Evolutionary Perspective of Drug Resistance in South America and Test Results of Suriname and Guyana Samples Analyzed by CDC

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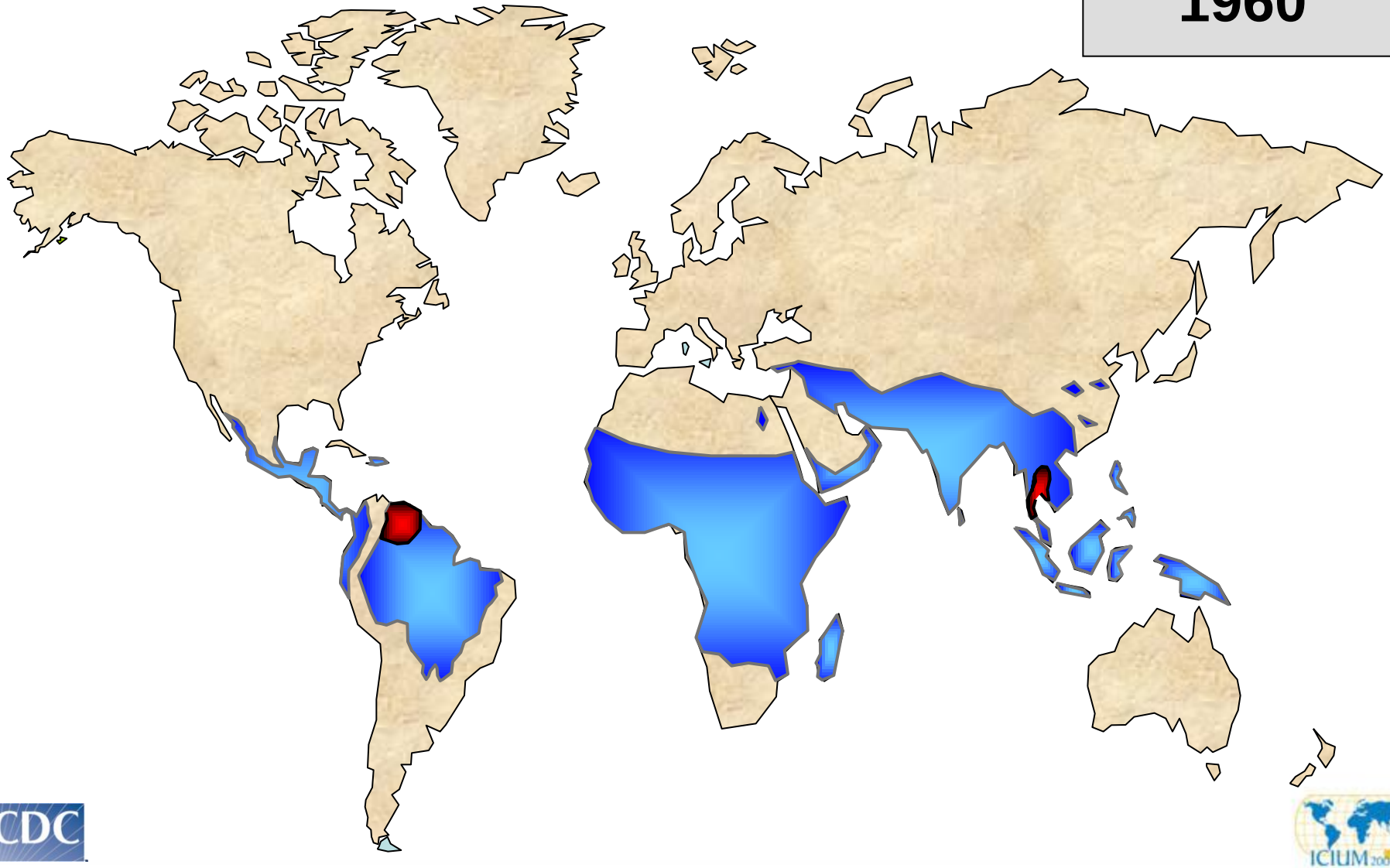
Kumar V. Udhayakumar, Ph.D
Malaria Branch
CDC, Atlanta, USA

