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Kelch13 mutations in *Plasmodium falciparum* and risk of spreading in Amazon basin countries

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Background: The first potential focus for artemisinin resistance in South America was recently confirmed with the presence of the C580Y mutation in the *Plasmodium falciparum* kelch 13 gene (*pfk13*) in Guyana.

Objectives: This study aimed to strengthen *pfk13* monitoring in the Amazon basin countries, to compile the available data and to evaluate the risk of spreading of mutations.

Methods: Sanger sequencing was done on 862 samples collected between 1998 and 2019, and a global map of *pfk13* genotypes available for this region was constructed. Then, the risk of spreading of mutations based on *P. falciparum* case importation between 2015 and 2018 within countries of the Amazon basin was evaluated.

Results: No additional *pfk13* C580Y foci were identified. Few mutations (0.5%, 95% CI = 0.3%–0.8%) in the propeller domain were observed in the general parasite population of this region despite a high proportion of K189T mutations (49.1%, 95% CI = 46.2%–52.0%) in the non-propeller domain. Case information revealed two patterns of intense human migration: Venezuela, Guyana and the Roraima State in Brazil; and French Guiana, Suriname and the Amapá State in Brazil.

Conclusions: There are few *pfk13* mutant foci, but a high risk of dispersion in the Amazon basin, mainly from the Guiana Shield, proportionate to mining activities. Therefore, access to prompt diagnosis and treatment, and continuous molecular monitoring is essential in these geographical areas.

Introduction

Drug resistance of *Plasmodium falciparum* has become a major concern worldwide. To delay its propagation, artemisinin combination therapy (ACT) has been recommended since 2001. However, in 2008, resistance emerged in Cambodia and spread throughout south-east Asia, favoured by resistance to partner drugs, piperaquine and mefloquine.[1,2] Studies from this region led to the identification of the *P. falciparum* kelch 13 gene (*pfk13*) as a molecular marker of artemisinin partial resistance.[3] Currently, more than 100 mutations located in the propeller domain of *pfk13* have

been identified worldwide, but only 9 of them have been validated as responsible for partial artemisinin resistance.[4] Currently, one mutation has quickly spread and has overcome the others in the eastern part of the Greater Mekong subregion, *pfk13* C580Y.[5]

In the Amazon basin countries, where most of the malaria cases occur, the first-line treatment against *P. falciparum* is artemether/lumefantrine, except in Peru where artesunate/mefloquine is used.[6] However, 6 years after its deployment, *pfk13* C580Y mutant parasites were reported (5.1%, *n* = 5/98) in Guyana and have in time reached up to 25.0% prevalence in 2016–17, in Region 1.[7,8] The selection of resistance occurred in parasites of South American genetic background and this mutation confers artemisinin resistance *in vitro*. Without threatening ACT efficacy if the partner drug is still efficient, the presence of this mutation exposes the partner drug to a quicker selection for resistant parasites. These findings also raise concerns about the risk of spread throughout the Amazon basin countries. In fact, mining, one of the main economic activities in the Guiana Shield, favours *P. falciparum* transmission and generates cross-border human migration, which could promote spreading of resistance in the subregion. Additionally, malaria transmission has increased in several countries of this continent, including Venezuela, Brazil, Colombia, Ecuador and Guyana.[6]

As WHO recommends *pfk13* genotyping to monitor artemisinin resistance, this study aimed to strengthen it in the Amazon basin and to construct a global map of *pfk13* genotypes by reviewing actual data available in published literature. It also assessed the risk of spreading of mutations within Guyana and between the different countries and subregions based on *P. falciparum* case migration patterns.

Methods

P. falciparum samples (*n* = 862) were collected in French Guiana (*n* = 475, 1998–2018), Brazil (Amazonas State, Manaus, *n* = 64, 2012–13), Guyana (*n* = 199, 2014–19) and Venezuela (Amazonas and Bolivar States, *n* = 124, 2016–18) from symptomatic patients infected by *P. falciparum* from only those who sought diagnosis and care.

The samples were collected as part of the malaria routine surveillance system implemented in French Guiana, Guyana and Venezuela. Healthcare facilities were in charge of collecting samples from anonymized *P. falciparum*-positive cases. Identification of individuals cannot be established. In accordance with WHO guidelines on ethical issues in public health surveillance, the sample collections were exempt from an ethical review since these interventions were part of routine monitoring of public health by the National Malaria Control Program of the Ministry of Public Health of each country (https://www.who.int/publications/i/item/who-guidelines-on-ethical-issues-in-public-health-surveillance). The analysis of the samples was also approved by the Environmental Protection Agency in the framework of the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization. Samples were registered by the French Ministry for Research (declaration number DC-2010–1223). Regarding Brazilian samples, this study was approved by the Brazilian National Committee of Ethics (CONEP) (349.211/2013).

