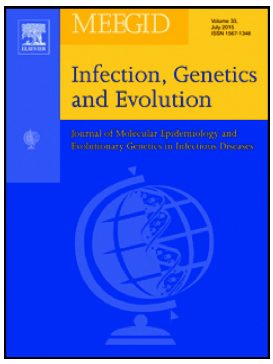

Marked variation in prevalence of malaria-protective human genetic polymorphisms across Uganda

Andrew Walakira, Stephen Tukwasibwe, Moses Kiggundu, Federica Verra, Patrick Kakeeto, Emmanuel Ruhamyankaka, Chris Drakeley, Grant Dorsey, Moses R. Kamya, Samuel L. Nsobya, Philip J. Rosenthal

PII: S1567-1348(17)30327-1
DOI: doi: 10.1016/j.meegid.2017.09.021
Reference: MEEGID 3277

To appear in: *Infection, Genetics and Evolution*

Received date: 6 June 2017
Revised date: 16 August 2017
Accepted date: 18 September 2017

Please cite this article as: Andrew Walakira, Stephen Tukwasibwe, Moses Kiggundu, Federica Verra, Patrick Kakeeto, Emmanuel Ruhamyankaka, Chris Drakeley, Grant Dorsey, Moses R. Kamya, Samuel L. Nsobya, Philip J. Rosenthal , Marked variation in prevalence of malaria-protective human genetic polymorphisms across Uganda, *Infection, Genetics and Evolution* (2017), doi: 10.1016/j.meegid.2017.09.021

Marked variation in prevalence of malaria-protective human genetic polymorphisms across Uganda

Andrew Walakira,[a] Stephen Tukwasibwe,[a] Moses Kiggundu,[a] Federica Verra,[b*] Patrick Kakeeto,[a] Emmanuel Ruhamyankaka,[a] Chris Drakeley,[b] Grant Dorsey,[c] Moses R. Kamya,[a,d] Samuel L. Nsobya,[a,e] Philip J. Rosenthal[c]

[a]Infectious Diseases Research Collaboration, Kampala, Uganda; [b]London School of Hygiene and Tropical Medicine, London, United Kingdom; [c]Department of Medicine, University of California, San Francisco, CA, USA; [d]Department of Medicine, Makerere University, Kampala, Uganda; [e]Department of Pathology, Makerere University, Kampala, Uganda

*Current address: Centre for Tropical Diseases, Sacro Cuore-Don Calabria Hospital, 37024 Negrar, Verona, Italy

Corresponding author: Philip J. Rosenthal, Box 0811, University of California, San Francisco 94143 USA; Phone: 1-415-206-8845; e-mail: philip.rosenthal@ucsf.edu

Abstract

A number of human genetic polymorphisms are prevalent in tropical populations and appear to offer protection against symptomatic and/or severe malaria. We compared the prevalence of four polymorphisms, the sickle hemoglobin mutation (β globin E6V), the α-thalassemia 3.7 kb deletion, glucose-6-phosphate dehydrogenase deficiency caused by the common African variant (G6PD A-), and the CD36 T188G mutation in 1,344 individuals residing in districts in eastern (Tororo), south-central (Jinja), and southwestern (Kanungu) Uganda. Genes of interest were amplified, amplicons subjected to mutation-specific restriction endonuclease digestion (for sickle hemoglobin, G6PD A-, and CD36 T188G), reaction products resolved by electrophoresis, and genotypes determined based on the sizes of reaction products. Mutant genotypes were common, with many more heterozygous than homozygous alleles identified. The prevalences (heterozygotes plus homozygotes) of sickle hemoglobin (28% Tororo, 25% Jinja, 7% Kanungu), α-thalassemia (53% Tororo, 45% Jinja, 18% Kanungu) and G6PD A- (29% Tororo, 18% Jinja, 8% Kanungu) were significantly greater in Tororo and Jinja compared to Kanungu ($p<0.0001$ for all three alleles); prevalences were also significantly greater in Tororo compared to Jinja for α-thalassemia ($p = 0.03$) and G6PD A- ($p<0.0001$). For the CD36 T188G mutation, the prevalence was significantly greater in Tororo compared to Jinja or Kanungu (27% Tororo, 17% Jinja, 18% Kanungu; $p = 0.0004$ and 0.0017, respectively). Considering ethnicity of study subjects, based on primary language spoken, the prevalence of mutant genotypes was lower in Bantu compared to non-Bantu language speakers, but in the Jinja cohort, the only study population with a marked diversity of language groups, prevalence did not differ between Bantu and non-Bantu speakers. These results indicate marked differences in human genetic features between populations in different regions of Uganda. These differences might be explained by both ethnic variation and by varied malaria risk in different regions of Uganda.

Keywords: Malaria, Plasmodium, Sickle, Thalassemia, G6PD, CD36

1. Introduction

Multiple human genetic polymorphisms are prevalent in tropical populations and offer protection against malaria, suggesting that these were selected in human populations due to protection against death from *Plasmodium falciparum* infection (Hedrick, 2011). As first proposed by Haldane, disadvantages of a homozygous mutation can be balanced by advantages to heterozygotes in protection against infectious diseases (Haldane, 1949). It has been suggested that malaria has offered the strongest evolutionary pressure of any infectious disease in recent human history (Kwiatkowski, 2005), and disorders of erythrocytes are the most common genetic disorders of humans (Weatherall, 2008).

