Evolution of artemisinin partial resistance in Ugandan malaria parasites

Supplementary Appendix

Melissa D. Conrad, PhD[1], Victor Asua, MSc[2,3], Shreeya Garg, BS[1], David Giesbrecht, PhD[4], Karamoko Niaré, PhD[4], Sawyer Smith, BS[4], Jane F. Namuganga, MHS[2], Thomas Katairo, MSc[2], Jennifer Legac, BS[1], Rebecca M. Crudale, BS[4] Patrick K. Tumwebaze, MSc[2], Samuel L. Nsobya, PhD[2], Roland A. Cooper, PhD[5], Moses R. Kamya, M.B.ChB, PhD[2,6], Grant Dorsey, MD, PhD[1], Jeffrey A. Bailey, MD, PhD[4], Philip J. Rosenthal, MD[1]

[1]University of California, San Francisco, CA, USA; [2]Infectious Diseases Research Collaboration, Kampala, Uganda; [3] University of Tübingen, Tübingen, Germany; [4]Brown University, Providence, RI, USA; [5]Dominican University of California, San Rafael, CA, USA; [6]Makerere University, Kampala, Uganda

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SECTION 1: SUPPLEMENTAL METHODS

GENETIC SURVEILLANCE OF *P. FALCIPARUM* IN UGANDA

Dried blood spots (DBS) were collected from consenting individuals aged 6 months-10 years of age (2016-2018) or older than 6 months of age (2019-2020) who were diagnosed with uncomplicated malaria using rapid diagnostic tests or microscopy at 10 (2016-17) or 16 (2018-21) Malaria Reference Centers (MRCs) across Uganda. We targeted collection of 50 samples per MRC in 2016-19 and 100 samples in 2020 and 2021, although some collections fell short due to low malaria incidence (Fig. 1, Table S2). Sample collections were performed primarily in June-July each year, although collection periods were extended in areas of low malaria prevalence.

DNA was extracted from DBS using Chelex 100 as previously described.[1] PfK13 sequences were determined using both dideoxy sequencing of *pfk13* and molecular inversion probe (MIP) technology[2,3] for samples collected in 2017-2020, and using only MIP assessments for samples collected in 2016 and 2021. Dideoxy sequencing, MIP capture, library preparation, and sequencing were performed as previously described.[1,2] For MIP data, MIPTools software (version 0.19.12.13) was used to organize raw sequencing data and to perform variant calling (https://github.com/bailey-lab/MIPTools). Individual genotypes were assigned for polymorphic sites that were covered by a minimum of 5 unique molecular identifiers (UMIs) and variants were required to have a genotype allele count ≥3 UMIs for alternate alleles and ≥2 UMIs for reference alleles. When available, dideoxy sequencing data were used to supplement missing MIP data. Raw sequencing reads from MIP assays are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the following accession numbers PRJNA880926 (2016 samples), PRJNA880930 (2017 samples), PRJNA655702 (2018 and 2019 samples), PRJNA880932 (2020 samples), and PRJNA880933 (2021 samples). Dideoxy sequencing data were previously published[1,4] and can be located at the following accession numbers: MH788997-MH789408 (2017 samples), MT857288-MT857721 (2018 and 2019 samples), and OP859158 - OP860241 (2020 samples).

PHYLOGENETIC ANALYSIS OF ISOLATES WITH PARTIAL RESISTANCE MARKERS

Microsatellites (Fig. 3A). To assess the origins of emerging PfK13 mutations in Uganda, we characterized 7 microsatellite loci flanking *pfk13* in Ugandan parasites. Parasites collected in 2017-2021 with PfK13 mutations of interest were paired with parasites lacking PfK13 mutations by collection period and site and genotyped at loci upstream (-0.15 kb, -3.7 kb, -6.36 kb, -31.9 kb) and downstream (+8.6 kb, +15.1 kb, +72.3 kb) of the *pfk13* gene using previously published Methods,[5] with some modifications. Briefly, 15 µL reactions contained Promega 2X Master Mix and 267 nM of the forward and reverse primers. Primers were as indicated in Table S1. Cycling parameters were: initial denaturation at 94°C for 2 min, followed by 5 cycles of denaturation at

94°C for 30 s, annealing at 50°C for 30 s, and extension at 60°C for 30 s, followed by 40 cycles at 94°C for 30 s, annealing at 45°C for 30 s, and extension at 60°C for 30 s, with no final extension cycle.

Fragment analysis was performed by Quinatara Biosciences (Hayward, CA) using a ROX 350 size standard, and allele calling was done using MicroSatellite Parameterized Analysis Tools (MicroSPAT), available at https://github.com/EPPIcenter/MicroSPAT. Relatedness was evaluated in samples that had genotypes called for at least 6 of the 7 loci genotyped and had only one microsatellite allele called for each locus. The meandistance.matrix function in the R package polysat was used to generate a distance matrix from microsatellite data.[6] A neighbor-joining tree was inferred using the njs() function in ape,[7] and was plotted using the ggtree package.[8]

Genome-wide Variants (Fig. 3B). To determine the origins of parasites with PfK13 mutations, we compared genotypes of Ugandan (collected in 2020) and southeast Asian (isolates from the *P. falciparum* Community Project, v6.0 (https://www.malariagen.net/resource/26), collected in 2008-2013) parasites with PfK13 polymorphisms of interest. Genotypes from Ugandan parasites were generated using a genome-wide MIP panel designed to capture overall levels of differentiation and relatedness,[2] and equivalent variant sites were extracted from available Pf6K genomes, for which variants were called using a malaria-optimized GATK4 pipeline. Data pruning included removing samples with >90% missingness, loci with >50% missingness, and variants with a read count below 3. Using both monoclonal and polyclonal samples containing PfK13 469Y, 469F, or 675V mutations, a distance matrix was generated in R[9] using RStudio[10] and the snpDiss() function in SNPRelate.[11] Hierarchical clustering of the distance matrix used the complete linkage method, and the dendrogram was plotted using ggtree.[8]

SECTION 2: SUPPLEMENTAL TABLES AND FIGURES

Table S1. Malaria Reference Center surveillance sites.

District	Health Facility	Site Description	Samples Collected Per Year						
			2016	2017	2018	2019	2020	2021	2022
Agago	Patongo HC III	IRS stopped	49	50	50	49	92	98	100
Amolatar	Amolatar HC IV	IRS ongoing	53	49	50	44	86	98	99
Arua	Opia HC III	No IRS	50	50	44	50	100	100	98
Hoima	Kigorobya HC IV	No IRS	-	-	50	50	100	100	96
Jinja	Bugembe HC IV	No IRS	51	49	47	50	97	100	100
Kaabong	Lokolia HC III	No IRS	-	-	50	50	103	100	99
Kanungu	Kihihi HC IV	No IRS	50	50	50	50	100	97	100
Kapchorwa	Kaserem HC IV	No IRS	-	-	0*	48	0*	95	100
Kasese	Karambi HC III	No IRS	-	-	26*	50	100	100	100
Katakwi	Toroma HC IV	IRS stopped	-	-	50	50	100	98	100
Koboko	Lobule HC III	No IRS	-	-	50	50	99	100	100
Kole	Aboke HC IV	IRS stopped	50	49	50	50	100	98	100
Lamwo	Padibe HC IV	IRS stopped	49	49	50	43	99	96	100
Mubende	Kasambya HC IV	No IRS	54	50	47	43	100	97	100
Rukiga**	Kamwezi HC IV	No IRS	50	0*	0*	15*	44*	100	100
Tororo	Nagongera HC IV	IRS ongoing	50	50	50	50	45*	99	100

Dash (-) indicates no samples were collected; HC = Health Centre; *Low transmission at time of sample collection prevented meeting targets; **Rukiga district was previously part of Kabale district.

Table S2. Microsatellite primer sequences.

Locus	Location	Primer	Primer Sequence
-0.15 kb	Pf3D7_13_v3:1726927-1727151	Forward	GAT TCC CTA TCA TAC GTC ATA G
		Reverse	FAM-ACA AGG CGT AAA TAT TCG TG
-3.7 kb	Pf3D7_13_v3:1730575-1730738	Forward	FAM- ATG AAA AGG AAC ATA CAC AC
		Reverse	GCA CAA ATC ACC ATA TTA TT
-6.36 kb	Pf3D7_13_v3:1733075-1733360	Forward	AAT TAG ACA GGA CTT GTT AAT G
		Reverse	FAM-GGA TAT GTT ATC CAA AAT GCA
-31.9 kb	Pf3D7_13_v3:1758942-1759150	Forward	FAM-AAT CAA TCA TGG AAA TTA TG
		Reverse	TTG TTC ATT TAA GGG TAT TT
+8.6 kb	Pf3D7_13_v3:1715937-1716208	Forward	ACG GGT TTC TGT ACG CCT CAC
		Reverse	HEX-CTA AAT TAG TGG CTT CTT CAT
+15.1 kb	Pf3D7_13_v3:1709549-1709698	Forward	CTT ACT TCT AGG AGA ATC CT
		Reverse	HEX-GAT AAC TAC TCA TCT CCA CA
+72.3 kb	Pf3D7_13_v3:1652456-1652695	Forward	HEX-ATG GCT CTA TAA TAG GAA GG
		Reverse	TGC AAA CTT TTT AGA CTT TT

Table S3. Prevalence of 4 key PfK13 mutations at 16 surveillance sites.

