Characterization of PbCARL mutations and their role in imidazolopiperazine resistance

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Point mutations in the endoplasmic reticulum (ER) and Golgi-associated transporters CARL, UGT, and ACT have been identified as key mediators of resistance to imidazolopiperazines, such as KAF156 (ganaplacide) and GNF179. In *Plasmodium falciparum*, the cyclic amine resistance locus (*pfcarl*) encodes a highly conserved protein characterized by seven transmembrane domains and precise localization to the cis-Golgi apparatus. Although its exact physiological role remains elusive, CARL is implicated in broader multidrug resistance mechanisms across different antimalarial classes. To investigate these dynamics in the *Plasmodium berghei* model, CRISPR-Cas9 was employed to introduce equivalent single nucleotide polymorphisms (SNPs) observed in *pfcarl* into the orthologous gene *pbcarl* (PBANKA_1216600). While attempts to introduce the P708L and V948L mutations indicated they were lethal to the parasite - a conclusion supported by the successful recovery of only silent control mutations (P708P and V948V) - the S921I substitution was successfully established. Phenotypic analysis of the S921I variant revealed no observable defects in gametocyte development or ookinete formation either *in vitro* or *in vivo*. However, the mutation appeared to impose a significant fitness cost; mutant parasites were rapidly outcompeted by wild-type strains within seven days and failed to demonstrate successful transmission. Most notably, while the S921I mutation conferred an insignificant 1.87-fold shift in sensitivity to dihydroartemisinin (DHA), it resulted in a profound 416-fold increase in resistance to GNF179. This confirms that CARL is a determinant of high-level resistance to this novel class of compounds, but critically this resistance appears to result in un-transmittable parasites.