Distribution of Chloroquine-resistant *P. falciparum*

Distribution of Malaria

1960



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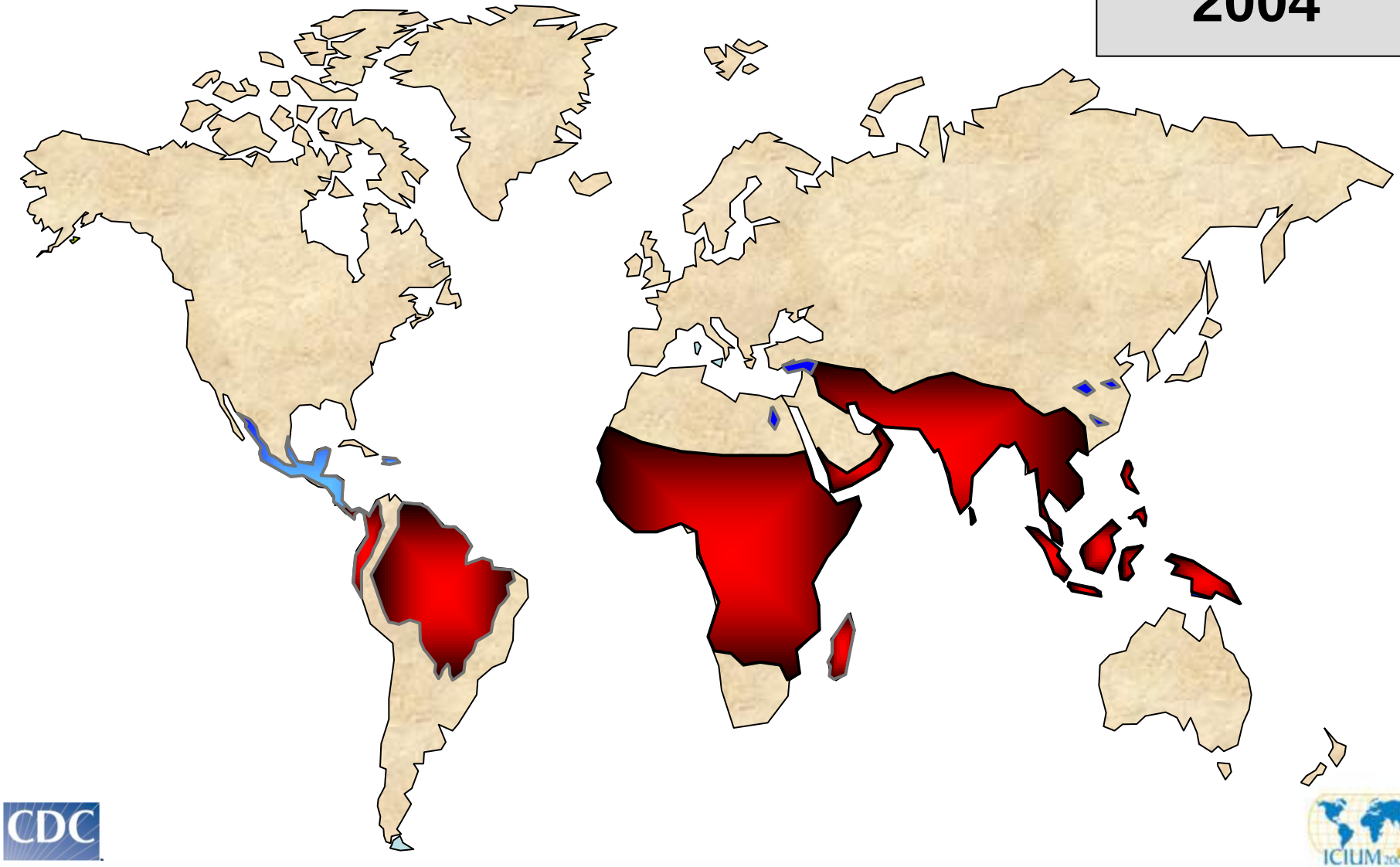
ICIUM 2004

Distribution of Chloroquine-resistant *P. falciparum*

Distribution of Malaria

2004



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ICIUM 2004

How did drug resistance mutations evolved and spread in the populations?

Molecular tools are useful to answer such questions

Molecular markers for drug resistance

- **Chloroquine (CQ):**

  Pfcrt: **Mutations at codons C72S, V73V, M74I, N75E, K76T**

- **Sulfadoxine + pyrimethamine (SP):**

  Pfdhps: **S436A/F/Y, A437G, K540E, A581G, A613S/T**

  Pfdhfr : **A16V, C50R, N51I, C59R, S108N, I164L**

- **Mefloquine (MQ), lumefantrine (LUM):**

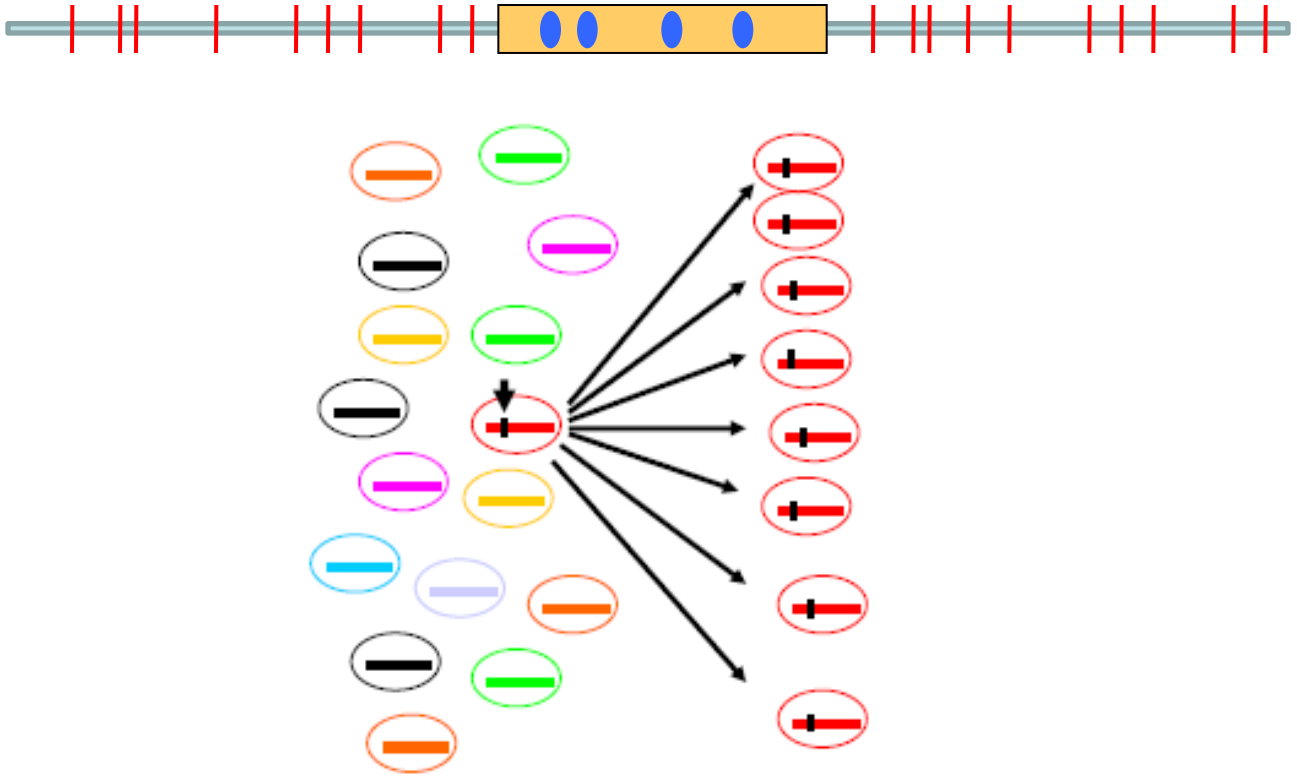
  Pfmdr1: **N86Y, Y184F, S1034C, N1042D, D1246Y**