District	Locus	2016	2017	2018	2019	2020	2021	2022	Trend
		#Mut/N, (%)	#Mut/N, (%)	#Mut/N, (%)	#Mut/N, (%)	#Mut/N, (%)	#Mut/N, (%)	#Mut/N, (%)	p-value
Agago	P441L	0/30 (0)	0/42 (0)	1/31 (3.2)	0/46 (0)	0/79 (0)	0/54 (0)	0/67 (0)	0.42
	C469Y	0/30 (0)	2/44 (4.5)	1/31 (3.2)	10/47 (21.3)	33/79 (41.8)	8/50 (16.0)	19/67 (28.4)	<0.0001
	A675V	0/31 (0)	2/44 (4.5)	1/35 (2.9)	8/46 (17.4)	13/79 (16.5)	7/52 (13.5)	9/68 (13.2)	0.009
	C469F	0/30 (0)	0/42 (0)	0/30 (0)	0/39 (0)	0/79 (0)	0/50 (0)	0/64 (0)	NA
	R561H	0/22 (0)	0/44 (0)	0/30 (0)	0/48 (0)	0/79 (0)	0/43 (0)	0/55 (0)	NA
Amolatar	P441L	0/28 (0)	0/48 (0)	0/4 (0)	0/26 (0)	0/85 (0)	0/49 (0)	0/13 (0)	NA
	C469Y	0/28 (0)	2/49 (4.1)	0/4 (0)	0/28 (0)	0/85 (0)	0/48 (0)	0/13 (0)	0.10
	A675V	0/30 (0)	0/49 (0)	0/27 (0)	0/27 (0)	0/85 (0)	1/50 (2.0)	0/13 (0)	0.27
	C469F	0/28 (0)	0/48 (0)	0/4 (0)	0/17 (0)	0/85 (0)	0/48 (0)	0/13 (0)	NA
	R561H	0/13 (0)	0/48 (0)	0/11 (0)	0/25 (0)	0/85 (0)	0/49 (0)	0/13 (0)	NA
Arua	P441L	0/31 (0)	0/42 (0)	0/20 (0)	0/24 (0)	0/81 (0)	0/69 (0)	1/76 (1.3)	0.25
	C469Y	0/31 (0)	0/45 (0)	0/20 (0)	0/24 (0)	0/81 (0)	2/68 (2.9)	6/76 (7.9)	0.003
	A675V	0/33 (0)	1/45 (2.2)	0/30 (0)	1/29 (3.4)	0/81 (0)	1/68 (1.5)	2/78 (2.6)	0.52
	C469F	0/31 (0)	0/43 (0)	0/20 (0)	0/10 (0)	0/81 (0)	0/68 (0)	0/70 (0)	NA
	R561H	0/29 (0)	0/43 (0)	0/24 (0)	0/24 (0)	0/81 (0)	0/56 (0)	0/67 (0)	NA
Hoima	P441L	-	-	0/42 (0)	1/79 (1.3)	0/92 (0)	0/82 (0)	0/60 (0)	0.36
	C469Y	-	-	0/42 (0)	0/83 (0)	8/92 (8.7)	0/82 (0)	0/60 (0)	0.82
	A675V	-	-	0/45 (0)	0/82 (0)	6/92 (6.5)	0/82 (0)	14/63 (22.2)	<0.0001
	C469F	-	-	1/42 (2.4)	0/47 (0)	0/92 (0)	0/82 (0)	0/60 (0)	0.07
	R561H	-	-	0/42 (0)	0/82 (0)	0/92 (0)	0/65 (0)	0/53 (0)	NA
Jinja	P441L	0/29 (0)	0/48 (0)	0/37 (0)	0/89 (0)	0/96 (0)	0/95 (0)	0/99 (0)	NA
	C469Y	0/29 (0)	0/48 (0)	0/37 (0)	0/91 (0)	0/96 (0)	0/95 (0)	0/99 (0)	NA
	A675V	0/29 (0)	0/48 (0)	0/40 (0)	0/90 (0)	0/96 (0)	0/95 (0)	1/99 (1.0)	0.20
	C469F	0/29 (0)	0/48 (0)	0/37 (0)	0/43 (0)	0/96 (0)	0/95 (0)	0/99 (0)	NA
	R561H	0/27 (0)	0/48 (0)	1/38 (2.6)	0/89 (0)	0/96 (0)	0/74 (0)	0/92 (0)	0.35
Kaabong	P441L	-	-	0/30 (0)	0/45 (0)	0/75 (0)	0/60 (0)	0/64 (0)	NA
	C469Y	-	-	2/30 (6.7)	0/45 (0)	13/75 (17.3)	7/57 (12.3)	10/64 (15.6)	0.04
	A675V	-	-	3/34 (8.8)	10/46 (21.7)	14/75 (18.7)	10/59 (16.9)	4/68 (5.9)	0.19
	C469F	-	-	0/29 (0)	0/31 (0)	0/75 (0)	3/57 (5.3)	0/60 (0)	0.36
	R561H	-	-	0/30 (0)	0/44 (0)	0/75 (0)	0/40 (0)	0/54 (0)	NA

Table S3 (continued)

District	Locus	2016	2017	2018	2019	2020	2021	2022	Trend
		#Mut/N, (%)	#Mut/N, (%)	p-value	#Mut/N, (%)	p-value	#Mut/N, (%)	#Mut/N, (%)	p-value
Kanungu	P441L	0/37 (0)	0/48 (0)	0/36 (0)	0/59 (0)	2/91 (2.2)	3/50 (6.0)	15/65 (23.1)	<0.0001
	C469Y	0/37 (0)	0/48 (0)	0/35 (0)	0/64 (0)	0/91 (0)	0/46 (0)	0/65 (0)	NA
	A675V	0/37 (0)	0/48 (0)	0/38 (0)	0/61 (0)	0/94 (0)	0/44 (0)	2/64 (3.1)	0.04
	C469F	0/37 (0)	0/48 (0)	1/36 (2.8)	0/40 (0)	4/91 (4.4)	2/46 (4.3)	0/65 (0)	0.39
	R561H	0/37 (0)	0/48 (0)	0/36 (0)	0/62 (0)	0/91 (0)	0/37 (0)	1/51 (2.0)	0.13
Kapchorwa	P441L	-	-	-	0/16 (0)	-	0/77 (0)	0/27 (0)	NA
	C469Y	-	-	-	0/16 (0)	-	1/77 (1.3)	2/27 (7.4)	0.11
	A675V	-	-	-	0/15 (0)	-	3/71 (4.2)	9/30 (30.0)	0.0004
	C469F	-	-	-	0/16 (0)	-	0/77 (0)	0/26 (0)	NA
	R561H	-	-	-	0/14 (0)	-	0/69 (0)	0/12 (0)	NA
Kasese	P441L	-	-	0/19 (0)	2/61 (3.3)	2/89 (2.2)	11/76 (14.5)	14/91 (15.4)	0.0001
	C469Y	-	-	0/19 (0)	0/61 (0)	0/89 (0)	0/78 (0)	8/91 (8.8)	0.0002
	A675V	-	-	0/24 (0)	0/64 (0)	0/92 (0)	0/75 (0)	3/92 (3.3)	0.02
	C469F	-	-	0/19 (0)	0/39 (0)	0/89 (0)	0/78 (0)	0/88 (0)	NA
	R561H	-	-	0/17 (0)	0/60 (0)	0/90 (0)	0/61 (0)	0/85 (0)	NA
Katakwi	P441L			0/40 (0)	0/55 (0)	2/87 (2.3)	0/58 (0)	0/78 (0)	0.78
	C469Y	-	-	2/40 (5.0)	3/61 (4.9)	12/87 (13.8)	7/58 (12.1)	18/78 (23.1)	0.0006
	A675V	-	-	1/45 (2.2)	6/57 (10.5)	6/88 (6.8)	9/58 (15.5)	14/75 (18.7)	0.003
	C469F	-	-	0/40 (0)	0/36 (0)	0/86 (0)	0/58 (0)	0/68 (0)	NA
	R561H	-	-	0/40 (0)	0/57 (0)	0/86 (0)	0/41 (0)	0/62 (0)	NA
Koboko	P441L	-	-	0/23 (0)	0/4 (0)	0/55 (0)	0/47 (0)	0/48 (0)	NA
	C469Y	-	-	0/23 (0)	0/4 (0)	2/55 (3.6)	1/46 (2.2)	1/48 (2.1)	0.71
	A675V	-	-	0/34 (0)	0/5 (0)	5/64 (7.8)	5/44 (11.4)	6/47 (12.8)	0.02
	C469F	-	-	0/23 (0)	0/4 (0)	0/54 (0)	0/46 (0)	0/47 (0)	NA
	R561H	-	-	0/26 (0)	0/3 (0)	0/62 (0)	0/27 (0)	0/35 (0)	NA
Kole	P441L	0/23 (0)	0/46 (0)	0/36 (0)	0/56 (0)	0/85 (0)	0/56 (0)	0/74 (0)	NA
	C469Y	1/23 (4.3)	1/47 (2.1)	0/36 (0)	3/56 (5.4)	12/85 (14.1)	0/54 (0)	9/74 (12.2)	0.04
	A675V	0/26 (0)	1/47 (2.1)	1/34 (2.9)	2/60 (3.3)	10/86 (11.6)	2/58 (3.4)	6/78 (7.7)	0.06
	C469F	0/23 (0)	0/47 (0)	0/36 (0)	0/39 (0)	0/85 (0)	0/54 (0)	0/72 (0)	NA
	R561H	0/22 (0)	0/47 (0)	0/36 (0)	0/57 (0)	0/84 (0)	0/42 (0)	0/37 (0)	NA

Table S3 (continued)

District	Locus	2016	2017	2018	2019	2020	2021	2022	Trend
		#Mut/N, (%)	#Mut/N, (%)	p-value	#Mut/N, (%)	p-value	#Mut/N, (%)	#Mut/N, (%)	p-value
Lamwo	P441L	0/33 (0)	0/42 (0)	0/34 (0)	0/49 (0)	0/84 (0)	0/58 (0)	0/53 (0)	NA
	C469Y	3/33 (9.1)	3/44 (6.8)	8/34 (23.5)	5/50 (10.0)	28/84 (33.3)	16/51 (31.4)	11/53 (20.8)	0.002
	A675V	2/37 (5.4)	5/44 (11.4)	1/36 (2.8)	6/49 (12.2)	16/84 (19.0)	12/53 (22.6)	8/53 (15.1)	0.01
	C469F	0/31 (0)	0/43 (0)	0/31 (0)	0/31 (0)	0/84 (0)	0/51 (0)	0/46 (0)	NA
	R561H	0/32 (0)	0/44 (0)	0/31 (0)	0/50 (0)	0/84 (0)	0/42 (0)	0/37 (0)	NA
Mubende	P441L	0/22 (0)	0/45 (0)	0/38 (0)	0/22 (0)	1/88 (1.1)	1/19 (5.3)	6/52 (11.5)	0.0004
	C469Y	0/26 (0)	1/46 (2.2)	0/36 (0)	0/22 (0)	3/88 (3.4)	0/19 (0)	0/52 (0)	0.99
	A675V	0/28 (0)	0/46 (0)	0/41 (0)	0/24 (0)	6/87 (6.9)	0/19 (0)	6/53 (11.3)	0.001
	C469F	0/26 (0)	0/45 (0)	4/38 (10.5)	0/22 (0)	0/88 (0)	0/19 (0)	0/52 (0)	0.18
	R561H	0/26 (0)	0/46 (0)	0/38 (0)	0/21 (0)	0/88 (0)	0/16 (0)	0/53 (0)	NA
Rukiga	P441L	0/35 (0)	-	-	0/26 (0)	0/39 (0)	1/83 (1.2)	0/60 (0)	0.66
	C469Y	0/35 (0)	-	-	0/26 (0)	0/39 (0)	0/72 (0)	1/46 (2.2)	0.29
	A675V	0/42 (0)	-	-	1/21 (4.8)	1/39 (2.6)	2/82 (2.4)	3/59 (5.1)	0.19
	C469F	8/42 (19.0)	-	-	0/14 (0)	11/39 (28.2)	29/72 (40.3)	23/60 (38.3)	0.005
	R561H	0/41 (0)	-	-	0/23 (0)	0/39 (0)	11/67 (16.4)	12/53 (22.6)	0.0001
Tororo	P441L	0/34 (0)	0/48 (0)	0/31 (0)	0/71 (0)	0/40 (0)	0/74 (0)	0/51 (0)	NA
	C469Y	0/34 (0)	0/48 (0)	0/31 (0)	0/71 (0)	0/40 (0)	3/73 (4.1)	5/51 (9.8)	0.0006
	A675V	0/37 (0)	1/48 (2.1)	0/43 (0)	0/73 (0)	0/41 (0)	3/74 (4.1)	9/57 (15.8)	0.0001
	C469F	1/35 (2.9)	1/48 (2.1)	0/31 (0)	0/44 (0)	0/40 (0)	0/73 (0)	0/48 (0)	0.056
	R561H	0/34 (0)	0/48 (0)	0/32 (0)	0/70 (0)	0/41 (0)	0/66 (0)	0/39 (0)	NA

Table S4. Prevalence of additional PfK13 propeller domain (codons 441-724) mutations, 2016-22*.

