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学　位　論　文　要　旨

Dissertation Summary

氏　　名　(Name) **BALIKAGALA BETTY**
論文名：
(Dissertation Title) **Clinical and Molecular evaluation of the therapeutic efficacy of the antimalarial drug artemether-lumefantrine.**

Malaria is a life threatening tropical disease, caused by protozoan parasites of the genus *Plasmodium*. Five species of plasmodia cause malaria in humans via the bite of female mosquitoes of the genus *Anopheles* during a blood meal. These include; *Plasmodium falciparum*, *P. vivax*, *P. malariae, P. ovale* and *P. knowlesi*. Of the five species, *P.falciparum* poses the greatest threat. This thesis focuses on *P.falciparum*. Nearly half of the world's population is at risk of malaria infection and 91 countries have ongoing malaria transmission. According to the latest 2016 WHO estimates, 212 million cases of malaria and 429 000 deaths were reported worldwide, Africa disproportionately hosting 90% of the malaria cases and 92% of malaria deaths. Several malaria control strategies involving vector control (i.e. the use of insecticide-treated mosquito nets (ITNs), indoor residual spraying (IRS) and early diagnosis and treatment with artemisinin-based combination therapy (ACT) have resulted in significant decline in malaria infections and death. Indeed between 2010 and 2015, the number of new malaria cases fell by 21% globally and malaria death rates fell by 29% globally and by 31% in the African Region.

The recent gain in malaria control is partly attributed to the wide-scale use of ACTs for the treatment of malaria in all endemic regions. Unfortunately these fragile gains are threatened by the emergence of artemisinin resistance. Currently artemisinin resistance has been confirmed in 5 countries in South East Asia (SEA). These include; Cambodia, Lao People's Democratic Republic, Myanmar, Thailand and Vietnam. Current studies in Africa show no evidence of artemisinin resistance. However, there is a possibility that the established resistance in South East Asia may invade the African continent, which faces the greatest burden of the disease, as previously observed in chloroquine and sulfadoxine/pyrimethamine resistance. Yet there are no treatment alternatives to the current ACT in the drug development pipeline.

In 2014, *Pfkelch13* gene (PF3D7_1343700) was identified as the molecular marker for tracking artemisinin resistance. Some mutations in this gene are associated with artemisinin resistance in South East Asia. In addition, genome wide association studies identified several single nucleotide polymorphisms (SNPs); D193Y in *ferredoxin* (*fd*), T484I in *multidrug resistance protein 2*+ (*mdr2*), V127M in the *apicoplast ribosomal protein S10* (*arps10*), I356T in *chloroquine-resistance transporter* (*crt*), V1157L in *protein phosphatase* (*pph*) and C1484F in *phosphoinositide-binding protein* (*pibp*) assumed to be background genetic changes for artemisinin resistance in South East Asia. In Africa, polymorphisms in these genes have been occasionally observed, but they are different from those reported in South East Asia. Making it unclear whether the existence of these mutations in Africa is a consequence of selection induced by the use of antimalarial drugs.

During treatment with a malaria drug, less susceptible parasites can be selected in the human body, a process termed *in vivo* selection. This is because the treatment creates drug concentration circumstances that are sufficient to kill susceptible, but not less susceptible parasites. Previous investigations revealed that artemether-lumefantrine (AL) treatment (an ACT widely used in the treatment of uncomplicated malaria selected for parasites harbouring alleles with K76 in the *Plasmodium falciparum* chloroquine-resistance transporter (*pfcrt*) gene and N86, 184F and D1246 in *Plasmodium falciparum* multidrug resistance gene 1 (*pfmdr1*). However, the possibility of similar *in vivo* selection has not been fully investigated in *pfkelch13* and the putative background genes.

Therefore, this study evaluated the therapeutic efficacy of AL, (the current first line treatment for uncomplicated malaria in Uganda), in Gulu Northern Uganda, a region of intense malaria transmission in May and October 2014. Individuals suspected to have malaria were consecutively enrolled from the outpatient department in St. Mary's hospital Lacor, one of the hospitals offering healthcare to the people in Gulu Northern Uganda. Before enrolling participants in the study, permission for participation (informed consent) was sought from each individual. Thereafter, the presence of malaria was confirmed by microscopy and species-specific polymerase chain reaction (PCR). Blood for parasite genotyping was also collected. Supervised administration of oral AL was performed for all recruited individuals and followed up on days 1, 2, 3, 7 and 28. On each follow up day, as at enrollment, the presence of malaria was confirmed microscopically and by species-specific PCR. Blood for parasite genotyping was also collected. At the end of the follow-up period, participants were assigned treatment outcomes according to WHO guidelines as: adequate clinical and parasitological response (ACPR), early treatment failure (ETF), late clinical failure (LCF) and late parasitological failure (LPF). Genotypes in the above mentioned genes were quantified at baseline and then comparatively analysed before drug administration and on days; 3, 7, and 28 days after treatment to determine evidence of *in vivo* selection for polymorphisms associated with AL resistance.