  Pfmdr1: **Increase in gene copy number**

Microsatellites: Tool for tracking resistant alleles

Random repeats (2-6 bp) of nucleotides scattered along the chromosome

Example: ATATATATATATATATATAT



<u>Selection</u>: Resistant alleles spread rapidly through population

Evolution of *P. falciparum* drug resistance associated *pfcrt* alleles

Chloroquine resistance:

Mita and Tanabe. JID 2012.

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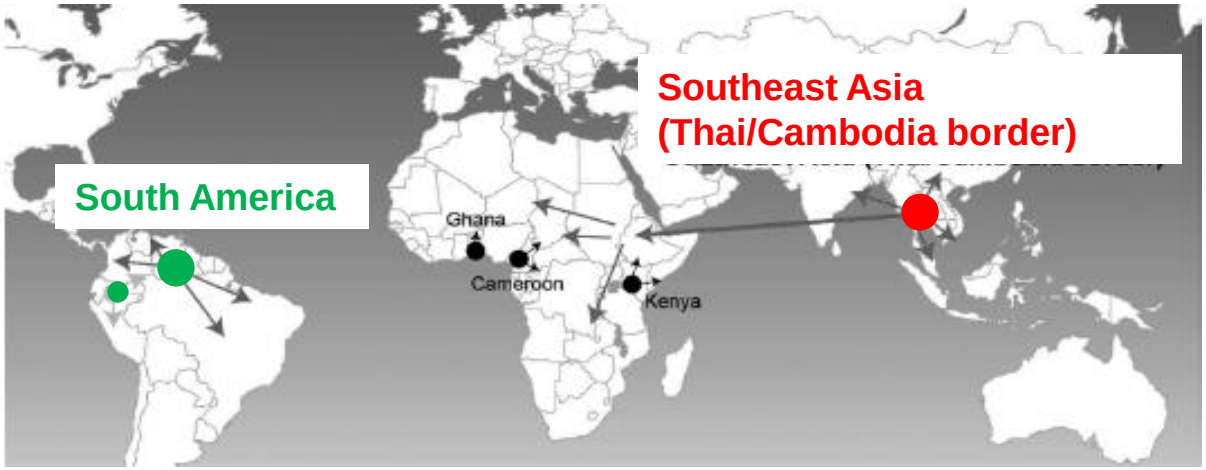
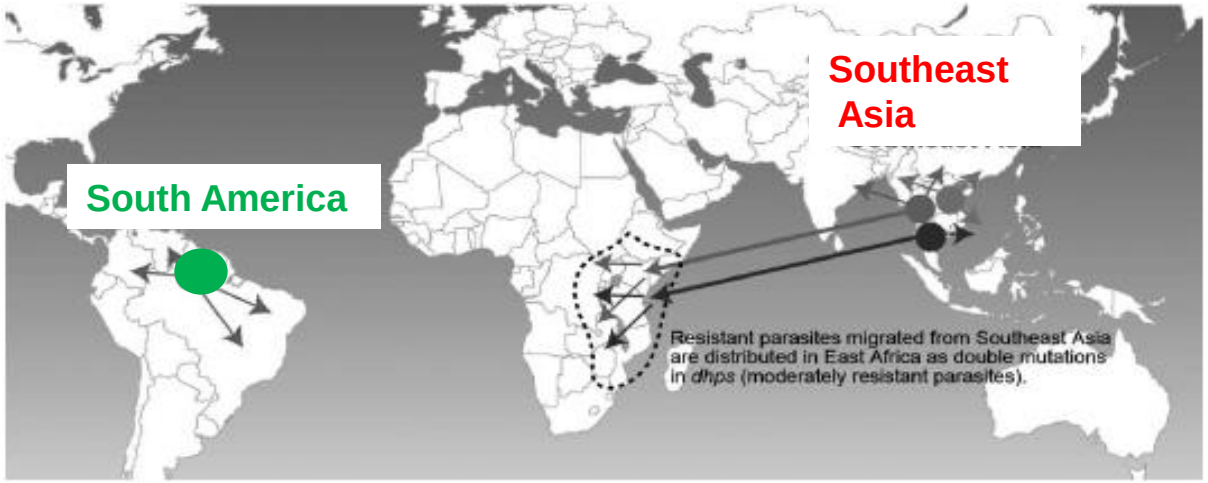
Evolution of *P. falciparum* SP resistance