Locus	District	Year	Mutation prevalence
P441A	Agago	2022	2/67 (3.0%)
	Amolatar	2021	1/45 (2.2%)
	Kaabong	2022	3/64 (4.7%)
F442L	Kanungu	2018	3/35 (8.6%)
		2019	2/24 (8.3%)
		2020	7/83 (8.4%)
		2021	1/42 (2.4%)
N490T	Jinja	2021	1/73 (1.4%)
	Kanungu	2020	1/94 (1.1%)
		2022	2/60 (3.3%)
	Katakwi	2020	1/87 (1.1%)
	Mubende	2019	1/21 (4.8%)
		2020	1/88 (1.1%)
	Rukiga	2019	2/24 (8.3%)
		2022	1/46 (2.2%)
V517I	Katakwi	2021	1/47 (2.1%)
	Tororo	2016	2/34 (5.9%)
S522C	Jinja	2022	1/99 (1.0%)
	Kole	2022	2/71 (2.8%)
G533A	Agago	2019	1/40 (2.5%)
		2020	1/80 (1.2%)
	Kanungu	2017	2/48 (4.2%)
	Kasese	2022	1/93 (1.1%)
	Mubende	2017	1/46 (2.2%)
R539T	Amolatar	2019	1/30 (3.3%)
	Hoima	2019	2/89 (2.2%)
	Kanungu	2020	1/94 (1.1%)
	Mubende	2017	1/46 (2.2%)
I540T	Kanungu	2017	1/48 (2.1%)
		2018	2/38 (5,3%)
		2020	2/94 (2.1%)
V555A	Kanungu	2022	5/51 (9.8%)
Y558C	Kaabong	2021	1/34 (2.9%)
	Kasese	2020	2/90 (2.2%)
		2021	3/53 (5.7%)
		2022	2/85 (2.4%)
	Mubende	2020	1/88 (1.1%)
A569V	Mubende	2022	8/53 (15.1%)
R575K	Kanungu	2021	1/43 (2.3%)
	Katakwi	2022	1/79 (1.3%)
A578S	Agago	2017	3/44 (6.8%)
		2019	5/56 (8.9%)
		2020	1/80 (1.2%)
		2022	2/68 (2.9%)
	Amolatar	2017	3/49 (6.1%)
		2021	1/50 (2.0%)

Table S4 (continued)

Locus	District	Year	Mutation prevalence
A578S (continued)	Arua	2017	1/45 (2.2%)
		2022	3/79 (3.8)
	Hoima	2020	5/92 (5.4%)
	Jinja	2016	2/34 (5.9%)
		2017	2/48 (4.2%)
		2022	1/99 (1.0%)
	Kaabong	2018	1/33 (3.0%)
		2020	2/75 (2.7%)
		2021	2/58 (3.4%)
	Kasese	2019	1/69 (1.4%)
		2020	2/93 (2.2%)
		2022	7/93 (7.5%)
	Katakwi	2018	1/45 (2.2%)
		2019	2/65 (3.1%)
		2020	2/88 (2.3%)
		2021	1/57 (1.8%)
	Koboko	2018	1/34 (2.9%)
	Kole	2019	1/65 (1.5%)
		2020	1/86 (1.2%)
		2022	1/77 (1.3%)
	Lamwo	2020	2/85 (2.4%)
	Mubende	2017	1/46 (2.2%)
		2020	1/88 (1.1%)
	Tororo	2016	1/37 (2.7%)
K607E	Agago	2019	1/54 (1.9%)
	Kanungu	2019	2/69 (2.9%)
		2020	3/93 (3.2%)
V637I	Hoima	2019	4/90 (4.4%)
	Katakwi	2022	3/78 (3.8%)
	Tororo	2020	1/41 (2.4%)
V666I	Arua	2016	1/30 (3.3%)
P667L	Mubende	2021	1/17 (5.9%)
P667S	Kasese	2022	3/92 (3.3%)
A675T	Tororo	2022	1/57 (1.8%)
F699C	Kasese	2022	5/88 (5.7%)
L713F	Kanungu	2021	2/37 (5.4%)
		2022	6/58 (10.3%)
	Mubende	2021	1/17 (5.9%)
		2022	3/52 (5.8%)
G718S	Rukiga	2022	1/56 (1.8%)
A724P	Kanungu	2016	1/33 (3.0%)
		2022	2/58 (3.4%)
	Kasese	2021	4/63 (6.3%)
		2022	7/88 (8.0%)

*All identified propeller domain (codons 442-724) mutations excluding codons 441(L), 469, 561, and 675, which are described in Table S3, are shown. Data for loci or districts with no mutants in any year are not shown.

Table S5. Prevalence of PfK13 mutations in codons upstream of propeller domain, 2016-22.

Variant	Codon	Site	Year	N	N WT	N Mut	% WT	% Mut
Lys9Arg	9	Jinja	2016	24	23	1	95.8	4.2
Glu23Gly	23	Kole	2016	17	16	1	94.1	5.9
Gly26Asp	26	Agago	2021	38	37	1	97.4	2.6
Phe45Tyr	45	Agago	2016	21	20	1	95.2	4.8
		Jinja	2016	25	24	1	96	4
Lys108Glu	108	Agago	2021	32	30	2	93.8	6.2
			2022	46	45	1	97.8	2.2
		Kaabong	2021	28	27	1	96.4	3.6
		Koboko	2022	29	28	1	96.6	3.4
		Lamwo	2016	23	22	1	95.7	4.3
			2021	34	33	1	97.1	2.9
			2022	36	34	2	94.4	5.6
		Rukiga	2022	45	44	1	97.8	2.2
Asp109Val	109	Mubende	2021	12	10	2	83.3	16.7
Gly112Glu	112	Katakwi	2022	40	39	1	97.5	2.5
		Lamwo	2022	36	35	1	97.2	2.8
		Rukiga	2022	45	44	1	97.8	2.2
		Tororo	2022	26	24	2	92.3	7.7
Leu116Ile	116	Kanungu	2016	23	22	1	95.7	4.3
Lys118Gln	118	Jinja	2022	91	90	1	98.9	1.1
Asn133Asp	133	Agago	2022	43	42	1	97.7	2.3
Ile134Thr	134	Kasese	2021	37	36	1	97.3	2.7
		Kole	2021	33	32	1	97	3
Asn135Tyr	135	Agago	2022	43	42	1	97.7	2.3
His136_Asn137del	136	Kole	2022	59	58	1	98.3	1.7
His136Asn	136	Agago	2016	18	16	2	88.9	11.1
		Jinja	2022	86	85	1	98.8	1.2
		Koboko	2022	27	25	2	92.6	7.4
Asn141_Asn142del	141	Agago	2021	31	30	1	96.8	3.2
		Arua	2022	43	40	3	93	7
		Hoima	2021	36	32	4	88.9	11.1
		Kaabong	2021	29	28	1	96.6	3.4
		Kasese	2022	69	65	4	94.2	5.8
Asn141_Asn142dup	141	Agago	2016	16	15	1	93.8	6.2
			2021	31	28	3	90.3	9.7
			2022	44	43	1	97.7	2.3
		Amolatar	2016	8	6	2	75	25
		Arua	2021	51	48	3	94.1	5.9
		Hoima	2021	36	31	5	86.1	13.9
			2022	23	20	3	87	13
		Jinja	2022	91	63	28	69.2	30.8

Table S5. (continued)

Variant	Codon	Site	Year	N	N WT	N Mut	% WT	% Mut
Asn141_Asn142dup (continued)	141	Kaabong	2021	29	25	4	86.2	13.8
			2022	40	38	2	95	5
		Kanungu	2022	32	24	8	75	25
		Kapchorwa	2021	28	27	1	96.4	3.6
		Kasese	2022	68	65	3	95.6	4.4
		Katakwi	2021	31	30	1	96.8	3.2
			2022	35	33	2	94.3	5.7
		Koboko	2022	26	24	2	92.3	7.7
		Kole	2016	12	11	1	91.7	8.3
			2021	33	32	1	97	3
			2022	58	55	3	94.8	5.2
		Lamwo	2021	30	29	1	96.7	3.3
			2022	28	24	4	85.7	14.3
		Mubende	2021	13	11	2	84.6	15.4
			2022	49	37	12	75.5	24.5
		Rukiga	2016	34	33	1	97.1	2.9
			2021	48	41	7	85.4	14.6
			2022	43	36	7	83.7	16.3
		Tororo	2016	23	21	2	91.3	8.7
			2021	57	54	3	94.7	5.3
Asn142del	142	Agago	2016	16	15	1	93.8	6.2
			2022	43	40	3	93	7
		Arua	2016	26	25	1	96.2	3.8
			2021	51	49	2	96.1	3.9
			2022	40	37	3	92.5	7.5
		Hoima	2022	23	22	1	95.7	4.3
		Jinja	2021	54	48	6	88.9	11.1
			2022	86	72	14	83.7	16.3
		Kaabong	2022	39	35	4	89.7	10.3
		Kanungu	2022	31	29	2	93.5	6.5
		Kapchorwa	2022	7	6	1	85.7	14.3
		Kasese	2022	68	57	11	83.8	16.2
		Koboko	2022	25	23	2	92	8
		Kole	2022	58	45	13	77.6	22.4
		Lamwo	2016	22	21	1	95.5	4.5
			2021	30	27	3	90	10
		Mubende	2016	21	20	1	95.2	4.8
			2022	41	33	8	80.5	19.5
		Rukiga	2016	34	31	3	91.2	8.8
			2022	40	39	1	97.5	2.5
		Tororo	2021	57	55	2	96.5	3.5
			2022	24	23	1	95.8	4.2

