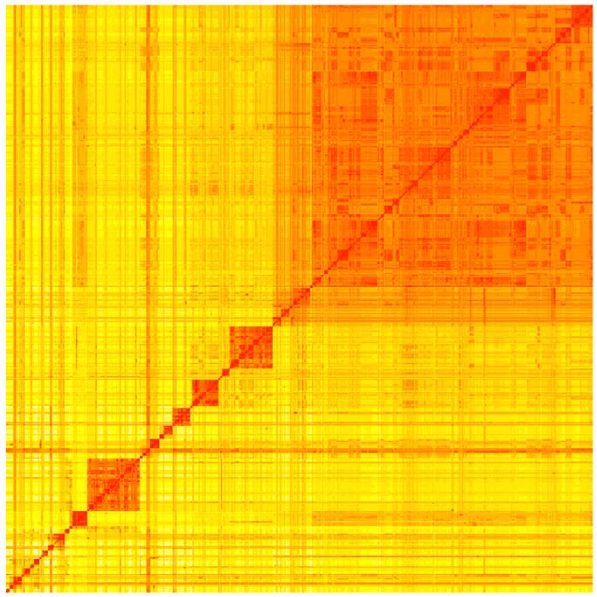
Imwong et al. (2017) Lancet ID

Multiple origins of kelch13 resistance alleles

- We identified 38 *kelch13* haplogroups
- The 580Y resistance allele was found in six different *kelch13* haplogroups
- **The most common was denoted the KEL1 lineage.**

