

Molecular surveillance to monitor
drug and diagnostic resistance in
Plasmodium falciparum in South and
Central America

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Monitoring Resistance in South America

- Drug resistant parasites are evolving independently in SA
- ACT has been in use since 2001
- Molecular surveillance is valuable and complementary to other tools

Molecular markers for drug resistance

❑ Chloroquine (CQ):

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

Pfmdr1: N86Y, Y184F, S1034C, N1042D, D1246Y

❑ Sulfadoxine + pyrimethamine (SP):

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

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

❑ Mefloquine (MQ), lumefantrine (LUM):

Pfmdr1: Increase in gene copy number

Pfmdr1: N86Y, Y184F, D1246Y

❑ Artemisinin:

PfATPase6???????- Extensive efforts underway.....

Chloroquine resistance is fixed

CVMET/CVMNT-B

SVMNT-A

CVMNT -A/B

Two haplotypes
SVMNT/CVMNT-A
CVMNT/CVMET-B



SP resistance is fixed in most of the Amazon except in Peru and the coast

dhfr

50R, 51I, 108N

51I, 108N, 164L

dhps

437G, 540E, 581G

Low /no resistance

In the coast

Dhfr-single/WT

Dhps-Single/WT

SP is used with ART
for treatment

Pfmdr1

(N86Y, Y184F, S1034C, N1042D, D1246Y)

