### **Abstract**

# **Background**

Although several studies have investigated the impact of reduced malaria transmission due to insecticide-treated bed nets (ITNs) on the patterns of morbidity and mortality, there is limited information on their effect on parasite diversity.

## **Methods**

Sequencing was used to investigate the effect of ITNs on polymorphisms in two genes encoding leading *Plasmodium falciparum* vaccine candidate antigens, the 19 kilodalton blood stage merozoite surface protein-1 (MSP-1<sub>19kDa</sub>) and the Th2R and Th3R T-cell epitopes of the pre-erythrocytic stage circumsporozoite protein (CSP) in a large community-based ITN trial site in western Kenya. The number and frequency of haplotypes as well as nucleotide and haplotype diversity were compared among parasites obtained from children <5 years old prior to the introduction of ITNs (1996) and after 5 years of high coverage ITN use (2001).

## **Results**

A total of 12 MSP-1<sub>19kDa</sub> haplotypes were detected in 1996 and 2001. The Q-KSNG-L and E-KSNG-L haplotypes corresponding to the FVO and FUP strains of *P. falciparum* were the most prevalent (range 32–37%), with an overall haplotype diversity of > 0.7. No MSP-1<sub>19kDa</sub> 3D7 sequence-types were detected in 1996 and the frequency was less than 4% in 2001. The CSP Th2R and Th3R domains were highly polymorphic with a total of 26 and 14 haplotypes, respectively detected in 1996 and 34 and 13 haplotypes in 2001, with an overall haplotype diversity of > 0.9 and 0.75 respectively. The frequency of the most predominant Th2R and Th3R haplotypes was 14 and 36%, respectively. The frequency of Th2R and Th3R haplotypes corresponding to the 3D7 parasite strain was less than 4% at both time points. There was no significant difference in nucleotide and haplotype diversity in parasite isolates collected at both time points.

### Conclusion

High diversity in these two genes has been maintained overtime despite marked reductions in malaria transmission due to ITNs use. The frequency of 3D7 sequence-types was very low in this area. These findings provide information that could be useful in the design of future malaria vaccines for deployment in endemic areas with high ITN coverage and in interpretation of efficacy data for malaria vaccines based on 3D7 parasite strains.