Assessing the Genetic Diversity of the Aldolase Genes of *Plasmodium falciparum* and *Plasmodium vivax* and Its Potential Effect on Performance of Aldolase-Detecting Rapid Diagnostic Tests[▽]

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Malaria-specific rapid diagnostic tests (RDTs) targeting aldolase show highly variable sensitivities. We assessed diversity in *Plasmodium falciparum* and *P. vivax* aldolases by sequencing the coding genes from parasites of various origins. The results show that aldolases are highly conserved, indicating that antigenic diversity is not a cause of variable RDT sensitivity.

Malaria-specific rapid diagnostic tests (RDTs) are lateral-flow immunochromatographic tests that detect specific antigens produced by malaria parasites. Malaria RDTs offer the potential to improve the accuracy of malaria diagnosis and case management, particularly when microscopic diagnosis is not available or is unreliable.

Many branded RDT products are now commercially available; some tests detect *Plasmodium falciparum* only, while others detect *P. falciparum* plus one or more of the three other species that commonly cause human malaria. The major target antigens in RDTs specific for *P. falciparum* are histidine-rich protein 2 (PfHRP2) and *Plasmodium* lactate dehydrogenase, while pan-specific *Plasmodium* lactate dehydrogenase and aldolase are targeted to detect the other three *Plasmodium* species.

Recently, we reported extensive diversity in PfHRP2 in isolates collected globally and demonstrated that this diversity affected the lower detection limits of two PfHRP2-detecting RDTs (2). We have also shown that the epitopes of anti-PfHRP2 monoclonal antibodies vary significantly in composition and frequency among different parasite isolates, resulting in antibodies that recognize different isolates at different strengths (11). These findings highlighted the potential effect of parasite genetic diversity on the performance of malaria RDTs and the need to investigate the degree of genetic diversity in antigens that are targeted by antibodies used in non-HRP2-detecting RDTs. Since a number of published studies have shown poor sensitivities of aldolase-detecting RDTs (4, 7, 9, 12, 14), genetic diversity is a plausible explanation.

Aldolase is a key enzyme in the glycolysis pathway in malaria parasites. Unlike higher vertebrates, with three tissue-specific aldolase isoenzymes (13), *P. falciparum* and *P. vivax* possess

only one aldolase isoenzyme (6, 10), similar to *Trypanosoma brucei* (5) and *Giardia lamblia* (8). The *P. falciparum* and *P. vivax* aldolases are both 369 amino acids long, and their nucleotide and amino acid sequences are relatively conserved (6). However, genetic variation within *P. falciparum* and *P. vivax* aldolases has not been examined systematically.

To determine the degree of diversity and the potential effect of diversity on the performance of aldolase-detecting RDTs, we examined and compared the *P. falciparum* and *P. vivax* aldolase gene sequences for 36 *P. falciparum* lines and 18 *P. vivax* isolates originating from geographically different areas. The origins of the *P. falciparum* lines (2) and the collection and origin of *P. vivax* samples (1) were described previously and are summarized in Table 1.

Genomic DNA was extracted from guanidine hydrochloride-preserved blood as previously described (3) and from frozen packed cells by using a QIAamp DNA mini kit (QIAGEN, Germany) following the manufacturer's instructions. Full-length *P. falciparum* and *P. vivax* aldolase genes were amplified by PCRs using gene-specific primers. PCRs were carried out in 50- μ l reaction mixtures containing 7.5 ng of each primer, 2.5 mM MgCl₂, 1.25 units of AmpliTaq Gold DNA polymerase (PE Applied Biosystems), a 200 μ M concentration of each deoxynucleoside triphosphate (Promega, Madison, Wis.), and buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3). For *P. falciparum* aldolase, the primers used were 5'-TGCCTGAATATA TGAATGCC-3' and 5'-GACATATTTCTTTTCATATCCT G-3', while for *P. vivax* aldolase, the primers were 5'-ATG GCCACTGGATCCG-3' and 5'-ACGTACTTCTTTTCGT AAAGGG-3'. PCR cycling conditions consisted of a 94°C denaturation step for 10 min followed by 40 cycles of amplification (94°C for 50 seconds, 50 seconds at 50°C for *P. falciparum* or 55°C for *P. vivax*, and 70°C for 1 min). PCR products were visualized following agarose gel electrophoresis, purified using a QIAquick PCR purification kit (QIAGEN, Valencia, CA), and then sequenced in an ABI PRISM 370 sequencer (Applied Biosystems, Inc., Foster City, CA).

