2013

Spatial distribution of G6PD deficiency variants across malaria-endemic regions

Rosalind E. Howes
University of Oxford, rosalind.howes@zoo.ox.ac.uk

Mewahyu Dewi
Eijkman-Oxford Clinical Research Unit, Jalan Diponegoro No. 69, Jakarta, Indonesia

Frederic B. Piel
University of Oxford

Wuelton M. Monteiro
Tropical Medicine Foundation Dr. Heitor Vieira Dourado, Manaus, Amazonas, Brazil

Katherine E. Battle
University of Oxford

See next page for additional authors

Follow this and additional works at: http://digitalcommons.unl.edu/publichealthresources

Authors

Rosalind E. Howes, Mewahyu Dewi, Frederic B. Piel, Wuelton M. Monteiro, Katherine E. Battle, Jane P. Messina, Anavaj Sakuntabhai, Thomas N. Williams, J. Kevin Baird, and Simon I. Hay

RESEARCH   **Open Access**

Spatial distribution of G6PD deficiency variants across malaria-endemic regions

Rosalind E Howes[1*], Mewahyu Dewi[2], Frédéric B Piel[3], Wuelton M Monteiro[4,5], Katherine E Battle[1], Jane P Messina[1], Anavaj Sakuntabhai[6], Ari W Satyagraha[7], Thomas N Williams[8,9], J Kevin Baird[2,10] and Simon I Hay[1]

Abstract

Background: Primaquine is essential for malaria control and elimination since it is the only available drug preventing multiple clinical attacks by relapses of *Plasmodium vivax*. It is also the only therapy against the sexual stages of *Plasmodium falciparum* infectious to mosquitoes, and is thus useful in preventing malaria transmission. However, the difficulties of diagnosing glucose-6-phosphate dehydrogenase deficiency (G6PDd) greatly hinder primaquine's widespread use, as this common genetic disorder makes patients susceptible to potentially severe and fatal primaquine-induced haemolysis. The risk of such an outcome varies widely among *G6PD* gene variants.

Methods: A literature review was conducted to identify surveys of *G6PD* variant frequencies among representative population groups. Informative surveys were assembled into two map series: (1) those showing the relative proportions of the different variants among G6PDd individuals; and (2) those showing allele frequencies of *G6PD* variants based on population surveys without prior G6PDd screening.

Results: Variants showed conspicuous geographic patterns. A limited repertoire of variants was tested for across sub-Saharan Africa, which nevertheless indicated low genetic heterogeneity predominated by the *G6PD A-*[202A] mutation, though other mutations were common in western Africa. The severe *G6PD Mediterranean* variant was widespread across western Asia. Further east, a sharp shift in variants was identified, with high variant heterogeneity in the populations of China and the Asia-Pacific where no single variant dominated.

Conclusions: *G6PD* variants exhibited distinctive region-specific distributions with important primaquine policy implications. Relative homogeneity in the Americas, Africa, and western Asia contrasted sharply with the heterogeneity of variants in China, Southeast Asia and Oceania. These findings will inform rational risk assessments for primaquine in developing public health strategies for malaria control and elimination, and support the future development of regionally targeted policies. The major knowledge gaps highlighted here strongly advocate for further investigation of *G6PD* variant diversity and their primaquine-sensitivity phenotypes.

Keywords: Glucose-6-phosphate dehydrogenase deficiency, G6PD, Genetic variants, Spatial distribution, Primaquine, *Plasmodium vivax*, Malaria elimination, Haemolysis

Background

The discovery of glucose-6-phosphate dehydrogenase deficiency (G6PDd) occurred during the clinical development of primaquine against the relapse of *Plasmodium vivax* malaria in American prisoner volunteers during and immediately after the Second World War [1]. The high prevalence of this inherited disorder among people at risk

of malaria [2], along with the practical difficulty of its diagnosis where almost all of them live [3], sharply limits the otherwise enormous public health importance and utility of primaquine in controlling and eliminating malaria [4-8]. This drug is currently the only licensed therapy active against the latent liver-stages of *P. vivax* responsible for multiple relapses in the weeks, months, and several years following a single infectious bite by an anopheline mosquito [9]. As with the primary infection, and despite the long-held dogma that considered vivax malaria clinically benign, each relapse carries risk of severe disease and

* Correspondence: rosalind.howes@zoo.ox.ac.uk
[1]Spatial Ecology and Epidemiology Group, Department of Zoology, University of Oxford, South Parks Road, Oxford, UK
Full list of author information is available at the end of the article

mortality [5]. Furthermore, primaquine is the only drug with activity against the mature transmission-stages of all *Plasmodium* species [4,10,11], giving it a role of undeniable importance in reducing transmission levels, most particularly in preventing the spread of artemisinin-resistant *Plasmodium falciparum* [12-15]. The recently published model-based geostatistical mapping study showed that G6PDd is prevalent across malaria-endemic countries, with an estimated median allele frequency in these regions of 8.0% (50% CI: 7.4-8.8) [2], thought to be driven by a selective advantage against life-threatening malaria [16-18]. Despite its prevalence and hindrance to malaria control, the spatial patterns of the genetically and clinically diverse *G6PD* mutations are poorly documented globally; this knowledge gap, highlighted by others [19], constitutes the focus of the present study. A better understanding of the spatial epidemiology of *G6PD* gene variants would support assessments towards safe delivery strategies to increase access to primaquine.

G6PD enzyme generates nicotinamide adenine dinucleotide phosphate (NADPH), which represents the primary defence against oxidative stresses in red blood cells (RBC). Mutations in the *G6PD* gene can destabilize the enzyme and reduce its activity levels, leaving cells vulnerable to damage from exogenous triggers, including certain foods, infections, and a range of drugs, that may lead to RBC lysis and acute haemolytic anaemia (AHA) [20,21]. The clinical burden of G6PDd was evaluated in the Global Burden of Disease Study 2010 [22,23], and includes a range of pathologies, notably neonatal jaundice and AHA. The clinical symptoms due to primaquine-induced haemolysis range from negligible to lethal [24,25] depending largely upon the variants involved and drug dosing.

At least 186 mutations have been characterized in the *G6PD* gene [26], although not all are polymorphic and of clinical significance. *G6PD* variants have been categorized into three types (Table 1) [21,25]. About half of the known variants appear to be sporadic mutations identified in only a handful of patients [27]. These rare variants, classified as Type 1, usually express very severe chronic disease – a pathology known as chronic non-spherocytic haemolytic anaemia (CNSHA) – which can result in lifelong dependency on blood transfusion [25].

Although numerous by type, these variants never reach polymorphic frequencies (prevalence ≥1%) and are thus not of serious public health concern. Type 2 variants, in contrast, do reach frequencies ≥1% and put individuals at significant risk of haemolytic crises. These Type 2 variants correspond to the Class II and III variants of the WHO-endorsed classification system [28,29]. Given their public health significance, being both polymorphic and clinically significant, Type 2 variants are the focus of the present study. Type 3 variants express phenotypically normal G6PD activity and present no clinical or public health concerns.

The *G6PD A-* variant from Africa has been the most thoroughly studied with respect to sensitivity to primaquine. This may be a consequence of being the first studied and the most accessible (in African American volunteers) during primaquine development [30]. This variant typically exhibits residual enzyme activity of about 5-10% of normal levels [31]. Primaquine toxicity in the *G6PD A-* volunteers was relatively mild and self-limiting [32]; *G6PD A-* individuals dosed with daily primaquine for four months have been observed to typically recover from relatively shallow AHA within about three weeks, despite continued daily dosing [33]. In contrast, the *G6PD Mediterranean* variant, which is common across southern Italy (particularly Sardinia) and the Arabian Peninsula [34], exhibits exceedingly low residual enzyme activity (<1% [35]) and predisposes individuals to favism [36,37] and clinically severe AHA following primaquine therapy [12,35,38,39]. *G6PD Mahidol* is the best-characterized Asian variant and is considered predominant across Myanmar and Thailand [40,41]. Enzyme activity in *G6PD Mahidol* individuals is reduced to 5-32% of normal levels [42], and its primaquine sensitivity phenotype lies between that of the *G6PD A-* and *Mediterranean* variants [43,44]. As for the balance of many dozens of other *G6PD* variants, residual enzyme activity is generally known for many [26], but the primaquine sensitivity phenotypes for any of these remains effectively unknown [4]. There may be an inverse correlation between residual enzyme activity and AHA sensitivity to primaquine (as roughly seen with the three variants discussed), but this is not yet firmly established.

Table 1 G6PDd variant types

Type	Residual enzyme activity	Population-level prevalence	Clinical significance	Primaquine-associated public health concern
Type 1	<10%	Sporadic (never polymorphic)	Severe and chronic: CNSHA	None – too rare
Type 2[§]	<1-50%	Polymorphic	Asymptomatic until exposed to an exogenous trigger	Yes – commonly inherited and cause individuals to be at risk of haemolysis
Type 3	Normal (>50%)	Polymorphic (wild-type)	None	None – G6PD normal

[§]Type 2 variants have previously been separated into Class II and Class III variants of the WHO-endorsed G6PDd variant classification [28,29]. For many variants, however, this subdivision is blurred and poorly defined [25].

The diversity of G6PDd phenotypes and genotypes, and our limited knowledge thereof, greatly compounds the difficulty of addressing the technical and practical limitations which this deficiency imposes on primaquine treatment for attacking the endemic malarias [45]. National authorities responsible for the prevention, control and treatment of endemic malaria naturally strive for evidence-based practices that minimize risk and maximize benefit. The complexity of the problem imposed by G6PDd – both by its genetic variability and its heterogeneous prevalence [2] – compounds the difficulty of developing practical and useful tools for assessing risk and benefit. The present study begins the task of characterizing *G6PD* variant spatial distribution and diversity, with the aim of contributing towards the evidence-based policies for primaquine treatment so essential to realizing the vision of malaria elimination.

Methods
The aim of this study was to map population surveys reporting the prevalence of the *G6PD* variants of greatest public health significance. A systematic literature review was conducted of online biomedical databases for all records referring to "G6PD" or "glucose-6-phosphate dehydrogenase" and then cross-referenced with previously published *G6PD* variant databases [26,46-50]. The study was limited to data from malaria-endemic countries [2,51,52], corresponding to where primaquine therapy is needed. Refer to Additional file 1 for more detailed descriptions of the methodology. The assembled datasets are available from the authors on request.