Pyrimethamine

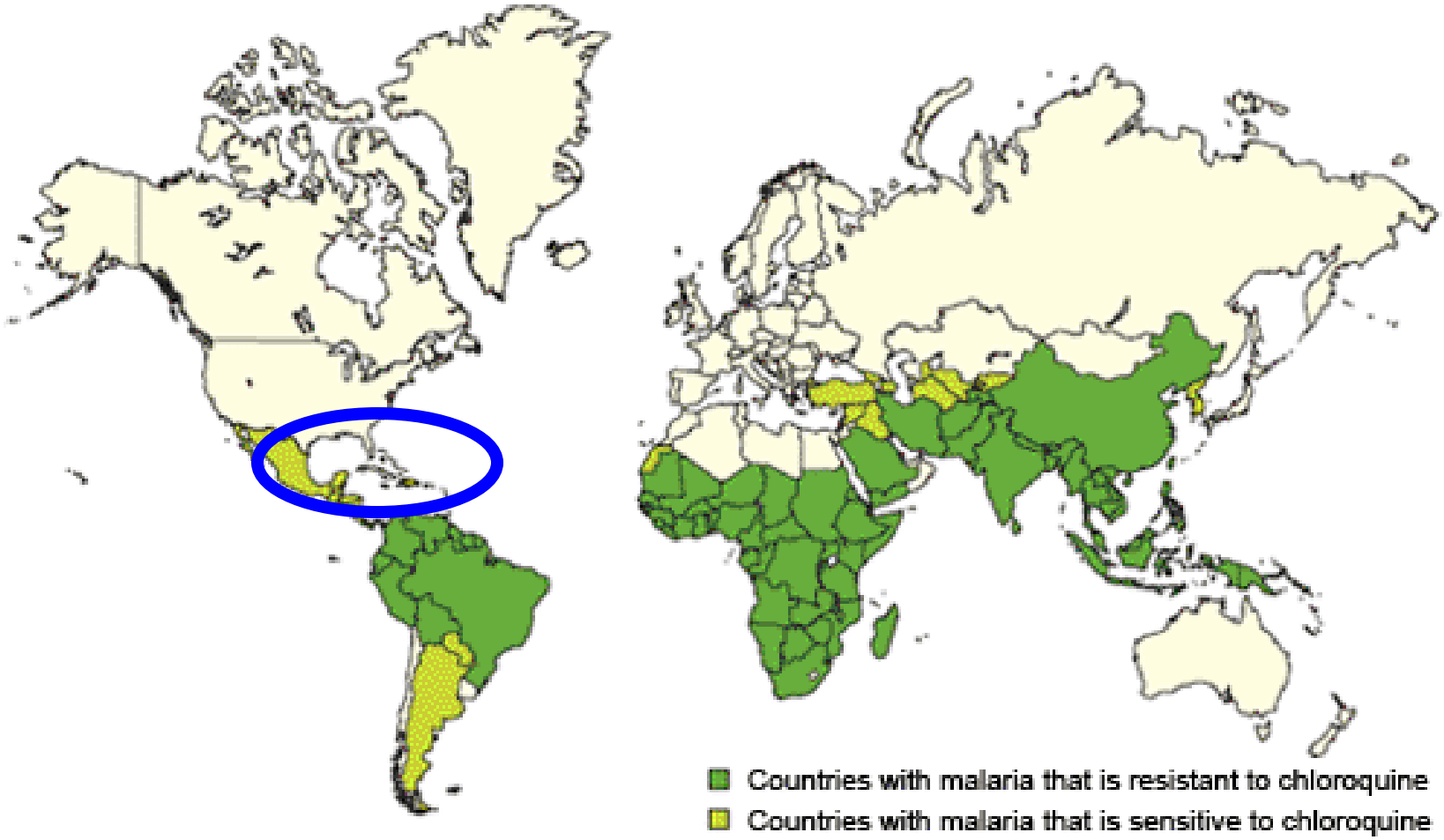


Sulfadoxine



Mita and Tanabe. JID 2012.

Why CQ/SP Resistance Not Spread to Central American Region?



http://publications.nigms.nih.gov/thenewgenetics/chapter4.html

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What are the key findings?