mdr1
β-lineage

mdr1
α-lineage

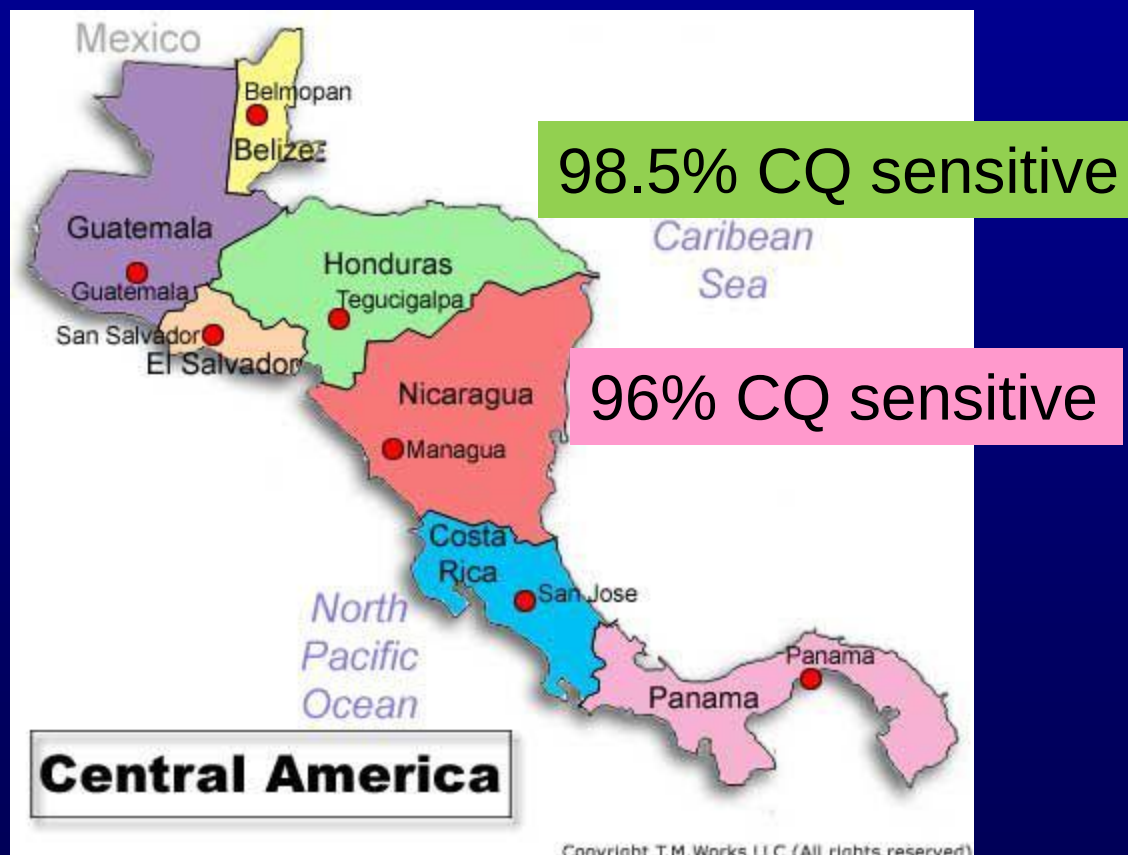
Quadruple/triple
mutants-fixed

Two haplotypes
(alpha and beta)

Copy number
increase in Venezuela
Suriname?
Not in Peru

MQ resisatnce?

Molecular surveillance for chloroquine resistance in Central America



Haiti- CQ and SP resistance genotypes 2010 samples

(preliminary analysis, Barnwell J et al unpublished)

<u>Molecular marker</u>	<u># samples</u>
CQ resistance	
WT (CVMNK)	105 (98.13%)
CVIET	2 (1.87%)
SP resistance	
<i>dhfr</i> WT	42(60.86%)
108N mutant	27(39.13%)
High resist. triple mutant	0 (0%)

(Total 345 blood spots, 118 +ve for 18S gene amplification)



Future Directions

A comprehensive molecular surveillance strategy is needed for the region (including for speciation)

Systematic surveillance –strategy may vary from region to region

Link all in vivo clinical trials with molecular marker analysis

Imported cases of malaria (travel history)



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Molecular Profile and Survey of HRP2 and HRP3 Genetic Deletions in South America

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Centers for Disease Control and Prevention**



Background - Rapid Diagnostic Tests (RDTs) and malaria diagnosis

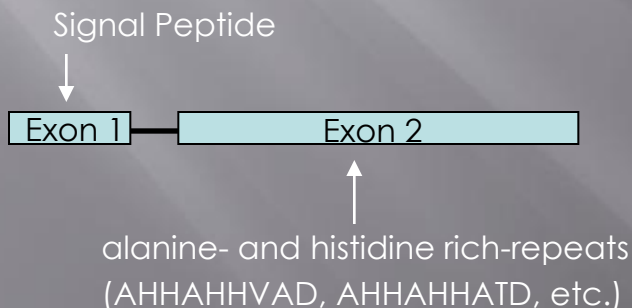
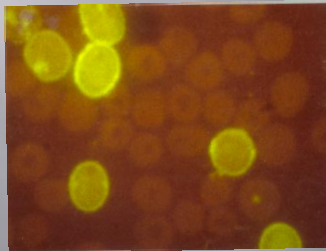


➤ Over the past two decades RDTs have become well accepted for malaria diagnosis in places where light microscopy is not available.

➤ RDTs are antigen-capture based assays detecting in whole blood:

1. *P. falciparum* histidine-rich protein-2 (HRP-2)
2. *Plasmodium* lactate dehydrogenase (pLDH)
3. *Plasmodium* aldolase enzyme

➤ HRP-2 is a stable soluble exported protein composed of largely histidine, alanine and aspartic acid amino acids arranged in a variety of tandem repeats.



Deletion of hrp-2 and hrp-3 genes in *P. falciparum* populations in the Peruvian Amazon

OPEN ACCESS Freely available online



A Large Proportion of *P. falciparum* Isolates in the Amazon Region of Peru Lack *pfhrp2* and *pfhrp3*: Implications for Malaria Rapid Diagnostic Tests

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- Detected during establishment of a specimen bank for RDT QA/QC and Performance Evaluation Program.
- Instituto de Medicina Tropical/Universidad Peruana Cayetano Heredia, AAMI/QIMR, FIND, WHO, CDC, and HTD (London)



Current and Retrospective Survey of hrp2/hrp3 deletions in the Amazon Basin

▣ Purpose:

- Determine the current extent of *P. falciparum* populations with deletions of the Histidine-rich protein 2 (HRP2) and Histidine-rich protein 3 (HRP3) genes in the Amazon Basin and adjoining areas of South America.
- Retrospectively determine the origin and population history of the HRP2 and HRP3 deletions in South America through archived samples.