DNA extraction was performed from patient blood samples (filter paper or EDTA tube) using the QIAmp® DNA mini kit (Qiagen, Germany) and following the manufacturer's protocol. The *pfk13* gene was amplified using nested PCR methods with primers previously described.[3] Double-strand sequencing of PCR-amplified products was performed by Eurofins (Paris, France) with the same primers used in nested PCR. Sequences were aligned with Geneious® v8.1.7 using the 3D7 *pfk13* sequence as a reference.

The *pfk13* analysed sequences from this study are available in the GenBank repository (accession numbers MW037842–MW038738).

Some isolates were phenotyped using the ring-stage survival assay as previously described.[9] The ring-stage survival assay was interpretable if the initial parasitaemia was greater than 0.25% and if the growth rate was greater than 2-fold per 48 h. Statistical significance between survival rates was calculated using Student's *t*-test.

We performed a literature review on the PubMed database to identify studies reporting *pfk13* genotyping in countries of the Amazon basin. Different searches with word combinations were used, including 'k13', 'kelch 13', '*Plasmodium falciparum*', 'artemisinin resistance', 'South America', 'Ecuador', 'Colombia', 'Peru', 'Bolivia', 'Brazil', 'Guyana', 'French Guiana', 'Suriname' and 'Venezuela'. When necessary, additional information (data collection, location etc.) were requested from the corresponding authors to properly localize the samples. A total of 3150 *pfk13* genotyping results were found in 16 publications and were used to construct a general distribution map of *pfk13* mutations in the Amazon basin (Table S1, available as Supplementary data at *JAC* Online).[7,8,10–23]

Cumulative numbers of *P. falciparum* mono or mixed infections from 2015 to 2018 reported by member states of PAHO/WHO were used to construct the malaria case migration maps. Countries report on the aggregate number of confirmed cases by species type, imported from other countries during a calendar year. Data were not available from Peru for 2015–17, Colombia for 2017, Ecuador for 2017 and 2018, and French Guiana for 2018. Data for cases imported from country A to country B were summed with those being imported from country B to country A, i.e. bidirectional movement of cases was calculated (Table S2). Aggregate data on the country from which cases were imported, reported at state level from Brazil, were sourced from a public database for the years 2015–18.[24] Data here correspond to unidirectional movement of cases from other countries to Brazilian states. To construct the map of migration of *P. falciparum* cases within Guyana, information from the Malaria Surveillance System of Guyana Ministry of Public Health was used for the years 2015–18. Data on place of diagnosis of confirmed cases were cross-tabulated with the place the person was 2 weeks before the date of diagnosis, used as a proxy for the putative place of infection. Data were aggregated to bidirectional movement between a set of localities, i.e. both those that were imported from locality A to locality B, as well as those that were putatively infected in locality B and were diagnosed in locality A.

Results

Limited genetic diversity of the *pfk13* gene in the Amazon basin countries

No mutation (non-synonymous or synonymous) was identified in the *pfk13* propeller domain (codons 441–704) of the 862 newly analysed samples (Table 1).

A map representing the distribution of *pfk13* mutations in the Amazon basin was constructed using the genotyping of these 862 samples and 3150 from previously published data (Figure 1 and Table S1). Regarding the *pfk13* propeller domain, a general mutant proportion of 0.5% (95% CI = 0.3%–0.8%) was observed in the Amazon basin countries. The mutation C580Y (TGT→T**A**T) was observed in 2010 and 2016–17 in Guyana (1.6%, 95% CI = 0.9%–2.3%), the mutation A481V (GCT→G**T**T) was observed in 2013 in Brazil (0.1%, 95% CI = 0.0%–0.3%) and the mutation A504D (GCT→G**A**T) was observed in 2018 in Colombia (0.1%, 95% CI = 0.0%–0.4%).

Among the 1171 *pfk13* non-propeller genotypes available from this study (*n* = 648; Table 1) and the literature (*n* = 523), a mutant proportion of 49.3% (95% CI = 46.4%–52.1%) was found in this

Table 1. *pfk13* polymorphism of parasites from the Amazon basin

	French Guiana 1998–2018	Brazil 2012–13	Guyana 2014–19	Venezuela 2016–18	Total
Total number of analysed isolates for non-propeller domain codons 50–440	274	63	199	112	648
WT	185	0	79	25	289
K189T	87	63	118	87	355
K/T 189[a]	0	0	2	0	2
K189N	1	0	0	0	1
R/K 255[a]	1	0	0	0	1
number of mutants (%)	88 (32.1)[a]	63 (100.0)	120 (60.3)[a]	87 (77.7)	359 (55.4)[a]
Total number of analysed isolates for propeller domain codons 441–704	475	64	199	124	862
WT	475	64	199	124	862

