An open dataset of Plasmodium vivax genome variation in 1,895 worldwide samples

MalariaGEN *Plasmodium vivax* Genome Variation Project

Contributing partner studies

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The *P. vivax* Genome Variation Project (https://www.malariagen.net/parasite/p-vivax-genome-variation) is coordinated by the MalariaGEN Resource Centre and connects multiple research groups which are primarily concerned with understanding the population genetics of *P. vivax*. The project is comprised of partner studies – independent studies undertaken in malaria endemic areas. Each partner study is unique with their own research objectives. They have agreed to contribute samples to the project on the understanding that this will not interfere with their research objectives. Prior to submitting samples, all partner studies complete a Partner Study Information Form that captures information about their study, and confirms that all relevant ethical and regulatory requirements have been met and that all stakeholders have agreed to contribute samples and data to the project. Each partner study is represented on the project website with a brief description of the study, and details of the study contact person, key associates and their affiliations. Below is a summary of the information presented at the time of publication.

1044-PF-KH-FAIRHURST

Genomics of parasite clearance and recrudescence rates in Cambodia

In field-based studies, Rick Fairhurst and colleagues investigated patient responses to artemisinin combination therapies (ACTs), in three Cambodian provinces, where artemisinin resistance is entrenched (Pursat), emerging (Preah Vihear), or uncommon (Ratanakiri). They provided samples from this study with the aim to identify genetic markers of antimalarial drug resistance, use them in real time to define frontline treatments at the provincial level, and eliminate multidrug-resistant malaria in the Greater Mekong Subregion. All three sites provided *Plasmodium falciparum* samples, with Pursat additionally providing samples from patients presenting with *Plasmodium vivax*. In related laboratory-based studies, researchers aimed to elucidate the molecular mechanisms of *P. falciparum* artemisinin and partner-drug resistance, to develop point-of care diagnostics to identify drug-resistant parasites, and discover new compounds to treat drug-resistant malaria episodes. For *P. vivax*, they investigated whether red blood cell polymorphisms protected against *P. vivax* malaria, *P. vivax*-infected erythrocyte binding to monocytes, reticulocyte invasion, immune responses to candidate vaccine antigens, and efficacy of chloroquine against *P. vivax* malaria.

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1046-PV-BR-FERRERIA

Developing the *Plasmodium Vivax* Genome Variation Project with partners in Brazil

Marcelo Ferreira and colleagues contributed to the early stages of the MalariaGEN *Plasmodium vivax* genome variation project by providing samples that were not themselves basis of a partner study, but were used in establishing our laboratory and analytical pipelines. For example, some samples were used to analyse early Illumina sequencing outputs or for preliminary analysis of population structure.

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1047-PV-LK-KARUNAWEERA

Developing the *Plasmodium Vivax* Genome Variation Project with partners in Sri Lanka

Nadira Karunaweera and colleagues contributed to the early stages of the MalariaGEN *Plasmodium vivax* genome variation project by providing samples that were not themselves basis of a partner study, but were used in establishing our laboratory and analytical pipelines. For example, some samples were used to analyse early Illumina sequencing outputs or for preliminary analysis of population structure.

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1049-PV-VN-BONI

Developing the *Plasmodium Vivax* Genome Variation Project with partners in Vietnam

The Oxford University Clinical Research Unit in Vietnam contributed to the early stages of the MalariaGEN *Plasmodium vivax* genome variation project by providing samples that were not themselves basis of a partner study, but were used in establishing our laboratory and analytical pipelines. For example, some samples were used to analyse early Illumina sequencing outputs or for preliminary analysis of population structure.

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1050-PV-PN-MUELLER

Developing the *Plasmodium Vivax* Genome Variation Project with partners in Papua New Guinea

Ivo Mueller and colleagues contributed to the early stages of the MalariaGEN *Plasmodium vivax* genome variation project by providing samples that were not themselves basis of a partner study, but were used in establishing our laboratory and analytical pipelines. For example, some samples were used to analyse early Illumina sequencing outputs or for preliminary analysis of population structure.

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1052-PF-TRAC-WHITE

Tracking Resistance to Artemisinin Collaboration (TRAC)

TRAC is investigating the scope and spread of parasite resistance to artemisinin-based therapies at sites across Asia and Africa. The first TRAC study has been completed. This multi-centre, open-label randomised trial studied the clearance rates of peripheral blood *P. falciparum* parasitaemias in patients with acute uncomplicated *falciparum* malaria treated with two different doses of artesunate. Findings were used to validate the recently discovered kelch13 marker of artemisinin resistance. Working with MalariaGEN, TRAC samples have been sequenced and analysed for features of population genetics and signatures of selection, and contributed to the genetic basis of a genome-wide associations study for genetic markers of artemisinin resistance. Where samples were found to contain *P. vivax* data, for example due to mixed infection, this data was contributed to the *P. vivax* Genome Variation project.

