Molecular surveillance of mutations in the Cytochrome b gene of *Plasmodium falciparum* in Gabon and Ethiopia

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Objectives

- To detect and determine the prevalence of Cytochrome b gene mutation associated with Atovaquone resistance in *P. falciparum* in the blood specimen collected from patients with uncomplicated malaria in Gabon and Ethiopia.

Methods

- Clinical material (blood) from both study sites
- DNA extraction using QIA amp® DNA Mini Kit
- Samples were analyzed using PCR and DNA sequencing to detect variation in the *cyt b* gene
- **Primers for Gabon** samples to amplify a 939 bp fragment containing the cyt b gene:
  - *Cytb1* (5' – CTCTATTAATTTAGTTAAAGCACAC – 3') and
  - *Cytb4* (5' – ACAGAATAATCTCTAGCACC - 3')
- Obtained fragments were analysed on a 1% agarose gel for purity
- Gels were stained with CYBR® GREEN I and visualized on a dark reader trans-illuminator

Methods

- Prior to sequencing, the amplified DNA was purified by a PCR purification kit
- DNA sequence was determined using Big Dye 1.1® and purified again by DNA grade sephadex®
- Strand separation was done on an Applied Biosystems Genetic Analyzer 3100®
- DNA sequences were finally analysed with the Bio-edit ® sequence alignment program to detect point mutations
- **Primers for Ethiopia** samples to amplify a 939 bp fragment containing the cyt b gene:
  - *CytbF* (5' – GGGTATGATACAGCATTAAAAATAC – 3') and
  - *Cytb4* resulting in a 349 bp fragment
- Here we were interested only in the 3' end of the gene since no mutations were detected in the 5' end in the Gabonese samples

Results

- 4/40 (10%) mutant types (4 various polymorphisms leading to an amino acid change from M to I in one single case) in Gabonese isolates
- 141/141 wild type isolates from Ethiopia

Results

Prevalence of wild and mutant type in the cytochrome b gene of *Plasmodium falciparum* isolates from Gabon and Ethiopia

country of origin	number of isolates	wild type	mutation[1,2]	amino acid change
Ethiopia	141	141	n. a.	n. a.
Gabon	40	36	T676A	---
			C689T	---
			T760G	---
			G925T	M to I

n.a. = not applicable
[1]all SNP were found in one sample each
[2]no mutations found in codon 268

Discussion & Conclusions

- initial reports of definite, or possible, resistance-conferring cyt b gene polymorphisms were in isolates from tx-failing travellers from Thailand and Nigeria (Tyr268Asn and Tyr268Ser) and in other codons (only *in vitro*) (Korsinsky et al 2000, Fivelman 2002)

- mutations in codon 268 have been recognized as potential markers to measure and control the emergence of resistance against A/P

- however, in vivo A/P resistance may not always be associated with cyt b mutations (Wichmann et al. 2004) since failure in drug action can also be resulting from metabolic diversion to the alternative respiratory pathway of the parasite (Thapar et al. 2005)
- no resistance-conferring mutations found *in vitro* and on sequencing of 37 isolates from Cameroon (Basco et al. 2003)

- none of 100 isolates from Northern Ghana exhibited resistance-conferring polymorphisms in codon 268 (Muehlen et al. 2004)

Future perspectives

- Many other findings are well in line with our own results.
- In the absence of drug pressure, spontaneous and possible resistance-conferring mutations are rare.
- However, a higher rate of resistance in the future is likely to occur under drug pressure
- Like aminoquinoline and sulpha drugs, possibly resistance-confirming polymorphisms (single, or in combination) will occur with almost certainty.