[a]Mixed genotype, included for the calculation of mutants.

domain (codons 50–440; Table S1). K189T (AAA→A**C**A) was the most prevalent. This non-synonymous mutation has been fixed in the Amazonas State of Brazil since 2012 (Figure 1 and Table S1). Its minimal proportion was observed in the Amapá and Pará States of Brazil (22.2%, 95% CI = 3.0%–41.4%). No difference in the survival rates of parasites carrying ($n = 24$) or not carrying ($n = 115$) this mutation has been observed; 0.90% ± 0.26% versus 0.71% ± 0.09% ($P = 0.43$) (Figure 2). The K189N (AAA→AA**T**) mutation was found in 0.4% (95% CI = 0.0%–1.1%) of samples ($n = 1$) from French Guiana and R255K (AGA→A**A**A) as a mixed genotype was also found, both without impact on the survival rate (0.25% ± 0.25% and 0.40% ± 0.4%, respectively).

Two major routes at risk of spreading *pfk13* mutations

To assess the risk of spreading mutant parasites, we chose as a proxy the number of *P. falciparum* mono and mixed infections imported between the different Amazon basin countries at the time of observation of *pfk13* C580Y in Guyana, i.e. the 2015–18 period.

Two important migration patterns of malaria cases in South America were identified, both linked to the Guiana Shield, the area recording the highest *P. falciparum* transmission in South America (Figure 3). The Guiana Shield encompasses Amazon forest areas of Guyana, Suriname, French Guiana and parts of Brazil, Colombia and Venezuela.

The major migration pattern includes Venezuela, Guyana and the Roraima State of Brazil. Over the 2015–18 period, 3417 *P. falciparum* cases were imported between Venezuela and Guyana in a bidirectional manner (54.9%, 95% CI = 53.2%–56.6%, imported from Guyana to Venezuela; Table S2). Near these hotspots for malaria transmission, the Roraima State of Brazil recorded 3305 cases imported from Venezuela overwhelmingly in miners (2477; i.e. 75.0%, 95% CI = 73.5%–76.4%) (Table 2 and Figure 3). These movements were mainly unidirectional, from Venezuela to Brazil (97.4%, 95% CI = 96.9%–97.9%; Table S2). Moreover, the migration pattern into Brazil has been changing from being mostly in Brazilian miners diagnosed in Boa Vista municipality, which is far from the international border, in 2015 (59.6%, 95% CI = 54.7%–64.6%; $n = 226/$ 379 cases imported in that year in Brazil) to increasing in Venezuelan nationals working as miners being diagnosed in

Pacaraima municipality right on the border with Venezuela in 2018 (44.4%, 95% CI = 41.9%–47.0%; $n = 660/1485$). Between Guyana and Brazil, 934 *P. falciparum* malaria cases were exchanged, 83.1% (95% CI = 80.7%–85.5%) being imported to Brazil (Table S2), mainly to the Roraima State (82.0%, 95% CI = 79.6%–84.5%; Table 2). These cases mainly occurred in Brazilian miners returning to Boa Vista municipality in Roraima (506 *P. falciparum* malaria cases).

Within Guyana, intense movement of people exists. In fact, 40.8% of the *P. falciparum* cases were diagnosed in a region different from where the patient was possibly infected (Table S3). Additionally, 31.0% of the cases were diagnosed and associated with a putative place of infection within the same region, but in two different localities. These movements were mainly from the hinterlands of Regions 1, 7 and 8 to the capital Georgetown on the coast (Figure 4).

The second major migration pattern, but proportionally much less important, involved Suriname, French Guiana and the northern states of Brazil (Figure 3). *P. falciparum* malaria case movements within this hotspot are generally unidirectional with Brazil and Suriname reporting imported cases from French Guiana. Over the 2015–18 period, 398 cases from French Guiana were reported imported in Brazil, mainly in Amapá (57.0%, 95% CI = 52.2%–61.9%), Maranhão (30.2%, 95% CI = 25.6%–34.7%) and Pará (7.5%, 95% CI = 4.9%–10.1%) States (Table 2 and Figure 3) with 70.9% (95% CI = 66.4%–75.3%) being in Brazilian miners ($n = 282$ cases). The last two states are farther removed geographically from French Guiana, but miners from these states work in the Guiana Shield area. In Suriname, cases from French Guiana represent 95.0% (95% CI = 92.9%–97.1%) (Table S2) of the parasite movements between those countries.