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1098-PF-ET-GOLASSA

The prevalence of asymptomatic carriage; emergence of parasite mutations conferring anti-malaria drug resistance; and G6PD deficiency in the human population, as possible impediments to malaria elimination in Ethiopia

Since 2004, Ethiopia has adopted a species-specific treatment policy for malaria: artemether-lumefantrine (AL) for the treatment of uncomplicated *P. falciparum* malaria and chloroquine (CQ) for *P. vivax* infections. *P. falciparum* and *P. vivax* are co-endemic in Ethiopia. Periodic assessment of mutant and susceptible genotypes would help towards a better understanding of the effects of the current regimens. In areas where *P. vivax* is endemic and primaquine is required for the radical cure, individual's G6PD status must be known before the recommendation of this drug. Indeed, G6PD-deficient individuals are at risk of haemolysis when exposed to primaquine and tafenoquine drugs. Apparently no measures are currently in place to ensure safe delivery of this drug within the context of G6PD deficiency risk in the country. Given the incomplete removal of CQ, co-transmission of *P. falciparum* and *P. vivax* in the country and use of primaquine for the radical cure of *P. vivax*, Lemu Golassa and colleagues are interested to explore the frequencies of *P. falciparum* clinical isolates carrying mutant and susceptible genotypes in Pfcrt and Pfmdr-1 genes and to determine the prevalence of G6PD deficiency among endemic people. This study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1102-PF-MG-RANDRIANARIVELOJOSIA

Genotyping *P. falciparum* and *P. vivax* in Madagascar

The island of Madagascar is geographically situated in the south western region of the Indian Ocean and amongst malaria-endemic countries, its situation is unique: historically, human migration has occurred from both Africa and Asia; Duffy negative people can be susceptible to *P. vivax*; there is an absence of *pfcrt* mutant *P. falciparum* despite the official use of chloroquine to treat malaria for six decades (1945 - 2005). This study is mainly investigating *Plasmodium* samples collected directly from patients with uncomplicated malaria, as well as tracking malaria parasites and genetic markers of drug resistance in these parasites. This long-term study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* across Africa.

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1128-PV-MULTI-GSK

A global survey of *P. vivax* genome variation in samples from two GSK phase 3 clinical trials of tafenoquine in Pv relapse/reinfection (trial names DETECTIVE and GATHER)

As a service to the *P. vivax* genomics scientific community, GSK made DNA from the DETECTIVE and GATHER trials available for whole genome sequencing and analysis. The whole genome sequence data was not used in any way as part of any clinical trials.

DETECTIVE was a prospective, double-blind, randomised, placebo-controlled, fully powered superiority trial comparing the safety and efficacy of tafenoquine 300 mg single dose to placebo when used in conjunction with chloroquine. Patients with uncomplicated *P. vivax* malaria and G6PD activity >70% were recruited from 8 sites in six countries (Brazil, Peru, Ethiopia, Thailand, Cambodia & the Philippines). A primaquine arm was included as a benchmark positive control (15 mg daily for 14 days). The primary endpoint was based upon the proportion of patients who were free from recurrence at 6 months (defined as *P. vivax* clearance without recurrent parasitaemia) with safety as a key secondary endpoint. This study was considered the pivotal trial supporting registration of tafenoquine with the US FDA. The study is described at https://www.nejm.org/doi/full/10.1056/NEJMoa1710775.

GATHER was a prospective, double-blind, randomized, controlled trial comparing the safety profile of tafenoquine 300mg single dose against primaquine 15mg daily for 14 days in patients presenting with uncomplicated *P. vivax* malaria and G6PD activity >40%. Participants were recruited from seven in sites in Peru, Brazil, Colombia, Vietnam, and Thailand. The primary endpoint of the GATHER study was haemoglobin decrease (>3.0 g per deciliter or ≥30% from baseline or to a level of <6.0 g per deciliter). A key secondary endpoint was freedom from recurrence of *P. vivax* parasitemia at 6 months. Safety data from the GATHER study was used to support registration of tafenoquine with the US

FDA. The study is described at https://www.nejm.org/doi/full/10.1056/NEJMoa1802537.

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1154-PV-TH-PRICE

Characterisation of drug resistance in *P. falciparum* and *P. vivax* populations from Indonesia and Thailand

Ric Price, Rintis Noviyanti and Francois Nosten are the principal investigators in a genome-wide study aiming to characterise the molecular profile of drug resistance-conferring variants in Thai and Indonesian parasite populations. The study entails genome-wide scans to identify novel resistance variants as well as characterising known variants in *P. falciparum* and *P. vivax* field isolates with ex vivo-determined drug sensitivity profiles for a range of antimalarial drugs. Samples are contributed by consenting patients attending local health centres and hospitals in Indonesia and Thailand. These studies are coordinated by Sarah Auburn and Jutta Marfurt.

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1157-PV-MULTI-PRICE

P. vivax SNP barcode for mapping parasite transmission and spread within and across borders: a vivaxGEN initiative

This project describes the genomic component within the broader vivaxGEN initiative. The vivaxGEN network comprises researchers and other key stakeholders from across the globe with a shared aim to develop novel molecular surveillance tools for *P. vivax* that provide clinically relevant information on emerging parasite adaptations, transmission dynamics, the major routes of parasite spread within and across borders, and the impact of local treatment policies on the dormant liver-stage reservoir. Researchers involved in this collaboration aim to identify markers through several separate genome-wide studies at each of the 14 sites/countries involved. Current studies include a multicenter collaborative effort to detect geographic markers for identifying and mapping imported *P. vivax* infections. Another study is underway to identify microhaplotype-based markers across the *P. vivax* genome that can be used as a parsimonious approach to characterize the identity by descent (IBD) between infection pairs. These and other markers will then be applied as a 'genetic barcode' that can be used at high sample throughput on a broad range of sample sources including DNA derived from blood spots on filter paper. To find out more about our work, visit the vivaxGEN site (http://menzies.edu.au/vivaxGEN). For interests in collaborating on any of the above projects, contact us at the emails below.

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