Basic Protocol

- ▣ Febrile patient, >5 yr., Microscopic diagnosis or pLDH RDT
- ▣ Single Pf infection, inform & consent
- ▣ 3 ml venous blood draw or FTA filter paper, information form, thick & thin film
- ▣ 3 aliquots of plasma and cellseach
- ▣ Molecularly analyse cell samples:
 - ▣ 1. test for species-specific rRNA
 - ▣ 2. Pf HRP2/3 and
 - ▣ 3. flanking genes
- ▣ Quantitatively assay plasma for HRP2

Genes of Interest in *hrp2* Locus

▣ HRP2:

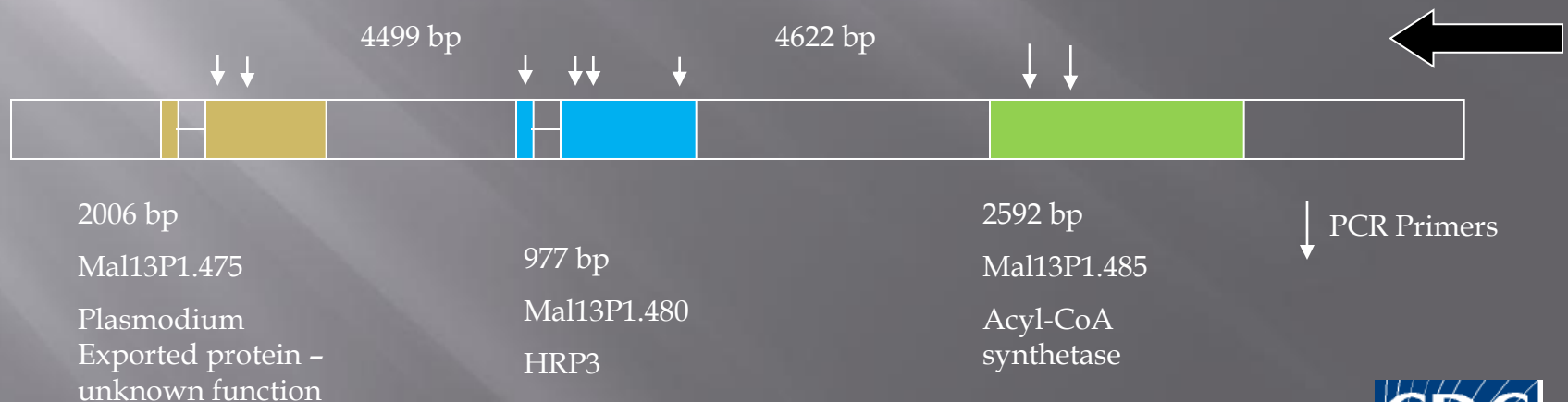
- Located on chromosome 7 (same chromosome as *Pfcr*t gene)
- Contains numerous histidine repeats (key trait exploited by RDTs for detection)
- Mal7P1_230 and Mal7P1_228 are the immediate upstream and downstream genes respectively.



Genes of Interest in *hrp3* Locus

▣ HRP3:

- Located on chromosome 13 near the telomeric region.
- Also contains numerous histidine repeats.
- Mal13P1_485 and Mal13P1_475 are the immediate upstream and downstream genes respectively.