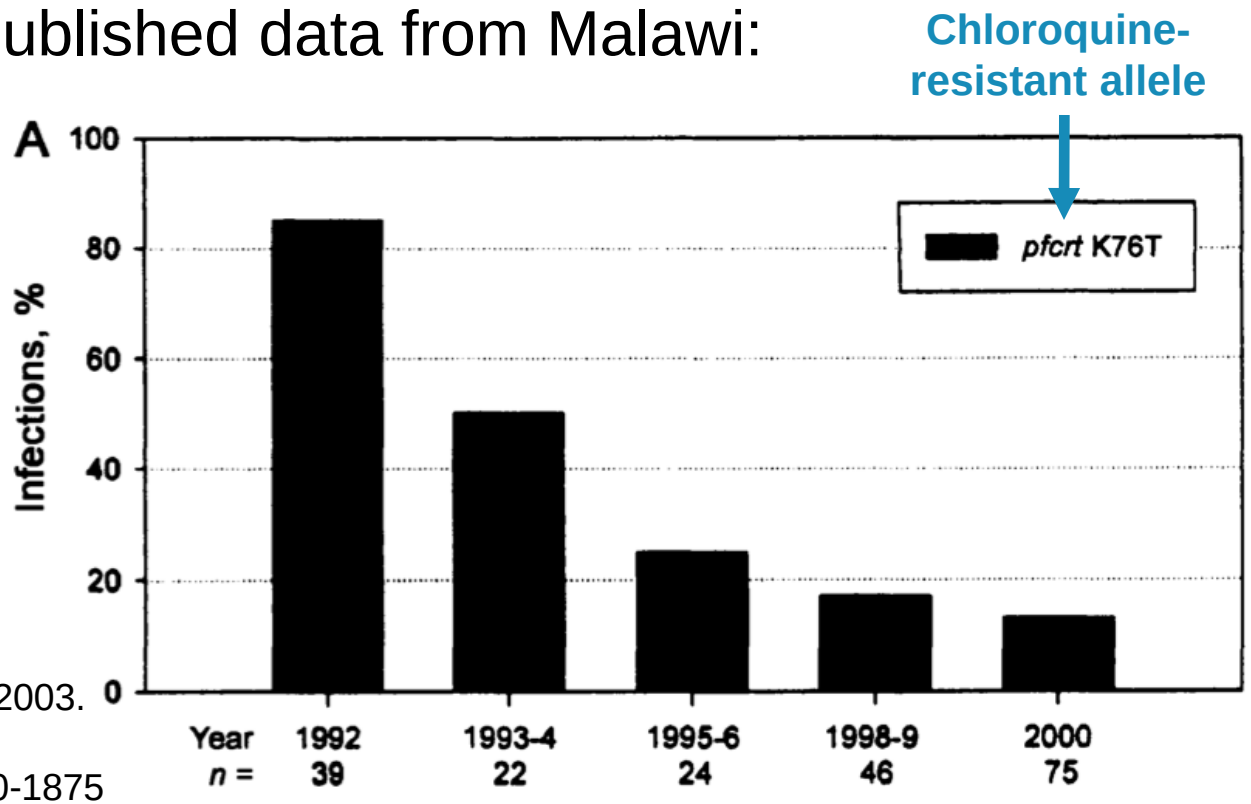
- **Historically drug resistance evolved and spread from limited number (4-5) of founder populations**
- **Resistance evolved independently and in parallel in South America and Southeast Asia**
- **Central American region remains a distinct ecological niche where resistance has not been established**
- **CQ and SP resistance spread to Africa from Southeast Asia**

Does resistance carry a fitness cost?

- Drugs target essential structures or functions
  - Targets often highly conserved
  - Mutations likely to reduce fitness
- If resistance reduces fitness, resistant parasites will be selected against when drug is absent
  - Inhibit spread of resistance (opposing selective forces in treated/untreated infections)
  - Cause decline of resistance after drug is retired
    - Facilitate drug cycling – reintroduction of retired antimalarials

Evidence for cost of resistance

- Resistance to chloroquine and SP have declined in some locations following drug policy change
  - Published data from Malawi:



Kublin *et al*. 2003. *J Infect Dis* 187(12):1870-1875

How is the price of resistance paid?

- Reduced transmission success
  - **Growth in human** or mosquito host
  - Establishment success in human or mosquito
- Shorter duration of infection in human host
  - Susceptibility to immune clearance
- Competitive disadvantage
  - **Competitive suppression**
  - **Competitive exclusion**

Fixation of resistant alleles in low endemic countries

- After retiring CQ, resistant genotypes declined in many parts of Africa whereas in South America and Southeast Asia resistant genotypes got fixed
- Why?
  - When new drugs were introduced resistant mutations already got fixed and no sensitive parasites available to compete?
  - Population structure and other ecological factors

What are the relevant messages?

- **Regional differences in the evolution and spread pattern of drug resistant alleles**
- **South American and Central American regions have distinct ecological niches (that is different from Southeast Asia and Africa)**
- **Consider this in policy making**

Some key points about ACT

Based on WHO recommendation artemisinin-based combination therapy (ACT) was adopted

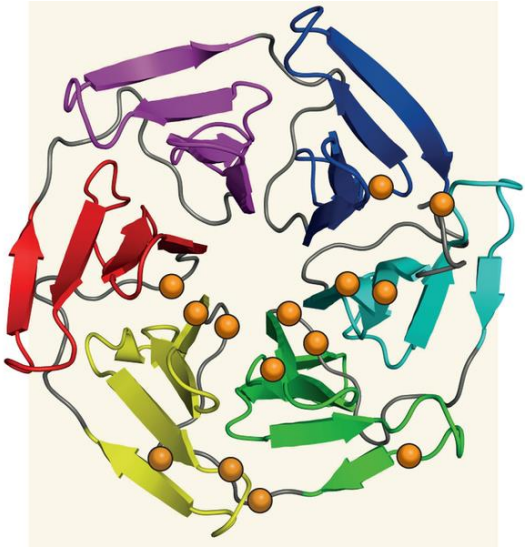
- **2000-Southeast Asia**
- **2001-South America (Peru, Venezuela)**
- **2006-Africa**
- **First artemisinin resistance reported in 2008 in Cambodia (partial resistance based on delayed clearance of parasites**
- **Therapeutic efficacy remains high in most parts of Southeast Asia despite evidence of artemisinin partial resistance**

