Malaria remains one of the most deadly infectious disease in sub-Saharan Africa accounting for high rates of mortality and morbidity especially in children less than 5 years. *Plasmodium falciparum (Pf)* parasite causes the most virulent form of malaria partially due to development of high levels of resistance against most anti-malaria drugs used. In malaria holoendemic areas of western Kenya, *P. falciparum* drug resistance has been noted since 1980s. Various genetic mutations have been identified and associated with *P. f* resistance to drugs, such as chloroquine (CQ), sulphadoxine-pyrimethamine (SP) and recently, Artemisinin-based combination therapy (ACT). Even though previous studies in Kanyawegi in western Kenya have addressed the presence of the genes associated with antimalarial resistance at one time point, the prevalence and temporal stability of the genes associated with ACT resistance in this malaria holoendemic population have not been reported. As such the temporal stability in the prevalence of *pfcrt* (K76T), *pfmdr1* (N86Y), *pfdhfr* (C59R) and *pfdhps* (K540E), and the association within and between the mutations that confer resistance against anti-malarials and those considered predictive of ACT treatment failure was investigated at the height of SP resistance just before administration of the ACT drug (July 2004), four weeks after administration of ACT (August 2004), and 5 years (July 2009) after SP withdrawal as front line treatment for uncomplicated malaria infections. A total of 95 paired retrospective blood samples from children under 5 years, confirmed positive for *P. f* were used. Using Polymerase Chain Reaction (PCR) and Nested PCR, *pfcrt* K76T, *pfmdr1* N86Y, *pfdhfr* (C59R) and *pfdhps* (K540E) genes were amplified and presence of mutations determined by gel electrophoresis after Restriction Fragment Length Polymorphism. Using chi-square analysis to determine the prevalence and Pearson's Correlation Co-efficient to determine the association between the genotypes and drug failures, the prevalence of *Pfdhps* wild type 540K increased from 14.7% (n =14/95) in July 2004 to 53.7% (n=51/95) in August 2004 (p=0.0004) and subsequently to 94.1% (16/17) in July 2009 (p=0.015). For *Pfdhfr* wild type C59, the prevalence in July 2004 was 0.0% (n =0/95) and 1.1% (n=1/95) (p=0.144) in August 2004 and 0.0% (n = 0/17) in July 2009. The prevalence of *Pfmdr1* wild type 86N was insignificant (p=0.223) from 0.0% (n=0/95) in July 2004 to 2.1% (n =2/95) in August 2004 and reduced to 0.0% (n = 0/17) in July 2009 (p = 0.759). Prevalence of *Pfcrt* wild type 76T remained constant in July and August 2004 at 2.1% (n=2/95) and rose to 5.9% (n=1/17) in 2009 (p=0.138). The prevalence of the K76T mutation was persistent in isolates from this highly holoendemic area indicating that selection for the mutant codon is in progress while results showed the absence of the *pfmdr1* N86Y isolates from both the baseline and the follow up isolates suggesting that the parasites harbouring this mutation are not widespread in this area and there was continued rise in the prevalence of the mutations associated with SP resistance while there was no association between the *pfcrt* K76T and *pfmdr1* N86Y pre- and post-adoption of ACT. Findings presented here suggest that resistant markers against CQ and SP have not faded and as such not recommended antimalarials in this *P.f* holoendemic region. This study will complement existing data on anti-malarial drug resistance monitoring and enhance future prediction of resistance levels that would be critical in informing anti-malarial drug policy aimed at reducing malaria-related morbidity and mortality.