Strong evidence supports balanced polymorphisms for sickle cell disease, α-thalassemia, and glucose-6-phosphate dehydrogenase (G6PD) deficiency, all of which are deleterious primarily in homozygotes, but appear to offer protection against severe malaria in heterozygotes, and are most prevalent in populations currently or historically at high risk of mortality from falciparum malaria (Kwiatkowski, 2005; Taylor and Fairhurst, 2014; Verra et al., 2009; Williams, 2006). The sickle hemoglobin mutation (β globin E6V) has a frequency of up to about 20% in populations in Africa, southern Europe, and India, and in multiple case-control studies the heterozygous AS genotype has been associated with over 70% protection against severe malaria (Kwiatkowski, 2005; Verra et al., 2009). α-thalassemia, due to deletion of one or more linked β globin genes, is very common in many tropical populations; the common variant in Africa contains a 3.7 kb deletion (Hedrick, 2011). α-thalassemia has been associated with marked protection against severe malaria in multiple studies from Africa (May et al., 2007; Williams et al., 2005) and elsewhere (Allen et al., 1997). G6PD deficiency is the most common enzyme deficiency in humans (Nkhoma et al., 2009). The common G6PD deficiency genotype in African populations is G6PD A- (V68M and N126D), which leads to an enzyme deficiency that is marked, but not as severe as with some other genetic variants (Town et al., 1992). G6PD A-

has been associated with protection against severe malaria in African populations (Guindo et al., 2007; Ruwende et al., 1995), although associations for this polymorphism have been less consistent than for sickle hemoglobin and α-thalassemia (Verra et al., 2009).

The human CD36 antigen is an integral membrane protein in many cell types and a member of the scavenger receptor family that imports fatty acids into cells (Canton et al., 2013). CD36 is also an endothelial receptor for binding of erythrocytes infected with *P. falciparum*; this cytoadhesion is believed to be an important feature of the virulence of falciparum malaria, due both to its prevention of clearance of infected erythrocytes by the spleen and to local effects of cytoadhering parasites (Newbold et al., 1999). Importantly, expression of CD36 is low in the brain, even in the setting of cerebral malaria (Silamut et al., 1999), suggesting that binding to CD36 is most relevant in non-cerebral forms of severe malaria. CD36 is also believed to be an important macrophage pattern recognition receptor that mediates innate recognition and clearance of infected erythrocytes (Cabrera et al., 2014). Considering our current understanding, CD36 expression might be seen to contribute to malaria severity, by mediating cytoadherence, or to help control malaria, via immune effects. Results with murine malaria models have been complex; mice with decreased CD36-mediated cytoadherence had decreased growth of *P. berghei* (Fonager et al., 2012), but CD36-deficient mice had increased risk of fatal *P. chabaudi* malaria (Patel et al., 2007). Considering human populations, many CD36 polymorphisms, including nonsense mutations that prevent expression of the protein, are common, particularly in African populations (Aitman et al., 2000). However, attempts to identify associations between common polymorphisms and malaria risk have led to inconsistent results, with evidence for enhancement of (Aitman et al., 2000; Omi et al., 2002), no effect (Fry et al., 2009), or protection from (Das et al., 2009; Omi et al., 2003; Pain et al., 2001; Sinha et al., 2008) severe malaria with different polymorphisms.

High prevalence of malaria-protective genetic polymorphisms is clearly associated with malaria endemicity, but prevalence varies among populations in endemic areas. Some of this difference can be explained by local malaria risk, as suggested by decreased prevalence of protective polymorphisms with increasing altitude in endemic countries (Hedrick, 2011). The prevalence of some protective polymorphisms has also been shown to vary between ethnic groups. In West Africa, the Fulani ethnic group has decreased susceptibility to malaria compared to Dogon populations (Bereczky et al., 2006), and the Fulani also have decreased prevalence of sickle hemoglobin (Nasr et al., 2008), α-thalassemia (Modiano et al., 2001a), and G6PD A- (Maiga et al., 2014; Modiano et al., 2001a) compared to other groups. In this case decreased malaria is likely not explained by genetic polymorphisms, but rather selective pressure for the balanced polymorphisms may have been lower in the Fulani due to decreased malaria incidence. In order to characterize the prevalence of key malaria-protective polymorphisms in Uganda, where malaria risk varies between regions of the country and ethnic diversity is great, we characterized polymorphisms in residents of three regions of the country.

2. Materials and methods

2.1. Study populations.

Cohorts were enrolled in three regions of Uganda (Kamya et al., 2015). The study sites were Nagongera Sub-county, Tororo District, a rural area with high transmission intensity in southeastern Uganda near the Kenyan border; Walukuba Sub-county, a peri-urban area near the city of Jinja and adjoining Lake Victoria in south-central Uganda; and Kihihi Sub-county, Kanungu District, a rural area with moderate transmission intensity in southwestern Uganda (Figure 1). To establish the cohorts, all households within the three sub-counties were enumerated and mapped, and randomly selected households that included at least one resident 0.5-10 years of age and one adult resident were enrolled, as previously described (Kamya et al., 2015). This study included cohort members reported previously and additional subjects

recruited into the cohorts after prior reports (Kamya et al., 2015; Rek et al., 2016). Cohort household adults provided the primary language spoken by the household. All cohort subjects provided a blood sample for genetic analysis. The parent cohort study and the genetic evaluations described in this report were approved by the Makerere University Research and Ethics Committee, the Uganda National Council for Science and Technology, and the University of California, San Francisco Committee on Human Research.

