bloodspots were collected for PCR analysis. Coartem® was given to 131 and 102 cases at Mkuzi and Ujiji and AQ/AS to 129 and 103 cases at the 2 sites respectively. Both drugs were given according to weight and under supervision. Significantly higher (p<0.0001) malarial parasite prevalence was found among those screened at Mkuzi (58.1%) compared to Ujiji (26.0%). In the Coartem® arm, adequate clinical and parasitological response (ACPR) by day 28 was 62.4% at Mkuzi and 84.7% at Ujiji. In the AQ+AS arm, ACPR by day 28 was 38.7% and 78.8% at Mkuzi and Ujiji respectively. Total treatment failures with Coartem by day 28 (not PCR corrected) were 37.6% at Mkuzi and 15.3% at Ujiji whilst failures in AQ+AS arm were 61.3% at Mkuzi and 21.2% at Ujiji. In the Coartem arm, ACPR by day 14 was 95.9% at Mkuzi and 97.8% at Ujiji. ACPR in AQ+AS arm by day 14 was 79.5% and 92.3% at Mkuzi and Ujiji respectively. Total treatment failure with Coartem by day 14 was 4.1% at Mkuzi and 2.2% at Ujiji; whilst failure in AQ+AS arm was 20.5% at Mkuzi and 7.4% at Ujiji. Despite similar ACPR with Coartem on day 14 at both sites, significantly higher (p=0.007) ACPR was seen at Ujiji (84.7%) compared to Mkuzi (62.4%). Significant improvement in mean Hb levels was seen on both days 14 and 28 in both treatment arms at both sites; but levels at day 28 were higher than day 14. High failures by day 28 despite excellent response by day 14 might be due to new infections; PCR corrected data will resolve this. Marked Hb recovery at both day 14 and 28 suggests malaria was the major cause of the initial anaemia. Data obtained here will be fed into the National Malaria Control Programme database for future use in reviewing anti-malarial drug policy in Tanzania.

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PHENOTYPING SENSITIVITY AND RESISTANCE TO CHLOROQUINE IN *PLASMODIUM VIVAX*: STUDIES AT SENTANI, NORTHEASTERN PAPUA, INDONESIA

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Chloroquine remains the first-line therapy for treatment of acute vivax malaria after 60 years of continuous use. Chloroquine-resistant *Plasmodium vivax*, first recorded in 1989, is now known to be a severe problem in much of eastern Indonesia, with more than half of infections exhibiting therapeutic failure. In support of efforts aimed at searching for mutations linked to resistance to chloroquine, we evaluated the therapeutic response to chloroquine in 73 subjects naturally infected by *P. vivax* in northeastern Papua, Indonesia. We phenotyped these infections as susceptible or resistant to chloroquine using a 28-day *in vivo* test format. 18 (25%) subjects had infections presumptively classified as resistant on the basis of persistent or recurrent parasitemia. 3 (17% of resistant infections) subjects had persistent parasitemia at Day 4 or had recurrent parasitemia by Day 7 and were considered early treatment failures. 7 (39%) had recurrent parasitemia by Day 14, and 8 (44%) Day 28. 55 (75%) subjects had no recurrent parasitemia diagnosed microscopically by Day 28 and were presumptively classified as having infections sensitive to chloroquine. The final step in the phenotyping process involves evaluating CQ+DCQ levels on day of recurrent parasitemia, as well as nested PCR on Day 28 samples from infections presumptively classified as sensitive. Those data are in process. Our study affirms prevalent resistance to chloroquine in this region of Indonesia, and we describe a simple, standardized system for phenotyping *P. vivax* infections as a first step in conducting genetic analysis of parasite genotypes linked to therapeutic responsiveness.

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IDENTIFICATION OF MOLECULAR MARKERS IN *PLASMODIUM FALCIPARUM* ISOLATES ASSOCIATED TO MEFLOQUINE AND ARTESUNATE DRUG RESITANCE IN THE PERUVIAN AMAZON BASIN