Survey inclusion criteria
The assembled library of sources was reviewed for population surveys of *G6PD* variants and those meeting a specific set of inclusion criteria (Figure 1) were identified. First, only surveys that could be geopositioned to at least the national level were included, and these were mapped to the highest spatial resolution available, ideally as point locations (e g, villages). Second, to ensure that survey samples were widely representative of the communities being studied, only surveys which provided apparently unbiased prevalence estimates were included: all case studies and patient groups, malaria patients (to avoid underestimating frequencies of *G6PD* variants which may provide a protective effect against malaria), family studies, and samples selected according to ethnic background were therefore excluded. Third, only surveys using molecular diagnostics were included to avoid the diagnostic uncertainty of surveys reliant on biochemical methods (see Additional file 1).

Surveys meeting these criteria were of two types depending upon the nature of the population samples surveyed and were mapped as two separate series:

Map series 1: variant proportion maps
Studies examining individuals who had been previously identified as phenotypically G6PDd from a population screening survey and were being followed-up for molecular diagnosis of underlying mutations were included in map series 1. Pie charts were chosen to display the relative proportions of the different variants identified in each of the surveys, with each colour-coded segment proportional to the variant's relative frequency across the study sample. Sample size was reflected in the relative sizes of the pie charts, which was transformed on a square-root scale to allow clear visualization.

It is important to note that studies did not always attempt to identify all the variants represented in the legends of the maps. For instance, if only *G6PD Mediterranean* was tested for among a sample of deficient individuals, those individuals who tested negative would be included in the "Other" category, regardless of whether they expressed one of the other variants listed in the legend or a completely different one. The absence of a specific variant from a sample can only be inferred if no "Other" samples are reported.
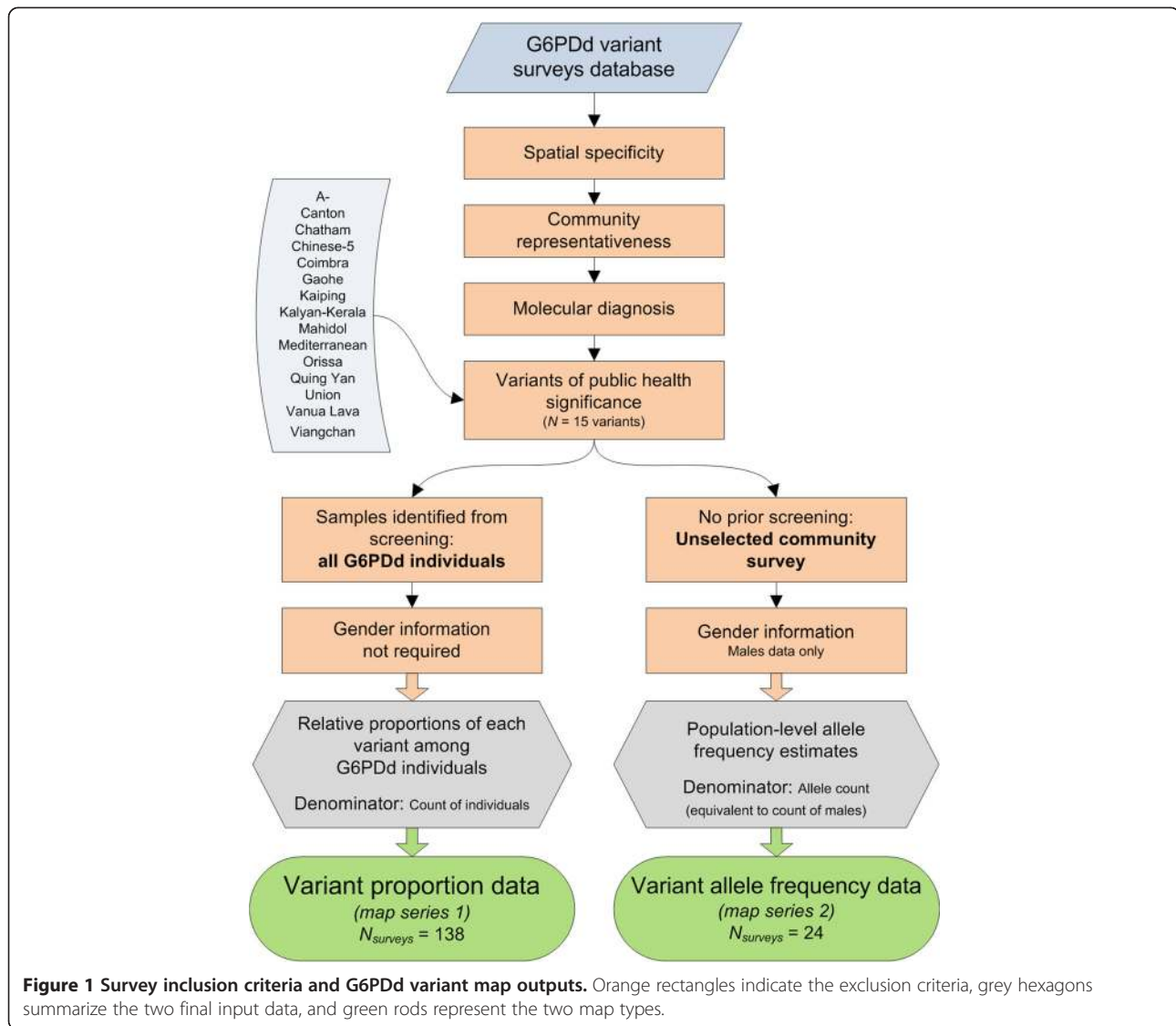
Map series 2: allele frequency maps
Surveys which investigated *G6PD* variants in cross-sectional population samples without prior phenotype screening were included in map series 2. These studies estimated the allele frequencies of selected *G6PD* variants at the population level and were mapped spatially using bar charts to represent the allele frequencies. This visualization conveyed the important concept that frequency estimates are only available for variants that were included in the diagnoses, underlining the importance of knowing which variants had been tested for. The variants tested for were named in the vertical bars of the bar charts. Empty spaces along the x-axis indicate that the named variant was tested for but not identified in the population sample. The size of the plots meant that in some cases, significant spatial uncertainty was introduced to their positioning on the map (Additional file 1).

The *G6PD* gene's X-linked inheritance means that allele frequencies correspond to frequencies among males. Heterozygous inheritance in females makes allele frequency estimates from female samples harder to calculate confidently as not all studies consistently distinguished heterozygotes from homozygotes. For reliability, therefore, only data from males were included in these variant allele frequency maps.

Variant inclusion criteria
Given the diversity of *G6PD* variants, for the purposes of this mapping study it was necessary to identify those variants that presented a primary public health concern. This study focussed on Type 2 variants which (i) express

Figure 1 Survey inclusion criteria and G6PDd variant map outputs. Orange rectangles indicate the exclusion criteria, grey hexagons summarize the two final input data, and green rods represent the two map types.

significantly reduced enzyme activity (<50%); (ii) are clinically significant by predisposing individuals to haemolytic anaemia; and, (iii) reach polymorphic allele frequencies at the population level (Table 1). Although 77 Type 2 variants have so far been described [26], the database of population survey data compiled in this study indicated that most variants were reported fewer than ten times (most fewer than three) and, when identified, were found to have low prevalence. A clear subset of variants was responsible for the majority of investigated G6PDd cases (Additional file 1); these were defined for the purposes of this study to be those variants reported from a minimum of ten localities across the malaria-endemic region. This ensured that the spatial distributions of the most significant variants could be legibly visualized in the maps. Fifteen variants met these criteria: *G6PD A-, Canton, Chatham, Chinese-5, Coimbra,*

Gaohe, Kaiping, Kalyan-Kerala, Mahidol, Mediterranean, Orissa, Quing Yan, Union, Vanua Lava, and *Viangchan* (Figure 1).

Results
The database
A total of 18,939 bibliographic sources were identified from keyword searches, of which 2,176 were considered likely to include spatial information about *G6PD* variants. Following their detailed review, 93 published and unpublished sources were identified which reported surveys eligible for inclusion in the maps (listed in the Additional file 2). From these sources (which could each report several surveys from multiple locations), 138 population surveys could be geolocated and met the criteria for map series 1, as these were of community samples which had undergone prior screening for phenotypic

G6PDd. Map series 2 was populated with 24 geolocated population surveys of community samples providing variant frequency estimates. Map series 1 data were predominantly from populations in Asia (123/138 surveys; 89%), while map series 2 data were almost exclusively from the Africa + region (Africa, Yemen and Saudi Arabia [53]: 20/24 surveys, or 83%). These database descriptors are summarized in Table 2.

Distribution of *G6PD* variants in malaria-endemic regions

The maps reveal conspicuous distinct geographical patterns in the distribution and prevalence of *G6PD* variants across regions. The two series of maps represent both the relative proportions of the variants responsible for phenotypic G6PD enzyme deficiency (Figures 2, 3, 4, 5, 6, 7) and the allele frequencies of selected variants (Figure 8 and Additional files 3, 4 and 5). Together, these maps revealed a number of clear patterns further described below: (i) a relatively low diversity of *G6PD* variants reported from populations of the Americas and Africa + regions, among whom variants of the *G6PD A-* phenotype are predominantly searched for and reported; similarly, *G6PD Mediterranean* was predominant in west Asia (from Saudi Arabia and Turkey to India); (ii) a sharp shift in variants identified east of India, showing no admixture with the common variants of west Asia; (iii) high variant diversity in east Asia and the Asia-Pacific region, with multiple variants co-occurring and no single variant being predominant.

G6PD variants in the Americas

Relatively few surveys were available from the Americas (nine in map series 1, one in map series 2). Figure 2A indicates that among G6PDd individuals, the predominant variant was *G6PD A-* (in the majority of cases encoded by the *G6PD A-^{202A}* mutation, though the *G6PD A-^{968C}* variant was common in some samples), identified in 91% of deficient individuals surveyed across the region (579 of 636 total G6PDd individuals

surveyed). Other variants identified included *G6PD Mediterranean*^{563T} and *Seattle*^{844C} (the latter was classified as part of the "Other" category as it was only reported twice, both instances from Brazil where it reached a prevalence of 13% (n = 2/15, [Additional file 2: S63] and 5% (n = 9/196, [S30])). A survey of Mexicans reported 61% of deficient cases being due to various *G6PD A-* variants, but did not test for any other variant (e g, *G6PD Mediterranean*), explaining the large proportion of "Other" variants [Additional file 2: S85].

A single community screening survey could be identified which investigated *G6PD* variant allele frequencies. This was in Acrelândia, Brazil, and searched for only one single-nucleotide polymorphism (SNP): *G6PD A-^{202A}*. An allele frequency of 6% (n = 509, [Additional file 2: S14]) was reported (see Additional file 3).