2.2. Laboratory reagents.

All PCR and restriction endonuclease reagents were from New England Biolabs, except as noted. Other reagents were from Sigma-Aldrich, or as noted. Molecular grade water was used in all reactions.

2.3. Sample collection and DNA purification.

Blood samples were collected into EDTA tubes, and DNA was purified from buffy coats using QIAamp DNA Mini Kits (Qiagen), following manufacturer's instructions with minor modifications. For each sample 300 µl of buffy coat was mixed with 20 µl of kit protease enzyme solution and then 200 µl of lysis buffer, the mixture was vortexed for 15 sec and incubated at 56°C for 10 min, and then 200 µl of absolute ethanol was added. The mixture was vortexed briefly and transferred to a QIAamp column, and the column was spun for 1 min at 8000 rpm. The column was then washed twice with wash buffer, and DNA was eluted by incubating with 80 µl of elution buffer at room temperature for 5 min followed by centrifugation at 8,000 rpm for 5 min.

2.4. Characterization of sickle hemoglobin.

As previously described (Modiano et al., 2001b), 2 µl of purified DNA was amplified in a 50 µL reaction consisting of 5 µl of PCR 10X Taq buffer, 1.0 µl each of 10 µM forward (5'-

AGGAGCAGGGAGGGCAGG A -3') and reverse (5'-TCCAAGGGTAGACCACCAGC-3') primers, 5 µL of each dNTP (2 mM), and 0.2 µl *Taq* DNA polymerase (5 U/µL). PCR conditions were 96°C for 5 min followed by 30 cycles of 96°C for 30 sec, 60°C for 1 min, and 72°C for 30 sec, followed by 72°C for 10 min. 15 µl of the PCR product was digested by incubating at 37°C for 3 h with 2 µL10X CutSmart buffer, 0.2 µL BSA, 1 µL *Mnl*I (5 U/µL) or 0.5 µL *Dde*I (10 U/µL) endonuclease, and 1.8 µL water. All PCR reactions described in this report included positive and negative controls. Reaction products were resolved in 3% agarose gels and stained with ethidium bromide, and sizes were determined based on DNA ladders. Expected fragment sizes were, for *Mnl*I reactions: HbAA: 173 bp, 109 bp, and 60 bp; HbCC/SS/SC: 173 bp, 109 bp, and 76 bp; HbAC/AS: 173 bp, 109 bp, 76bp, and 60 bp; and for *Dde*I reactions: HbSS: 331 bp; HbSC: 130 bp, 201 bp, and 331 bp; HbCC: 201 bp and 130 bp; HbAS: 130bp, 201bp, and 331bp; HbAC: 130 bp and 201 bp.

2.5. Characterization of α-thalassemia 3.7 kb deletion.

As previously described (Liu et al., 2000), 1.5 ul of purified DNA was amplified in a 25 µl reaction consisting of 12.5 µl HotStart *Taq* DNA polymerase mix containing dNTPs (Qiagen), 0.5 µl forward primer (5'-AAGTCCACCCCTTCCTTCCTCACC-3'), 0.2 µl reverse primer 1 (5'-ATGAGAGAAATGTTCTGGCACCTGCACTTG-3'), 0.2 µl reverse primer 2 (5'-TCCATCCCCTCCTCCCGCCCCTGCCTTTTC-3'; each primer 25 µM), 1.25 µl DMSO, and 3.75 µl glycine betaine (5 M). PCR conditions were 95°C for 16 min, followed by 35 cycles of 95°C for 60 sec, 62°C for 60 sec, 72°C for 150 sec, followed by 72°C for 10 min. Amplicons were resolved in 1% agarose gels and stained with ethidium bromide. Wild type (αα/αα) contained a single 2,213 bp product, heterozygotes (carriers, αα/-$\alpha^{3.7}$) products of 2,213 bp and 1,963 bp, and homozygotes (-$\alpha^{3.7}$/-$\alpha^{3.7}$) a 1,963 bp product.

2.6. Characterization of G6PD A-.

As previously described (Fanello et al., 2008), 2 µL of purified DNA was amplified in a 30 µL reaction containing 3 µl of 10X Taq buffer, 1 µL each of 10 µM forward (5'-CTGGCCAAGAAGAAG ATCTACCC-3') and reverse (5'-GAGAAAACGCAGCAGAGCACAG 3') primers, 3 µl of each dNTP (2 mM), and 0.2 µl *Taq* DNA polymerase (5 U/µL). PCR conditions were 95°C for 5 min, followed by 30 cycles of 95°C for 40 sec, 64°C for 40 sec, and 72°C for 40 sec, followed by 72°C for 10 min. 15 µl of the PCR product was digested by incubating at 37°C for 3 h with 2 µl 10X CutSmart buffer, 0.2 µl BSA, 0.3 µl *Nla*III (10 U/uL), and 2.5 µL water. Reaction products were resolved in 2.5% agarose gels and stained with ethidium bromide. Wild type contained fragments of 300 bp and 150 bp; heterozygotes fragments of 300 bp, 150 bp, and 140 bp; and homozygotes (and hemizygous males) fragments of 150 bp and 140 bp.

