Mutation in *P. falciparum* BTB/POZ domain of K13 confers artemisinin resistance

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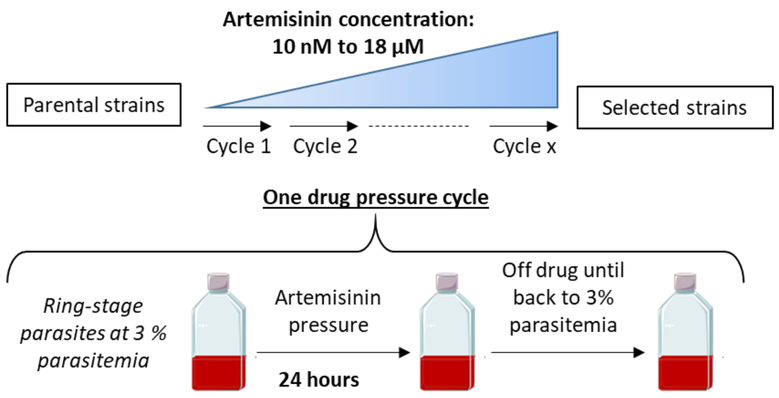
Partial artemisinin (ART) resistance, is defined in patients as a delayed parasite clearance following artemisinin-based treatment and is conferred by non-synonymous mutations in the Kelch beta-propeller domain of the *Plasmodium falciparum* k13 (*pfk13*) gene (1,2,3). Here, we carried out *in vitro* selection over a 1-year period on a West African *P. falciparum* strain isolated from Mali under a dose escalating and sequencing artemisinin regimen. The field parasite isolate SMT010 (randomly chosen) was collected in 2010. This isolate was not associated with any clinical failure after an ACT treatment course, displayed a wild-type *pfk13* sequence and an *in vitro* RSA$^{0\text{-}3h}$ survival rate < 1% (4).

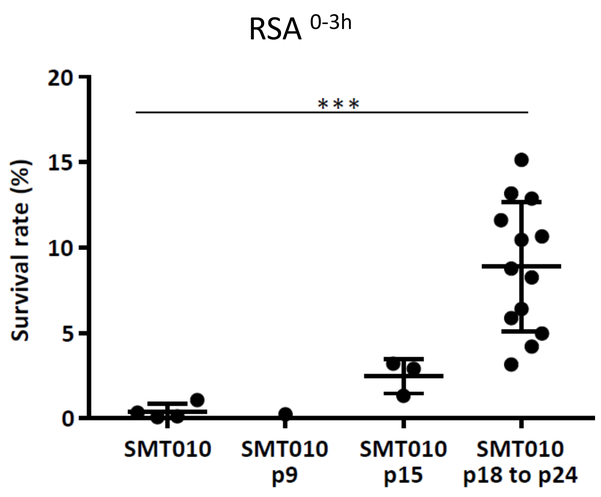
ART-sensitive strain SMT010

Drug pressure



Protocol used for F32-ART selection, the 1st in vitro selected artemisinin resistant line with the M476I K13 mutation (5)

Elevated survival rate after 18 drug pressure cycles

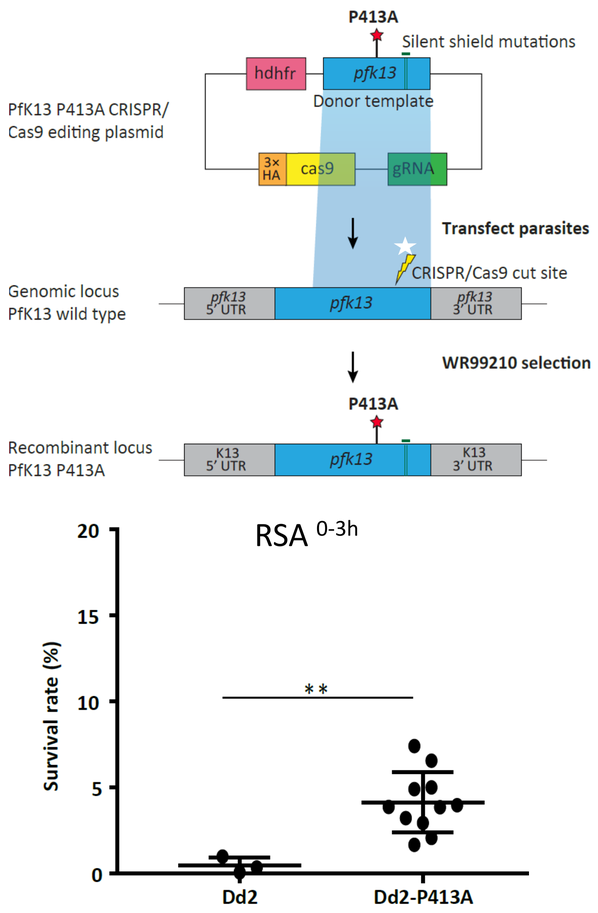


11 SNPs in SMT010p19 *vs* the parental strain

Gene ID	Gene name	SNPs in SMT010p19
PF3D7_0410000	erythrocyte vesicle protein 1	K294E, E286V, T290R, N296H
PF3D7_0411200	PP-loop family protein	K1056I
PF3D7_0710200	conserved protein unknown fonction	N693S
PF3D7_1343700	**kelch protein K13**	**P413A**
PF3D7_1362700	conserved protein unknown fonction	D1080N
PF3D7_1366400	rhoptry protein RHOP148	T285I
PF3D7_1425600	zinc finger protein	H1497N, N1505H