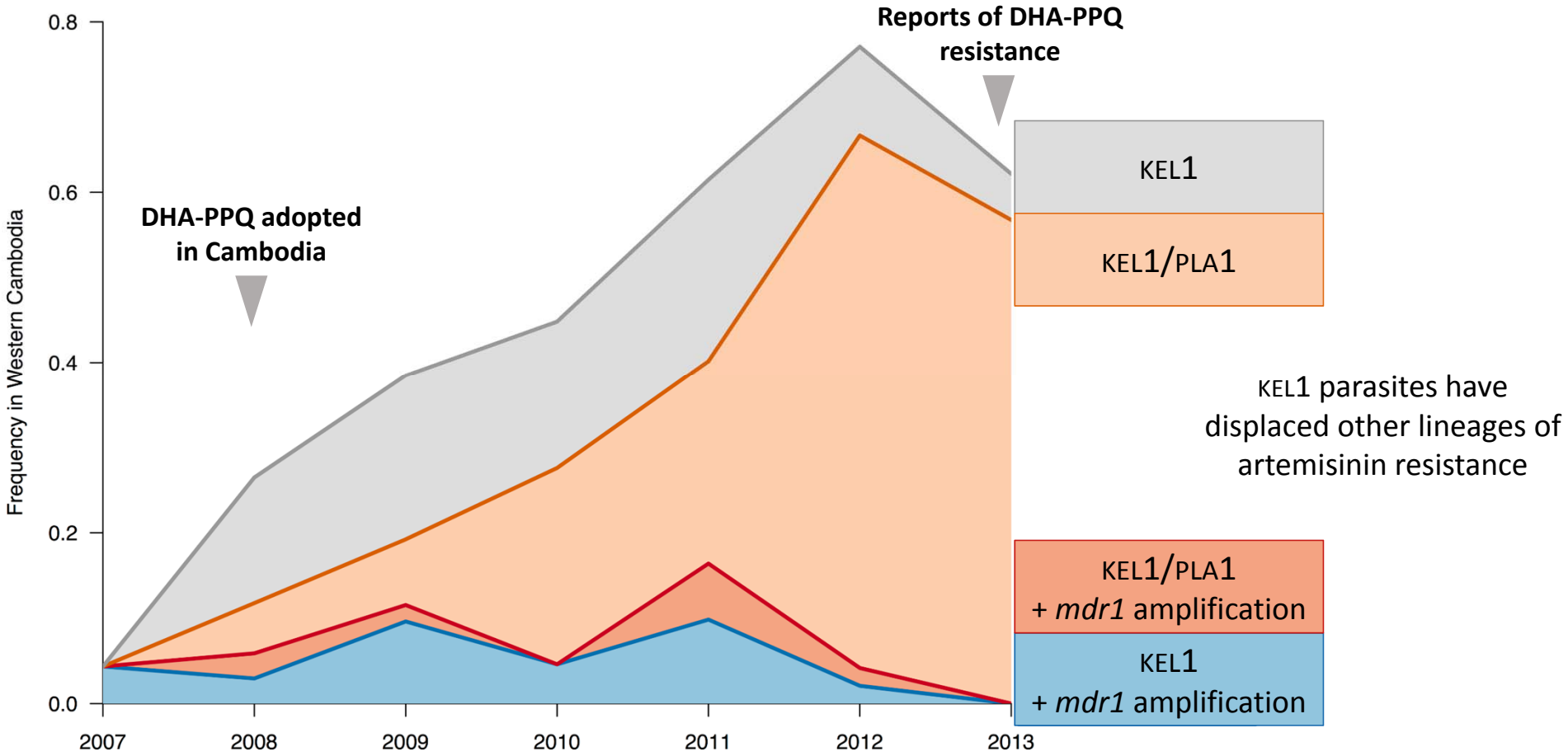


Amato R. *et al.* (2017) Lancet ID (in press)



Statistical chromosome painting to estimate the level of shared ancestry between samples

KEL1 + PLA1 → Winning Combination



KEL1 parasites have displaced other lineages of artemisinin resistance

Amato R. *et al.* (2017) Lancet ID (in press)

Impact of Genomic Epidemiology

- Patterns of Population Structure reveal drug resistance dynamics
- GWAS allows identification of genetic markers of resistance
- Ancestry analysis allows identification of lineages and co-lineages

What do these mean for control and elimination?

- Markers for monitoring and mapping drug resistance
- Markers to monitor specific “strains”
- Epidemiological patterns associated with emergence of resistance

Can this be implemented into routine surveillance?

The GenRe-Mekong Project


WHO/Harri Anenden

Symptomatic Patients

Health Centres

Control and Elimination Policy

Country Partner (e.g. National Malaria Control Programme)

Genetic Report Cards

Dried Blood Spots (DBS)