Peru-Results for hrp2/hrp3 genotype profiling (2003 - 2008)

Area	No.	<i>pflhrp2</i> - PCR		<i>pflhrp3</i> - PCR	
		Pos (%)	Neg (%)	Pos (%)	Neg (%)
Iquitos	233	135 (58)	98 (42)	44 (19)	189 (81)
Condorcanqui	14	0 (0)	14 (100)	4 (28.5)	10 (71.5)
Yurimaguas	42	32(76)	10 (24)	18 (43)	24 (57)
Combined	289	167 (58)	122(42)	66 (23)	223 (77)

Peru Update

Coastal Peru

2001 (10)
100% HRP2(+)
100% HRP3(+)

Loreto/Iquitos

2010 (73)
[HRP2(-)]
(31/73 = **42.5%**)
[HRP3(-)]
(48/73 = 65.8%)



○ = 2010
○ = 2001

Coastal

4/70 (6.0%)
HRP2 (-)
31/70 (44.3%)
HRP3 (-)

Choco

1/7 (14.3%)
HRP3 (-)

Buenaventura

1/28 (3.6%)
HRP2 (-)
10/28 (36%)
HRP3 (-)

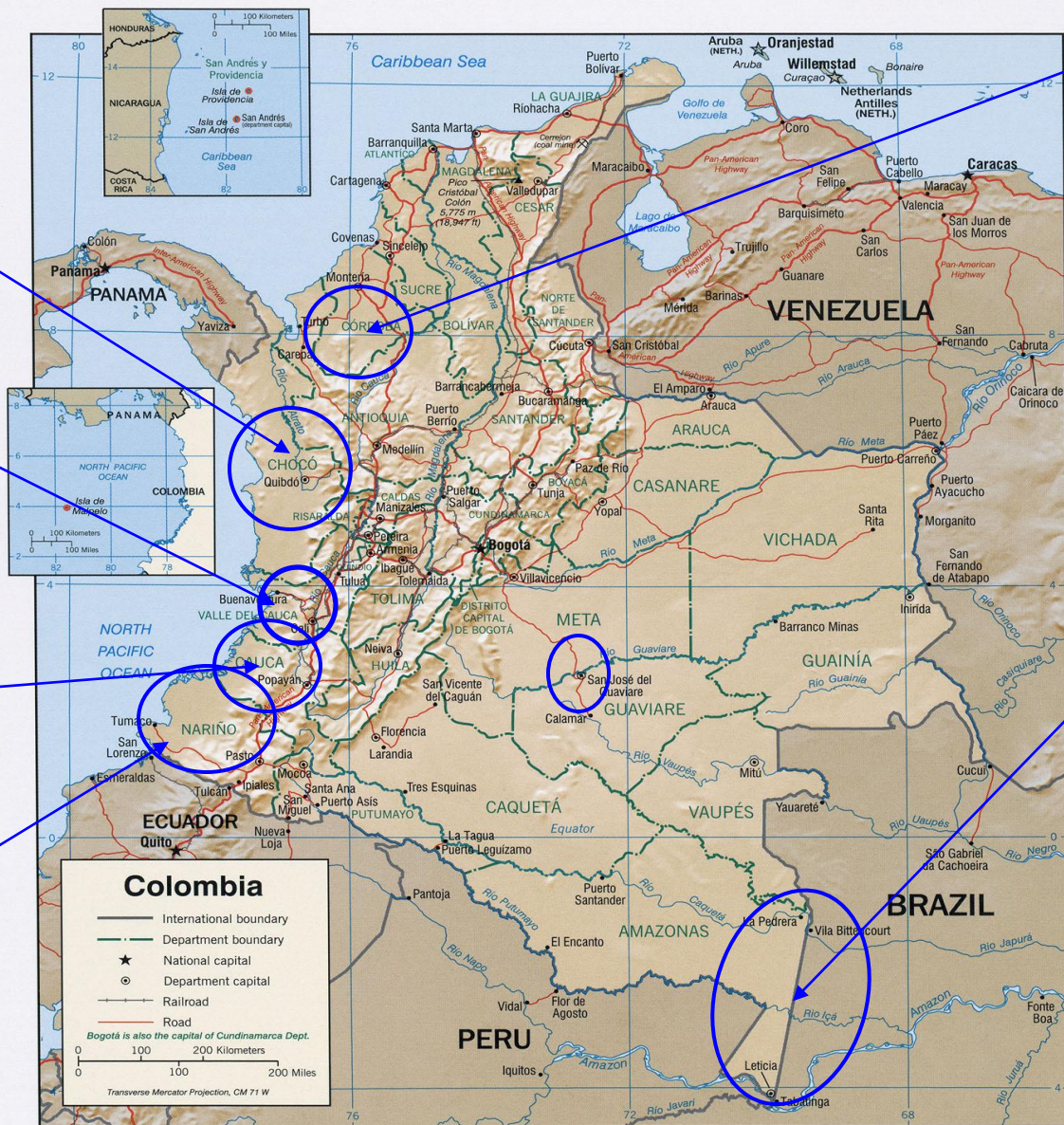
Cauca

1/6 (16.7%)
HRP2 (-)

Tumaco, Narino

2/19 (10.5%)
HRP2 (-)
10/19 (53%)
HRP3 (-)

Colombia 2006 - 2009 Samples



Cordoba

HRP2 (-)
0/10 (0.0%)
HRP3 (-)
10/10 (100%)

Red circle = 2010
Blue circle = 2006
- 2009

Amazonas

HRP2 (-)
8/20 (40.0%)
HRP3 (-)
10/20 (50%)
HRP2/3 (-)
8/20 (40%)

Colombia 2010 Samples

Total confirmed

0/39 (0.0%)
HRP2 (-)
17/39 (43.6%)
HRP3 (-)
? 14 samples

Buenaventura

0/11 (0.0%)
HRP2 (-)
4/11 (36.4%)
HRP3 (-)

Tumaco, Narino

0/22 (0.0%)
HRP2 (-)
7/22 (31.8%)
HRP3 (-)

Cordoba

HRP2 (-)
0/6 (0.0%)
HRP3 (-)
6/6 (100%)

○ = 2010
○ = 2006
- 2009



Brazil

Itaituba (1999)