2.7. Characterization of CD36 T188G.

As previously described (Das et al., 2009), 2 µl of purified DNA was amplified in a 50 µL reaction with 5 µl of 10X Taq buffer, 1 µL each of 10 µM forward (5'-CTATGCTGTATTTGAATCCGACGTT-3') and reverse (5'-CTGTGCTACTGAGGTTATTTACTC-3') primers, 5 µl of each dNTP (2 mM), and 0.2 µl *Taq* DNA polymerase (5 U/µL). PCR conditions were 95°C for 3 min, followed by 40 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min, followed by 72°C for 10 min. 15 µl of the PCR product was digested by incubating at 37°C for 5 h with 3 µl 10X CutSmart buffer, 1 µl *Nde*I (10 U/uL), and 11 µL water. Reaction products were resolved in 2% agarose and stained with ethidium bromide. Wild type contained fragments of 148 bp and 64 bp; heterozygotes fragments of 212 bp, 148 bp, and 64 bp; and mutants a single 212 bp fragment.

2.8. Data analysis.

Outcomes for each tested genotype were categorized as wild type, heterozygous, or homozygous. For α-thalassemia the αα/αα genotype represents wild type, αα/-$\alpha^{3.7}$ the

heterozygote (silent carrier), and $-\alpha^{3.7}/-\alpha^{3.7}$ the homozygous mutation. For G6PD A-, homozygous females and hemizygous males were considered homozygotes. Prevalences of polymorphisms were compared using the Fisher's exact test, with comparisons either between wild type and heterozygotes/homozygotes combined or between wild type and only heterozygotes. P-values <0.05 were considered statistically significant.

3. Results

3.1. Study sites and populations.

Study populations were members of cohorts enrolled from a rural area of Tororo District in southeastern Uganda; a peri-urban area of Jinja District, in south-central Uganda; and a rural area of Kanungu District in southwestern Uganda (Figure 1; Table 1). Out of the 1,344 subjects enrolled, 44% were children under 5 years of age, 32% 5-10 years, and 24% above 18 years. Prior reports defined malaria transmission, prevalence, and incidence in the three cohorts, as summarized in Table 1 (Kamya et al., 2015; Rek et al., 2016). The sites differed markedly, with very high transmission intensity, parasite prevalence, and malaria incidence in Tororo District, lower levels of all of these parameters in Kanungu District, and the lowest levels in Jinja District. Of note, malaria transmission was considerably greater in earlier surveys of Jinja District, and transmission has since decreased greatly in Tororo District after a campaign of indoor residual spraying of insecticide that was launched in 2014 (Katureebe et al., 2016). Historically, it appears that malaria transmission intensity followed the rank order Tororo > Jinja > Kanungu (Yeka et al., 2012). It is anticipated that various differences between study sites impact on malaria transmission intensity; our goal in this study was to compare human genetic polymorphisms that may have been selected under differential malaria selection pressures at the sites.

3.2. Comparative prevalence of human genetic polymorphisms at three sites in Uganda.

DNA from cohort subjects was analyzed for the prevalence of four polymorphisms associated with protection against malaria: the sickle hemoglobin mutation (β globin E6V), α-thalassemia, G6PD deficiency caused by the common African variant (G6PD A-; V68M and N126D), and the CD36 T188G mutation (which introduces a stop codon in exon 10). For all studied sites and alleles, >95% of samples were successfully analyzed. For all of these polymorphisms, mutant genotypes were common, with many more heterozygous than homozygous alleles identified (Table 2). HbC (β globin E6K) was not identified in any samples. Marked differences were identified in prevalences of polymorphisms of interest at the three study sites. For sickle hemoglobin, α-thalassemia, and G6PD deficiency, prevalences of wild type were lowest in Tororo and highest in Kanungu; for CD36 T188G prevalence of wild type was lowest in Tororo, and the same in Jinja and Kanungu (Table 3; Figure 2). Consideration of any two polymorphisms together yielded associations similar to those seen for individual polymorphisms, with prevalences of wild type generally lowest in Tororo and highest in Kanungu (Supplemental Table 1).

3.3. Comparative prevalence of human genetic polymorphisms among different ethnic groups.

Uganda has a highly diverse population, and it was of interest to determine if differences in the prevalences of genetic polymorphisms were associated with the ethnicity of different populations. Ethnicity was defined based on the primary language spoken by the household, with stratification for speakers of Bantu and non-Bantu (Nilotic and Central Sudanic) languages. Each study population included a range of ethnicities, but the Tororo cohort was primarily composed of speakers of Dhopadhola, a Nilotic language; the Jinja cohort included a mixture of Bantu and non-Bantu speakers, and the Kanungu cohort contained nearly all Bantu speakers (primarily Bakiga and Banyarwanda; Supplemental Table 2). The four studied polymorphisms were all more common in non-Bantu speakers, with differences in prevalence statistically significant for all but the CD36 T188G mutation (Table 3). This result suggests that differences

in prevalence between Ugandan sites were largely explained by major ethnic differences among the study populations. However, when prevalences were compared among ethnic groups in Jinja, the only site with large numbers of both Bantu and non-Bantu speakers, no differences in prevalence of any of the studied polymorphisms were seen between Bantu and non-Bantu speakers (Supplemental Table 3). Thus, both ethnicity and place of residence appear to have contributed to the observed differences in prevalence of studied traits.