G6PD variants in Africa, Yemen and Saudi Arabia (Africa+)

Very few studies (*n* = 6) investigating the variants of known G6PDd individuals were identified from the Africa + region (Figure 3). Where available, both in West Africa and the western Indian Ocean islands, surveys indicated *G6PD A-^{202A}* to be the predominant variant; in Saudi Arabia, the *G6PD Mediterranean* variant was responsible for 78% of G6PDd cases in two surveys in the western coastal city of Jeddah [Additional file 2: S6,S28].

Surveys investigating population-level frequencies of specific *G6PD* variants were more common (Figure 8 and Additional file 4). These consistently looked for SNPs of *G6PD A-*, notably focussed on the *G6PD A-^{202}* locus. Frequencies of this SNP were over 20% in surveys from Nigeria and Côte d'Ivoire in West Africa, and found at 19% in coastal Kenyan populations. The *G6PD A-^{968C}* mutation encodes a similar phenotype to *G6PD A-^{202A}* and was found to be the more common of the two in West African populations (in The Gambia and Senegal, for instance [Additional file 2: S21,S25]); despite this, only six of 20 surveys across Africa + included the *G6PD A-^{968}* SNP in their diagnoses. Large population surveys in East Africa (n = 311 in Uganda [Additional file 2: S38] and n = 2,756 in Kenya [Additional file 2: S76]) found no evidence of the *G6PD A-^{968C}* mutation despite frequencies of 12 and 19% of the *G6PD A-^{202A}* mutation, respectively.

Most population surveys (70%) in the Africa + region tested the *G6PD A-^{376}* locus. Although this mutation has barely any clinical effect, it is commonly co-inherited with mutations at other loci, together encoding the range of *G6PD A-* variants (Figure 8). The *G6PD A-^{376G}* variant had very high prevalence among sub-Saharan African populations, and in all cases affected over 10% of individuals surveyed; further, in a subset of ten out of 16 of these surveys, its frequency reached 25-50%. Exceptions to this high *G6PD A-^{376G}* prevalence were

Table 2 Summary of input data according to map type

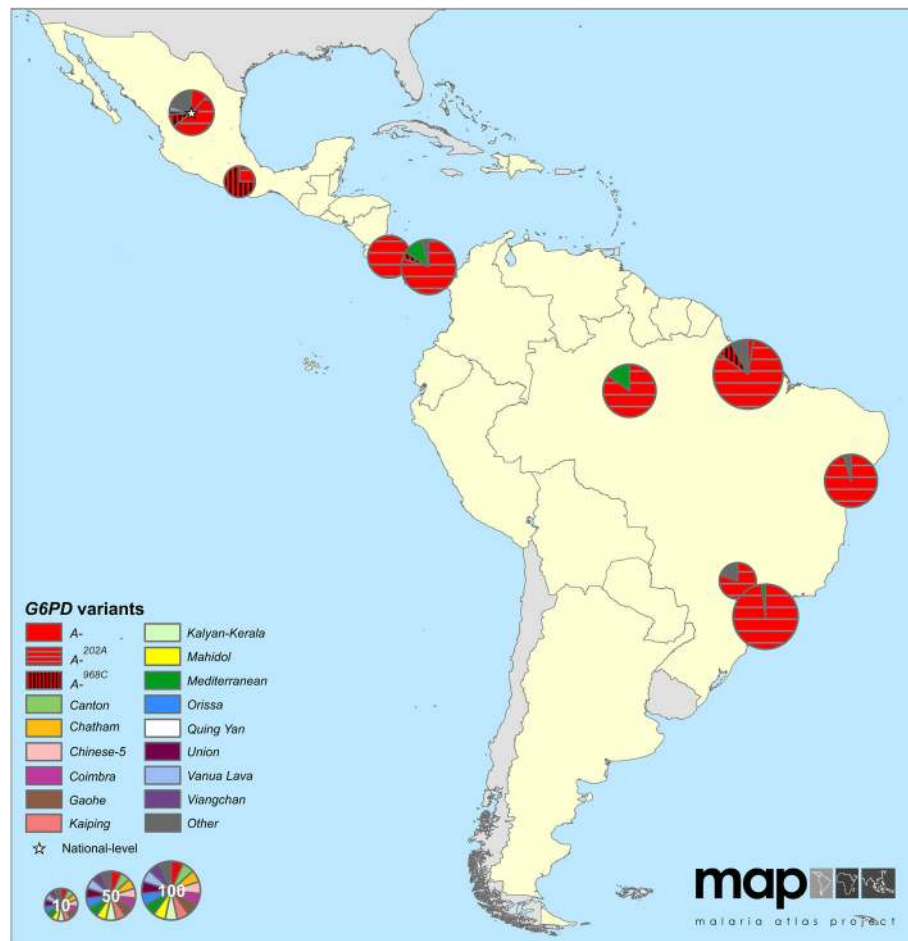|		Africa+	Americas	Asia	Global
		(Malaria-endemic countries only)			
Map series 1 Variant proportion maps	$N_{surveys}$	6	9	123	138
	$N_{countries}$	5	4	16	25
	$N_{G6PDd\ indivs}$	258	636	5,278	6,172
	Mean sample size	43	71	43	45
Map series 2 Variant frequency maps	$N_{surveys}$	20	1	3	24
	$N_{countries}$	13	1	2	16
	$N_{male\ indivs}$	6,796	509	448	7,753
	Mean sample size	340	509	149	323

Figure 2 G6PDd variant proportion map: Americas (map series 1). Map includes nine surveys with a mean sample size of 71 (range: 8-196); one survey was mapped at the national level. Pie charts represent individuals previously identified as G6PDd. Sample size is reflected in the size of the pie charts, which is transformed for clarity on a square-root scale. Surveys which could only be mapped to the country-level are indicated by a white star; this applied to seven surveys globally. Spatial duplicates from independent studies were mapped with a "jitter" of 0.5-1 decimal degrees in their latitude or longitude coordinates to allow visualization of the multiple charts. Malaria-endemic countries in the region mapped are shown with a yellow background; white backgrounds indicate endemic countries outside the region in focus; grey backgrounds represent malaria-free countries. G6PDd variants that could not be diagnosed were reported as "Other".

two surveys (n = 46 and 55) from northern Sudan, which reported low (<5%) prevalence [Additional file 2: S41]; the *G6PD A-²⁰²ᴬ* mutation was not detected in these samples.

G6PD variants in Asia and Asia-Pacific

Greatest *G6PD* variant diversity globally was across the Asia and Asia-Pacific regions (Figures 4, 5, 6, 7) where up to ten variants were reported to co-occur within single populations. Furthermore, significant proportions of "Other" cases were frequently reported in the variant proportion maps (map series 1), indicating that genetic diversity is greater than represented by the pie charts in these maps.

From Turkey to Pakistan (Figure 5), *G6PD Mediterranean* was predominant, identified in 728 of 895 G6PDd indivi-

duals examined (81%). Two variants, *G6PD Kalyan-Kerala⁹⁴⁹ᴬ* and *Orissa¹³¹ᴳ*, were reported exclusively from Indian populations. On the Indian sub-continent, these two variants and the *G6PD Mediterranean* variant represented the majority (88% of n = 555 G6PDd individuals tested) of deficiency cases, though notable proportions (up to 80%) of "Other" cases were also reported from eastern and southern India.

East of India, a completely different set of variants emerged (Figure 6). *G6PD Mahidol⁴⁸⁷ᴬ* predominated across Myanmar, with 98 of 117 G6PDd individuals (84%) diagnosed across 14 locations as carrying this variant. Variants common among G6PDd individuals in southeast China were largely unique to these populations. Of the 2,883 G6PDd cases from China, the commonly diagnosed variants included *G6PD Kaiping¹³⁸⁸ᴬ*
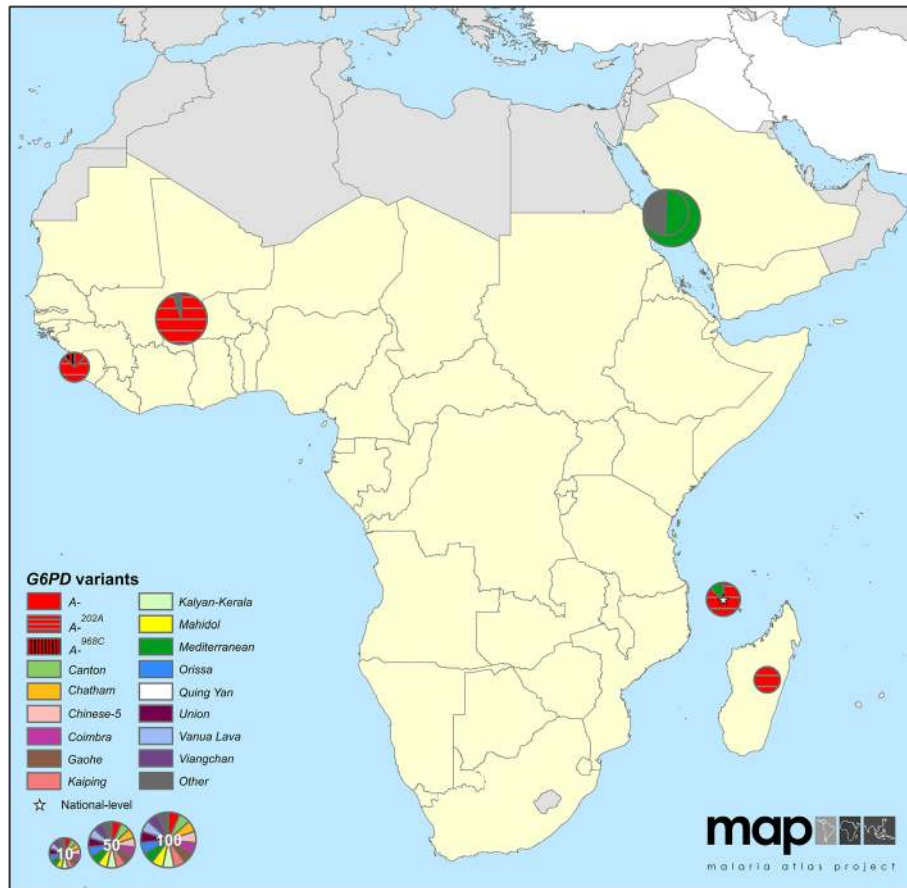
Figure 3 G6PDd variant proportion map: Africa+ (map series 1). Map includes six surveys with a mean sample size of 43 (range: 5-110). One survey was mapped at the national level. (See Figure 2 for full legend).
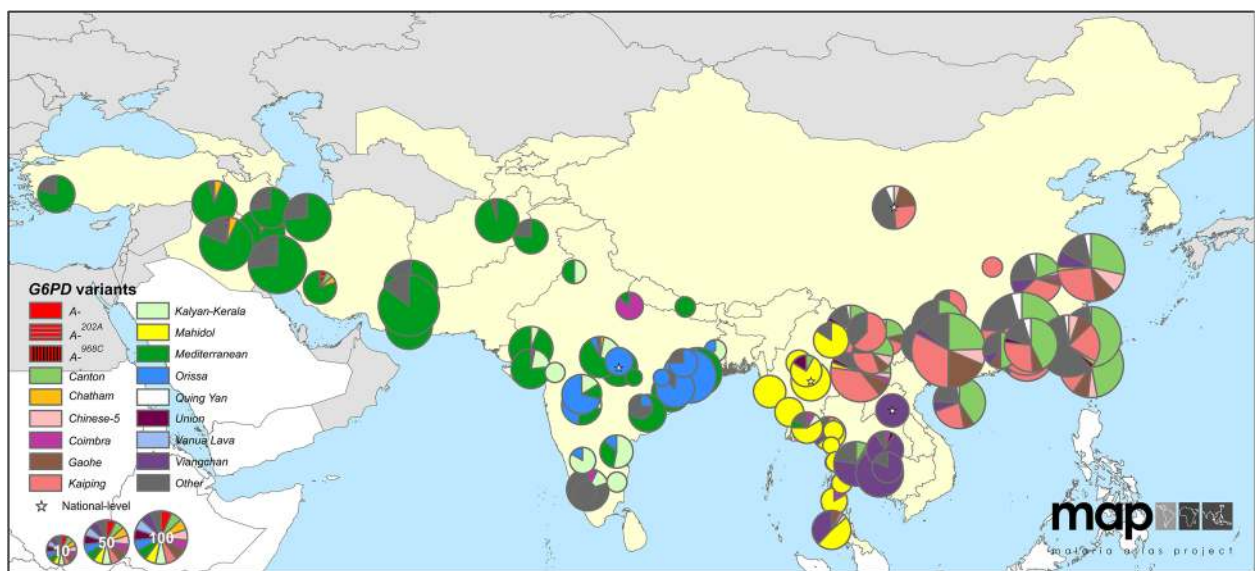