In the west of the Amazon basin, 510 cases from Venezuela (Table S2) have been reported imported in Colombia during this period, most likely in Colombian states bordering Venezuela, and 743 cases from Peru to the Amazonas State of Brazil (Figure 3 and Table 2). These later were mostly limited to municipalities sharing a border with the Loreto State of Peru and related to economic activities like agriculture and fishing in adjoining areas (51.5%, 95% CI = 48.0%–55.1%; $n = 383$ cases). Data from Peru were only available for 1 of the 4 years analysed, making it difficult to ascertain the level of migration, but migration of malaria cases was in both directions, as indicated by the fact that over 32.6%
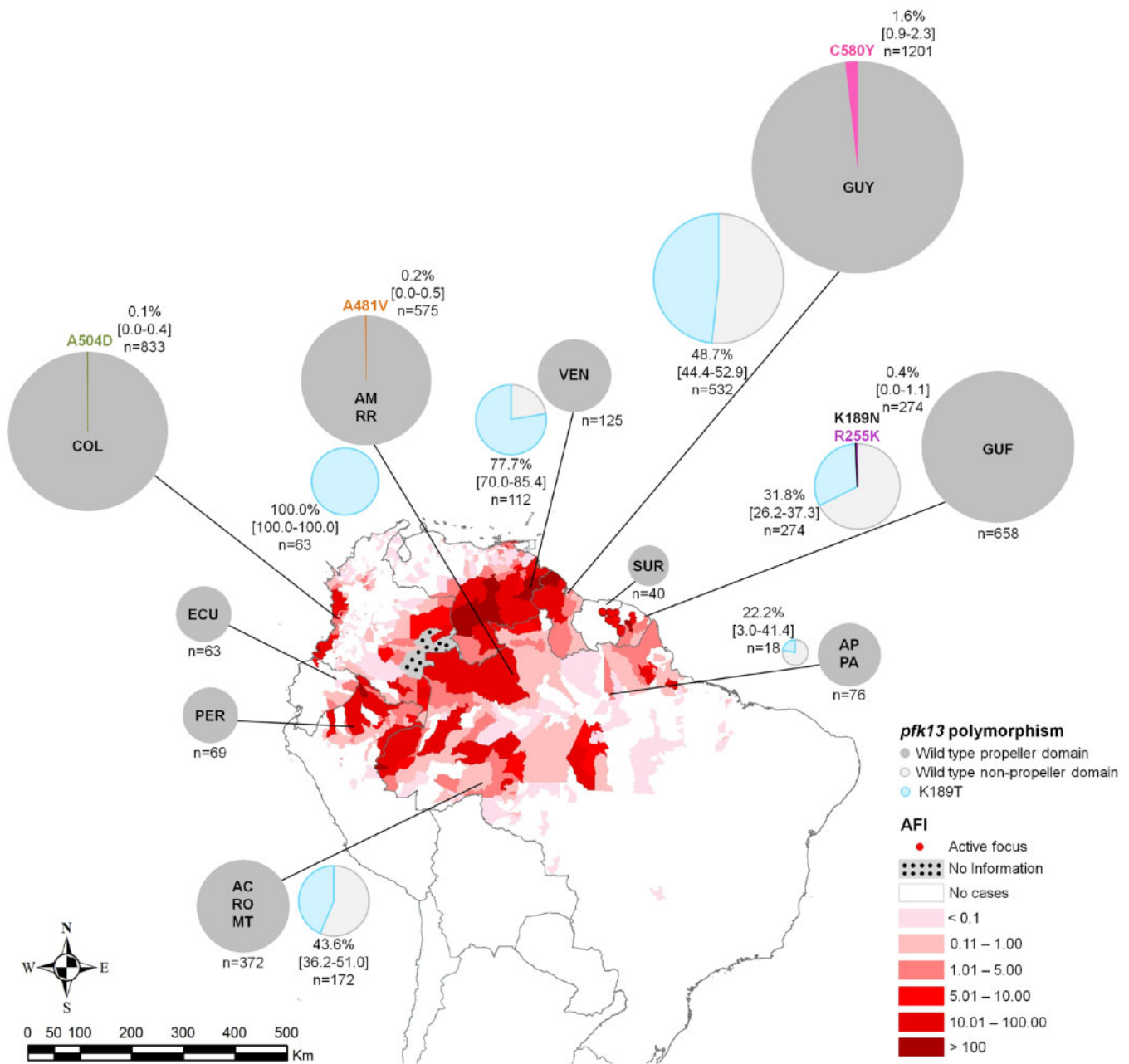
Figure 1. Distribution of *pfk13* mutations in the Amazon basin between 1983 and 2019. Pie charts represent *pfk13* allele frequencies per country. The total number of analysed samples is shown with the percentage and the 95% CI (in brackets) when a mutation was identified. Results are shown in the map of the Annual *Falciparum* Incidence (AFI) in the Americas region in 2017 (Annual Country Reports to PAHO/CDENT/Malaria). For Brazil, results are regrouped by closely located states. Notably, most Colombian and Ecuadorian samples (mutant included) represented here were collected on the Pacific coast (99.8% and 93.7%, respectively) and not in the Amazon region of the country. Brazilian states: AC, Acre; AM, Amazonas; AP, Amapá; MT, Mato Grosso; PA, Pará; RO, Rondônia; and RR, Roraima. Countries: COL, Colombia; ECU, Ecuador; GUF, French Guiana; GUY, Guyana; PER, Peru; SUR, Suriname; and VEN, Venezuela.