4. Discussion

We characterized the prevalences of four common human genetic polymorphisms, each previously associated with protection against severe malaria, in three regions of Uganda. All four polymorphisms demonstrated similar patterns, with highest prevalence in Tororo District, in eastern Uganda and, except for CD36 T188G, lowest prevalence in Kanungu District, in southwestern Uganda. Considering ethnicity of study populations, prevalence of the polymorphisms was greatest among non-Bantu speakers. However, in Jinja District, the only site with a highly diverse ethnic make-up, prevalence of the polymorphisms was not associated with ethnicity. Our results indicate marked variations in the prevalences of malaria-protective human genetic polymorphisms between populations in different regions of Uganda and suggest that these variations might be explained both by ethnic differences and by varied malaria risk in different regions of the country.

The three Ugandan study sites differed greatly in recent malaria transmission intensity (Kamya et al., 2015; Katureebe et al., 2016). Tororo District has had very high transmission intensity recorded, with entomologic inoculation rates >300 infectious bites per year, although very recently transmission intensity has decreased due to an intensive IRS campaign. Jinja District has had decreasing transmission intensity, presumably due to both improved utilization of ITNs and malaria case management, and to effects of urbanization. Kanungu District has intermediate levels of malaria transmission intensity. However, appreciation of malaria

transmission prior to recent intensive control efforts and urbanization suggests that the rank order for historical transmission intensity for these sites is Tororo > Jinja > Kanungu (Yeka et al., 2012). Thus, our measured prevalences of genetic polymorphisms followed a pattern similar to that of historical malaria transmission intensity, with the prevalence of all four studied polymorphisms highest in Tororo, the region with the highest transmission intensity, potentially explained by the greatest selective pressure for mutations that protect against lethal malaria at this site.

Our study also collected information on the primary language of study households, a proxy for ethnicity, and thus it offered the opportunity to assess associations between ethnicity and genetic polymorphisms. A large literature has demonstrated that, in studies across populations, linguistic trees correspond remarkably well to genetic trees (Cavalli-Sforza, 1997). In our case, there were strong associations between language and genetic polymorphisms. The prevalence of sickle hemoglobin, a-thalassemia, and G6PD deficiency were all greatest in non-Bantu speakers. Major movements of different African populations appear to have occurred over about the last 3,000-8,000 years (Beltrame et al., 2016). Our results suggest that non-Bantu speakers, in our study consisting of speakers of Nilotic and Central Sudanic languages, whom appear to have originated from the area of modern Sudan and South Sudan, experienced greater malaria risk than did Bantu-speaking groups, who are believed to have originated around modern Cameroon and Nigeria (Beltrame et al., 2016). A number of dating studies suggest that sickle hemoglobin and G6PD deficiency arose within the last few thousand years (Hedrick, 2011). The marked differences in prevalences of malaria-protective polymorphisms between ethnic groups are consistent with our recent understanding of the evolution of *P. falciparum*, with the appreciation that this parasite crossed from gorillas to humans quite recently, probably within the last 10,000 years (Loy et al., 2017). Thus, it is plausible that modern differences in the prevalence of malaria-protective human genetic polymorphisms are due to differences in malaria risk over the last few thousand years.

In summary, we identified marked variation in the prevalences of human genetic polymorphisms in residents of three different regions of Uganda. These differences may be due, in part, to differences in historical malaria risk between these regions and to genetic differences between the ethnic groups principally inhabiting these regions. The large differences in prevalences of balanced polymorphisms between harmful homozygous and protective heterozygous mutations suggest a profound impact of fatal falciparum malaria on the human genome.

Acknowledgements. We thank study participants for their generous cooperation and study staff for their expert management of the cohorts. We thank Emmanuel Arinaitwe and Elijah Kakande for help with data collection; Victor Asua and Elizabeth Namirembe for laboratory assistance; and Melissa Conrad and Michelle Roh for advice concerning figures. This study (AI089674 and AI075045) and trainees contributing to the study (TW007375) were supported by funding from the National Institutes of Health. The funder had no direct role in study design; the collection, analysis, and interpretation of data; the writing of this report; or the decision to submit the article for publication.

Conflicts of interest: none.

References

Aitman, T.J., Cooper, L.D., Norsworthy, P.J., Wahid, F.N., Gray, J.K., Curtis, B.R., McKeigue, P.M., Kwiatkowski, D., Greenwood, B.M., Snow, R.W., Hill, A.V., Scott, J., 2000. Malaria susceptibility and CD36 mutation. Nature 405, 1015-1016.

Allen, S.J., O'Donnell, A., Alexander, N.D., Alpers, M.P., Peto, T.E., Clegg, J.B., Weatherall, D.J., 1997. alpha+-Thalassemia protects children against disease caused by other infections as well as malaria. Proc Natl Acad Sci U S A 94, 14736-14741.

Beltrame, M.H., Rubel, M.A., Tishkoff, S.A., 2016. Inferences of African evolutionary history from genomic data. Curr Opin Genet Dev 41, 159-166.