Table S5. (continued)

Variant	Codon	Site	Year	N	N WT	N Mut	% WT	% Mut
Asn142dup	142	Agago	2016	16	15	1	93.8	6.2
			2021	30	29	1	96.7	3.3
			2022	43	41	2	95.3	4.7
		Arua	2016	26	22	4	84.6	15.4
			2021	51	50	1	98	2
		Hoima	2021	10	7	3	70	30
		Jinja	2016	12	11	1	91.7	8.3
			2021	52	49	3	94.2	5.8
		Kaabong	2021	24	22	2	91.7	8.3
			2022	41	38	3	92.7	7.3
		Kanungu	2016	23	21	2	91.3	8.7
		Kasese	2022	69	66	3	95.7	4.3
		Koboko	2021	18	17	1	94.4	5.6
			2022	26	24	2	92.3	7.7
		Kole	2016	13	11	2	84.6	15.4
			2021	33	32	1	97	3
			2022	60	52	8	86.7	13.3
		Lamwo	2016	22	21	1	95.5	4.5
			2021	30	29	1	96.7	3.3
			2022	34	28	6	82.4	17.6
		Mubende	2016	21	20	1	95.2	4.8
			2022	42	30	12	71.4	28.6
		Rukiga	2022	42	40	2	95.2	4.8
		Tororo	2016	22	21	1	95.5	4.5
Leu143_Thr149delinsAsnAsnLeu ThrAlaAsnAsnIleSer	143	Kole	2022	58	57	1	98.3	1.7
Leu143delinsAsnAsnVal	143	Agago	2021	30	27	3	90	10
			2022	45	39	6	86.7	13.3
		Arua	2022	42	40	2	95.2	4.8
		Hoima	2022	28	23	5	82.1	17.9
		Jinja	2022	86	85	1	98.8	1.2
		Kaabong	2021	24	23	1	95.8	4.2
			2022	42	36	6	85.7	14.3
		Kapchorwa	2022	9	7	2	77.8	22.2
		Kasese	2022	69	67	2	97.1	2.9
		Katakwi	2021	31	30	1	96.8	3.2
			2022	40	34	6	85	15
		Koboko	2021	18	17	1	94.4	5.6
			2022	26	21	5	80.8	19.2
		Kole	2021	33	32	1	97	3
			2022	61	58	3	95.1	4.9

Table S5. (continued)

Variant	Codon	Site	Year	N	N WT	N Mut	% WT	% Mut
Leu143delinsAsnAsnVal (continued)	143	Lamwo	2016	23	21	2	91.3	8.7
			2021	30	27	3	90	10
			2022	30	26	4	86.7	13.3
		Tororo	2021	55	54	1	98.2	1.8
			2022	26	22	4	84.6	15.4
Leu143delinsAsnVal	143	Arua	2022	41	40	1	97.6	2.4
Thr144Ser	144	Agago	2022	43	42	1	97.7	2.3
Thr149Ser	149	Arua	2021	51	50	1	98	2
		Kasese	2022	68	66	2	97.1	2.9
		Katakwi	2022	35	34	1	97.1	2.9
		Kole	2016	13	12	1	92.3	7.7
			2021	33	32	1	97	3
		Lamwo	2021	30	29	1	96.7	3.3
		Tororo	2016	23	21	2	91.3	8.7
Leu172Phe	172	Jinja	2022	93	92	1	98.9	1.1
Ala175Ser	175	Arua	2016	28	27	1	96.4	3.6
Ser182Thr	182	Kaabong	2021	34	33	1	97.1	2.9
			2022	44	43	1	97.7	2.3
		Kanungu	2016	27	26	1	96.3	3.7
		Kole	2022	64	63	1	98.4	1.6
		Lamwo	2022	35	34	1	97.1	2.9
		Mubende	2016	24	23	1	95.8	4.2
		Rukiga	2021	56	55	1	98.2	1.8
		Tororo	2016	27	26	1	96.3	3.7
			2021	58	57	1	98.3	1.7
Leu187Phe	187	Lamwo	2016	29	28	1	96.6	3.4
Lys189Asn	189	Agago	2016	19	18	1	94.7	5.3
			2021	32	31	1	96.9	3.1
			2022	50	45	5	90	10
		Arua	2022	50	48	2	96	4
		Hoima	2022	31	18	13	58.1	41.9
		Jinja	2021	44	42	2	95.5	4.5
		Kanungu	2016	27	26	1	96.3	3.7
			2022	29	27	2	93.1	6.9
		Kasese	2021	45	43	2	95.6	4.4
			2022	73	72	1	98.6	1.4
		Katakwi	2022	39	38	1	97.4	2.6
		Koboko	2021	21	18	3	85.7	14.3
		Kole	2016	15	14	1	93.3	6.7
			2022	64	62	2	96.9	3.1
		Lamwo	2016	29	28	1	96.6	3.4
			2022	35	33	2	94.3	5.7

Table S5. (continued)

Variant	Codon	Site	Year	N	N WT	N Mut	% WT	% Mut
Lys189Asn (continued)	189	Mubende	2016	24	22	2	91.7	8.3
			2022	50	43	7	86	14
		Rukiga	2016	38	37	1	97.4	2.6
			2022	46	45	1	97.8	2.2
		Tororo	2021	59	58	1	98.3	1.7
			2022	26	24	2	92.3	7.7
Lys189Thr	189	Agago	2016	19	13	6	68.4	31.6
			2021	32	20	12	62.5	37.5
			2022	50	40	10	80	20
		Amolatar	2021	19	17	2	89.5	10.5
		Arua	2016	28	8	20	28.6	71.4
			2021	53	30	23	56.6	43.4
			2022	50	28	22	56	44
		Hoima	2021	34	20	14	58.8	41.2
			2022	31	20	11	64.5	35.5
		Jinja	2016	14	9	5	64.3	35.7
			2021	60	41	19	68.3	31.7
			2022	93	17	76	18.3	81.7
		Kaabong	2021	36	24	12	66.7	33.3
			2022	44	25	19	56.8	43.2
		Kanungu	2016	27	17	10	63	37
			2021	9	7	2	77.8	22.2
			2022	29	22	7	75.9	24.1
		Kapchorwa	2021	19	7	12	36.8	63.2
		Kasese	2021	45	36	9	80	20
			2022	73	39	34	53.4	46.6
		Katakwi	2021	39	31	8	79.5	20.5
			2022	39	27	12	69.2	30.8
		Koboko	2021	21	9	12	42.9	57.1
			2022	28	16	12	57.1	42.9
		Kole	2016	15	3	12	20	80
			2021	34	20	14	58.8	41.2
			2022	64	36	28	56.2	43.8
		Lamwo	2016	29	19	10	65.5	34.5
			2021	34	23	11	67.6	32.4
			2022	35	18	17	51.4	48.6
		Mubende	2016	24	13	11	54.2	45.8
			2021	13	8	5	61.5	38.5
			2022	50	33	17	66	34
		Rukiga	2016	38	31	7	81.6	18.4
			2021	56	44	12	78.6	21.4
			2022	46	30	16	65.2	34.8

Table S5. (continued)

Variant	Codon	Site	Year	N	N WT	N Mut	% WT	% Mut
Lys189Thr (continued)	189	Tororo	2016	27	16	11	59.3	40.7
			2021	57	42	15	73.7	26.3
			2022	26	20	6	76.9	23.1
Ile205Thr	205	Kanungu	2022	29	27	2	93.1	6.9
		Rukiga	2021	54	53	1	98.1	1.9
			2022	46	44	2	95.7	4.3
Glu208Lys	208	Rukiga	2021	69	67	2	97.1	2.9
Asn217His	217	Agago	2022	62	61	1	98.4	1.6
		Hoima	2022	52	47	5	90.4	9.6
		Jinja	2021	84	83	1	98.8	1.2
		Katakwi	2022	64	63	1	98.4	1.6
		Kole	2022	74	72	2	97.3	2.7
		Mubende	2021	15	14	1	93.3	6.7
		Tororo	2022	40	39	1	97.5	2.5
Arg255Lys	255	Agago	2016	28	24	4	85.7	14.3
			2021	53	51	2	96.2	3.8
			2022	65	61	4	93.8	6.2
		Arua	2016	29	28	1	96.6	3.4
			2021	70	63	7	90	10
			2022	77	66	11	85.7	14.3
		Hoima	2021	84	82	2	97.6	2.4
		Jinja	2022	99	98	1	99	1
		Kaabong	2021	59	53	6	89.8	10.2
			2022	64	61	3	95.3	4.7
		Kanungu	2016	35	34	1	97.1	2.9
		Kapchorwa	2021	77	75	2	97.4	2.6
		Kasese	2021	73	71	2	97.3	2.7
			2022	93	85	8	91.4	8.6
		Katakwi	2021	54	51	3	94.4	5.6
			2022	76	72	4	94.7	5.3
		Koboko	2022	45	40	5	88.9	11.1
		Kole	2021	57	55	2	96.5	3.5
			2022	76	70	6	92.1	7.9
		Lamwo	2016	33	27	6	81.8	18.2
			2021	54	51	3	94.4	5.6
			2022	48	46	2	95.8	4.2
		Rukiga	2021	82	80	2	97.6	2.4
			2022	61	60	1	98.4	1.6
		Tororo	2016	30	28	2	93.3	6.7
			2021	72	71	1	98.6	1.4
			2022	52	48	4	92.3	7.7
Leu258Met	258	Agago	2022	65	62	3	95.4	4.6

Table S5. (continued)