(95% CI = 29.2%–35.9%) (*n* = 242/743) of the infections imported in Brazil were in Brazilian nationals who had travelled to Peruvian territory. Of note, no cases were reported imported in Brazil from Ecuador within the same period.

In the same period, eight *P. falciparum* cases were reported imported in the Amazon basin countries from countries in south and south-east Asia. Six cases in Brazil were imported from the

Philippines (*n* = 2), India (*n* = 2), Malaysia (*n* = 1) and Indonesia (*n* = 1), and two cases in Ecuador were imported from India.

Discussion

The selection of parasites bearing the *pfk13* C580Y mutation and resistant *in vitro* to artemisinin derivatives in Guyana has recently
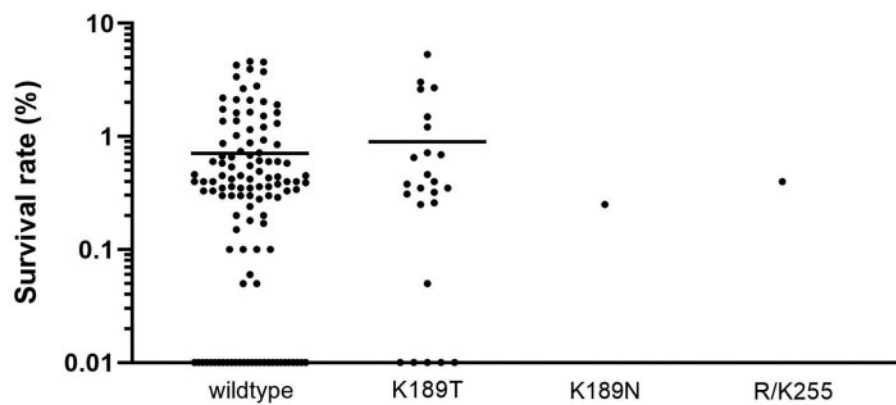
Figure 2. Ring-stage survival assays for parasites from French Guiana. The survival rate of ring-stage parasites *pfk13* WT (*n* = 115), *pfk13* K189T (*n* = 24), *pfk13* K189N (*n* = 1) and *pfk13* R/K255 (mixed genotype, *n* = 1) after a 6 h pulse of 700 nM dihydroartemisinin as measured by microscopy 66 h later. Horizontal bars represent mean±SEM percentage survival.
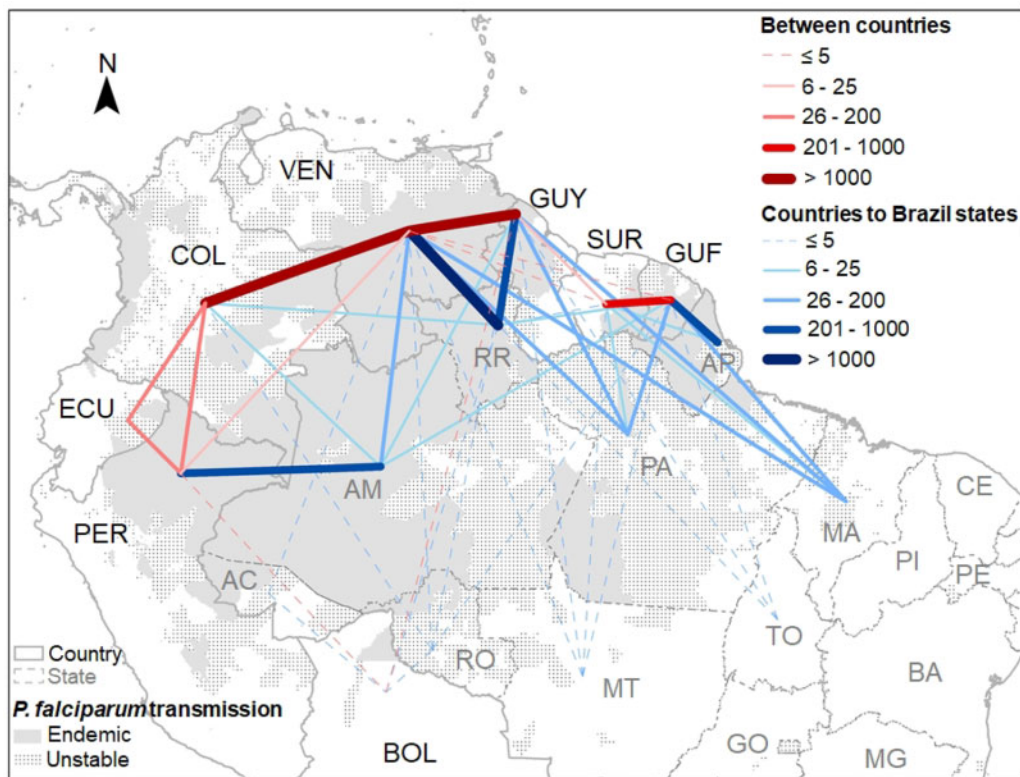