Bereczky, S., Dolo, A., Maiga, B., Hayano, M., Granath, F., Montgomery, S.M., Daou, M., Arama, C., Troye-Blomberg, M., Doumbo, O.K., Farnert, A., 2006. Spleen enlargement and genetic diversity of Plasmodium falciparum infection in two ethnic groups with different malaria susceptibility in Mali, West Africa. Trans R Soc Trop Med Hyg 100, 248-257.

Cabrera, A., Neculai, D., Kain, K.C., 2014. CD36 and malaria: friends or foes? A decade of data provides some answers. Trends Parasitol 30, 436-444.

Canton, J., Neculai, D., Grinstein, S., 2013. Scavenger receptors in homeostasis and immunity. Nat Rev Immunol 13, 621-634.

Cavalli-Sforza, L.L., 1997. Genes, peoples, and languages. Proc Natl Acad Sci U S A. 94, 7719-7724.

Das, A., Das, T.K., Sahu, U., Das, B.P., Kar, S.K., Ranjit, M.R., 2009. CD36 T188G gene polymorphism and severe falciparum malaria in India. Trans R Soc Trop Med Hyg 103, 687-690.

Fanello, C.I., Karema, C., Avellino, P., Bancone, G., Uwimana, A., Lee, S.J., d'Alessandro, U., Modiano, D., 2008. High risk of severe anaemia after chlorproguanil-dapsone+artesunate antimalarial treatment in patients with G6PD (A-) deficiency. PLoS One 3, e4031.

Fonager, J., Pasini, E.M., Braks, J.A., Klop, O., Ramesar, J., Remarque, E.J., Vroegrijk, I.O., van Duinen, S.G., Thomas, A.W., Khan, S.M., Mann, M., Kocken, C.H., Janse, C.J., Franke-Fayard, B.M., 2012. Reduced CD36-dependent tissue sequestration of Plasmodium-infected erythrocytes is detrimental to malaria parasite growth in vivo. J Exp Med 209, 93-107.

Fry, A.E., Ghansa, A., Small, K.S., Palma, A., Auburn, S., Diakite, M., Green, A., Campino, S., Teo, Y.Y., Clark, T.G., Jeffreys, A.E., Wilson, J., Jallow, M., Sisay-Joof, F., Pinder, M., Griffiths, M.J., Peshu, N., Williams, T.N., Newton, C.R., Marsh, K., Molyneux, M.E., Taylor, T.E., Koram, K.A., Oduro, A.R., Rogers, W.O., Rockett, K.A., Sabeti, P.C., Kwiatkowski, D.P., 2009. Positive selection of a CD36 nonsense variant in sub-Saharan Africa, but no association with severe malaria phenotypes. Hum Mol Genet 18, 2683-2692.

Guindo, A., Fairhurst, R.M., Doumbo, O.K., Wellems, T.E., Diallo, D.A., 2007. X-linked G6PD deficiency protects hemizygous males but not heterozygous females against severe malaria. PLoS Med 4, e66.

Haldane, J.B.S., 1949. The rate of mutation of human genes. Hereditas 35, 267-273.

Hedrick, P.W., 2011. Population genetics of malaria resistance in humans. Heredity (Edinb) 107, 283-304.

Kamya, M.R., Arinaitwe, E., Wanzira, H., Katureebe, A., Barusya, C., Kigozi, S.P., Kilama, M., Tatem, A.J., Rosenthal, P.J., Drakeley, C., Lindsay, S.W., Staedke, S.G., Smith, D.L., Greenhouse, B., Dorsey, G., 2015. Malaria transmission, infection, and disease at three sites with varied transmission intensity in Uganda: implications for malaria control. Am J Trop Med Hyg 92, 903-912.

Katureebe, A., Zinszer, K., Arinaitwe, E., Rek, J., Kakande, E., Charland, K., Kigozi, R., Kilama, M., Nankabirwa, J., Yeka, A., Mawejje, H., Mpimbaza, A., Katamba, H., Donnelly, M.J., Rosenthal, P.J., Drakeley, C., Lindsay, S.W., Staedke, S.G., Smith, D.L., Greenhouse, B., Kamya, M.R., Dorsey, G., 2016. Measures of Malaria Burden after Long-Lasting Insecticidal

Net Distribution and Indoor Residual Spraying at Three Sites in Uganda: A Prospective Observational Study. PLoS Med 13, e1002167.

Kwiatkowski, D.P., 2005. How malaria has affected the human genome and what human genetics can teach us about malaria. Am J Hum Genet 77, 171-192.

Liu, Y.T., Old, J.M., Miles, K., Fisher, C.A., Weatherall, D.J., Clegg, J.B., 2000. Rapid detection of alpha-thalassaemia deletions and alpha-globin gene triplication by multiplex polymerase chain reactions. Br J Haematol 108, 295-299.

Loy, D.E., Liu, W., Li, Y., Learn, G.H., Plenderleith, L.J., Sundararaman, S.A., Sharp, P.M., Hahn, B.H., 2017. Out of Africa: origins and evolution of the human malaria parasites Plasmodium falciparum and Plasmodium vivax. Int J Parasitol 47, 87-97.