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TABLE 1. Origins of *P. falciparum* and *P. vivax* isolates and SNPs in aldolases

Organism and region/country of origin	Line/isolate name ^a	SNP (bp position [change])
<i>P. falciparum</i>		
Africa		
Burkina Faso	CARDI	
Cameroon	CM232	
Liberia	TRACI	
Sudan	106/1	
	123/4	
Zambia	ZM105	
	3D7*	
West Pacific	AN101	
PNG	AN143	
	FCQ33	
	FCQ41	
	FCQ64	
Solomon Islands	SJ44	
	S55	
	N70	
	ROV11	
	ROV15	
Southeast Asia		
Vietnam	V2	
	V4	
	V8	
	V10	
	V11	
Thailand	GA3	
	INDO20	
	W2	
	K1*	512 (T to A) ^b
Indonesia	ARSO1	
	ARSO2	
Malaysia	CAMP	
Philippines	PH1	174 (A to G)
	PH4	
	Palawan7	
	Palawan18	
	Palawan130	174 (A to G)
South America		
Brazil	PX403	
	PX413	
	S34/89	
Ecuador	ECU1110	
<i>P. vivax</i>		
West Pacific		
PNG	Poker	
	Luke	
	AMRU2	
	WDK*	1066 (C to G) ^b
Vanuatu	005	
	008	
	011	
Southeast Asia and Asia		
Vietnam	A10	
	A11	
	A13	
	A14	
	A15	
Philippines	X061	615 (A to G)
	X028	
	S002	
China	J53	
	J54	615 (A to G)
	J55	615 (A to G)
	J58	

^a *, reference sequence available in GenBank.^b Nonsynonymous change.

Comparison of the DNA sequences of the *P. falciparum* aldolase genes from 36 different strains originating from eight different regions showed a high level of conservation, with a synonymous single nucleotide polymorphism (SNP) at nucleotide 174 (A to G) observed in only two isolates. Both parasites with this SNP, PH1 and Palawan130, originated from the Philippines. The remaining 34 sequences were identical to those of strains FCBP (GenBank accession no. M2881) and 3D7 (PlasmoDB accession no. NP_702314) but different from that of K1 (GenBank accession no. AAA29473) at nucleotide 512, resulting in an amino acid change (I to N). Notably, however, when the 34 sequences were aligned with another *P. falciparum* aldolase sequence (GenBank accession no. AF179421 [FCC1]), greater sequence divergence was observed, with 13 distinct SNPs, five of which were synonymous and eight of which resulted in an amino acid change (data not shown). There are two possible explanations for this difference. First, the differences may be due to a sequencing error in the FCC1 aldolase sequence. Alternatively, FCC1 has a rare form of aldolase that is different from those in other parasite lines. Since the aldolase from the same parasite line was sequenced by a different laboratory more recently and reported to be identical to other published aldolases (15), it is most likely that the divergent sequence deposited in GenBank contains sequence errors.

The aldolase genes from 18 *P. vivax* isolates from five different regions were sequenced and compared. A single synonymous SNP at base pair 615, from A to G, was observed in 3 of the 16 isolates. Two of these isolates (J54 and J55) were from China, while isolate X061 was from the Philippines. Alignment with the previously published *P. vivax* WDK sequence (6) (GenBank accession no. AF247063) showed a SNP at bp 1,066 resulting in an amino acid change of P to A.

In contrast to PfHRP2, both the *P. falciparum* and *P. vivax* aldolase isoenzymes appear to be highly conserved among the parasite lines and isolates we examined, which originated from geographically distinct areas. No insertions/deletions or non-synonymous changes that are likely to affect the binding efficiency of anti-aldolase antibodies were observed in the aldolases of 36 *P. falciparum* and 18 *P. vivax* isolates. Although we cannot completely rule out the possibility that some strains of *P. falciparum* or *P. vivax* may possess a form of aldolase that has profound amino acid changes, our data suggest that it is highly unlikely. The conservation of the sequence potentially makes this enzyme a consistent target for detection by malaria-specific rapid diagnostic tests detecting *P. falciparum* and *P. vivax* infections in different regions. The overall limited sequence variation observed in both *P. falciparum* and *P. vivax* aldolases suggests that antigen variation is an unlikely cause of variation in the sensitivity of aldolase-detecting RDTs for either species. Reports of low sensitivities of aldolase-detecting RDTs are therefore more likely to be due to low antigen concentrations, to a single/small number of epitopes per parasite, or to other factors affecting RDT quality.

Nucleotide sequence accession numbers. The novel nucleotide sequences of the *P. falciparum* and *P. vivax* aldolases reported in this paper have been deposited in the GenBank database under accession numbers DQ874471 to DQ874473.

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