Variant	Codon	Site	Year	N	N WT	N Mut	% WT	% Mut
Leu258Met (continued)	258	Amolatar	2016	27	20	7	74.1	25.9
		Arua	2016	29	28	1	96.6	3.4
		Jinja	2016	23	21	2	91.3	8.7
		Kaabong	2021	59	58	1	98.3	1.7
		Kanungu	2016	35	32	3	91.4	8.6
			2022	64	61	3	95.3	4.7
		Kapchorwa	2021	77	76	1	98.7	1.3
		Kasese	2021	69	67	2	97.1	2.9
			2022	93	85	8	91.4	8.6
		Katakwi	2021	54	53	1	98.1	1.9
		Koboko	2022	45	44	1	97.8	2.2
		Kole	2021	56	54	2	96.4	3.6
			2022	76	75	1	98.7	1.3
		Mubende	2016	27	26	1	96.3	3.7
		Tororo	2021	72	69	3	95.8	4.2
Glu270Lys	270	Kasese	2022	92	89	3	96.7	3.3
Gln271His	271	Arua	2021	62	61	1	98.4	1.6
		Kole	2016	18	17	1	94.4	5.6
Lys332Arg	332	Kole	2022	68	67	1	98.5	1.5
Asp343His	343	Rukiga	2022	55	54	1	98.2	1.8
Thr348Ile	348	Hoima	2021	59	54	5	91.5	8.5
		Kaabong	2022	54	51	3	94.4	5.6
		Kanungu	2022	53	42	11	79.2	20.8
		Kapchorwa	2021	37	36	1	97.3	2.7
		Kasese	2021	57	56	1	98.2	1.8
			2022	82	80	2	97.6	2.4
		Lamwo	2021	46	45	1	97.8	2.2
			2022	45	40	5	88.9	11.1
		Mubende	2021	14	12	2	85.7	14.3
			2022	50	44	6	88	12
		Rukiga	2021	68	54	14	79.4	20.6
			2022	55	44	11	80	20
		Tororo	2021	63	59	4	93.7	6.3
			2022	38	37	1	97.4	2.6
Pro419Ser	419	Jinja	2022	99	46	53	46.5	53.5
		Kanungu	2021	51	50	1	98	2
		Kasese	2021	80	78	2	97.5	2.5
			2022	93	91	2	97.8	2.2
		Lamwo	2022	56	55	1	98.2	1.8
		Rukiga	2021	84	83	1	98.8	1.2
Leu428Phe	428	Mubende	2021	18	16	2	88.9	11.1

Table S5. (continued)

Variant	Codon	Site	Year	N	N WT	N Mut	% WT	% Mut
Glu433Asp	433	Agago	2021	48	47	1	97.9	2.1
		Arua	2022	76	72	4	94.7	5.3
		Kasese	2022	91	87	4	95.6	4.4
		Lamwo	2021	54	53	1	98.1	1.9
		Tororo	2022	51	50	1	98	2

Table S6. Individual sample microsatellite haplotypes. The table includes haplotypes for samples described in Fig 3A. "NA" indicates missing data. Microsatellite alleles comprising the most prevalent haplotype for each PfK13 mutation are highlighted in colors specific for the PfK13 genotype. When the same allele appeared in haplotypes for different PfK13 mutations, the allele was highlighted in color appropriate for that mutation. When these alleles appeared in WT samples, alleles were highlighed in the color assigned to 469F or 469Y.

Sample ID	Site	Year	Microsatellite Locus							PfK13 Genotype
			neg31.9	neg6.36	neg3.7	neg0.15	pos8.6	pos15.1	pos72.3	
KS-06-22	Kasese	2021	203	279	142	194	280	138	232	441L
KS-06-61	Kasese	2021	203	288	142	194	282	138	240	441L
KS-06-77	Kasese	2021	203	288	142	194	282	138	240	441L
KB-05-03	Rukiga	2020	213	276	156	204	272	141	244	469F
KB-05-20	Rukiga	2020	213	276	156	202	NA	141	244	469F
KB-05-37	Rukiga	2020	213	276	156	202	NA	141	244	469F
KB-05-38	Rukiga	2020	213	276	156	204	NA	141	244	469F
KB-05-39	Rukiga	2020	213	276	156	192	272	141	244	469F
KB-05-41	Rukiga	2020	213	276	156	204	272	141	244	469F
KB-05-42	Rukiga	2020	213	276	156	204	272	141	244	469F
KB-05-44	Rukiga	2020	213	276	156	204	NA	141	244	469F
KB-06-02	Rukiga	2021	215	276	156	204	272	141	244	469F
KB-06-19	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-20	Rukiga	2021	213	276	156	NA	272	141	244	469F
KB-06-22	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-29	Rukiga	2021	213	276	156	204	272	141	227	469F
KB-06-36	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-37	Rukiga	2021	213	276	156	204	272	141	227	469F
KB-06-46	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-64	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-68	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-79	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-80	Rukiga	2021	213	276	156	204	270	141	244	469F
KB-06-81	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-87	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-89	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-95	Rukiga	2021	213	276	156	204	272	141	244	469F
KB-06-96	Rukiga	2021	213	276	156	204	272	141	227	469F
KN-06-15	Kanungu	2021	207	267	151	188	294	141	234	469F
KN-06-72	Kanungu	2021	213	276	156	204	272	141	244	469F
AG-02-18	Agago	2017	227	273	156	194	NA	138	236	469Y
AG-02-29	Agago	2017	227	273	151	194	280	NA	236	469Y
AG-03-13	Agago	2018	227	NA	151	194	280	138	236	469Y
AG-05-13	Agago	2020	227	273	151	194	280	138	236	469Y
AG-05-44	Agago	2020	227	273	151	194	280	138	236	469Y
AG-05-59	Agago	2020	227	273	151	194	280	138	240	469Y
AG-05-69	Agago	2020	227	273	151	194	280	NA	240	469Y

Table S6 (continued)

Sample ID	Site	Year	Microsatellite Locus							PfK13 Genotype
			neg31.9	neg6.36	neg3.7	neg0.15	pos8.6	pos15.1	pos72.3	
AG-05-81	Agago	2020	227	273	NA	194	280	138	236	469Y
AG-06-40	Agago	2021	227	273	151	194	NA	138	240	469Y
AG-06-48	Agago	2021	209	273	151	194	280	NA	236	469Y
AR-06-85	Arua	2021	227	273	151	194	280	138	236	469Y
HO-05-37	Hoima	2020	227	273	151	194	280	138	236	469Y
HO-05-38	Hoima	2020	227	273	151	194	280	138	236	469Y
HO-05-43	Hoima	2020	227	273	151	194	280	138	236	469Y
HO-05-49	Hoima	2020	227	273	151	194	280	138	236	469Y
HO-05-50	Hoima	2020	227	273	151	194	280	138	236	469Y
KBG-05-27	Kaabong	2020	227	273	151	194	280	138	236	469Y
KBG-05-37	Kaabong	2020	227	273	NA	194	280	138	249	469Y
KBG-05-89	Kaabong	2020	227	273	NA	194	280	138	249	469Y
KBG-06-08	Kaabong	2021	227	273	151	194	280	138	236	469Y
KBG-06-25	Kaabong	2021	227	273	151	194	280	138	249	469Y
KBG-06-70	Kaabong	2021	227	273	151	NA	280	138	230	469Y
KBG-06-96	Kaabong	2021	227	273	151	194	280	138	236	469Y
KBK-06-90	Koboko	2021	227	273	151	194	280	138	249	469Y
KO-05-24	Kole	2020	227	273	151	194	280	138	232	469Y
KTK-03-48	Katakwi	2018	227	NA	151	194	280	138	236	469Y
KTK-05-28	Katakwi	2020	227	276	151	194	280	132	242	469Y
KTK-05-97	Katakwi	2020	227	273	151	194	280	138	242	469Y
KTK-06-18	Katakwi	2021	199	273	170	NA	278	138	240	469Y
KTK-06-69	Katakwi	2021	227	273	170	194	280	138	249	469Y
LA-02-04	Lamwo	2017	227	NA	151	194	280	138	236	469Y
LA-03-08	Lamwo	2018	196	279	151	194	280	138	238	469Y
LA-03-13	Lamwo	2018	205	NA	151	194	280	138	236	469Y
LA-05-07	Lamwo	2020	227	273	151	194	280	138	236	469Y
LA-05-24	Lamwo	2020	227	273	151	194	280	141	234	469Y
LA-05-40	Lamwo	2020	227	273	151	194	280	138	236	469Y
LA-05-54	Lamwo	2020	227	273	151	194	280	138	236	469Y
LA-05-55	Lamwo	2020	227	273	151	194	280	138	240	469Y
LA-05-62	Lamwo	2020	205	273	151	194	280	138	236	469Y
LA-05-74	Lamwo	2020	NA	273	151	194	280	138	236	469Y
LA-05-75	Lamwo	2020	203	273	151	192	280	138	246	469Y
LA-05-81	Lamwo	2020	227	273	151	NA	280	138	236	469Y
LA-05-94	Lamwo	2020	227	273	151	192	280	138	236	469Y
LA-06-03	Lamwo	2021	207	276	156	208	276	150	249	469Y
LA-06-84	Lamwo	2021	205	273	170	194	280	138	249	469Y
TO-06-20	Tororo	2021	227	273	151	192	280	138	236	469Y
TO-06-48	Tororo	2021	227	273	NA	194	280	138	249	469Y
KB-06-04	Rukiga	2021	209	276	149	192	261	144	234	561H

Table S6 (continued)

Sample ID	Site	Year	Microsatellite Locus							PfK13 Genotype
			neg31.9	neg6.36	neg3.7	neg0.15	pos8.6	pos15.1	pos72.3	
KB-06-09	Rukiga	2021	205	276	162	194	276	138	223	561H
KB-06-18	Rukiga	2021	205	276	162	194	276	138	225	561H
KB-06-27	Rukiga	2021	205	276	162	194	274	138	223	561H
KB-06-78	Rukiga	2021	205	276	162	194	276	138	223	561H
AG-02-12	Agago	2017	219	276	149	204	272	138	246	675V
AG-03-40	Agago	2018	219	NA	149	204	272	138	246	675V
AG-04-12	Agago	2019	219	NA	149	204	272	138	236	675V
AG-04-40	Agago	2019	219	276	149	204	272	138	246	675V
AG-05-15	Agago	2020	219	276	149	204	272	138	244	675V
AG-05-16	Agago	2020	219	276	149	204	274	138	242	675V
AG-05-47	Agago	2020	227	270	146	198	272	138	252	675V
AG-05-48	Agago	2020	219	276	149	204	272	138	246	675V
AG-05-75	Agago	2020	219	276	149	204	NA	138	240	675V
AG-06-06	Agago	2021	219	276	149	204	272	138	246	675V
AG-06-33	Agago	2021	NA	276	149	204	272	138	246	675V
AM-06-12	Amolatar	2021	221	276	149	204	272	138	246	675V
HO-05-05	Hoima	2020	219	276	149	204	272	138	246	675V
HO-05-09	Hoima	2020	219	276	149	204	272	138	246	675V
HO-05-10	Hoima	2020	219	276	149	204	272	138	246	675V
KAP-06-18	Kapchorwa	2021	219	276	149	204	272	138	246	675V
KAP-06-23	Kapchorwa	2021	221	276	149	204	272	138	234	675V
KB-05-35	Rukiga	2020	203	276	154	200	284	138	225	675V
KB-06-74	Rukiga	2021	201	285	151	192	266	138	234	675V
KB-06-99	Rukiga	2021	201	285	151	208	264	138	234	675V
KBG-04-27	Kaabong	2019	219	NA	149	204	272	138	234	675V
KBG-04-46	Kaabong	2019	219	276	149	204	272	138	246	675V
KBG-05-30	Kaabong	2020	207	276	149	NA	272	138	234	675V
KBG-05-68	Kaabong	2020	207	276	149	204	272	138	244	675V
KBG-05-80	Kaabong	2020	219	276	149	204	272	138	246	675V
KBG-05-92	Kaabong	2020	219	276	149	204	272	138	246	675V
KBG-06-18	Kaabong	2021	NA	276	149	204	273	138	246	675V
KBG-06-19	Kaabong	2021	219	276	149	204	NA	138	246	675V
KBG-06-28	Kaabong	2021	219	276	149	204	272	138	249	675V
KBG-06-33	Kaabong	2021	219	276	149	204	272	138	246	675V
KBG-06-60	Kaabong	2021	209	276	149	204	272	138	246	675V
KBG-06-72	Kaabong	2021	221	276	149	204	272	138	246	675V
KBK-05-36	Koboko	2020	219	276	149	204	272	138	246	675V
KO-05-08	Kole	2020	219	276	NA	204	272	138	249	675V
KO-05-29	Kole	2020	219	276	149	204	272	138	246	675V
KO-05-31	Kole	2020	219	276	149	204	272	138	246	675V
KO-05-42	Kole	2020	219	276	149	204	272	138	NA	675V