Figure 4 G6PDd variant proportion map: Asia (map series 1). Map includes 87 surveys with a mean sample size of 53 (range: 1-532). Four surveys were mapped to the national level. (See Figure 2 for full legend).
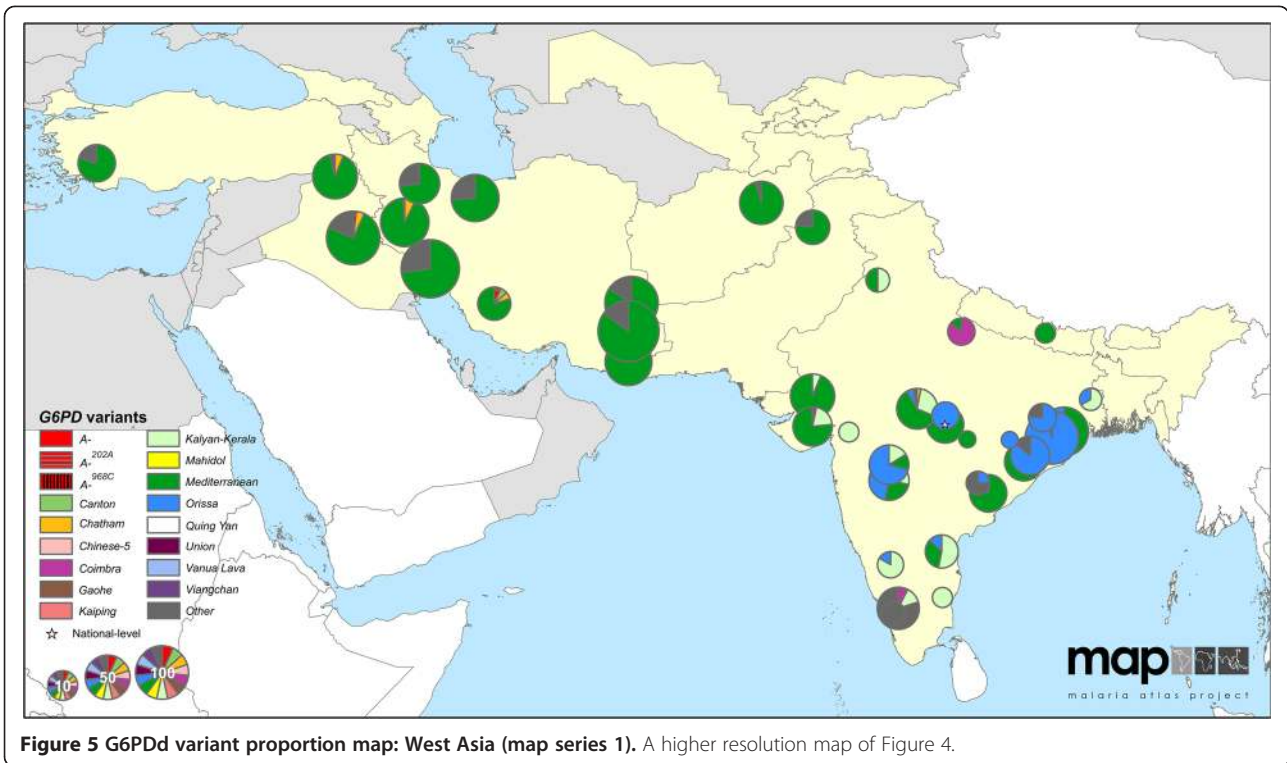
Figure 5 G6PDd variant proportion map: West Asia (map series 1). A higher resolution map of Figure 4.
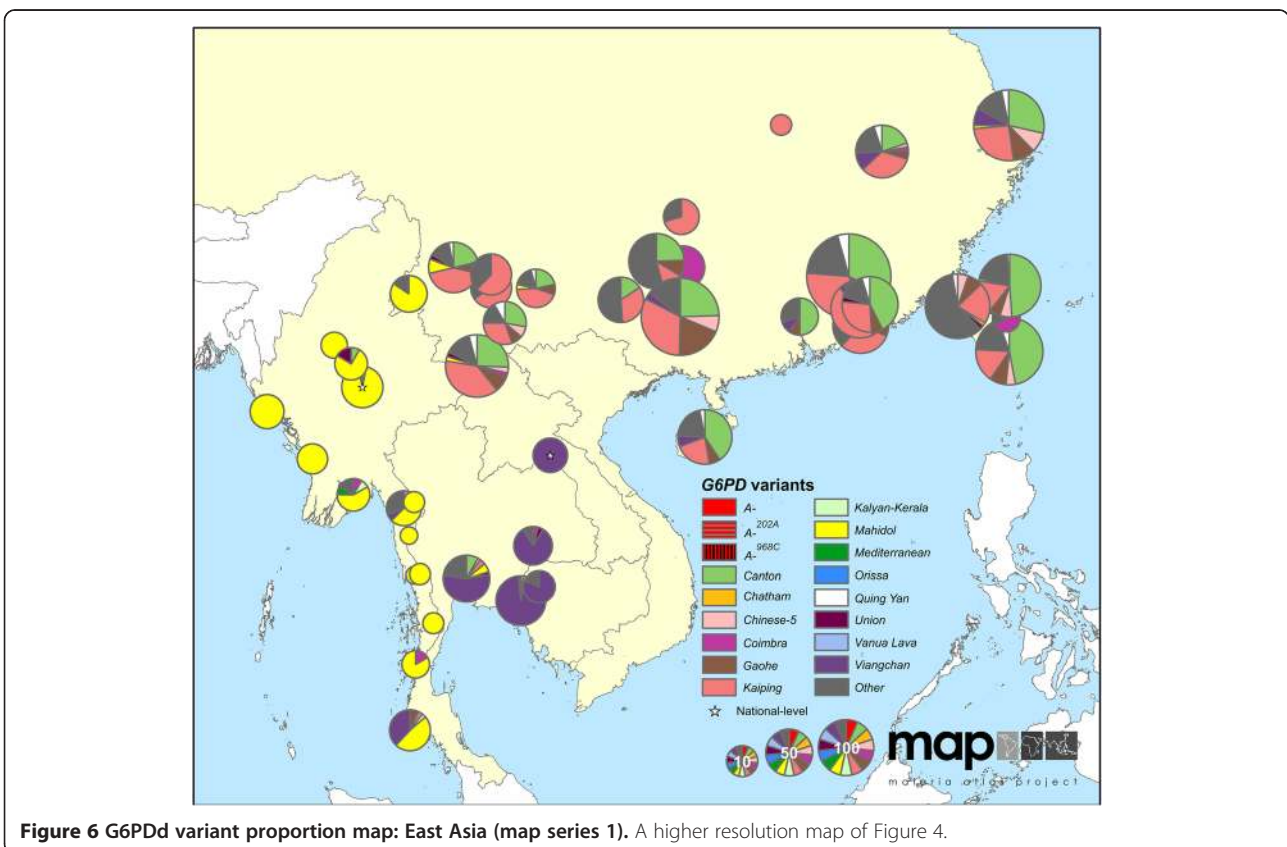
Figure 6 G6PDd variant proportion map: East Asia (map series 1). A higher resolution map of Figure 4.
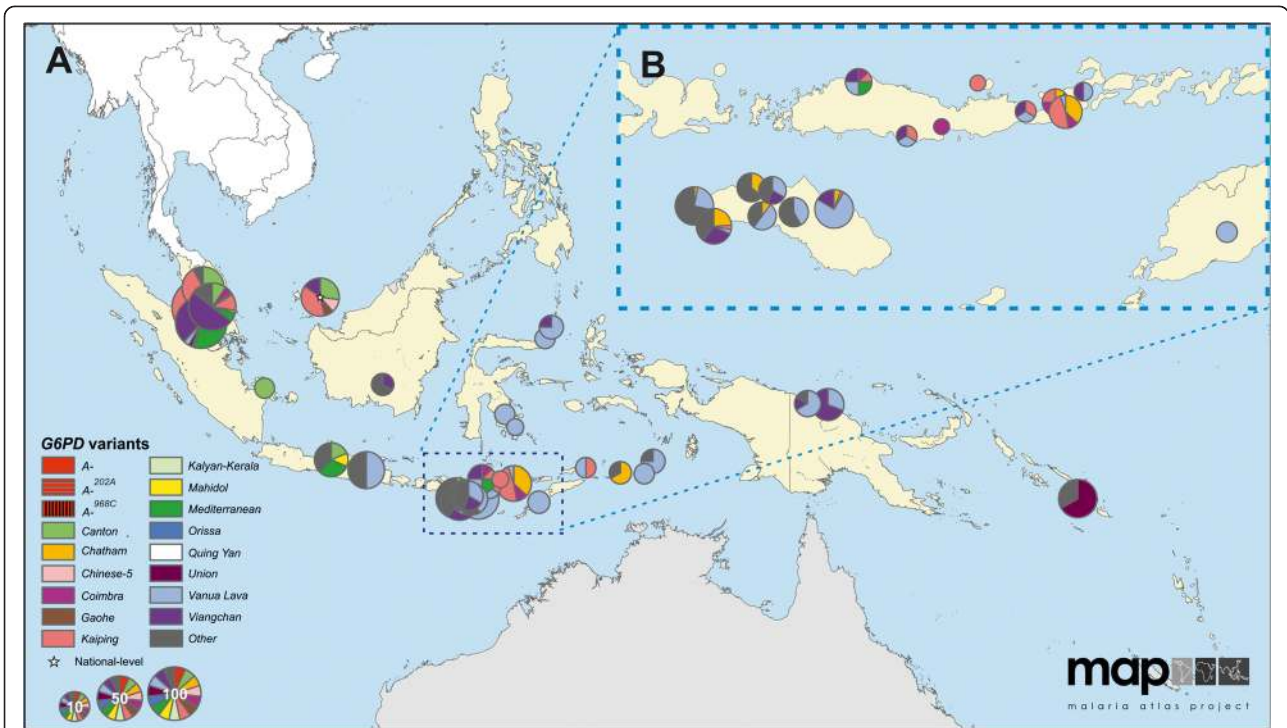
Figure 7 G6PDd variant proportion map: Asia-Pacific (map series 1). Panel **A** show the full map, which includes 36 surveys with a mean sample size of 18 (range: 1-128). One survey was mapped at the national level. The East Nusa Tenggara province islands identified by the dotted box are shown at higher resolution in Panel **B**. (See Figure 2 for full legend).
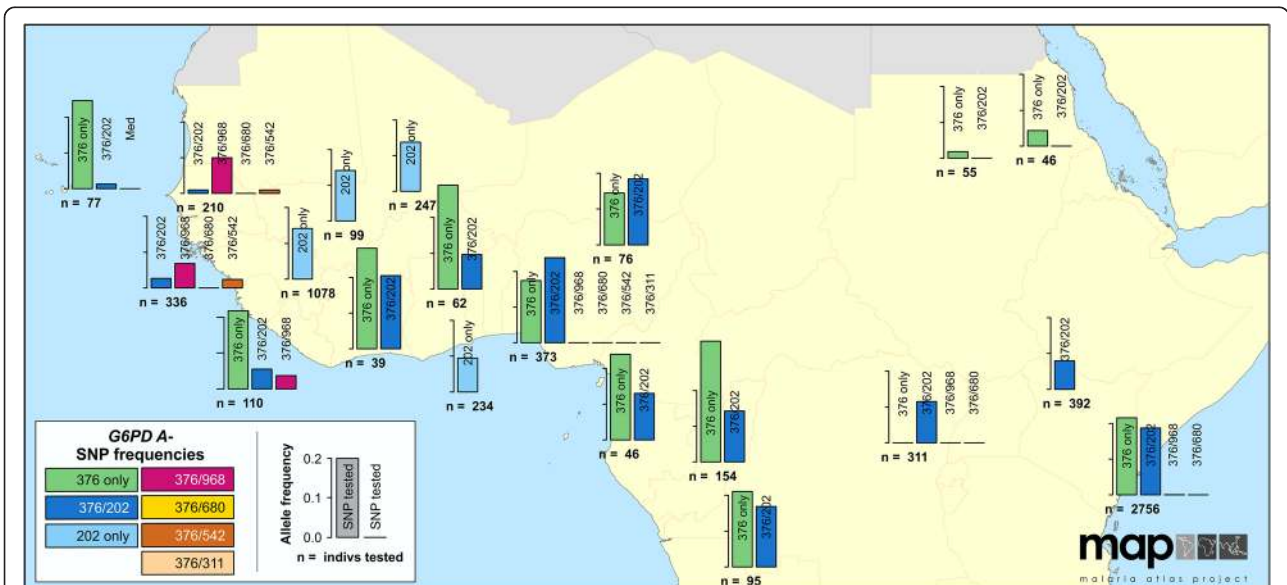
Figure 8 *G6PD A-* variant SNP frequency map for Africa (map series 2). Bar charts representing the frequencies of *G6PD* variants. The variants that were tested for in each location are listed above the *x*-axis. Empty spaces along the *x*-axis indicate that the named variant was tested for but not identified in the population sample. Survey locations are mapped to closest approximation at the point of origin of the plots and exact locations shown in Additional file 4. Sample size is listed under each plot. Equivalent maps of the Americas and Asia are Additional files 3 and 5.