1/10 (10.0%)

HRP2 (-)

1/10 (10.0%)

Porto Velho (1999)

2/22 (9.0%)

HRP2 (-)

2/16 (12.5%)

HRP3 (-)



Marabá (1999)

1/14 (7.0%)

HRP2 (-)

7/14 (50%)

HRP3 (-)

○ = 2010

○ = 1999

Goianaesia
(2010)

0/14 (0.0%)

HRP2 (-)

14/14 (100%)

HRP3 (-)



Guyana (2010)
 100 samples
 To be tested

Bolivia (2010)
 Riberalta
 Guyaramerin
 0/25 (0%)
 HRP2 (-)
 18/25 (72%)
 HRP3 (-)
 Uncertain 2?



Suriname
 0/23 (0%)
 HRP2 (-)
 0/23 (0%)
 HRP3 (-)
 Uncertain 2?

2010
 Retrospective



Training Activities

- ▣ Gustavo A. Fontecha-Sandoval, Universidad Nac. Autonoma de (Honduras) and Meisy E. Mendoza-Montoya, Laboratorio de Malaria, Tegucigalpa, Honduras (Jan 22-Feb 23, 2011)
- ▣ Lucia Ortiz-Batsche and Maria E. Castellanos-Reynosa, Univasidad de Valle of Guatemala, Guatemala (March 21 to April 17, 2010)

Training Activities (Cont.)

- ▣ Claribel Murillo-Solano, International Center for Medical Research CIDEIM, Cali, Columbia (Feb 14, 2010 to March 27, 2010)
- ▣ Molecular Training Workshop for the detection of HRP2/3 genetic deletions, Instituto Evandro Chagas, Belem, Brazil (Aug 30th-Sep 10th, 2010)
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- ▣ On site training in Bolivia and Guyana (Kumar and Alex Macedo)- March 2010

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