Molecular markers of resistance

doi:10.1038/nature12876

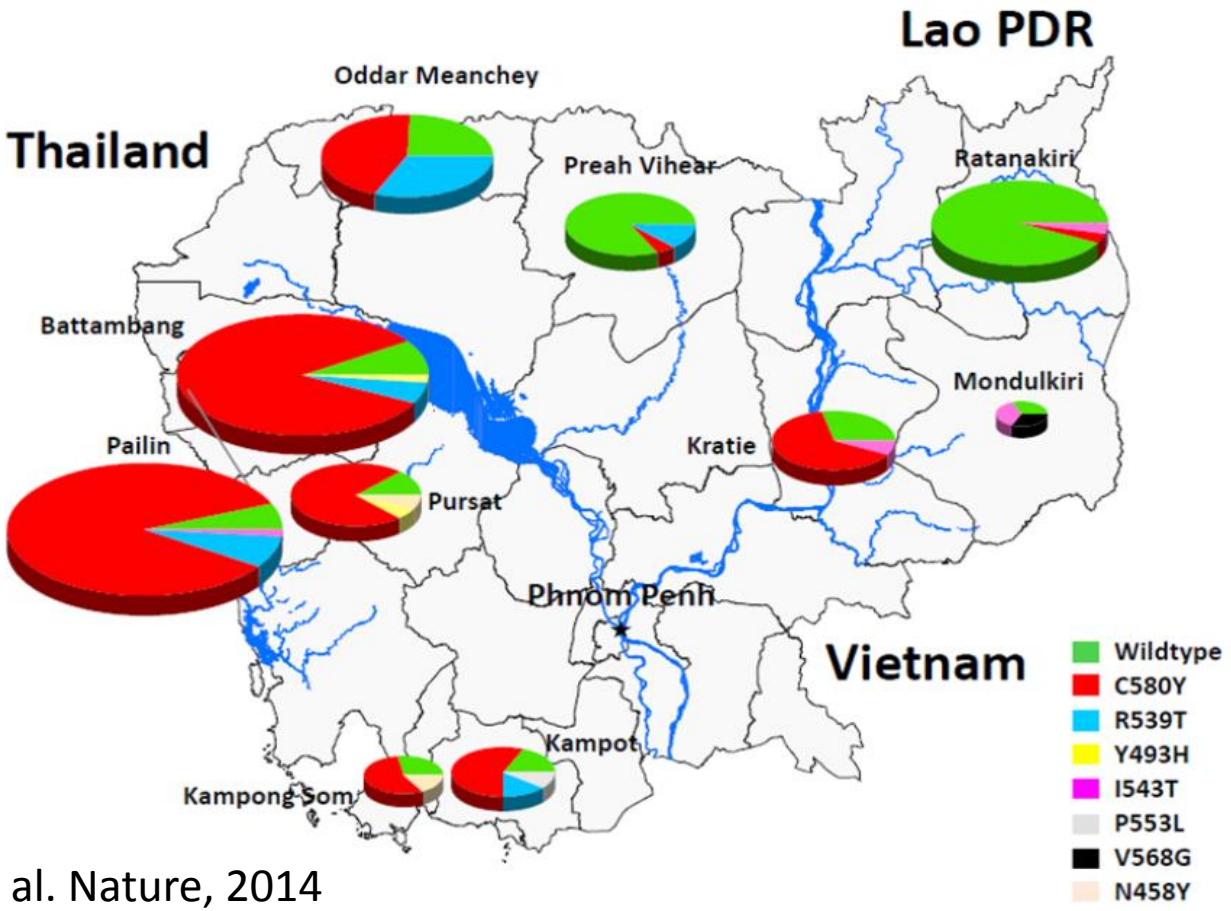
A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria

Frédéric Ariey[1,2]†, Benoit Witkowski[3], Chanaki Amaratunga[4], Johann Beghain[1,2]†, Anne-Claire Langlois[1,2], Nimol Khim[3], Saorin Kim[3], Valentine Duru[3], Christiane Bouchier[5], Laurence Ma[5], Pharath Lim[3,4,6], Rithea Leang[6], Socheat Duong[6], Sokunthea Sreng[6], Seila Suon[6], Char Meng Chuor[6], Denis Mey Bout[7], Sandie Ménard[8]†, William O. Rogers[9], Blaise Genton[10], Thierry Fandeur[1,3], Olivo Miotto[11,12,13], Pascal Ringwald[14], Jacques Le Bras[15], Antoine Berry[8]†, Jean-Christophe Barale[1,2]†, Rick M. Fairhurst[4]*, Françoise Benoit-Vical[16,17]*, Odile Mercereau-Puijalon[1,2]* & Didier Ménard[3]*