Table S6 (continued)

Sample ID	Site	Year	Microsatellite Locus							PfK13 Genotype
			neg31.9	neg6.36	neg3.7	neg0.15	pos8.6	pos15.1	pos72.3	
KTK-04-18	Katakwi	2019	219	276	149	204	272	138	246	675V
KTK-04-27	Katakwi	2019	219	NA	149	204	272	138	252	675V
KTK-04-34	Katakwi	2019	219	276	149	204	272	138	246	675V
KTK-05-66	Katakwi	2020	219	276	149	204	272	138	246	675V
KTK-05-77	Katakwi	2020	219	276	149	204	272	138	246	675V
KTK-06-46	Katakwi	2021	219	276	149	NA	272	138	249	675V
KTK-06-70	Katakwi	2021	219	276	NA	204	272	138	249	675V
KTK-06-73	Katakwi	2021	219	276	149	204	272	138	236	675V
LA-02-24	Lamwo	2017	219	276	149	204	272	138	246	675V
LA-02-43	Lamwo	2017	219	276	149	204	272	138	246	675V
LA-04-43	Lamwo	2019	219	276	149	204	272	138	246	675V
LA-05-25	Lamwo	2020	219	276	149	204	272	138	234	675V
LA-05-30	Lamwo	2020	219	276	149	204	272	138	246	675V
LA-05-39	Lamwo	2020	219	276	149	NA	272	138	246	675V
LA-05-70	Lamwo	2020	219	276	149	204	272	138	240	675V
LA-05-79	Lamwo	2020	219	276	149	NA	272	138	246	675V
LA-05-82	Lamwo	2020	219	276	149	204	NA	138	246	675V
LA-05-86	Lamwo	2020	219	276	149	NA	272	138	242	675V
LA-05-99	Lamwo	2020	219	276	149	204	272	138	230	675V
LA-06-55	Lamwo	2021	219	276	149	NA	272	138	249	675V
LA-06-77	Lamwo	2021	219	276	NA	204	272	138	249	675V
MU-05-04	Mubende	2020	201	288	156	190	266	138	230	675V
MU-05-77	Mubende	2020	203	276	154	200	NA	138	254	675V
MU-05-83	Mubende	2020	203	276	154	200	284	138	227	675V
TO-06-55	Tororo	2021	211	276	149	204	272	138	246	675V
TO-06-93	Tororo	2021	219	276	149	204	272	138	246	675V
AG-06-34	Agago	2021	221	279	151	204	272	141	249	675V
KBG-06-07	Kaabong	2021	219	276	149	188	272	NA	246	675V
KBG-06-93	Kaabong	2021	201	273	158	204	284	141	242	558C
MU-06-86	Mubende	2021	211	273	158	194	260	135	240	578S
KS-06-16	Kasese	2021	209	273	154	202	286	141	246	724P
KS-06-42	Kasese	2021	209	273	154	202	286	141	246	724P
KS-06-43	Kasese	2021	209	273	154	202	286	141	246	724P
KS-06-51	Kasese	2021	209	273	154	202	286	141	246	724P
AG-02-47	Agago	2017	211	276	148	194	267	141	234	WT
AG-04-05	Agago	2019	211	276	142	204	NA	141	240	WT
AG-04-11	Agago	2019	207	267	154	194	NA	138	232	WT
AG-04-13	Agago	2019	203	NA	164	202	272	138	244	WT
AG-04-39	Agago	2019	205	276	158	208	274	138	240	WT
AG-05-01	Agago	2020	205	273	160	202	276	144	232	WT
AG-05-02	Agago	2020	211	276	151	216	256	138	234	WT

Table S6 (continued)

Sample ID	Site	Year	Microsatellite Locus							PfK13 Genotype
			neg31.9	neg6.36	neg3.7	neg0.15	pos8.6	pos15.1	pos72.3	
AG-05-06	Agago	2020	211	273	158	202	289	141	242	WT
AG-05-22	Agago	2020	205	270	158	198	270	144	234	WT
AG-05-24	Agago	2020	219	276	149	204	272	138	252	WT
AG-05-36	Agago	2020	207	279	149	212	278	138	236	WT
AG-05-41	Agago	2020	203	273	162	194	276	138	230	WT
AG-05-49	Agago	2020	205	NA	158	206	284	138	236	WT
AG-05-56	Agago	2020	211	267	154	194	289	141	230	WT
AG-05-57	Agago	2020	203	282	148	196	264	138	238	WT
AG-05-62	Agago	2020	201	285	151	194	264	135	234	WT
AG-05-63	Agago	2020	201	273	151	194	264	132	242	WT
AG-05-66	Agago	2020	213	279	151	204	278	141	230	WT
AG-05-76	Agago	2020	207	267	154	194	261	144	249	WT
AG-05-89	Agago	2020	199	267	156	194	264	141	227	WT
AG-06-15	Agago	2021	213	276	158	198	274	141	240	WT
AG-06-30	Agago	2021	205	276	149	200	259	138	248	WT
AG-06-46	Agago	2021	201	273	148	194	267	141	246	WT
AG-06-47	Agago	2021	203	270	149	194	276	138	238	WT
AG-06-60	Agago	2021	203	288	170	194	264	138	236	WT
AG-06-87	Agago	2021	209	279	149	188	280	138	234	WT
AM-05-02	Amolatar	2020	205	276	146	216	286	150	238	WT
AR-05-12	Arua	2020	205	279	172	216	267	138	249	WT
AR-05-43	Arua	2020	211	NA	156	186	261	138	230	WT
AR-05-57	Arua	2020	207	262	162	208	280	138	246	WT
AR-05-66	Arua	2020	233	276	148	194	276	138	238	WT
AR-05-72	Arua	2020	211	273	151	194	282	138	240	WT
HO-05-06	Hoima	2020	215	276	154	192	276	125	254	WT
HO-05-08	Hoima	2020	203	273	NA	202	274	138	249	WT
JI-05-07	Jinja	2020	213	285	166	202	289	138	259	WT
JI-05-12	Jinja	2020	201	270	151	194	272	138	238	WT
JI-05-30	Jinja	2020	199	276	166	194	286	141	230	WT
JI-05-36	Jinja	2020	199	276	166	194	286	141	230	WT
JI-05-51	Jinja	2020	199	276	166	194	286	141	230	WT
JI-05-57	Jinja	2020	199	276	166	194	286	141	230	WT
JI-05-74	Jinja	2020	207	267	160	214	282	138	236	WT
KAP-06-07	Kapchorwa	2021	203	276	154	204	266	138	242	WT
KAP-06-62	Kapchorwa	2021	203	267	142	194	294	144	234	WT
KB-05-01	Rukiga	2020	211	267	156	194	282	138	236	WT
KB-05-02	Rukiga	2020	201	273	158	202	270	141	248	WT
KB-05-04	Rukiga	2020	221	276	154	194	264	141	230	WT
KB-05-05	Rukiga	2020	207	276	148	194	270	138	227	WT
KB-05-09	Rukiga	2020	201	273	158	202	270	141	248	WT

Table S6 (continued)

Sample ID	Site	Year	Microsatellite Locus							PfK13 Genotype
			neg31.9	neg6.36	neg3.7	neg0.15	pos8.6	pos15.1	pos72.3	
KB-05-11	Rukiga	2020	219	270	149	204	274	141	244	WT
KB-05-12	Rukiga	2020	217	270	149	204	NA	141	244	WT
KB-05-13	Rukiga	2020	213	267	158	194	289	141	240	WT
KB-05-16	Rukiga	2020	199	276	149	216	270	138	249	WT
KB-05-30	Rukiga	2020	207	276	154	194	NA	138	242	WT
KB-05-40	Rukiga	2020	201	273	156	202	270	141	248	WT
KB-06-05	Rukiga	2021	203	276	170	194	286	138	230	WT
KB-06-06	Rukiga	2021	205	282	164	NA	272	144	248	WT
KB-06-11	Rukiga	2021	203	276	170	194	286	138	230	WT
KB-06-13	Rukiga	2021	203	276	170	NA	286	138	230	WT
KB-06-14	Rukiga	2021	203	276	170	194	286	138	230	WT
KB-06-24	Rukiga	2021	203	276	170	194	286	138	230	WT
KB-06-31	Rukiga	2021	203	276	170	NA	286	138	230	WT
KB-06-33	Rukiga	2021	201	276	149	190	272	141	254	WT
KB-06-53	Rukiga	2021	199	279	154	198	276	138	249	WT
KB-06-56	Rukiga	2021	213	276	148	208	260	138	242	WT
KB-06-63	Rukiga	2021	225	273	148	194	276	138	234	WT
KB-06-73	Rukiga	2021	209	267	156	194	264	141	242	WT
KB-06-98	Rukiga	2021	209	288	NA	194	260	138	230	WT
KBG-03-03	Kaabong	2018	223	NA	158	194	282	141	236	WT
KBG-03-25	Kaabong	2018	205	NA	160	194	286	138	240	WT
KBG-04-03	Kaabong	2019	225	270	158	194	292	138	249	WT
KBG-04-35	Kaabong	2019	205	NA	158	206	274	138	242	WT
KBG-04-40	Kaabong	2019	211	273	158	194	284	141	242	WT
KBG-04-49	Kaabong	2019	209	279	158	194	267	141	238	WT
KBG-05-13	Kaabong	2020	207	279	158	194	267	141	246	WT
KBG-05-56	Kaabong	2020	215	NA	158	202	270	141	234	WT
KBG-05-57	Kaabong	2020	199	NA	158	206	273	138	238	WT
KBG-05-66	Kaabong	2020	209	273	154	194	267	138	254	WT
KBG-05-73	Kaabong	2020	209	273	148	194	260	138	236	WT
KBG-05-84	Kaabong	2020	205	276	151	204	267	138	236	WT
KBG-06-22	Kaabong	2021	203	276	148	194	267	144	230	WT
KBG-06-23	Kaabong	2021	203	276	151	198	272	147	234	WT
KBG-06-59	Kaabong	2021	NA	276	148	194	274	138	236	WT
KBG-06-73	Kaabong	2021	205	273	158	194	282	141	249	WT
KBG-06-76	Kaabong	2021	211	279	154	194	280	138	230	WT
KBG-06-79	Kaabong	2021	203	276	142	194	260	138	252	WT
KBG-06-88	Kaabong	2021	203	282	148	202	272	135	249	WT
KN-05-11	Kanungu	2020	199	276	148	202	276	138	234	WT
KN-05-87	Kanungu	2020	201	270	154	204	260	138	236	607E
KN-06-36	Kanungu	2021	211	276	162	194	276	138	242	WT