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Plasmodium falciparum malaria was treated in the Peruvian Amazon Basin for over 40 years with Chloroquine (CQ). Increased levels of treatment failure due to CQ resistance in 1997 forced the Peruvian Ministry of Health to change the first line therapy to Sulfadoxine-Pyrimethamine (SP). After only a few years SP resistance had developed. Therefore an Artemisinin Combination Therapy (ACT) based on Artemisin (AS) and Mefloquine (MQ) was implemented in 2003. The *Plasmodium falciparum multidrug resistance gene 1* (*Pfmdr1*) has been a candidate for CQ resistance and other chemically unrelated drugs, MQ and AS included. Recently, the *Plasmodium falciparum ATPase 6* gene (*Pfatp6*) has been proposed as the molecular target for Artemisinin based compounds. This study attempts to identify potential molecular markers within these genes associated with resistance to AS and/or MQ. Both genes were amplified by PCR in 161 *Plasmodium falciparum* samples from the Peruvian Amazon Basin taken in the years 1999 (*n*=104) and 2006 (*n*=58). Length variation was assed in 6 microsatellite markers loci flanking *Pfmdr1*. Molecular analysis was done by sequencing the *Pfmdr1* SNP 86, 184, 1034, 1042 and 1246; and by sequencing *Pfatp6* gene coding region to identify novel mutations. *Pfmdr1* alleles N86, 184F and 1042D were fixed in the Amazon Basin. Alleles S1034C and D1246Y were negatively selected by MQ+AS. Genetic diversity around *Pfmdr1* in 1999 (Hz=0.3397±0.21) was lower than in 2006 (Hz=0.4343±0.26). Polymorphisms found in the *Pfatp6* gene in 1999 were L402V, S466N, C(tgc)1030C(tgt) and a G deletion in codon 884; in 2006 were A630S and V1168I. The simple mutant 884 genotype was selected by the MQ+AS treatment. Genotype 402/630/1168 was only present in 2006. Mutant alleles in *Pfmdr1* at codons 1034, 1042 and 1246 selected by CQ would benefit the MQ+AS treatment. The presence of the 1042D allele could render isolates sensible for MQ. Addition of the other mutations could increase this sensibility. Also, mutant alleles in *Pfatp6* at codons 630 and 1168 could be potential molecular markers for MQ or/ and AS resistance.

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HITCHHIKING AND SELECTIVE SWEEPS OF *PLASMODIUM FALCIPARUM* SULFADOXINE AND PYRIMETHAMINE RESISTANT ALLELES IN CAMEROON

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Drug treatment of *Plasmodium falciparum* infections has led to the selection for resistant mutant alleles. Sulfadoxine pyrimethamine (SP) resistance is encoded by a number of mutations in the genes dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*). Here, we have genotyped mutations in *dhfr* and *dhps*, and have characterized microsatellite loci around *dhfr* on chromosome 4 and *dhps* on chromosome 8 as well as neutral markers on chromosomes 2 and 3 in 332 samples from Yaoundé, Cameroon. Our goals were to investigate the effects of selection on *dhfr* and *dhps* in this population, determine

the genetic relationships among *dhfr* and *dhps* alleles, and test the single origin hypothesis of highly pyrimethamine resistant alleles in a population from central Africa. Only 5% of the samples had wildtype *dhfr* or *dhps* alleles. This population shows strong linkage disequilibrium between the markers surrounding *dhfr* and *dhps* independently and little linkage within or between the neutral markers on chromosomes 2 and 3 - the result of strong selection on *dhfr* and *dhps*. The Cameroonian population shows skewed haplotype frequencies and a reduction of variation for mutant *dhfr* and *dhps* alleles, both are characteristics of selective sweeps occurring in this population. The previously reported Southeast Asian triple mutant *dhfr* haplotype is the most predominant in this sample set, but we also find additional independent, local haplotypes at low frequency. We also find multiple haplotypes for *dhps* mutant alleles; thus there have been multiple, independent originations of the mutant *dhfr* and *dhps* alleles in this population. This indicates that selection may act differently on *dhfr* and *dhps* within a population. These results yield support for the use of microsatellite markers to track resistant parasites in populations with a great amount of genetic diversity. In addition, this study demonstrates the signature of strong natural selection in a population with a great amount of recombination.