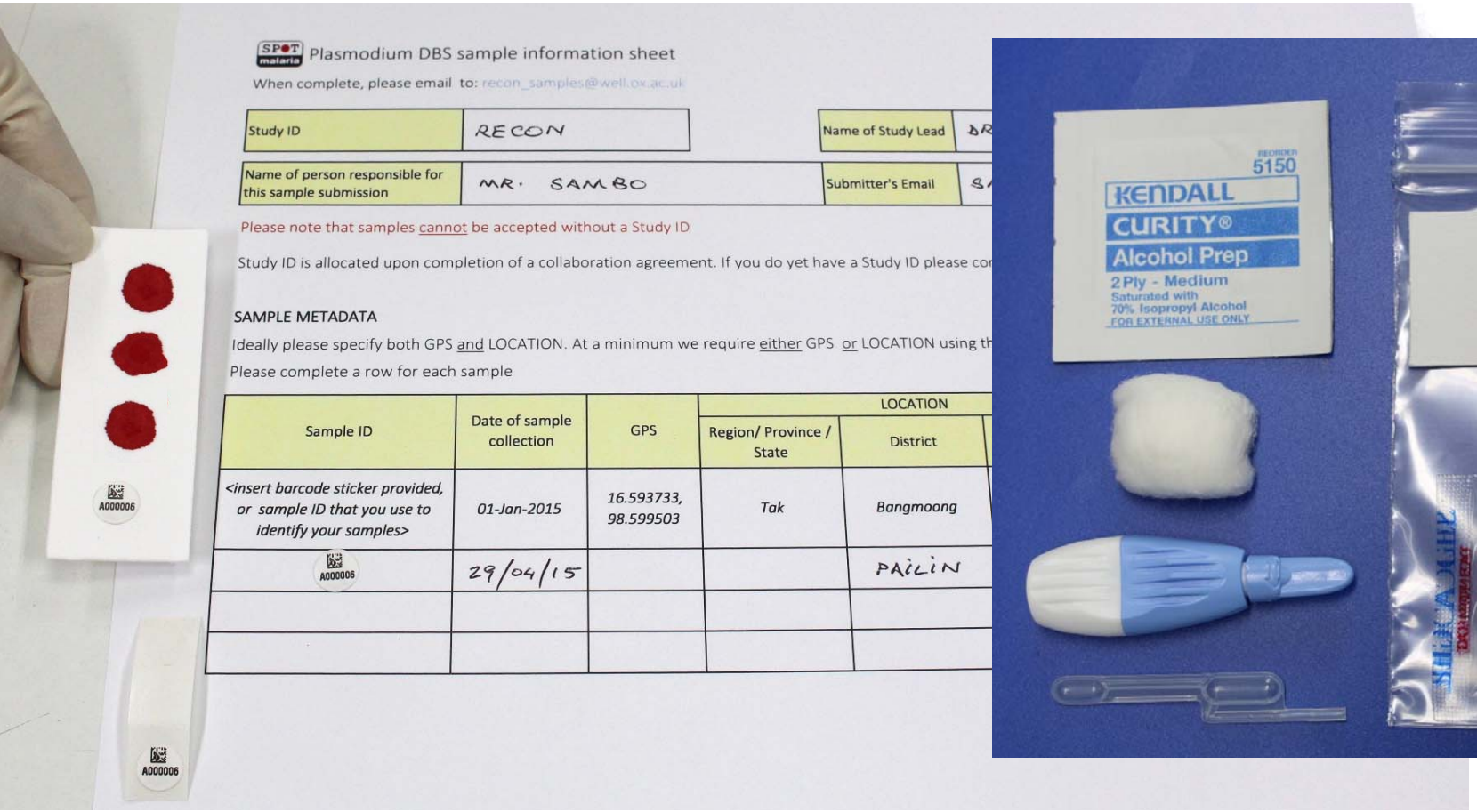
DNA Extraction

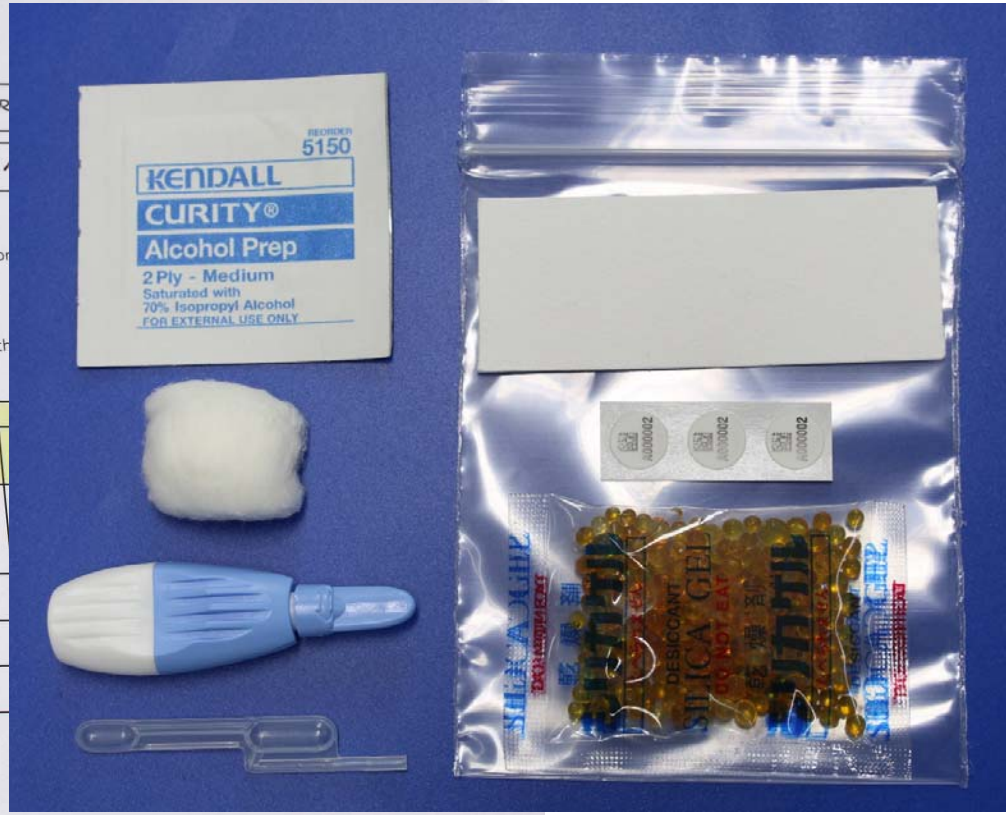
Sample Processing

Reporting

SPOT malaria GenRe MEKONG

Standardized Collection Kits





Genetic Report Cards

- Fast turnaround product for malaria control programs
- Simple to understand and analyze, with documentation and training
  - Can be summarized, mapped, aggregated, reported
- Integrates via barcodes
  - With NMCP patient data
  - With travel surveys
- Per-sample drug resistance markers
- Per-site, per-drug summaries