(total G6PDd cases: 827; 29% across China), *Canton*[1376T] (792 cases; 27%), *Gaohe*[95G] (265 cases; 9%), *Chinese-5*[1024T] (104 cases; 4%) and *Quing Yan*[392T] (87 cases; 3%). The distribution of *G6PD Viangchan*[871A] was diffuse, reportedly common from Laos (where examination of 15 G6PDd individuals all carried this variant [Additional file 2: S34]) and Cambodia (reported from 61 out of 64 G6PDd individuals [Additional file 2: S43]) to Papua New Guinea (where a sample of 13 G6PDd individuals included nine with this variant [Additional file 2: S33]).

Variants across the Asia-Pacific region were also highly heterogeneous (Figure 7). The common pattern emerging from the 36 surveys of deficient individuals identified from this region ($n_{indivs} = 635$) was the diversity of variants and the heterogeneity of their prevalence among different populations. Furthermore, it should be noted that 20 of these surveys tested fewer than ten G6PDd individuals, limiting the diversity that would be captured and thus the representativeness of these reports. The greatest diversity identified in a single survey was from the Malaysian neonatal screening programme in Kuala Lumpur, which recorded ten variants from an investigation of 86 G6PDd newborns [Additional file 2: S4]. The short spatial-scale heterogeneity of this co-occurring variant diversity is illustrated by a set of surveys across the East Nusa Tenggara province of Indonesia (Figure 7B). Amid this co-occurring variant heterogeneity, the *G6PD Vanua Lava*[383C] variant was the most common variant reported from Indonesian studies (identified in 84 out of 249 G6PDd individuals tested).

Only three allele frequency surveys (map series 2) could be identified from the Asia region (of 24 globally) [Additional file 2: S66,S83]. *G6PD Mahidol* was most commonly tested for, with allele frequencies of 12% and 18% ($n_{tested} = 353$ and 11, respectively; Additional file 5). Two surveys in Thailand tested for a broader range of variants; one found five variants (*G6PD Canton, Kaiping, Mahidol, Union* and *Viangchan*; $n_{tested} = 84$) at allele frequencies of 1-2%; and the other, with a smaller sample size ($n_{tested} = 11$), identified only the *G6PD Mahidol* variant [Additional file 2: S66].

Discussion

Over a third of the world's population lives at risk of *P. vivax* infection [14,52,54]. Limited evidence underpins estimates of clinical cases, but these have been estimated at 70-400 million annually [55,56], including potentially severe illness and death [5,24,57,58]. In the context of malaria elimination, therapy must target all infections, including asymptomatic and submicroscopic blood-stage infections, dormant liver-stage hypnozoites as well as clinical cases [59,60]. One of the many consequences of a half-century of neglect of *P. vivax* has been the failure to address the primaquine toxicity problem with G6PDd

and thereby solve the real problem of lack of access to primaquine for almost all malaria patients. No non-toxic therapeutic alternatives exist, and existing G6PDd diagnostics are largely impractical in point-of-care settings [4,6,21,45,56].

Fear of harm also leads to avoidance of primaquine as a *P. falciparum* transmission-blocking agent. The single transmission-blocking 0.75 mg/kg dose, consistently inconsequential in *G6PD A-* volunteers, reliably caused 20-30% drops in haematocrit in healthy Europeans with *G6PD Mediterranean* [39]. This risk, acknowledged at least 30 years ago, was only acted upon in 2012 with the WHO offering a recommendation for a lower dose of primaquine (0.25 mg/kg) gametocytocide alongside *P. falciparum* blood schizontocidal treatment with no requirement for prior G6PDd testing [10,11,61-63]. Nonetheless, many nations, in particular in Africa where there has been an almost complete lack of experience with primaquine [61] and where G6PDd prevalence is commonly greater than 10% [2], still resist use of this drug. An understanding of the therapeutic risks from primaquine in relation to the *G6PD* variants predominant in each region will contribute to the body of evidence on which to base policy.

Access to safe therapy either requires an alternative non-haemolytic drug or a practical means to identify G6PDd *P. vivax* malaria patients in remote, impoverished sites. Neither is likely in the short-term and improved use of primaquine promises immediately applicable and useful prospects [21]. This aim demands refined knowledge of the spatial epidemiology and primaquine-sensitivity phenotypes of *G6PD* variants. Development of primaquine treatment strategy could exploit, for example, the total dose effect (whereby therapeutic efficacy is dependent upon the total drug dose irrespective of the regimen duration [64-66]), to tailor population/region-specific dosing parameterized according to the highest dose safely tolerated by individuals with the most vulnerable local *G6PD* variant.

G6PD variants in Africa

The G6PDd phenotype has long been considered homogenous across African populations, with much of the pre-molecular era literature reporting *G6PD A-* from electrophoresis assays across the continent [34]. The early investigations of "primaquine sensitivity" among *G6PD A-* volunteers found primaquine-induced haemolysis to be mild and self-limiting [1,30,64,67-69]. Nevertheless, the haemolytic susceptibility of this "mild" variant has been observed through severe reactions requiring transfusion [70], as well as through the failure of the Lapdap (chlorproguanil-dapsone) anti-malarial trials [71,72], and as haemolysis induced by the ingestion of fava beans [73], a pathology previously thought to only be triggered by more severe variants [74]. Haemolysis associated with

G6PD A-, while perhaps less severe than with other variants in some circumstances or settings, ought not be universally characterized as "mild" as this risks leading to misplaced confidence in a relatively cavalier application of the drug.

