Mutation underlying resistance of *Plasmodium berghei* to atovaquone in the quinone binding domain 2 (Qo2) of the cytochrome b gene

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Abstract

The anti-malarial agent atovaquone specifically targets the cytochrome bc1 complex and inhibits the parasite respiration. Resistance to this drug, a coenzyme Q analogue, is associated with mutations in the mitochondrial cytochrome b gene. We previously reported atovaquone resistant mutations in *Plasmodium berghei*, in the first quinone binding domain (Qo1) of the cytochrome b gene (M133I and L144S) with V284F in the sixth transmembrane domain. However, in *P. falciparum* the most common mutations are found in the Qo2 region. To obtain a better model for biochemical and genetic studies, we have now extended our study to isolate a wider range of *P. berghei* resistant strains, in particular those in the Qo2. Here we report four new mutations (Y268N, Y268C, L271V and K272R), all in the Qo2 domain. Two of these mutations are convergent to codon 268 (n802–804) drug-induced mutation in *P. falciparum.*

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The emergence of drug-resistant strains of *Plasmodium falciparum* within the last few decades has caused major problems in malaria treatment and control in many endemic countries. New affordable drugs that target different biochemical pathways in the malaria parasite are needed. Atovaquone, a hydroxy-1, 4-naphthoquinone, is an anti-malaria that shares structural similarity with protozoan ubiquinone, a coenzyme involved in the mitochondrial electron transport [1, 2]. It is effective against chloroquine-resistant strains of *P. falciparum,* and is a major component of Malarone™ (a fixed combination of atovaquone and proguanil). This drug collapses mitochondrial membrane potential in *Plasmodium* spp [3], and is suggested to act by competitive binding with ubiquinone at the quinone binding domain of the quinol-cytochrome c reductase of the mitochondrial respiratory chain (bc1 complex, also referred to as complex III). Mutations conferring atovaquone resistance were identified in the mitochondrial cytochrome b (cytb) gene of *P. berghei* [4], *P. yoelii* [5], *P. falciparum* [6], *Pneumocystis carinii* [7], and *Toxoplasma gondii* [8]. In *Plasmodium* spp, 10 mutations, M133I, L144S, 1258M, F267I, Y268C/N/S, L271F/V, K272R, P275T, G280D, and V284F had been documented, mostly located in the quinone binding domain 2 (Qo2). Significantly, mutations affecting codon 268 (n802–804) of the cytb gene in the Qo2 domain, have been reported also in *P. falciparum* isolates collected from malaria-infected individuals in Africa, associated with the use of, and in some cases with demonstrated treatment-failure against Malarone [9–11], leading to the suggestion for its use as a molecular marker for atovaquone resistance in the field isolates [12]. The two main *P. berghei* mutations reported previously [4], M133I and L144S, were all located in the quinone binding domain 1 (Qo1), these mutations confer up to 1000-fold