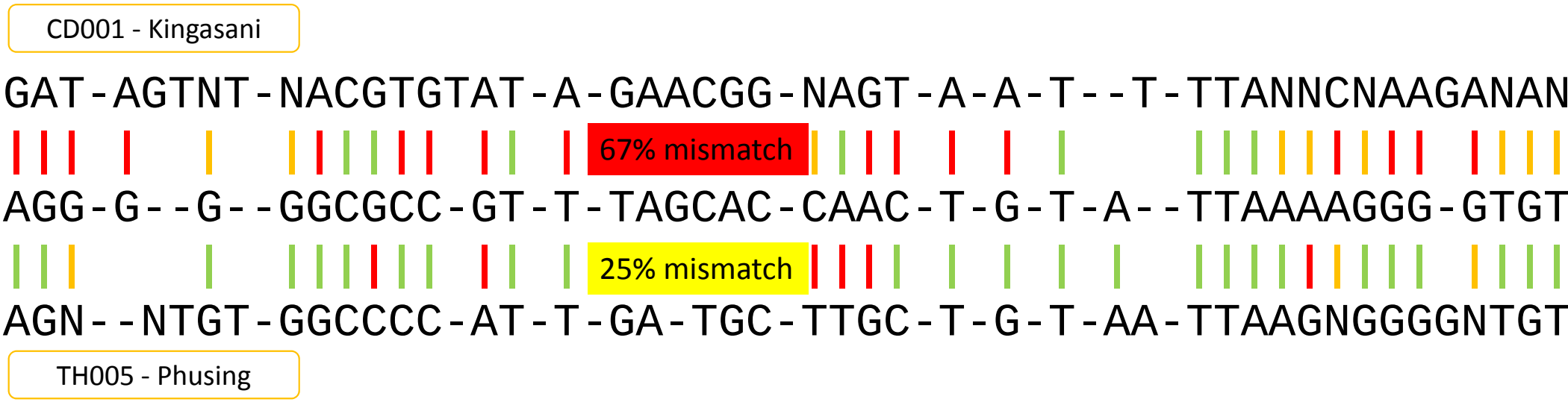
ID	COI	Species	K13	DHFR	MDR1	CRT	DHPS	EXO	K13 PGB
PM0023-C	1	Pf	wt	NCSI	NFD	-	SAKAA	D	NIDTV
PM0026-C	2	Pf	wt	IRNL	YFD	CVIET	AAKAA	D	NIDTV
PM0103-C	2	Pf	wt	IRNL	YFD	CVMNK	AAKAA	D	NIDIV
PM0113-C	1	Pf	I416M	NCSI	YFD	CVIET	AAKAA	D	NIDTV
PM0114-C	2	Pf	589V	IRNL	NFD	CVIET	AGKAA	D	NIDTV
PM0115-C	1	Pf	wt	IRNL	NYD	CVIET	AGKAA	D	NIDTV
PM0117-C	1	Pf	wt	NCSI	NYD	CVIET	AGKAA	D	NTDTV
PM0123-C	2	Pf	wt	NCSI	YFD	CVIET	AGKAA	D	NTDTV
PM0124-C	1	Pf	wt	IRNL	NYD	CVIET	AGKAA	D	SIDTV
PM0127-C	1	Pf	wt	IRNL	NYD	CVMNK	AGKAA	D	NIDTV
PM0129-C	1	Pf	wt	NCSI	NFD	CVMNK	SGKAA	D	NIDTV
PM0132-C	1	Pf	645N	NCSI	NFD	CVMNK	SGKAA	D	NIDTV
PM0133-C	1	Pf	wt	NCSI	NFD	CVIET	AGKAA	D	NIDTV
PM0134-C	2	Pf	wt	IRNL	YFD	CVIET	AAKAA	D	NTDTV
PM0136-C	2	Pf	548G	NCSI	YFD	CVIET	AGKAA	D	NIDTV
PM0138-C	3	Pf	N537I	IRNL	YFD	CVIET	AGKAA	D	NTDTV
PM0139-C	4	Pf	wt	IRNL	YFD	CVIET	AAKAA	D	NTDTV
PM0142-C	1	Pf	wt	IRNL	NYD	CVIET	AGKAA	D	NIDTV
PM0144-C	2	Pf	wt	IRNL	YFD	CVIET	AAKAA	D	NIDTV
PM0145-C	1	Pf	wt	NCSI	YFD	CVIET	AGKAA	D	NTDTV
PM0146-C	1	Pf	wt	NCSI	YYY	CVIET	AGKAA	D	NIDTV
PM0147-C	2	Pf	wt	NCSI	NFD	CVIET	AGKAA	D	NIDTV