Molecular analyses of *G6PD* variants among Africans revealed a diversity of SNPs within the umbrella of the *G6PD* A- phenotype [75]. The data, here mapped spatially, indicated a transition of SNP predominance from west (*G6PD* A-^{968C} polymorphism) to east (*G6PD* A-^{202A} widespread and *G6PD* A-^{968C} apparently absent). However, expansion of the relatively small number of surveys (n = 20) may reveal more complex distributions. Furthermore, the true diversity of *G6PD* variants cannot be discerned from the assembled surveys; it is not possible to know what proportion of genetic diversity is reflected in the map (Figure 3) because the diagnoses are limited to assessing the presence or absence of a limited number of anticipated SNPs, without any prior identification of phenotypically deficient hosts (as in map series 1). Full gene sequencing is the only reliable way of identifying all gene variants in the population. Genetic diversity within the umbrella *G6PD* A- phenotype may explain the spectrum of expression levels observed [70]: inheritance of a single SNP may not be sufficient to explain the observed phenotype. If these patterns of co-occurring SNP diversity encoding variable *G6PD* A- phenotypes are widely observed, a haplotype-based approach which allows for the co-inheritance of multiple SNPs may be required if the use of molecular diagnostics is to the scaled-up, as seems to be increasingly common [75]. Despite its obvious limitations to field-based applications, a gene-wide haplotype perspective would also allow increased compatibility with the rapidly expanding human genome sequencing efforts which are cataloguing human genetic diversity [76,77].

Although demand for primaquine has historically been low across Africa [78], it was Ethiopia that reported the highest number of *P. vivax* cases globally in 2011 (665,813 cases; [79]). G6PDd prevalence has been estimated at 1.0% in Ethiopia (50% CI: 0.7-1.5) [2], though no molecular information exists to indicate which variants may be responsible. Furthermore, beyond these areas with acknowledged endemic *P. vivax*, the status of transmission in human populations previously considered refractory to infection [78] is being brought into question by several lines of evidence [80-86]. The use of primaquine for *P. falciparum* control may also increase following the new WHO gametocytocide guidelines [63]. The elimination agenda includes African nations, with most located at the fringes of entrenched holo-endemic transmission [87,88], and the pressure to apply primaquine both as a hypnozoitocide for *P. vivax* and *Plasmodium ovale*, and as a gametocytocide for *P. falciparum* will increase with further progress towards zero transmission. A more detailed and targeted understanding of African *G6PD* epidemiology is necessary beyond the opportunistic surveys presented in this paper.

G6PD variants in West Asia

The *G6PD Mediterranean* variant, appearing predominant across southwest Asia and exceedingly common in much of India (especially western regions), is known to be highly sensitive to AHA induced by primaquine. This wide distribution and dominance of *G6PD Mediterranean* presents a significant hurdle to the malaria elimination programmes in West Asia, which include Azerbaijan, Georgia, Iran, Iraq, Kyrgyzstan, Saudi Arabia, Tajikistan, Turkey, and Uzbekistan [89]. These nations must consider the relatively high risk of serious harm caused by unintentional dosing of G6PDd patients in need of malaria therapy. Implementation of G6PDd screening prior to primaquine radical cure therapy would greatly mitigate such risks and accelerate progress to malaria elimination in this region.

Although *G6PD Mediterranean* was common across Indian populations, two other variants were also frequently reported which were indigenous to populations from this subcontinent: *G6PD Kalyan-Kerala* and *Orissa*. Little is known of the primaquine sensitivity phenotypes of these two variants. Since almost half of the global population at risk of *P. vivax* malaria occurs in this single nation [52,79,90] studies characterizing such sensitivity would ultimately prove very useful in planning towards elimination.

G6PD in East Asia and the West Pacific

The maps of eastern Asia and the western Pacific present the most complex picture of *G6PD* variants globally, coinciding with regions of heterogeneous and high *P. vivax* endemicity [52]. Almost all the common variants of public health concern globally were reported from this region. Reasons for this genetic diversity are unclear, but it is interesting that *P. falciparum* parasites (postulated to be selective agents of G6PDd [16]) have been found to show a greater degree of population structure with lower genetic relatedness between populations in Asia than across Africa [91]. As well as this overall diversity, the structure of *G6PD* variant heterogeneity with multiple variants co-occurring is starkly different from other areas where single variants predominate.

Despite large variant diversity across this region where many countries now target elimination (thus require primaquine therapy) [89], only one truly Asian variant has been examined in relation to AHA sensitivity to primaquine. *G6PD Mahidol*, common across Myanmar and parts of Thailand (Figure 6), exhibits residual G6PD enzyme activity ranging between 5-32% [42]. A handful of small primaquine sensitivity studies examining this variant have been conducted in Thailand, reporting very

mild to moderate sensitivity to both 14-day and eight weekly primaquine regimens [40,92,93], as well as to single-dose regimens of *P. falciparum* transmission blocking therapy [94]. However, the *G6PD Mahidol* primaquine sensitivity phenotype must not be assumed to be representative of the region. As emphasized by the WHO Expert Review Group on primaquine in 2012 [62,63], far greater evidence-based understanding of these phenotypes among the many common Asian variants is urgently needed. The identity and distribution of variants as sensitive as *G6PD Mediterranean* in the Asia-Pacific region remains unexplored and unknown. This lack of understanding threatens patients and programmes with a more aggressive roll-out of primaquine therapy. In populations of such high heterogeneity, a dual approach of phenotypic screening followed by variant analysis of residual enzyme activity may be required to identify such risks.

The danger of under-diagnosing deficiency by using molecular identification alone (i e, the data informing map series 2) is illustrated by comparison of the two types of maps (Figure 2C and Additional file 5). If only a selected subset of perceived "common" variants are used in population screening surveys, a proportion of deficient individuals may be missed and put at risk from primaquine therapy: only those variants that are looked for can be identified. The maps presented here also repeatedly highlight considerable proportions of "Other" *G6PD* variants. These correspond to an unknown haemolytic risk, and hint towards an ever greater diversity of variants. Only full gene sequencing will allow full characterization of the diversity in this polymorphic gene.

Conclusions

Both the failure to treat *P. vivax* infections and the treatment itself carry some risk of severe clinical complications [5,24,62] (Recht J, Ashley EA, White NJ:8-aminoquinolines safety review for WHO primaquine ERG, unpublished). Each repeated episode of acute *P. vivax* malaria carries possibilities of delayed or improper diagnosis, improper treatment, onward transmission, serious illness, and death [5,24]. Likewise, primaquine-induced AHA in G6PDd patients may provoke renal failure and require multiple transfusions for recovery [24,43]. Improper clinical management of primaquine-induced haemolysis may lead to death [24], although the relative risks of this remain poorly documented and are likely to be rare. Similarly, scaled-up application of low-dose transmission-blocking primaquine against *P. falciparum* will likely emerge as a key strategy towards elimination, especially in the context of artemisinin-resistant *P. falciparum* malaria. Evidence-based assessment of the risks incurred with primaquine therapy in any given region is essential for the development of rational strategies to minimize harm caused by either the parasite or the drug used to attack it. The maps presented in this article offer a first glimpse at the distribution of variants of public health significance across malaria-endemic regions. Their implications are discussed with respect to the regionally specific issues associated with the ramping up of primaquine therapy on the strategic road to elimination.

Additional files

> **Additional file 1: Supplementary methods.**
>
> **Additional file 2: Sources from which the datapoints in the maps were identified.**
>
> **Additional file 3: Map series 2: Americas.** Bar charts represent population surveys which examined the frequencies of selected *G6PD* variants in representative population samples. These population groups had not undergone prior G6PDd phenotype screening. The variants which were tested for in each location are listed above the *x*-axis. Survey locations are indicated by nearby black stars. Sample size is stated under each plot.
>
> **Additional file 4: Map series 2: Africa.** (A) Bar charts represent population surveys which examined the frequencies of selected *G6PD* variants in representative population samples. These population groups had not undergone prior G6PDd phenotype screening. The variants which were tested for in each location are listed above the *x*-axis. Sample size is stated under each plot. Survey locations are mapped to closest approximation at the point of origin of the plots; exact survey locations are shown in Panel B. (Panel A is reproduced from Figure 8 in the main manuscript).
>
> **Additional file 5: Map series 2: Asia.** Bar charts represent population surveys which examined the frequencies of selected *G6PD* variants in representative population samples. These population groups had not undergone prior G6PDd phenotype screening. The variants which were tested for in each location are listed above the *x*-axis. Survey locations are indicated by nearby black stars. Sample size is stated under each plot.

Author details

[1]Spatial Ecology and Epidemiology Group, Department of Zoology, University of Oxford, South Parks Road, Oxford, UK. [2]Eijkman-Oxford Clinical Research Unit, Jalan Diponegoro No. 69, Jakarta, Indonesia. [3]Evolutionary Ecology of Infectious Disease Group, Department of Zoology, University of Oxford, South Parks Road, Oxford, UK. [4]Tropical Medicine Foundation Dr. Heitor Vieira Dourado, Manaus, Amazonas, Brazil. [5]University of the State of Amazonas, Manaus, Amazonas, Brazil. [6]Institut Pasteur, Unité de Génétique Fonctionnelle des Maladies Infectieuses, Paris, France. [7]Eijkman Institute for Molecular Biology, Jakarta, Indonesia. [8]Kenya Medical Research Institute/Wellcome Trust Programme, Centre for Geographic Medicine Research-Coast, Kilifi District Hospital, Kilifi, Kenya. [9]Department of Medicine, Imperial College, St Mary's Hospital, London, UK. [10]Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK.