Table S6 (continued)

Sample ID	Site	Year	Microsatellite Locus							PfK13 Genotype
			neg31.9	neg6.36	neg3.7	neg0.15	pos8.6	pos15.1	pos72.3	
KO-04-30	Kole	2019	205	276	148	194	276	138	234	WT
KO-05-15	Kole	2020	211	273	149	194	280	141	249	WT
KO-05-70	Kole	2020	209	276	149	192	272	141	246	WT
KO-05-95	Kole	2020	205	273	NA	198	267	138	249	WT
KO-06-57	Kole	2021	205	276	174	198	292	150	232	WT
KS-05-01	Kasese	2020	205	276	162	194	276	138	254	WT
KS-05-07	Kasese	2020	207	276	151	194	266	138	246	WT
KS-05-29	Kasese	2020	217	267	154	194	NA	144	230	WT
KS-05-38	Kasese	2020	211	267	154	194	282	138	234	WT
KS-05-64	Kasese	2020	211	267	154	194	278	138	236	WT
KS-06-55	Kasese	2021	217	276	160	198	264	138	254	WT
KS-06-57	Kasese	2021	203	273	149	198	272	138	230	WT
KS-06-85	Kasese	2021	199	288	149	194	280	138	256	WT
KTK-03-30	Katakwi	2018	207	NA	149	216	282	141	240	WT
KTK-03-49	Katakwi	2018	207	NA	154	194	291	138	242	WT
KTK-04-02	Katakwi	2019	209	156	156	204	272	141	238	WT
KTK-04-26	Katakwi	2019	192	NA	149	194	267	138	242	WT
KTK-05-01	Katakwi	2020	205	276	NA	208	272	141	249	WT
KTK-05-24	Katakwi	2020	219	267	149	194	280	141	246	WT
KTK-05-45	Katakwi	2020	201	273	149	211	274	147	248	WT
KTK-05-67	Katakwi	2020	215	270	148	202	286	138	254	WT
KTK-05-98	Katakwi	2020	207	276	154	206	272	141	234	WT
KTK-06-37	Katakwi	2021	209	273	149	196	289	141	254	WT
KTK-06-53	Katakwi	2021	199	273	170	NA	276	138	249	WT
KTK-06-91	Katakwi	2021	201	285	170	194	291	138	240	WT
LA-02-02	Lamwo	2017	203	273	156	204	264	138	236	WT
LA-02-32	Lamwo	2017	213	276	156	194	276	138	244	WT
LA-02-47	Lamwo	2017	211	285	170	190	280	144	236	WT
LA-03-06	Lamwo	2018	192	NA	149	194	267	138	232	WT
LA-04-21	Lamwo	2019	205	270	156	194	272	135	252	WT
LA-04-25	Lamwo	2019	201	273	162	194	282	138	236	WT
LA-04-32	Lamwo	2019	211	NA	149	206	286	138	244	WT
LA-04-45	Lamwo	2019	211	NA	158	204	294	138	246	WT
LA-05-03	Lamwo	2020	203	276	158	190	270	138	232	WT
LA-05-08	Lamwo	2020	203	267	160	194	264	138	234	WT
LA-05-10	Lamwo	2020	199	273	154	208	274	144	238	WT
LA-05-13	Lamwo	2020	201	270	149	198	276	141	249	WT
LA-05-17	Lamwo	2020	196	279	156	202	294	138	234	WT
LA-05-23	Lamwo	2020	223	273	156	198	272	138	254	WT
LA-05-35	Lamwo	2020	211	276	160	211	NA	138	230	WT
LA-05-36	Lamwo	2020	217	276	158	198	264	141	242	WT

Table S6 (continued)

Sample ID	Site	Year	Microsatellite Locus							PfK13 Genotype
			neg31.9	neg6.36	neg3.7	neg0.15	pos8.6	pos15.1	pos72.3	
LA-05-52	Lamwo	2020	223	276	156	204	272	141	242	WT
LA-05-57	Lamwo	2020	203	273	149	202	284	141	244	WT
LA-05-59	Lamwo	2020	205	273	149	202	266	138	240	WT
LA-05-66	Lamwo	2020	215	279	156	194	266	144	234	WT
LA-05-89	Lamwo	2020	201	276	151	194	269	141	232	WT
LA-06-15	Lamwo	2021	217	276	158	NA	266	141	217	WT
LA-06-24	Lamwo	2021	201	273	162	198	266	138	238	WT
LA-06-44	Lamwo	2021	217	282	NA	194	274	132	249	WT
LA-06-58	Lamwo	2021	205	276	151	212	280	132	242	WT
LA-06-95	Lamwo	2021	209	279	170	194	270	132	236	WT
MU-05-09	Mubende	2020	203	276	149	NA	267	141	230	WT
MU-05-20	Mubende	2020	205	276	162	194	276	138	223	WT
MU-05-44	Mubende	2020	201	276	NA	194	286	138	249	WT
MU-05-56	Mubende	2020	205	273	149	194	294	138	244	WT
MU-06-56	Mubende	2021	205	267	156	194	264	141	249	WT
TO-05-07	Tororo	2020	203	285	149	194	282	138	242	WT
TO-05-14	Tororo	2020	203	NA	148	194	274	147	244	WT
TO-05-28	Tororo	2020	207	156	156	204	272	141	240	WT
TO-05-43	Tororo	2020	209	270	160	194	276	138	227	WT
TO-06-26	Tororo	2021	211	282	149	194	282	144	246	WT
TO-06-34	Tororo	2021	196	267	154	194	282	138	240	WT
TO-06-37	Tororo	2021	211	282	149	200	282	144	246	WT
TO-06-92	Tororo	2021	205	279	151	NA	276	138	240	WT

Table S7. Prevalence of key PfK13 mutations stratified by epidemiological categories

Year	Sites with resurgence following withdrawal of IRS[a]	Sites with sustained IRS			Sites with no history of IRS		
	Prevalence[b]	Prevalence[b]	ΔP[c] (95% CI)	p	Prevalence[b]	ΔP[c] (95% CI)	p
2016	6/75 (8.0%)	0/45 (0.0%)	8.0% (1.8-14.1%)	0.05	1/148 (0.7%)	7.3% (.01-13.6%)	0.003
2017	14/135 (10.4%)	3/96 (3.1%)	7.3% (1.0-13.5%)	0.04	2/185 (1.1%)	9.3% (3.9-14.6%)	<0.001
2018	14/132 (10.6%)	0/33 (0%)	10.6% (5.4-15.9%)	0.05	9/236 (3.8%)	6.8% (0.1-12.6%)	0.01
2019	35/188 (18.6%)	0/93 (0%)	18.6% (13.1-24.2%)	<0.001	14/397 (3.5%)	15.1% (9.2-20.9%)	<0.001
2020	120/333 (36.0%)	0/125 (0%)	36.0% (30.9-41.2%)	<0.001	76/704 (10.8%)	25.2% (19.6-30.9%)	<0.001
2021	48/158 (30.4%)	7/112 (6.3%)	24.1% (15.7-32.6%)	<0.001	76/487 (15.6%)	14.8% (6.9-22.6%)	<0.001
2022	72/225 (32.0%)	9/49 (18.4%)	13.6% (1.2-26.1%)	0.06	111/536 (20.7%)	11.3% (4.3-18.3%)	0.001

[a] Reference group

[b] Presence of C469Y, A675V, C469F, R561H, or P441L mutation (only samples with results for all 5 mutations included)