Excellent early response to AL treatment was observed in almost all patients. Only one (1.6%) child showed microscopically residual parasites (delayed parasite clearance) on day 3 after treatment. However, this prevalence of day 3 parasite positive individuals in the present study was less than 5% or 10%, which are the benchmarks for artemisinin-resistance. Confirming the absence of artemisinin resistance in Africa. PCR-confirmed day 3 parasite positivity after AL treatment was much higher than microscopically confirmed positivity; 22.9%, versus 1.6%. Also, individuals that were parasite positive by PCR on day 3 had significantly higher enrollment parasitaemia compared to the PCR negative group, suggesting that parasite biomass before treatment may be associated with treatment success and PCR

parasite-positive outcome on day 3 after treatment. Similar to the excellent early treatment response, artemether-lumefantrine treatment was very effective with PCR adjusted efficacy of 95.2%. Only three individual failed on treatment by developing new malaria infections within 28 days after initial treatment.

The molecular analysis revealed that among 146 isolates obtained before treatment, wild-type alleles were observed in 98.6% of isolates in *pfkelch13* and in all isolates in the six putative background genes except at position I356T in *pfcrt*, which had 2.4% of isolates as mixed infections. *In vivo* selection analysis revealed that *pfkelch13* mutations were not observed in the parasite positive samples on day 3, 7 and 28, consistent with the recent observations from Kenyan children. Also, no selection of putative six non-synonymous polymorphisms was observed (Figure 1), suggesting that these genetic changes may not be responsible for parasite persistence in the present study. In contrast, *Pfcrt* K76 and *Pfmdr1* N86/D1246 were observed in all recurrent parasites (Figure 2). The prevalence of *Pfmdr1* Y184F (33.3%) in the recurrent parasites was higher than baseline (14%), although not statistically significant. These observations support the potential selection of *Pfcrt* K76 and *Pfmdr1* N86/Y184F/D1246 haplotype after AL treatment. *In vivo* selection of these mutations would increase these allele prevalence in the parasite population.

In conclusion, this study demonstrated that AL treatment remains of high efficacy for the treatment of *P. falciparum* malaria after 8 years of use in a region of high malaria transmission in Uganda. Mutations in *pfkelch13* and the six background genes may not play an important role in the *in vivo* selection after artemether-lumefantrine treatment in Uganda. Different mechanisms might rather be associated with the existence of parasites after treatment.