Genetic Report Cards

- Parasite Drug Resistance profiles
  - Artemisinin: K13 allele, predisposing background
  - ACT partner drugs:
    Piperaquine, Mefloquine, Amodiaquine, Lumefantrine
  - Key antimalarials: Chloroquine
  - Key antimalarials: Sulfadoxine, Pyrimethamine
- Co-infecting Species Detection
  - *P. vivax*
  - *P. knowlesi*
- Complexity of Infection Estimates

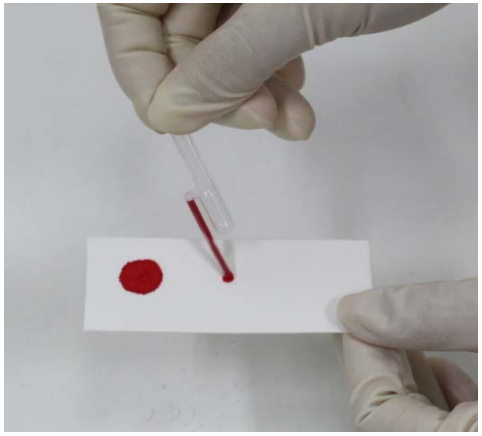
Genetic Barcodes

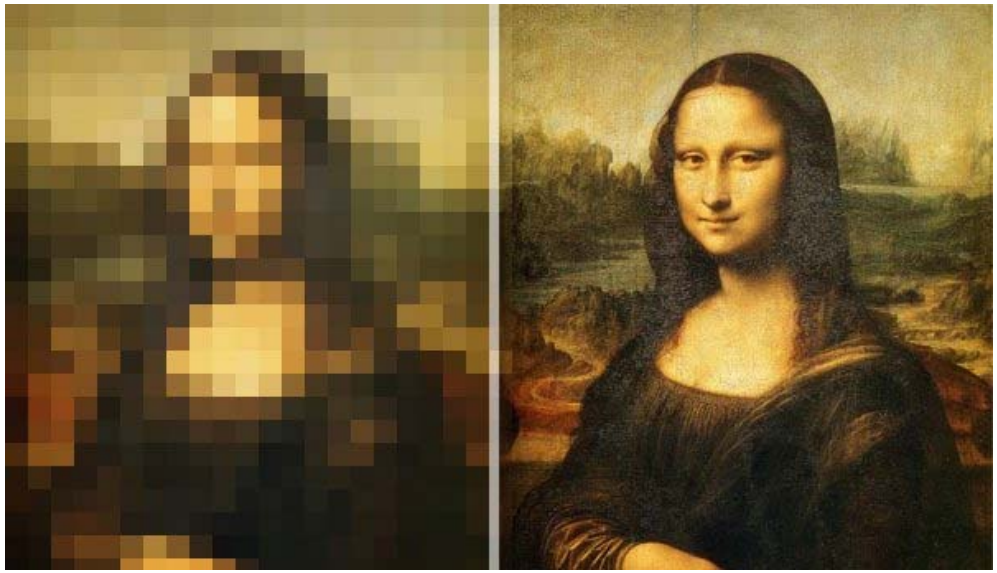
- We routinely genotype 101 *barcoding* variations
  - Recrudescence vs Reinfection
  - Complexity of Infection
  - Identification of expanding strains
  - Characterization of population diversity
- Concrete examples
  - Expansion of current wave of ART-R parasites across region
  - Detection of treatment failures