References

1. Carson PE, Flanagan CL, Ickes CE, Alving AS: **Enzymatic deficiency in primaquine-sensitive erythrocytes.** *Science* 1956, **124**:484–485.
2. Howes RE, Piel FB, Patil AP, Nyangiri OA, Gething PW, Hogg MM, Battle KE, Padilla CD, Baird JK, Hay SI: **G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map.** *PLoS Med* 2012, **9**:e1001339.
3. The malERA Consultative Group on Diagnoses and Diagnostics: **A research agenda for malaria eradication: diagnoses and diagnostics.** *PLoS Med* 2011, **8**:e1000396.
4. Baird JK, Surjadjaja C: **Consideration of ethics in primaquine therapy against malaria transmission.** *Trends Parasitol* 2011, **27**:11–16.
5. Baird JK: **Evidence and implications of mortality associated with acute** *Plasmodium vivax* **malaria.** *Clin Microbiol Rev* 2013, **26**:36–57.
6. Baird JK: **Reinventing primaquine for endemic malaria.** *Expet Opin Emerg Drugs* 2012, **17**:439–444.
7. White NJ: **The role of anti-malarial drugs in eliminating malaria.** *Malar J* 2008, **7**(Suppl 1):S8.
8. The malERA Consultative Group on Drugs: **A research agenda for malaria eradication: drugs.** *PLoS Med* 2011, **8**:e1000402.
9. White NJ: **Determinants of relapse periodicity in** *Plasmodium vivax* **malaria.** *Malar J* 2011, **10**:297.
10. Bousema T, Drakeley C: **Epidemiology and infectivity of** *Plasmodium falciparum* **and** *Plasmodium vivax* **gametocytes in relation to malaria control and elimination.** *Clin Microbiol Rev* 2011, **24**:377–410.
11. White NJ: **Primaquine to prevent transmission of falciparum malaria.** *Lancet Infect Dis* 2012, **13**:175–181.
12. WHO: *Guidelines for the treatment of malaria, second edition.* Geneva: World Health Organization; 2010.
13. WHO: *Global plan for artemisinin resistance containment (GPARC).* Geneva: WHO; 2011.
14. White NJ, Qiao LG, Qi G, Luzzatto L: **Rationale for recommending a lower dose of primaquine as a** *Plasmodium falciparum* **gametocytocide in populations where G6PD deficiency is common.** *Malar J* 2012, **11**:418.
15. Maude RJ, Socheat D, Nguon C, Saroth P, Dara P, Li G, Song J, Yeung S, Dondorp AM, Day NP, White NJ, White LJ: **Optimising strategies for** *Plasmodium falciparum* **malaria elimination in Cambodia: primaquine, mass drug administration and artemisinin resistance.** *PLoS One* 2012, **7**:e37166.
16. Greene LS: **G6PD deficiency as protection against falciparum-malaria: an epidemiologic critique of population and experimental studies.** *Yearb Phys Anthropol* 1993, **36**:153–178.
17. Kwiatkowski DP: **How malaria has affected the human genome and what human genetics can teach us about malaria.** *Am J Hum Genet* 2005, **77**:171–192.
18. Beutler E: **G6PD: population genetics and clinical manifestations.** *Blood Rev* 1996, **10**:45–52.
19. Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar DK, Alonso PL, del Portillo HA: **Key gaps in the knowledge of** *Plasmodium vivax*, **a neglected human malaria parasite.** *Lancet Infect Dis* 2009, **9**:555–566.
20. Cappellini MD, Fiorelli G: **Glucose-6-phosphate dehydrogenase deficiency.** *Lancet* 2008, **371**:64–74.
21. Howes RE, Battle KE, Satyagraha AW, Baird JK, Hay SI: **G6PD deficiency: Global distribution, genetic variants and primaquine therapy.** *Adv Parasitol* 2013, **81**:133–201.
22. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basanez MG, Baxter A, Bell ML, Benjamin EJ, *et al*: **Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the global burden of disease study 2010.** *Lancet* 2012, **380**:2197–2223.
23. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, *et al*: **Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010.** *Lancet* 2012, **380**:2095–2128.
24. Lacerda MV, Fragoso SC, Alecrim MG, Alexandre MA, Magalhaes BM, Siqueira AM, Ferreira LC, Araujo JR, Mourao MP, Ferrer M, Castillo P, Martin-Jaular L, Fernandez-Becerra C, del Portillo H, Ordi J, Alonso PL, Bassat Q: **Postmortem characterization of patients with clinical diagnosis of** *Plasmodium vivax* **malaria: to what extent does this parasite kill?** *Clin Infect Dis* 2012, **55**:e67–e74.
25. Luzzatto L: **Glucose-6-phosphate dehydrogenase deficiency.** In *Nathan and Oski's Hematology of Infancy and Childhood.* 7th edition. Edited by Orkin SH, Nathan DG, Ginsburg D, Look AT, Fisher DE, Lux SE. Philadelphia: Saunders; 2009.
26. Minucci A, Moradkhani K, Hwang MJ, Zuppi C, Giardina B, Capoluongo E: **Glucose-6-phosphate dehydrogenase (G6PD) mutations database: review of the "old" and update of the new mutations.** *Blood Cells Mol Dis* 2012, **48**:154–165.
27. Mason PJ, Vulliamy TJ: **Glucose-6-phosphate dehydrogenase (G6PD) deficiency: genetics.** In *Encyclopedia of Life Sciences.* Chichester: John Wiley & Sons, Ltd; 2005.
28. WHO Working Group: **Glucose-6-phosphate dehydrogenase deficiency.** *Bull World Health Organ* 1989, **67**:601–611.
29. Yoshida A, Beutler E, Motulsky AG: **Human glucose-6-phosphate dehydrogenase variants.** *Bull World Health Organ* 1971, **45**:243–253.
30. Hockwald RS, Arnold J, Clayman CB, Alving AS: **Toxicity of primaquine in Negroes.** *JAMA* 1952, **149**:1568–1570.
31. Beutler E: **Glucose-6-phosphate dehydrogenase deficiency.** *N Engl J Med* 1991, **324**:169–174.
32. Dern RJ, Beutler E, Alving AS: **The hemolytic effect of primaquine. II. The natural course of the hemolytic anemia and the mechanism of its self-limited character.** *J Lab Clin Med* 1954, **44**:171–176.
33. Alving AS, Johnson CF, Tarlov AR, Brewer GJ, Kellermeyer RW, Carson PE: **Mitigation of the haemolytic effect of primaquine and enhancement of its action against exoerythrocytic forms of the Chesson strain of** *Plasmodium vivax* **by intermittent regimens of drug administration: a preliminary report.** *Bull World Health Organ* 1960, **23**:621–631.
34. Cavalli-Sforza LL, Menozzi P, Piazza A: *The History and Geography of Human Genes.* Princeton, New Jersey: Princeton University Press; 1994.
35. Piomelli S, Corash LM, Davenport DD, Miraglia J, Amorosi EL: **In vivo lability of glucose-6-phosphate dehydrogenase in Gd^{A-} and Gd^{Mediterranean} deficiency.** *J Clin Invest* 1968, **47**:940–948.

36. Meloni T, Forteleoni G, Dore A, Cutillo S: Favism and hemolytic anemia in glucose-6-phosphate dehydrogenase-deficient subjects in North Sardinia. *Acta Haematol* 1983, **70**:83–90.
37. Luisada A: *Favism. JAMA* 1940, **115**:632–632.
38. Beutler E, Duparc S: Glucose-6-phosphate dehydrogenase deficiency and antimalarial drug development. *Am J Trop Med Hyg* 2007, **77**:779–789.
39. Clyde DF: Clinical problems associated with the use of primaquine as a tissue schizontocidal and gametocytocidal drug. *Bull World Health Organ* 1981, **59**:391–395.
40. Buchachart K, Krudsood S, Singhasivanon P, Treeprasertsuk S, Phophak N, Srivilairit S, Chalermrut K, Rattanapong Y, Supeeranuntha L, Wilairatana P, Brittenham G, Looareesuwan S: Effect of primaquine standard dose (15 mg/day for 14 days) in the treatment of vivax malaria patients in Thailand. *Southeast Asian J Trop Med Public Health* 2001, **32**:720–726.
41. Matsuoka H, Wang J, Hirai M, Arai M, Yoshida S, Kobayashi T, Jalloh A, Lin K, Kawamoto F: Glucose-6-phosphate dehydrogenase (G6PD) mutations in Myanmar: G6PD Mahidol (487G > A) is the most common variant in the Myanmar population. *J Hum Genet* 2004, **49**:544–547.
42. Louicharoen C, Patin E, Paul R, Nuchprayoon I, Witoonpanich B, Peerapittayamongkol C, Casadement I, Sura T, Laird NM, Singhasivanon P, Quintana-Murci L, Sakuntabhai A: Positively selected G6PD-Mahidol mutation reduces *Plasmodium vivax* density in Southeast Asians. *Science* 2009, **326**:1546–1549.
43. Burgoine KL, Bancone G, Nosten F: The reality of using primaquine. *Malar J* 2010, **9**:376.
44. Laosombat V, Sattayasevana B, Chotsampancharoen T, Wongchanchailert M: Glucose-6-phosphate dehydrogenase variants associated with favism in Thai children. *Int J Hematol* 2006, **83**:139–143.
45. Baird JK: Elimination therapy for the endemic malarias. *Curr Infect Dis Rep* 2012, **14**:227–237.
46. Mason PJ, Bautista JM, Gilsanz F: G6PD deficiency: the genotype-phenotype association. *Blood Rev* 2007, **21**:267–283.
47. Mourant AE, Kopec AC, Domaniewska-Sobczak K: *The distribution of the human blood groups and other polymorphisms.* London: Oxford University Press; 1976.
48. Livingstone FB: *Frequencies of hemoglobin variants: thalassemia, the glucose-6-phosphate dehydrogenase deficiency, G6PD variants and ovalocytosis in human populations.* New York: Oxford University Press; 1985.
49. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E: The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. *Blood Cells Mol Dis* 2009, **42**:267–278.
50. Singh S: Distribution of certain polymorphic traits in populations of the Indian peninsula and South Asia. *Isr J Med Sci* 1973, **9**:1225–1237.
51. Gething PW, Patil AP, Smith DL, Guerra CA, Elyazar IR, Johnston GL, Tatem AJ, Hay SI: A new world malaria map: *Plasmodium falciparum* endemicity in 2010. *Malar J* 2011, **10**:378.
52. Gething PW, Elyazar IR, Moyes CL, Smith DL, Battle KE, Guerra CA, Patil AP, Tatem AJ, Howes RE, Myers MF, George DB, Horby P, Wertheim HFL, Price RN, Mueller I, Baird JK, Hay SI: A long neglected world malaria map: *Plasmodium vivax* endemicity in 2010. *PLoS Negl Trop Dis* 2012, **6**:e1814.
53. Hay SI, Guerra CA, Gething PW, Patil AP, Tatem AJ, Noor AM, Kabaria CW, Manh BH, Elyazar IR, Brooker S, Smith DL, Moyeed RA, Snow RW: A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLoS Med* 2009, **6**:e1000048.
54. Battle KE, Gething PW, Elyazar IR, Moyes CL, Sinka ME, Howes RE, Guerra CA, Price RN, Baird KJ, Hay SI: The global public health significance of *Plasmodium vivax*. *Adv Parasitol* 2012, **80**:1–111.
55. Hay SI, Guerra CA, Tatem AJ, Noor AM, Snow RW: The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect Dis* 2004, **4**:327–336.
56. Mendis K, Sina BJ, Marchesini P, Carter R: The neglected burden of *Plasmodium vivax* malaria. *Am J Trop Med Hyg* 2001, **64**:97–106.
57. Singh H, Parakh A, Basu S, Rath B: *Plasmodium vivax* malaria: is it actually benign? *J Infect Pub Health* 2011, **4**:91–95.
58. Mahgoub H, Gasim GI, Musa IR, Adam I: Severe *Plasmodium vivax* malaria among Sudanese children at New Halfa Hospital, Eastern Sudan. *Parasit Vectors* 2012, **5**:154.
59. Douglas NM, Nosten F, Ashley EA, Phaiphun L, van Vugt M, Singhasivanon P, White NJ, Price RN: *Plasmodium vivax* recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. *Clin Infect Dis* 2011, **52**:612–620.