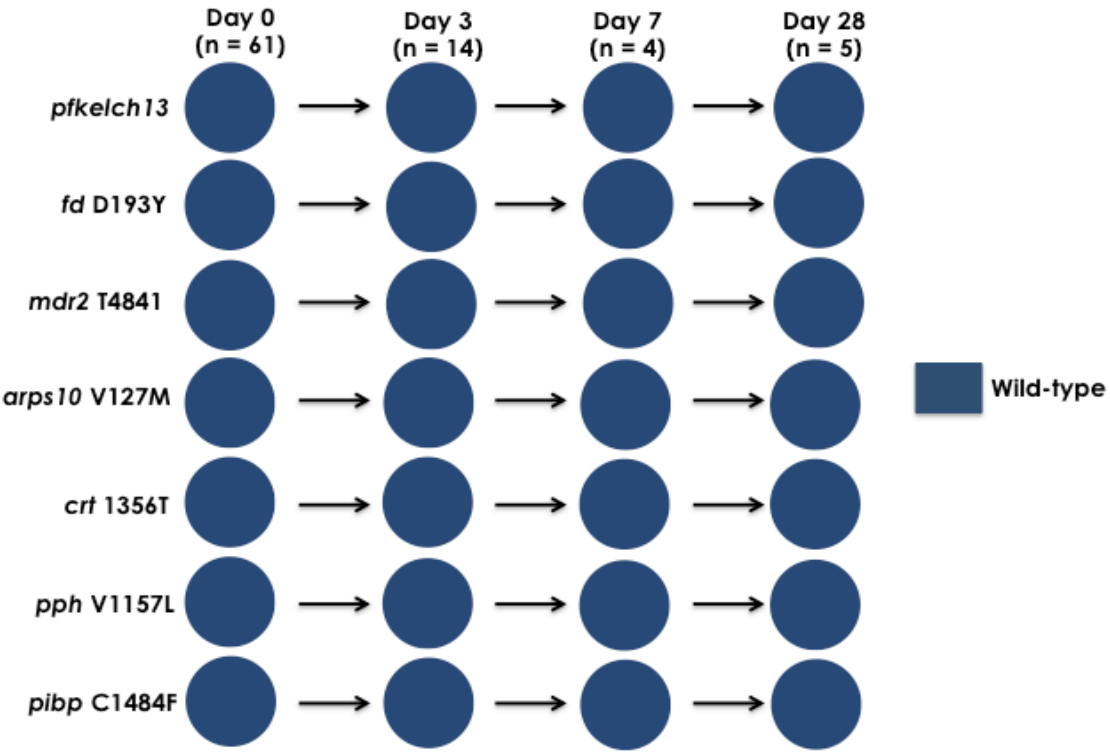

Figure 1. Allele prevalence in *pfkelch13, fd, mdr2, arps10, crt, pph* and *pibp* among 61 isolates collected before and after artemether-Lumefantrine treatment.

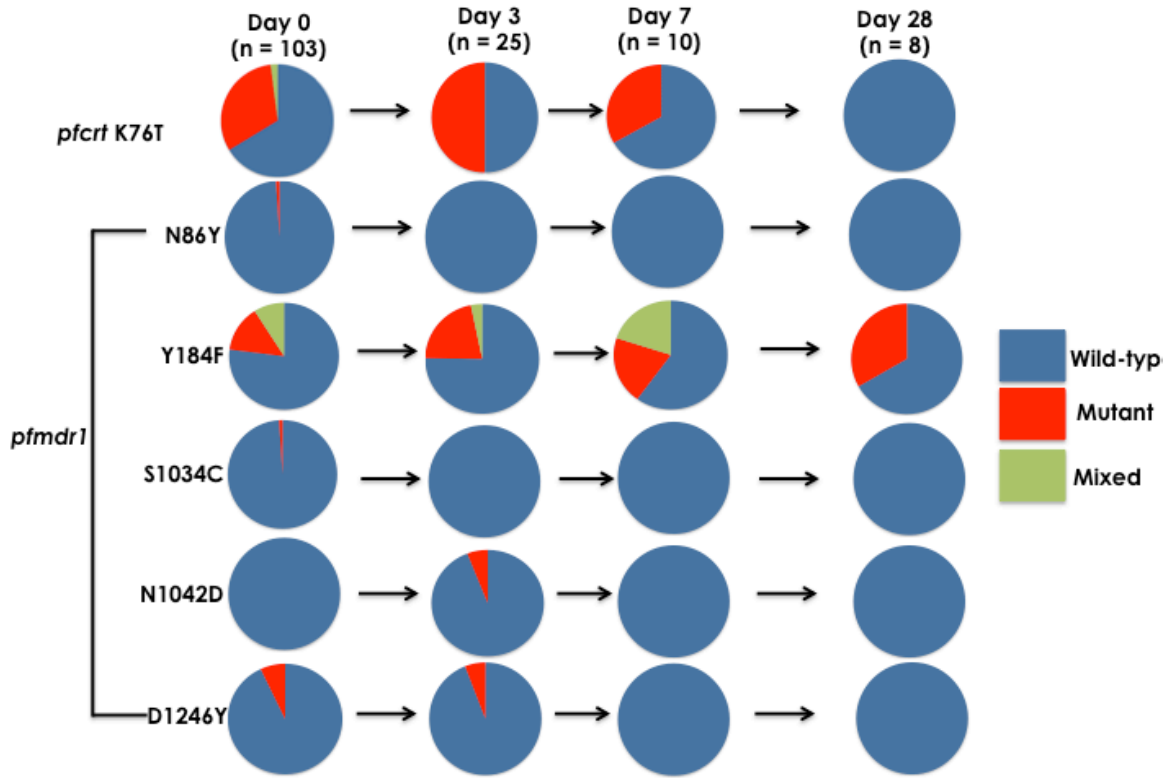

Figure 2. Allele prevalence in *pfcrt* K76T and *pfmdr1*among 103 isolates collected before and after Artemether-Lumefantrine treatment.