Figure 3. Map of *P. falciparum* infections imported between countries in the Amazon basin, 2015–18. Number of *P. falciparum* mono and mixed infections imported by country/state of diagnosis and putative country of infection. Each country/state is represented by a standardized location not linked with the putative contamination place. Lines in the map represent the cumulative numbers of infections recorded during the 2015–18 period. Lines between countries include bidirectional data, while lines from countries to Brazilian states are unidirectional (imported in Brazil). The width and colour of the lines are proportional to the number of imported cases. The grey *P. falciparum* transmission layer is based on the Annual *Falciparum* Incidence (AFI) reported during the 2015–18 period. Unstable corresponds to AFI <1 for ≥1 year and endemic (stable) corresponds to AFI >1 for all years. Countries: BOL, Bolivia; COL, Colombia; ECU, Ecuador; GUF, French Guiana; GUY, Guyana; PER, Peru; SUR, Suriname; and VEN, Venezuela. Brazilian states: AC, Acre; AM, Amazonas; AP, Amapá; BA, Bahia; CE, Ceará; GO, Goiás; MA, Maranhão; MG, Minas Gerais; MT, Mato Grosso; PA, Pará; PI, Piauí; PE, Pernambuco; RO, Rondônia; RR, Roraima; and TO, Tocantins.

been confirmed.[8] This situation raised serious concerns about the risk of mutant parasites spreading or their presence in different unknown foci in the Amazon basin. No propeller mutation was observed in the analysed samples. A mutant parasite, A481V, was previously reported in Manaus/Brazil. This mutation was identified as being associated with delayed parasite clearance in south-east

Table 2. Number of *P. falciparum* and mixed infections imported in Brazil (per state of diagnosis) by country of origin of infection, 2015–18