High-Resolution Data from DBS

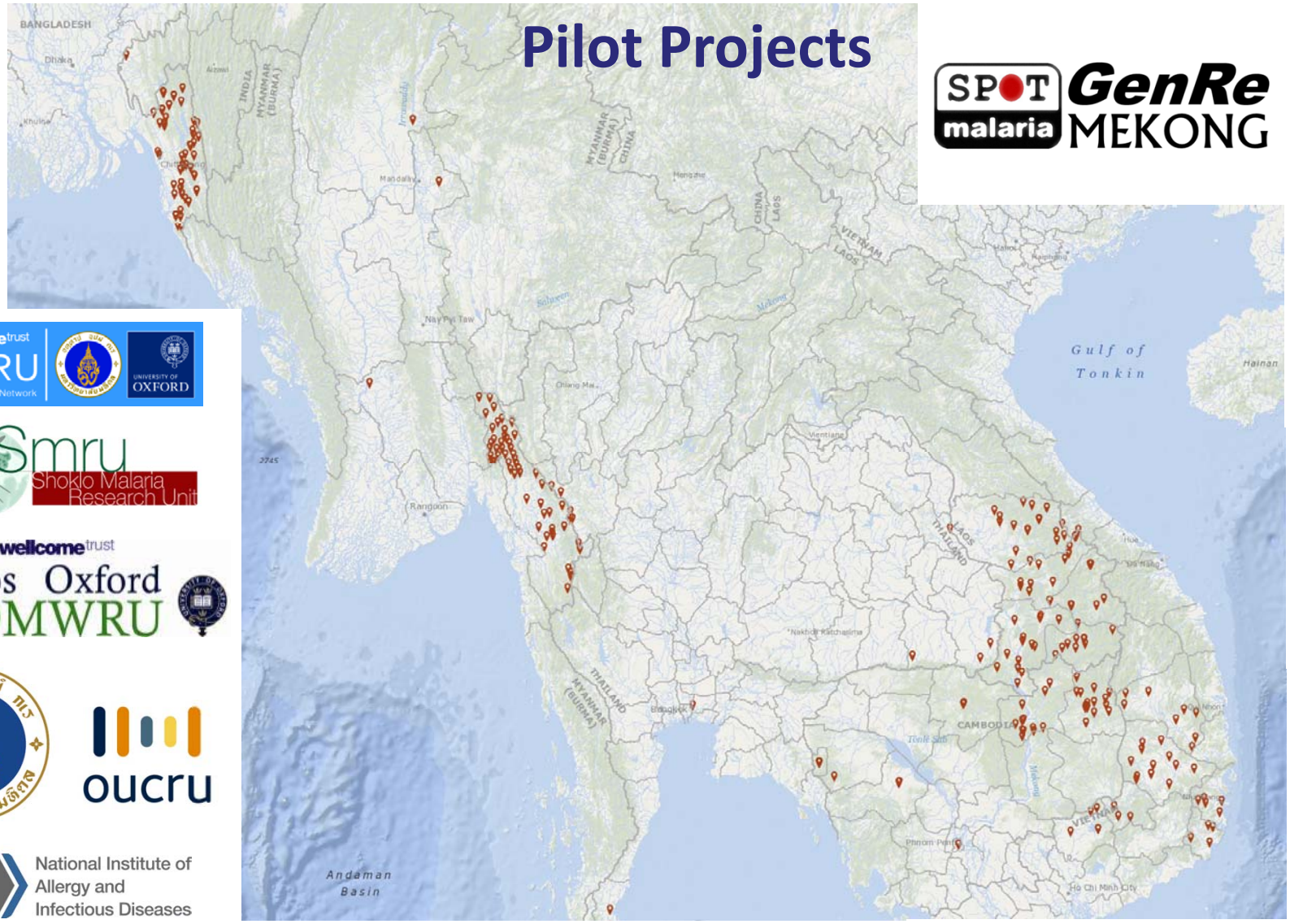
- Selective Whole-Genome Amplification
  - Allows enrichment of parasite DNA vs. human DNA
  - Reliably enables whole-genome sequencing (WGS) from DBSs





- Enable high-resolution epidemiology analyses
  - Reliable identification of imported cases
  - Modelling routes of gene flow
  - GWAS of parasites with phenotypic data
  - Study of haplotypes at candidate sites
  - Study of emerging markers or regions under selection

Pilot Projects



SPOT malaria GenRe MEKONG

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Smru Shoklo Malaria Research Unit

wellcome trust Laos Oxford LOMWRU

oucru

NIH National Institute of Allergy and Infectious Diseases

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- Rick Fairhurst
- Chanaki Amaratunga
- Seila Suon
- Sokunthea Sreng

All TRAC Collaborators

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- Bangladesh: **NMCP**
- Cambodia: **CNM**
- Lao PDR: **CMPE**
- Myanmar: **DSMRC**
- Thailand: **Ubon Provincial Health**
- Vietnam: **IMPE-QN**
- Vietnam: **NIMPE**

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BILL & MELINDA GATES foundation

MRC Medical Research Council

UKaid from the British people