[c] ΔP = absolute difference in prevalence compared to reference group

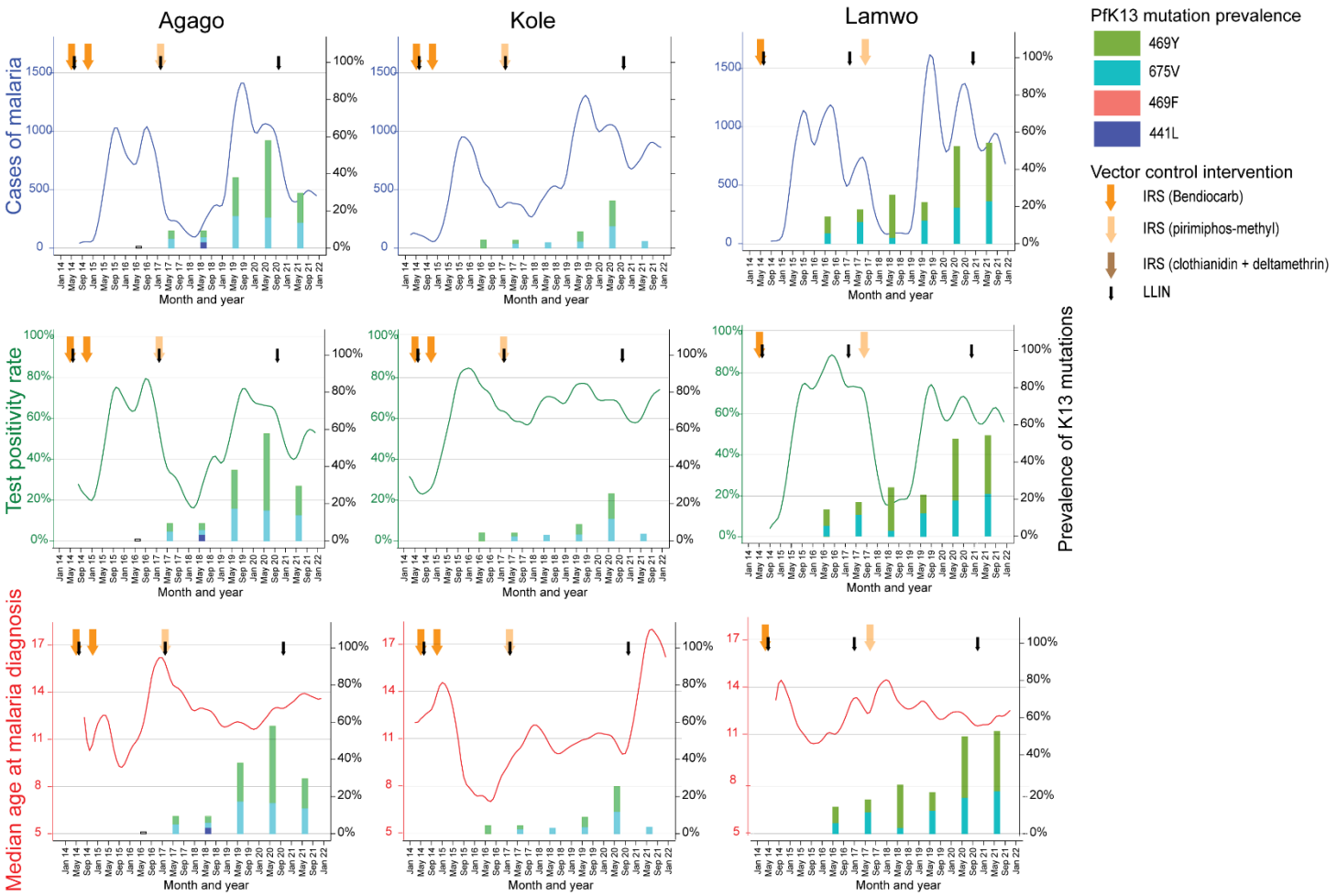

Figure S1. Malaria metrics and prevalence of PfK13 mutations at sites that received IRS in 2010-2014 and 2017. Malaria incidence, test positivity rate, median age of patients at time of malaria and mutation prevalence are shown for sites that received IRS every 6 months from 2010-14 and once in 2017. The malaria metrics were assessed monthly and are displayed with lowess smoothing. Large arrows indicate times of IRS, as defined in the key. Small arrows indicate times of LLIN distributions. Histogram bars indicate prevalences of PfK13 mutations (right axis). Lines represent malaria metrics (left axis).

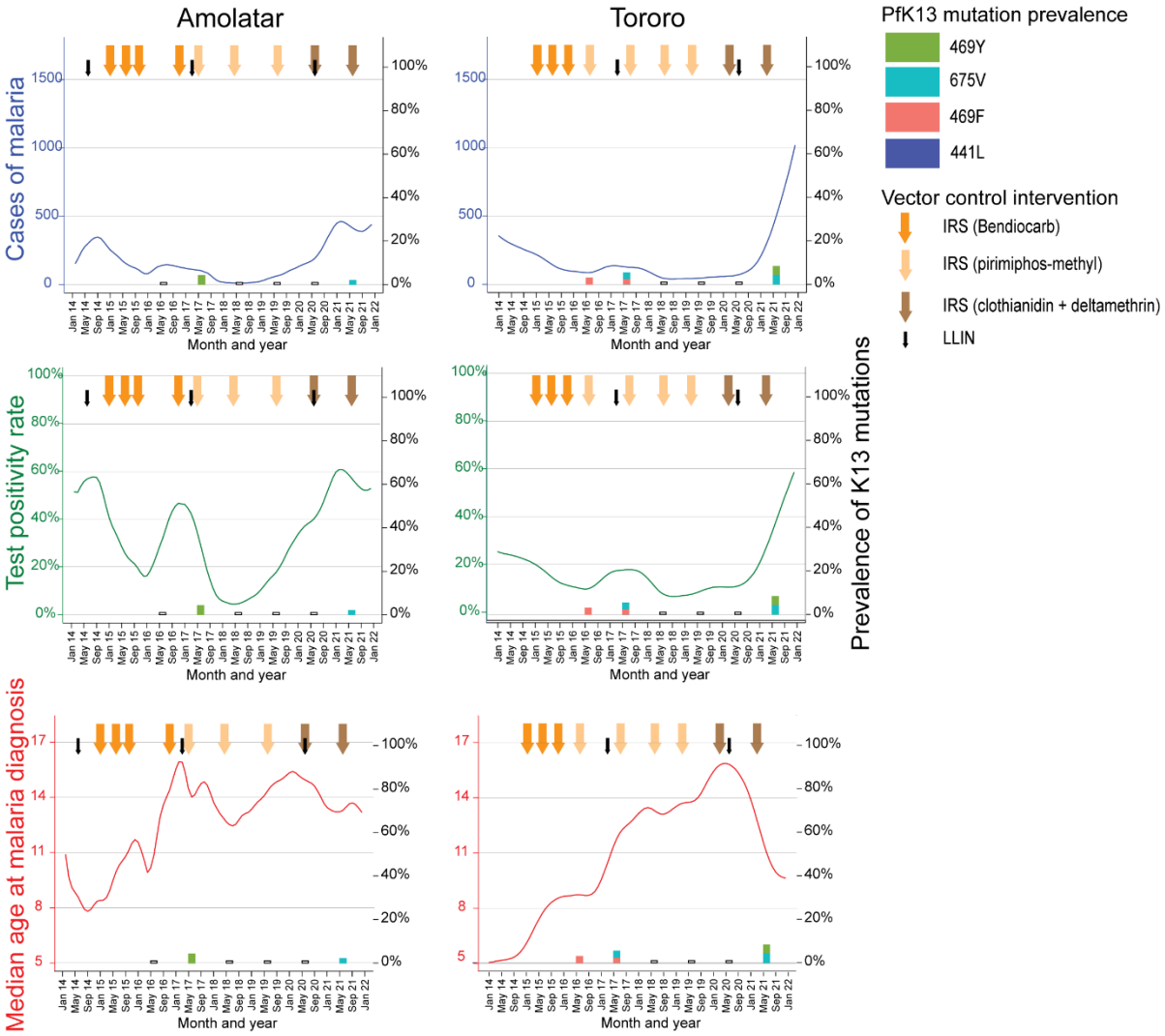

Figure S2. Malaria metrics and prevalence of PfK13 mutations at sites that received IRS in 2015-2022. Malaria incidence, test positivity rate, median age of patients at time of malaria and mutation prevalence are shown for sites that received IRS beginning in 2015. The malaria metrics were assessed monthly and are displayed with lowess smoothing. Large arrows indicate times of IRS, as defined in the key. Small arrows indicate times of LLIN distributions. Histogram bars indicate prevalences of PfK13 mutations (right axis). Lines represent malaria metrics (left axis).

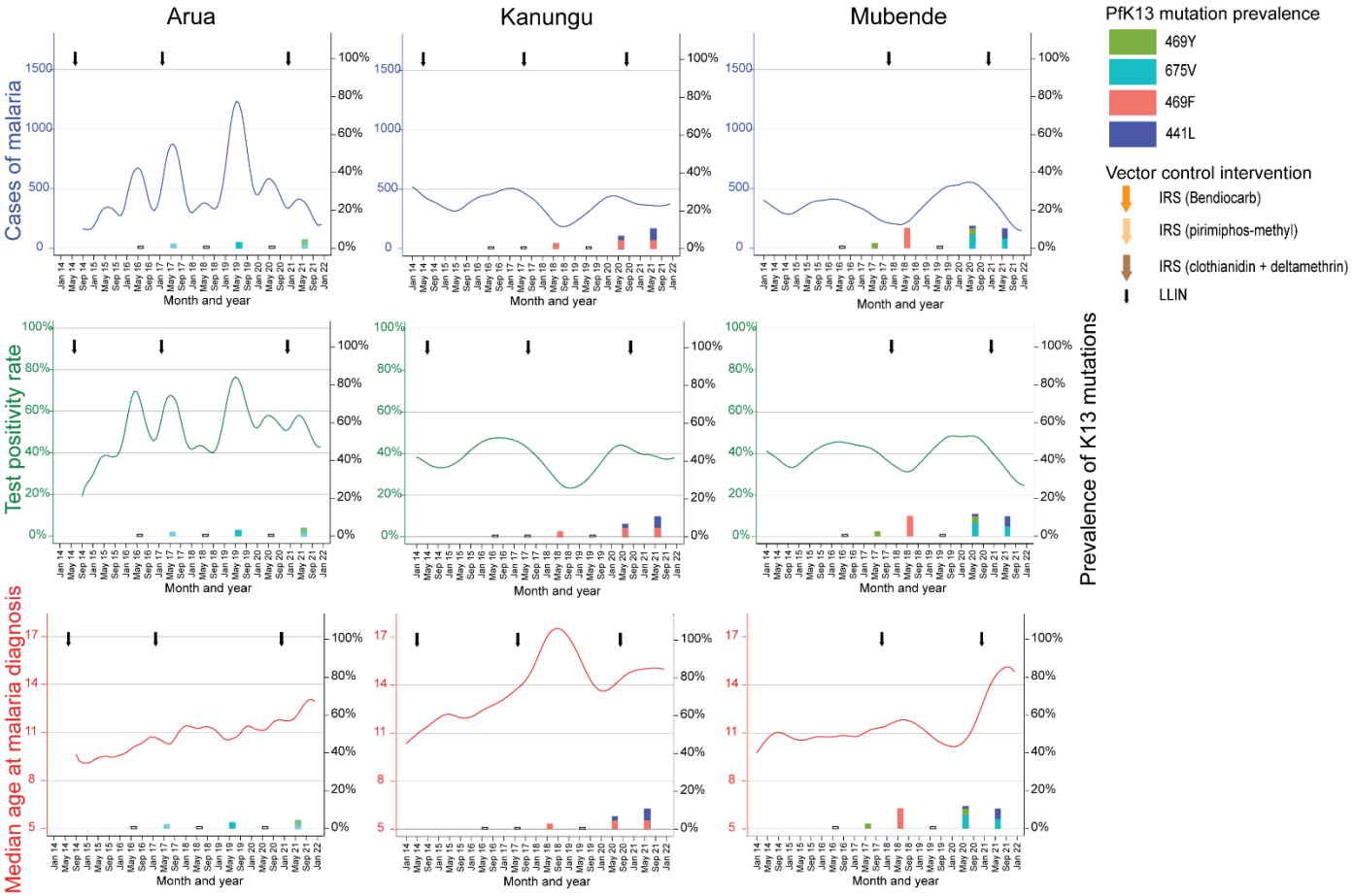

Figure S3. Malaria metrics and prevalence of PfK13 mutations at sites that have no history of IRS. Malaria incidence, test positivity rate, median age of patients at time of malaria and mutation prevalence are shown for sites that did not receive IRS. The malaria metrics were assessed monthly and are displayed with lowess smoothing. Large arrows indicate times of IRS, as defined in the key. Small arrows indicate times of LLIN distributions. Histogram bars indicate prevalences of PfK13 mutations (right axis). Lines represent malaria metrics (left axis).

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