60. Harris I, Sharrock WW, Bain LM, Gray KA, Bobogare A, Boaz L, Lilley K, Krause D, Vallely A, Johnson ML, Gatton ML, Shanks GD, Cheng Q: A large proportion of asymptomatic *Plasmodium* infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: challenges for malaria diagnostics in an elimination setting. *Malar J* 2010, **9**:254.
61. Eziefula AC, Gosling R, Hwang J, Hsiang MS, Bousema T, Von Seidlein L, Drakeley C, On behalf of the Primaquine in Africa Discussion Group: Rationale for short course primaquine in Africa to interrupt malaria transmission. *Malar J* 2012, **11**:360.
62. Malaria Policy Advisory Committee Meeting: *WHO Evidence Review Group Report: the safety and effectiveness of single dose primaquine as a P. falciparum gametocytocide.* Geneva: World Health Organization; 2012.
63. Global Malaria Programme WHO: *Updated WHO Policy Recommendation: Single dose Primaquine as a gametocytocide in Plasmodium falciparum malaria.* Geneva: World Health Organization; 2012.
64. Edgcomb JH, Arnold J, Yount EH Jr, Alving AS, Eichelberger L, Jeffery GM, Eyles D, Young MD: Primaquine, SN 13272, a new curative agent in vivax malaria; a preliminary report. *J Natl Malar Soc* 1950, **9**:285–292.
65. Alving AS, Hankey DD, Coatney GR, Jones R Jr, Coker WG, Garrison PL, Donovan WN: Korean vivax malaria. II. Curative treatment with pamaquine and primaquine. *Am J Trop Med Hyg* 1953, **2**:970–976.
66. Coatney GR, Alving AS, Jones R Jr, Hankey DD, Robinson DH, Garrison PL, Coker WG, Donovan WN, Di Lorenzo A, Marx RL, Simmons IH: Korean vivax malaria. V. Cure of the infection by primaquine administered during long-term latency. *Am J Trop Med Hyg* 1953, **2**:985–988.
67. Flanagan CL, Schrier SL, Carson PE, Alving AS: The hemolytic effect of primaquine. VIII. The effect of drug administration on parameters of primaquine sensitivity. *J Lab Clin Med* 1958, **51**:600–608.
68. Beutler E: The hemolytic effect of primaquine and related compounds: a review. *Blood* 1959, **14**:103–139.
69. Alving AS, Craige B, Pullman TN, Whorton CM, Jones R, Eichelberger L: Procedures used at Stateville Penitentiary for the testing of potential antimalarial agents. *J Clin Invest* 1948, **27**:2–5.
70. Shekalaghe SA, ter Braak R, Daou M, Kavishe R, van den Bijllaardt W, van den Bosch S, Koenderink JB, Luty AJ, Whitty CJ, Drakeley C, Sauerwein RW, Bousema T: In Tanzania, hemolysis after a single dose of primaquine coadministered with an artemisinin is not restricted to glucose-6-phosphate dehydrogenase-deficient (G6PD A-) individuals. *Antimicrob Agents Chemother* 2010, **54**:1762–1768.
71. Luzzatto L: The rise and fall of the antimalarial Lapdap: a lesson in pharmacogenetics. *Lancet* 2010, **376**:739–741.
72. Pamba A, Richardson ND, Carter N, Duparc S, Premji Z, Tiono AB, Luzzatto L: Clinical spectrum and severity of hemolytic anemia in glucose 6-phosphate dehydrogenase-deficient children receiving dapsone. *Blood* 2012, **120**:4123–4133.
73. Galiano S, Gaetani GF, Barabino A, Cottafava F, Zeitlin H, Town M, Luzzatto L: Favism in the African type of glucose-6-phosphate dehydrogenase deficiency (A-). *BMJ* 1990, **300**:236.
74. Mehta AB: Glucose-6-phosphate dehydrogenase deficiency. *Postgrad Med J* 1994, **70**:871–877.
75. Clark TG, Fry AE, Auburn S, Campino S, Diakite M, Green A, Richardson A, Teo YY, Small K, Wilson J, Jallow M, Sisay-Joof F, Pinder M, Sabeti P, Kwiatkowski DP, Rockett KA: Allelic heterogeneity of G6PD deficiency in West Africa and severe malaria susceptibility. *Eur J Hum Genet* 2009, **17**:1080–1085.
76. 1000 Genomes - A Deep Catalog of Human Genetic Variation. In. www.1000genomes.org.
77. International HapMap Project. In. http://hapmap.ncbi.nlm.nih.gov.
78. Howes RE, Patil AP, Piel FB, Nyangiri OA, Kabaria CW, Gething PW, Zimmerman PA, Barnadas C, Beall CM, Gebremedhin A, Menard D, Williams TN, Weatherall DJ, Hay SI: The global distribution of the Duffy blood group. *Nat Commun* 2011, **2**:266.
79. WHO: *World Malaria Report 2012.* Geneva: World Health Organization; 2012.
80. Boyd MF: *An Introduction to Malariology.* Cambridge, MA: Harvard University Press; 1930.
81. Guerra CA, Howes RE, Patil AP, Gething PW, Van Boeckel TP, Temperley WH, Kabaria CW, Tatem AJ, Manh BH, Elyazar IR, Baird JK, Snow RW, Hay SI: The international limits and population at risk of *Plasmodium vivax* transmission in 2009. *PLoS Negl Trop Dis* 2010, **4**:e774.

82. Ryan JR, Stoute JA, Amon J, Dunton RF, Mtalib R, Koros J, Owour B, Luckhart S, Wirtz RA, Barnwell JW, Rosenberg R: **Evidence for transmission of** *Plasmodium vivax* **among a duffy antigen negative population in Western Kenya.** *Am J Trop Med Hyg* 2006, **75:**575–581.

83. Culleton R, Ndounga M, Zeyrek FY, Coban C, Casimiro PN, Takeo S, Tsuboi T, Yadava A, Carter R, Tanabe K: **Evidence for the transmission of** *Plasmodium vivax* **in the Republic of the Congo, West Central Africa.** *J Infect Dis* 2009, **200:**1465–1469.

84. Menard D, Barnadas C, Bouchier C, Henry-Halldin C, Gray LR, Ratsimbasoa A, Thonier V, Carod JF, Domarle O, Colin Y, Bertrand O, Picot J, King CL, Grimberg BT, Mercereau-Puijalon O, Zimmerman PA: *Plasmodium vivax* **clinical malaria is commonly observed in duffy-negative malagasy people.** *Proc Natl Acad Sci USA* 2010, **107:**5967–5971.

85. Mendes C, Dias F, Figueiredo J, Mora VG, Cano J, De Sousa B, Do Rosario VE, Benito A, Berzosa P, Arez AP: **Duffy negative antigen is no longer a barrier to** *plasmodium vivax*–**molecular evidences from the African West Coast (Angola and Equatorial Guinea).** *PLoS Negl Trop Dis* 2011, **5:**e1192.

86. Wurtz N, Mint Lekweiry K, Bogreau H, Pradines B, Rogier C, Ould Mohamed Salem Boukhary A, Hafid JE, Ould Ahmedou Salem MS, Trape JF, Basco LK, Briolant S: **Vivax malaria in mauritania includes infection of a duffy-negative individual.** *Malar J* 2011, **10:**336.

87. Feachem RG, Phillips AA, Hwang J, Cotter C, Wielgosz B, Greenwood BM, Sabot O, Rodriguez MH, Abeyasinghe RR, Ghebreyesus TA, Snow RW: **Shrinking the malaria map: progress and prospects.** *Lancet* 2010, **376:**1566–1578.

88. Cotter C, Sturrock HJ, Hsiang MS, Liu J, Phillips AA, Hwang J, Gueye CS, Fullman N, Gosling RD, Feachem RG: **The changing epidemiology of malaria elimination: new strategies for new challenges.** *Lancet* 2013, **382**(9895):900–911.

89. UCSF Global Health Group and Malaria Atlas Project: *Atlas of Malaria-Eliminating Countries.* University of California, San Francisco: The Global Health Group, Global Health Sciences; 2011.

90. Hay SI, Gething PW, Snow RW: **India's invisible malaria burden.** *Lancet* 2010, **376:**1716–1717.

91. Manske M, Miotto O, Campino S, Auburn S, Almagro-Garcia J, Maslen G, O'Brien J, Djimde A, Doumbo O, Zongo I, Ouedraogo JB, Michon P, Mueller I, Siba P, Nzila A, Borrmann S, Kiara SM, Marsh K, Jiang H, Su XZ, Amaratunga C, Fairhurst R, Socheat D, Nosten F, Imwong M, White NJ, Sanders M, Anastasi E, Alcock D, Drury E, *et al*: **Analysis of** *Plasmodium falciparum* **diversity in natural infections by deep sequencing.** *Nature* 2012, **487:**375–379.

92. Takeuchi R, Lawpoolsri S, Imwong M, Kobayashi J, Kaewkungwal J, Pukrittayakamee S, Puangsa-art S, Thanyavanich N, Maneeboonyang W, Day NP, Singhasivanon P: **Directly-observed therapy (DOT) for the radical 14-day primaquine treatment of** *Plasmodium vivax* **malaria on the Thai-Myanmar border.** *Malar J* 2010, **9:**308.

93. Myat Phone K, Myint O, Aung N, Aye Lwin H: **The use of primaquine in malaria infected patients with red cell glucose-6-phosphate dehydrogenase (G6PD) deficiency in Myanmar.** *Southeast Asian J Trop Med Public Health* 1994, **25:**710–713.

94. Song J, Socheat D, Tan B, Dara P, Deng C, Sokunthea S, Seila S, Ou F, Jian H, Li G: **Rapid and effective malaria control in Cambodia through mass administration of artemisinin-piperaquine.** *Malar J* 2010, **9:**57.