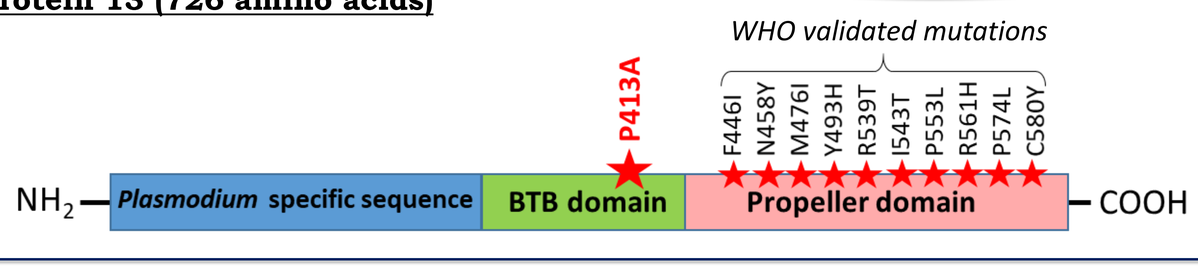
P413A mutation is located outside the propeller domain.

Gene editing in *P. falciparum* Dd2 strain

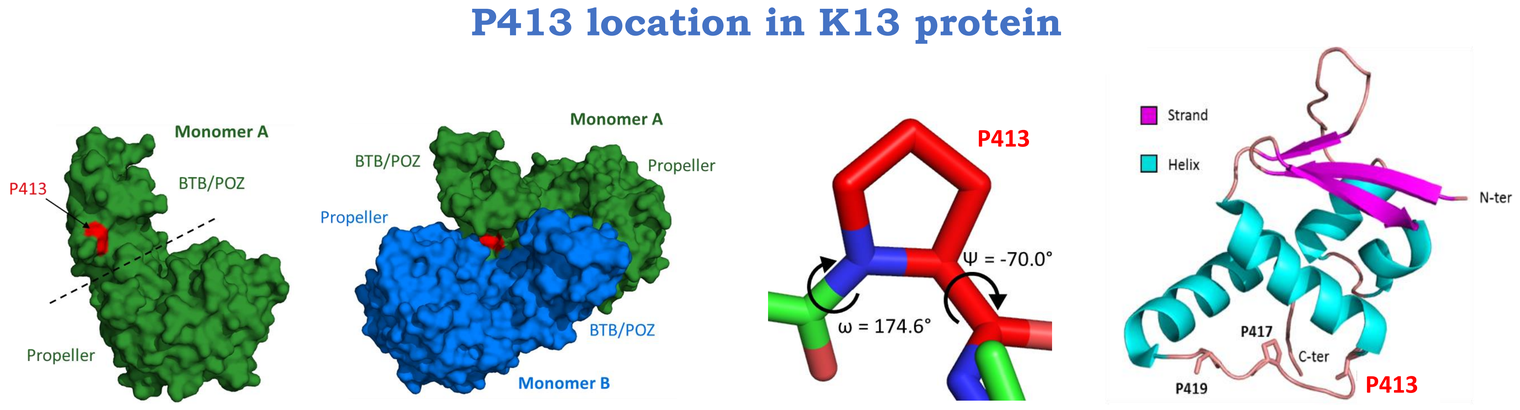


P413A mutation confers artemisinin resistance.

Kelch protein 13 (726 amino acids)



P413 location in K13 protein



P413 is exposed at the protein surface of each of the two monomers.

P413 adopts a *cis*-conformation (torsion angles phi = -70.0°, omega = 174.6°) and forms a turn at the extremity of one of the helix of the BTB/POZ domain.

At the sequence level, the P413A mutation was predicted to have a destabilizing effect on protein stability with a folding free energy change (DDG) of –1.53 as calculated by the machine learning-based SAAFEC-SEQ algorithm, suggesting that P413A has an impact on PfK13 protein structure during the folding process.

In silico prediction of a destabilizing effect of P413A during the protein folding process.

Neither P413A nor other missense mutations at position 413 have been reported so far. To date, only 1 non-synonymous mutation located in the BTB/POZ domain of PfK13 has been reported in clinical isolates, D353Y (found in five isolates from Vietnam), and associated with a parasite clearance half-life (PCt1/2) < 5 h after ACT treatment (6). The cyclic structure of proline's side chain induces exceptional conformational rigidity (7). Such a rigidity may be reduced in the P413A mutant structure, since the turn normally formed by P413 may likely be lost during the folding process. Rather, a machine learning-based algorithm at the sequence level revealed that P413A mutation has a destabilizing effect on protein stability. Such an effect could result in lower PfK13 abundance, a known driver of ART resistance in R539T and C580Y mutants (8,9).

These results, together with structural studies of the protein, demonstrate that the propeller domain is not the sole *in vitro* mediator of PfK13-mediated artemisinin resistance and highlight the importance of monitoring for mutations throughout PfK13.

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