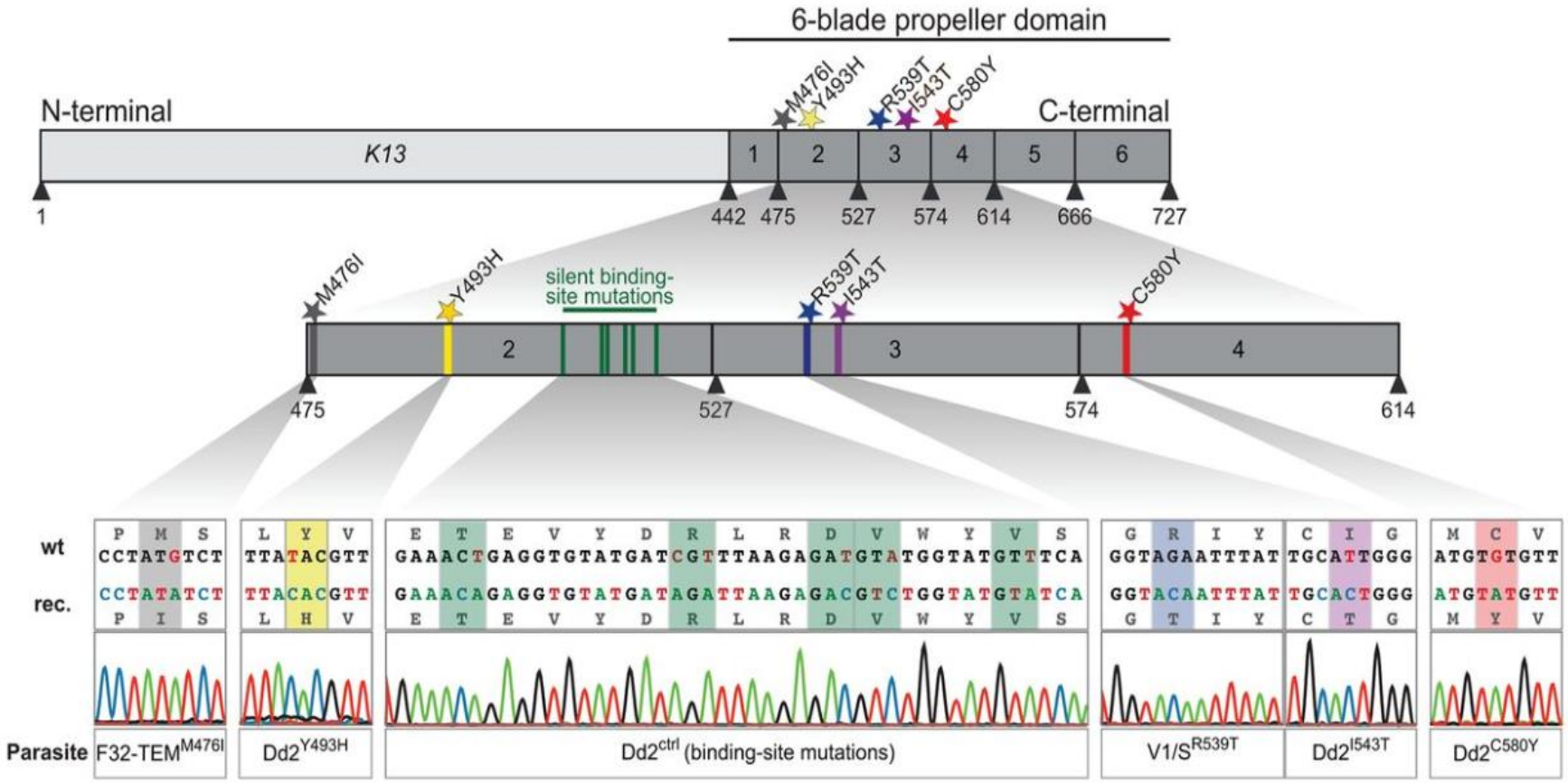
Mutations in the propeller domain of the Kelch protein K13

K13-propeller domain mutations in SEA are associated with delayed parasite clearance and ring stage survival assay



Ariey et al. Nature, 2014

Genetic experiments confirm that K13 mutations confer resistance



Straimer et al. Science express, 2015.

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K13 results from Suriname and Guyana

Suriname 2014 :

- **41 samples from ACT TES study**

Guyana 2014:

- **73 samples from artesunate TES study**

Guyana 2010:

- **100 samples from HRP2 surveillance study**

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K13 propeller domain sequencing of Suriname samples from 2014 TES study

Total number of samples	Positive for *P. falciparum*	Samples amplified for K13 propeller domain	Samples with K13 wild type Only	Samples with K13 580Y mutation only	Samples with mixed K13 580Y mutant and C580 wild type
41	40	40 (100%)	40	0	0

None of the samples had artemisinin resistance associated K13 mutation

Drug resistance profile of Suriname samples from 2014

- **Chloroquine (CQ): *Pfcrt* 72S, 76T**
- **Mefloquine (MQ): *Pfmdr-1* copy number: (1/40) 2 copies**
- **Pyrimethamine: *Pfdhfr:* 50R, 51I, 108N**

K13 propeller domain sequencing of Guyana samples from 2014 artemisinin TES study

Total number of samples	Positive for *P. falciparum*	Samples amplified for K13 propeller domain	Samples with K13 wild type Only	Samples with K13 580Y mutation only	Samples with mixed K13 580Y mutant and C580 wild type
73	73	73 (100%)	73	0	0

None of the samples had artemisinin resistance associated K13 mutation

CDC

K13 propeller domain sequencing of Guyana samples collected in 2010 for HRP2 surveillance study

Total number of samples	Positive for *P. falciparum*	Samples amplified for K13 propeller domain	Samples with K13 wild type Only	Samples with K13 580Y mutation only	Samples with mixed K13 580Y mutant and C580 wild type
100	98	98 (100%)	93 (94.9%)	3 (3.06%)	2 (2.04%)

5.1% of samples had artemisinin resistance associated K13 580Y mutation

This is the most common mutation in Southeast Asia

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Profile of K13 linked microsatellites of Guyana samples from 2010

South America	-31.9	-6.36	-3.74	-0.15	K13	3.4	8.6	15.1	72.3
Guyana hap 1	204	277	171	206	580Y	139	262	144	244
Guyana hap 2	204	277	171	206	580Y	139	262	144	240

If the mutation share ancestry, their microsatellite profile should appear similar

SE Asia	-31.9	-6.36	-3.74	-0.15	K13	3.4	8.6	15.1	72.3
Cambodia1	201	282	148	194	580Y	122	265	138	240
Cambodia2	201	282	148	194	580Y	131	289	138	238
Thailand1	201	282	148	194	580Y	131	288	138	248
Thailand2	201	282	148	194	580Y	131	278	138	248
Thailand3	201	282	148	194	580Y	117	278	138	248

In Guyana all five 580Y mutant samples had nearly identical haplotype suggesting their common origin and distinct from Southeast Asian 580Y alleles