State	Country							
	Bolivia	Colombia	Guyana	French Guiana	Peru	Suriname	Venezuela	Total
Acre	3	0	0	0	0	0	1	4
Amazonas	0	15	13	9	743	0	94	874
Amapá	0	0	0	227	0	6	0	233
Maranhão	0	0	82	120	0	13	100	315
Mato Grosso	0	0	2	2	0	1	2	7
Pará	0	0	37	30	0	7	57	131
Rondônia	3	1	2	3	0	0	1	10
Roraima	1	6	628	6	0	3	3305	3949
Tocantins	0	0	2	1	0	0	1	4
Total	7	22	766	398	743	30	3561	5527
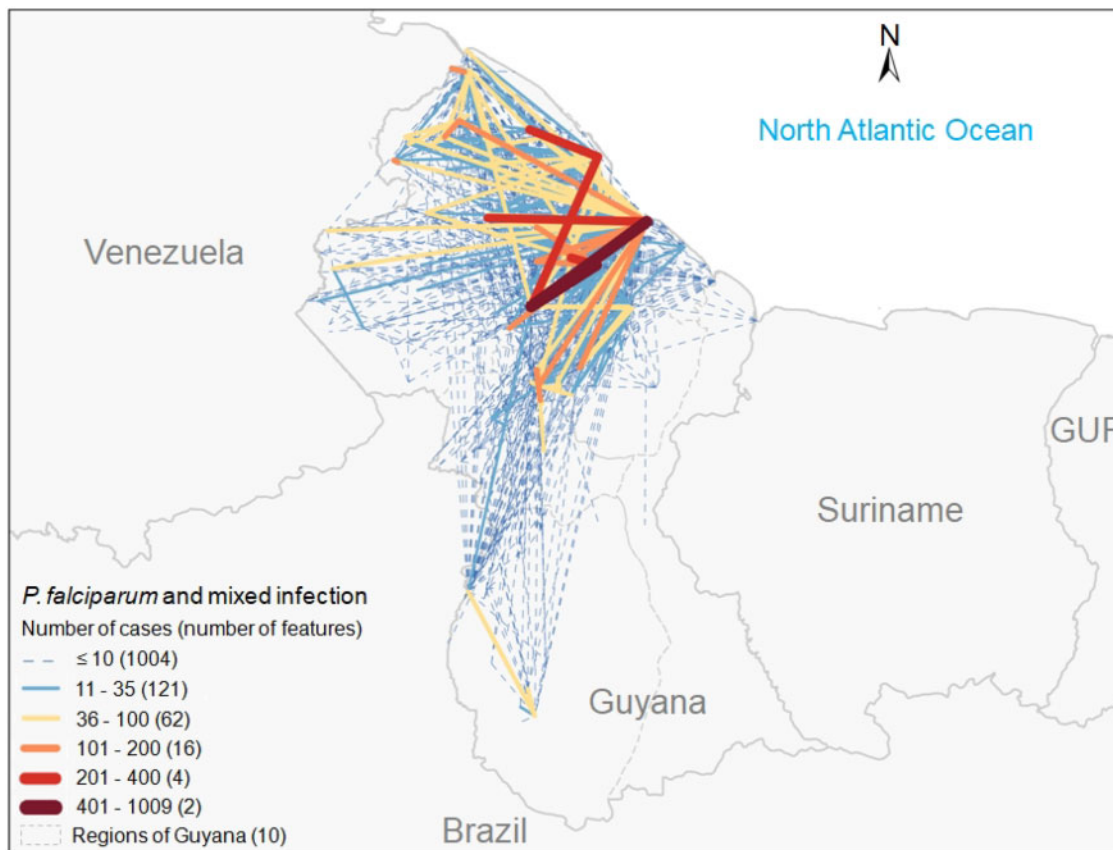
Figure 4. Number of *P. falciparum* mono and mixed infection cases by place of diagnosis and putative place of infection 2 weeks prior to diagnosis in Guyana, 2015–18. Lines represent a set of localities wherein one is where a patient's smear was taken for diagnosis and the second is the place where the patient self-reported they were 2 weeks prior to diagnosis. The width and colour of the lines represent the number of cases of *P. falciparum* mono and mixed infections. GUF, French Guiana.

Asia.[25] Unfortunately, no clinical data were associated with this sample. The A504D mutation observed in a Colombian sample was described once without associated phenotypic data.[20]

Globally, K189T was identified at a relatively high proportion in the Amazon basin (49.1%, 95% CI = 46.2%–52.0%). This mutation showed a similar prevalence in Africa, but was rarely described in south-east Asia.[25–28] Some *pfk13* K189T isolates have been associated with delayed parasite clearance time, but without clear correlation with artemisinin resistance.[25,29,30] Survival rates observed in isolates carrying this mutation and collected in French Guiana

suggested a natural polymorphism at this position. R255K, previously identified in Bangladesh, at the China–Myanmar border and also in Africa, was not linked to artemisinin resistance.[25,30–32]

Hosts of parasites disperse them; usually within a limited area by mosquitoes, especially in forests, but farther away by humans, who can move between endemic regions. Mobile populations greatly contribute to malaria dispersion and eventually to the spread of antimalarial drug resistance. Firstly, we excluded the risk of imported *pfk13* mutated parasites from south-east Asia in our region, as over the 2015–18 period only eight *P. falciparum* cases have been imported and recorded in the Amazon basin countries and none of those was from the Greater Mekong subregion, the hotspot for artemisinin resistance. Regionally, based on an analysis of reported imported cases and identified endemic regions, we inferred two principal areas at risk of spreading of resistance in the Amazon basin: one between Venezuela, Guyana and the Roraima State in Brazil and the second between Suriname, French Guiana and the Amapá State in Brazil (Figure 3).

The current crisis in Venezuela contributed to a huge displacement of people and increase in malaria cases in the country.[6] Several people have migrated to neighbouring countries and have consequently contributed to an increase in malaria cases there.[33–35] Besides this political crisis, Venezuelan miners are involved in mining activities in Guyana and *vice versa*. It is likely that most of this movement has originated in the two major mining regions of Guyana, Regions 1 and 7 (where the mutation *pfk13* C580Y was observed), and Sifontes municipality in Venezuela, which was the epicentre of malaria transmission during 2015–18.[36] Linked to this bidirectional malaria case exchange, the Roraima State was the epicentre for malaria case importation and recorded 71.4% of the imported cases in Brazil, with specific routes of migration coming from Venezuela or Guyana.[37,38]