Maiga, B., Dolo, A., Campino, S., Sepulveda, N., Corran, P., Rockett, K.A., Troye-Blomberg, M., Doumbo, O.K., Clark, T.G., 2014. Glucose-6-phosphate dehydrogenase polymorphisms and susceptibility to mild malaria in Dogon and Fulani, Mali. Malar J 13, 270.

May, J., Evans, J.A., Timmann, C., Ehmen, C., Busch, W., Thye, T., Agbenyega, T., Horstmann, R.D., 2007. Hemoglobin variants and disease manifestations in severe falciparum malaria. JAMA 297, 2220-2226.

Modiano, D., Luoni, G., Sirima, B.S., Lanfrancotti, A., Petrarca, V., Cruciani, F., Simpore, J., Ciminelli, B.M., Foglietta, E., Grisanti, P., Bianco, I., Modiano, G., Coluzzi, M., 2001a. The lower susceptibility to Plasmodium falciparum malaria of Fulani of Burkina Faso (west Africa) is associated with low frequencies of classic malaria-resistance genes. Trans R Soc Trop Med Hyg 95, 149-152.

Modiano, D., Luoni, G., Sirima, B.S., Simpore, J., Verra, F., Konate, A., Rastrelli, E., Olivieri, A., Calissano, C., Paganotti, G.M., D'Urbano, L., Sanou, I., Sawadogo, A., Modiano, G., Coluzzi, M., 2001b. Haemoglobin C protects against clinical Plasmodium falciparum malaria. Nature 414, 305-308.

Nasr, A., Elghazali, G., Giha, H., Troye-Blomberg, M., Berzins, K., 2008. Interethnic differences in carriage of haemoglobin AS and Fcgamma receptor IIa (CD32) genotypes in children living in eastern Sudan. Acta Trop 105, 191-195.

Newbold, C., Craig, A., Kyes, S., Rowe, A., Fernandez-Reyes, D., Fagan, T., 1999. Cytoadherence, pathogenesis and the infected red cell surface in Plasmodium falciparum. Int J Parasitol 29, 927-937.

Nkhoma, E.T., Poole, C., Vannappagari, V., Hall, S.A., Beutler, E., 2009. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood Cells Mol Dis 42, 267-278.

Omi, K., Ohashi, J., Naka, I., Patarapotikul, J., Hananantachai, H., Looareesuwan, S., Tokunaga, K., 2002. Polymorphisms of CD36 in Thai malaria patients. Southeast Asian J Trop Med Public Health 33 Suppl 3, 1-4.

Omi, K., Ohashi, J., Patarapotikul, J., Hananantachai, H., Naka, I., Looareesuwan, S., Tokunaga, K., 2003. CD36 polymorphism is associated with protection from cerebral malaria. Am J Hum Genet 72, 364-374.

Pain, A., Urban, B.C., Kai, O., Casals-Pascual, C., Shafi, J., Marsh, K., Roberts, D.J., 2001. A non-sense mutation in Cd36 gene is associated with protection from severe malaria. Lancet 357, 1502-1503.

Patel, S.N., Lu, Z., Ayi, K., Serghides, L., Gowda, D.C., Kain, K.C., 2007. Disruption of CD36 impairs cytokine response to Plasmodium falciparum glycosylphosphatidylinositol and confers susceptibility to severe and fatal malaria in vivo. J Immunol 178, 3954-3961.

Rek, J., Katrak, S., Obasi, H., Nayebare, P., Katureebe, A., Kakande, E., Arinaitwe, E., Nankabirwa, J.I., Jagannathan, P., Drakeley, C., Staedke, S.G., Smith, D.L., Bousema, T., Kamya, M., Rosenthal, P.J., Dorsey, G., Greenhouse, B., 2016. Characterizing microscopic and submicroscopic malaria parasitaemia at three sites with varied transmission intensity in Uganda. Malar J 15, 470.

Ruwende, C., Khoo, S.C., Snow, R.W., Yates, S.N., Kwiatkowski, D., Gupta, S., Warn, P., Allsopp, C.E., Gilbert, S.C., Peschu, N., et al., 1995. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. Nature 376, 246-249.

Silamut, K., Phu, N.H., Whitty, C., Turner, G.D., Louwrier, K., Mai, N.T., Simpson, J.A., Hien, T.T., White, N.J., 1999. A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. Am J Pathol 155, 395-410.

Sinha, S., Qidwai, T., Kanchan, K., Anand, P., Jha, G.N., Pati, S.S., Mohanty, S., Mishra, S.K., Tyagi, P.K., Sharma, S.K., Indian Genome Variation, C., Venkatesh, V., Habib, S., 2008. Variations in host genes encoding adhesion molecules and susceptibility to falciparum malaria in India. Malar J 7, 250.

Taylor, S.M., Fairhurst, R.M., 2014. Malaria parasites and red cell variants: when a house is not a home. Curr Opin Hematol 21, 193-200.

Town, M., Bautista, J.M., Mason, P.J., Luzzatto, L., 1992. Both mutations in G6PD A- are necessary to produce the G6PD deficient phenotype. Hum Mol Genet 1, 171-174.

Verra, F., Mangano, V.D., Modiano, D., 2009. Genetics of susceptibility to Plasmodium falciparum: from classical malaria resistance genes towards genome-wide association studies. Parasite Immunol 31, 234-253.