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GENETIC ANALYSIS OF THE RETURN OF CHLOROQUINE SENSITIVE *PLASMODIUM FALCIPARUM* PARASITES TO LAMBARÉNÉ, GABON

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Chloroquine resistance is mediated by the K76T allele of the *Plasmodium falciparum* chloroquine resistance transporter gene (*pfcrt)* and is highly prevalent throughout Africa. Due to a genetic sweep the chromosomal haplotype surrounding the *pfcrt* locus is largely conserved in resistant parasites. Recently it has been observed that cessation of chloroquine use can lead to a return of sensitive parasites. The exact genetic mechanism of this phenomenon however remains unclear. Here we attempt to address this question via haplotype analysis in the setting of the formerly 100% resistant area of Lambaréné, Gabon, where chloroquine was removed from the national treatment guidelines in 2003. We screened parasite DNA from 90 samples obtained in 2005/06 and 54 samples obtained in 2007 for the sensitive *pfcrt* allele and found 1 sensitive isolate in 2005/6 and 3 in 2007. Sequence analysis revealed that all 4 sensitive isolates carried the same sensitive allele. Chromosomal haplotype analysis with microsatellite markers revealed two different sensitive haplotypes: one without similarities to the resistant haplotype and one that is identical to the resistant haplotype extending to approximately 20kb upstream of the *pfcrt* gene at which point the sequences start to diverge. This suggests that the sensitive allele is incrossing into the resistant population. In addition analysis of 145 resistant samples obtained over a time period from 1995 to 2007 revealed a decreasing prevalence of the dominant resistant haplotype from 79% in 1995-96 to 58% in 2005-2007. Removal of chloroquine from the national treatment guidelines in Gabon appears to have coincided with reintroduction of the sensitive *pfcrt* allele by immigration and incrossing.

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HERITABILITY OF PARASITE CLEARANCE TIME FOLLOWING ARTEMISININ COMBINATION THERAPY

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Malaria parasites in some SE Asian locations show slow clearance after treatment with Artemisinin Combination Therapy (ACT). This has lead to alarm about the evolution of resistant parasites. But are parasite genes really responsible? To answer this question we measured the heritability of parasite clearance time in parasites from the Thai-Burma border. We measured clearance time at 24 or 6 hr intervals in 185 single clone parasite infections following ACT treatment, and characterised parasites by genotyping 423 microsatellites markers distributed at ~50kb intervals through the genome. We found many clonally identical or closely related parasites in this population sample. We therefore asked whether such parasites tend to show similar clearance times, using methods analogous to those used in human twin and pedigree studies. While we found a wide range of clearance times, there was no significant impact of parasite genes on clearance time. In contrast, *in vitro* resistance to a variety of drugs showed strong heritability in the same parasite collection, demonstrating that we have adequate power to detect genetic effects with this study design. These results provide a cautionary tale about the dangers of using *in vivo* phenotypes for case-control association studies because these may be influenced by multiple factors. We conclude that variation in clearance times is not influenced by parasite genotype on the Thailand-Burma border. However, we caution that this conclusion may not hold in other locations, such as the Thailand/Cambodia border, where unusually slow clearance times have been reported.

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CHARACTERISTICS OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN AGED 6-59 MONTHS IN THE KASSENA NANKANA DISTRICT OF NORTHERN GHANA

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An effective malaria vaccine remains the most viable option to effectively fight malaria. Such a vaccine will possibly target children between the ages of 6 to 59 months since this is the group mostly affected by malaria. Uncomplicated malaria could be a potential trial endpoint. Proper identification and understanding of the seasonal variation of uncomplicated malaria cases in the Kassena Nankana District could also lead to improvement in diagnosis and early management of malaria cases in the district. This study therefore, describes age-specific levels of uncomplicated malaria as well as the seasonal variations of its infection within the Kassena Nankana District of northern Ghana. This study was conducted in the Kassena Nankana District of Northern Ghana, an impoverished rural area with hyper endemic transmission of malaria. Children between the ages of 6- 59 months presenting at a district hospital and four health centres within the district with clinical symptoms suggestive of uncomplicated or mild malaria were recruited into this study.1642 were enrolled. Analysis was done using stata 9.0. Age specific levels of infection and seasonality of transmission were calculated. 80% of study participants were between 6 and 24 months old.58% had auxiliary temperature of ≥ 37.5 while the remaining 42% reported history of fever within the last 24 hours preceding the interview. Other presenting symptoms were shaking chills or rigor, (45%), vomiting, (64%). 99.88% of study participants were diagnosed with *Plasmodium falciparum*.The remaining 0.12% of the cases were due to *P. malariae* infection. Most of the cases were recruited between the months of June and September, coinciding with the onset and intensity of rain fall. In conclusion, malaria prevention and treatment programmes will have to give special attention

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Hitchhiking and selective sweeps of *Plasmodium falciparum* sulfadoxine and pyrimethamine resistant alleles in Cameroon

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