In the Amazon basin, the high prevalence of *P. falciparum* cases observed in mining areas is a concern.[39,40] Miners represent a highly mobile population, as mines are generally located far from living areas and people move according to the quantity and the value of the gold they can find with limited trouble with police. During their life, miners generally move within two to three countries or different places within their own country.[38,41] They move between Suriname, French Guiana and the Amapá State.[41–43] However, the number of *P. falciparum* malaria cases moving within this area is lower (*n* = 651 for the analysed period) compared with the Venezuela–Guyana–Roraima triangle (*n* = 7350), as both malaria transmission and human migration intensity are lower.[44,45] However, 12.3% of Brazilian imported cases occurred in Maranhão (*n* = 315), Amapá (*n* = 233) and Pará (*n* = 131). Those cases in the Amapá State (*n* = 227) and in the Maranhão State (*n* = 120) were mainly from French Guiana, because of the vicinity and the geographical origins of the miners, respectively.[41] Maranhão is considered as a non-endemic region except for sporadic transmission or in small areas bordering Pará with 0.4% of the Brazilian Amazon *P. falciparum* cases (*n* = 72/16 987 malaria cases in 2019[46]). Therefore, mutation spread is unlikely throughout this state.

In Guyana, the malaria surveillance system is effective and allows detailed tracing of patients and putative infection places within the country. Mining abounds on both a medium and a large scale, as well as on an artisanal scale, in Regions 1, 7 and 8. However, miners mostly live in the coastal area, which is non-endemic. They access health centres on the coast upon their return from 2–3 month stints in the mines. Therefore, they are frequently diagnosed in the Malaria Clinic in Georgetown or at other diagnostic posts en route in other regions. The situation is similar in Suriname and French Guiana, where miners work in remote areas and are generally diagnosed upon their return to Paramaribo or Cayenne, respectively. In French Guiana, miners could also be diagnosed at the closest health centre or cross the international borders into Suriname and Brazil, depending on the presence of the police in the area.

This analysis of the risk of spreading of mutations within the Amazon basin is based on malaria cases imported between countries/areas as a proxy of parasite movements. This approach is the result of the malaria risk encountered by the people exposed to the disease who have contracted and taken it with them to another country/areas. But it has limitations. First, the risk of spread and/or emergence of resistance is related to the number of people exposed to malaria, but also to their use of antimalarial drugs, as well as other variables, including drug quality, adherence, drug absorption etc. Second, the suggested maps represent the patterns but not precisely the path taken by people during their journey. These paths could include additional stops during the journey, in transmission areas, with a risk of mutant dispersal. Third, our approach is based on cases captured by surveillance systems, but in some areas access to malaria diagnosis is low and self-treatment rates are high. Therefore, cases are not identified and even less reported in this context, but selection for resistance and the risk of resistance spreading could be high.

With these additional *pfk13* genotype results and this evaluation of the malaria case movements within the Amazon basin countries, we conclude that the *pfk13* mutation associated with artemisinin resistance *in vitro* in South America appears to be limited to Guyana.

Between 2010 and 2016, *pfk13* C580Y did not really increase in prevalence in Guyana and it was not observed in samples collected in 2018 and 2019, despite the fact that it confers *in vitro* artemisinin resistance.[8] In western Cambodia it has displaced other mutations because of the KEL1/PLA1 lineage, which is resistant to artemisinins, but also piperaquine.[30,47,48] The high level of efficacy of lumefantrine in the Guiana Shield associated with the negative impact on parasite growth could explain this low level of spread.[8,20,21] However, as soon as resistance to the partner drug appears, the risk of artemisinin resistance spreading will be high, first within the country itself and then among the neighbouring countries or the areas linked by human migration, i.e. Venezuela and Roraima. This risk of spread will also be proportionate to the intensity of mining activities and inversely proportionate to the level of care offered to miners. With this in mind, countries will benefit by reinforcing malaria diagnosis and treatment availability within the mining sites deep in the forest. Practical deployment must be adapted according to the legal status of the mines and the miners and the regulation constraints of each country. When it exists, the private sector is an important partner and in the case of small-scale mining, which is often illegal, community health workers could be deployed, as is the case in Suriname, or a self-diagnosis and treatment approach could be considered, as in French Guiana.[49,50] Moreover, regulation of sale of antimalarial drugs and use is key in these settings, as erratic use exists and commercialization of monotherapies of artemisinin is not strictly regulated in all countries of the region.[8,51] Finally, molecular surveillance is

crucial, particularly in Venezuela, which is experiencing a malaria outbreak with deficient control and because the analysed sample size in this study was limited [*n* = 124, i.e. 0.1% (95% CI = 0.1%–0.1%) of the 196 166 *P. falciparum* cases reported in 2016–18[6]].

Transparency declarations

M.-P.A., P.S., J.S.F.A. and A.M.A. are staff members of the World Health Organization. The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the World Health Organization.

All other authors: none to declare.

Supplementary data

Tables S1 to S3 are available as Supplementary data at *JAC* Online.

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