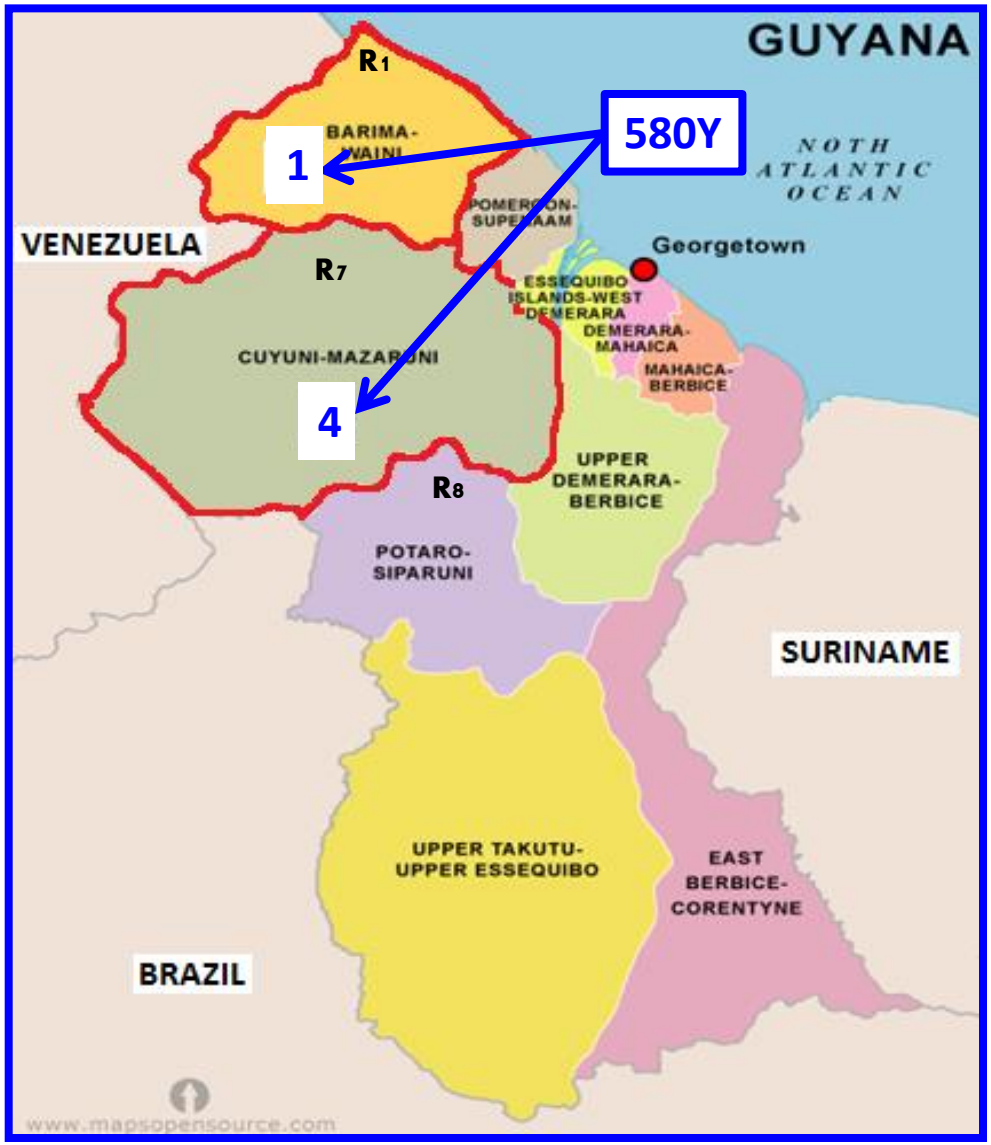
Guyana 2010: All 580Y mutant samples had CQ and SP resistant background

		K13	*Pfcrt*		*Pfdhfr*			*Pfmdr-1*			*Pfmdr-1*
Samples	Region	580	72	76	50	51	108	184	1042	1246	Copy number
Guyana 1	7	Y	S	T	R	I	N	F	D	Y	2
Guyana 2	7	Y	S	T	R	I	N	F	D	Y	1
Guyana 3	7	Y/C	S	T	R	I	N	F	D	Y	1
Guyana 4	1	Y/C	S	T	R	I	N	F	D	Y	1
Guyana 5	7	Y	S	T	R	I	N	F	D	Y	1

K13 580Y (artemisinin resistance) positive samples had CQ resistant, SP resistant and triple mutant mdr1 allele background (multi drug resistant background)

One sample with 2 copies of mdr1 (mefloquine resistance)

Travel history of 580Y mutant positive subjects



580Y positive subjects: Four visited Region 7 and one visited Region 1

Malaria transmission is endemic in Barima-Waini (R1), Cuyuni-Mazaruni (R7) and Potaro-Siparuni (R8); these areas are popular among immigrant workers due to gold mining and logging.

R: Regions

Summary of results

- No K13 was detected in Suriname and Guyana TES samples
- 5/98 samples collected from Guyana 2010 HRP2 surveillance study had K13 580Y mutation. This is the most common K13 mutation found in Southeast Asia.
- K13 580Y alleles had nearly identical haplotype suggesting their common origin. This haplotype is genetically unrelated to 580Y allele found in Southeast Asia suggesting local evolution of this allele in South America.
- Subjects with K13 580Y mutation in Guyana traveled to Region 7 (4 subjects) or Region 1 (one subject) bordering Venezuela.

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Obrigada!