Weatherall, D.J., 2008. Genetic variation and susceptibility to infection: the red cell and malaria. Br J Haematol 141, 276-286.

Williams, T.N., 2006. Human red blood cell polymorphisms and malaria. Curr Opin Microbiol 9, 388-394.

Williams, T.N., Wambua, S., Uyoga, S., Macharia, A., Mwacharo, J.K., Newton, C.R., Maitland, K., 2005. Both heterozygous and homozygous alpha+ thalassemias protect against severe and fatal Plasmodium falciparum malaria on the coast of Kenya. Blood 106, 368-371.

Yeka, A., Gasasira, A., Mpimbaza, A., Achan, J., Nankabirwa, J., Nsobya, S., Staedke, S.G., Donnelly, M.J., Wabwire-Mangen, F., Talisuna, A., Dorsey, G., Kamya, M.R., Rosenthal, P.J., 2012. Malaria in Uganda: challenges to control on the long road to elimination: I. Epidemiology and current control efforts. Acta Trop 121, 184-195.

Figure legends

Figure 1. Study sites. Districts and subcounties are labelled and shaded in gray and black, respectively.

Figure 2. Prevalence of studied polymorphisms at the three study sites.

Table 1. Description of study sites.

	Study site		
Characteristics of sites	**Tororo**	**Jinja**	**Kanungu**
Location	Southeastern	South-central	Southwestern
Setting	Rural	Peri-urban	Rural
Altitude	695-1,443 m	1,102-1,500 m	886-1,329 m
Number of study subjects[1]			
Children	340	321	365
Adults	106	114	98
Total	446	435	463
Malaria indicators (children)[2]			
Entomological inoculation rate per year	310	2.8	32.0
Parasite prevalence	28.7%	7.4%	9.3%
Malaria incidence per year	2.81	0.43	1.43

[1]The cohorts consisted of children up to 11 years of age and adults over 18 years of age.
[2]These results are from Kamya et al., 2015.

Table 2. Overall prevalence (%) of human genetic polymorphisms at 3 sites in Uganda.

Polymorphism	N	Wild type	Heterozygous	Homozygous
HbS	1,321	1,060 (80.2)	256 (19.4)	5 (0.4)
α-Thalassemia	1,284	794 (61.8)	420 (32.7)	70 (5.5)
G6PD deficiency	1,323	1,083 (81.9)	166 (12.5)	74 (5.6)
CD36 T188G	1,334	1,061 (79.5)	263 (19.7)	10 (0.7)

Table 3. Comparative prevalence (%) of polymorphisms of interest at 3 sites in Uganda.

Site	Polymorphism				P-value[1]		
	N	WT	Heterozygous	Homozygous	T vs. J	T vs. K	J vs. K
	HbS						
Tororo	434	313 (72)	119 (27)	2 (1)	0.442		
Jinja	429	320 (75)	106 (24)	3 (1)		<0.0001	
Kanungu	458	427 (93)	31 (7)	0			<0.0001
	α-Thalassemia						
Tororo	414	196 (47)	190 (46)	28 (7)	0.0327		
Jinja	430	236 (55)	156 (37)	38 (8)		<0.0001	
Kanungu	440	362 (82)	74 (17)	4 (1)			<0.0001
	G6PD deficiency						
Tororo	438	310 (71)	80 (18)	48 (11)	<0.0001		
Jinja	432	355 (82)	58 (14)	19 (4)		<0.0001	
Kanungu	453	418 (92)	28 (6)	7 (2)			<0.0001
	CD36 T188G						
Tororo	442	324 (73)	114 (26)	4 (1)	0.0004		
Jinja	433	360 (83)	67 (16)	6 (1)		0.0017	
Kanungu	459	377 (82)	82 (18)	0			0.724

[1]P-values for comparisons of prevalences in Tororo (T), Jinja (J), and Kanungu (K) Districts were determined for comparisons of wild type (WT) vs. heterozygous + homozygous genotypes using the Fisher's exact test. Consideration of WT vs. only heterozygous genotypes identified the same comparisons as statistically significant.

Table 4. Comparative prevalence (%) of polymorphisms in Bantu and non-Bantu language speakers.

Language	Polymorphism				P-value[1]
	N	WT	Heterozygous	Homozygous	
	HbS				
Bantu	692	595 (86.0)	96 (13.9)	1 (0.1)	<0.0001
Non-Bantu	472	358 (75.8)	109 (23.1)	5 (1.1)	
	α-Thalassemia				
Bantu	676	487 (72.0)	169 (25.0)	20 (3.0)	<0.0001
Non-Bantu	461	230 (49.9)	196 (42.5)	35 (7.6)	
	G6PD deficiency				
Bantu	689	606 (87.1)	65 (9.4)	18 (2.6)	<0.0001
Non-Bantu	464	342 (73.7)	79 (17.0)	43 (9.3)	
	CD36 T188G				
Bantu	652	542 (83.1)	106 (16.3)	4 (0.6)	0.094
Non-Bantu	430	340 (79.1)	86 (20.0)	4 (0.9)	

[1]P-values for comparisons of prevalences between language groups were determined for comparisons of wild type (WT) vs. heterozygous + homozygous genotypes using the Fisher's exact test. Consideration of WT vs. only heterozygous genotypes identified the same comparisons as statistically significant.

Fig. 1

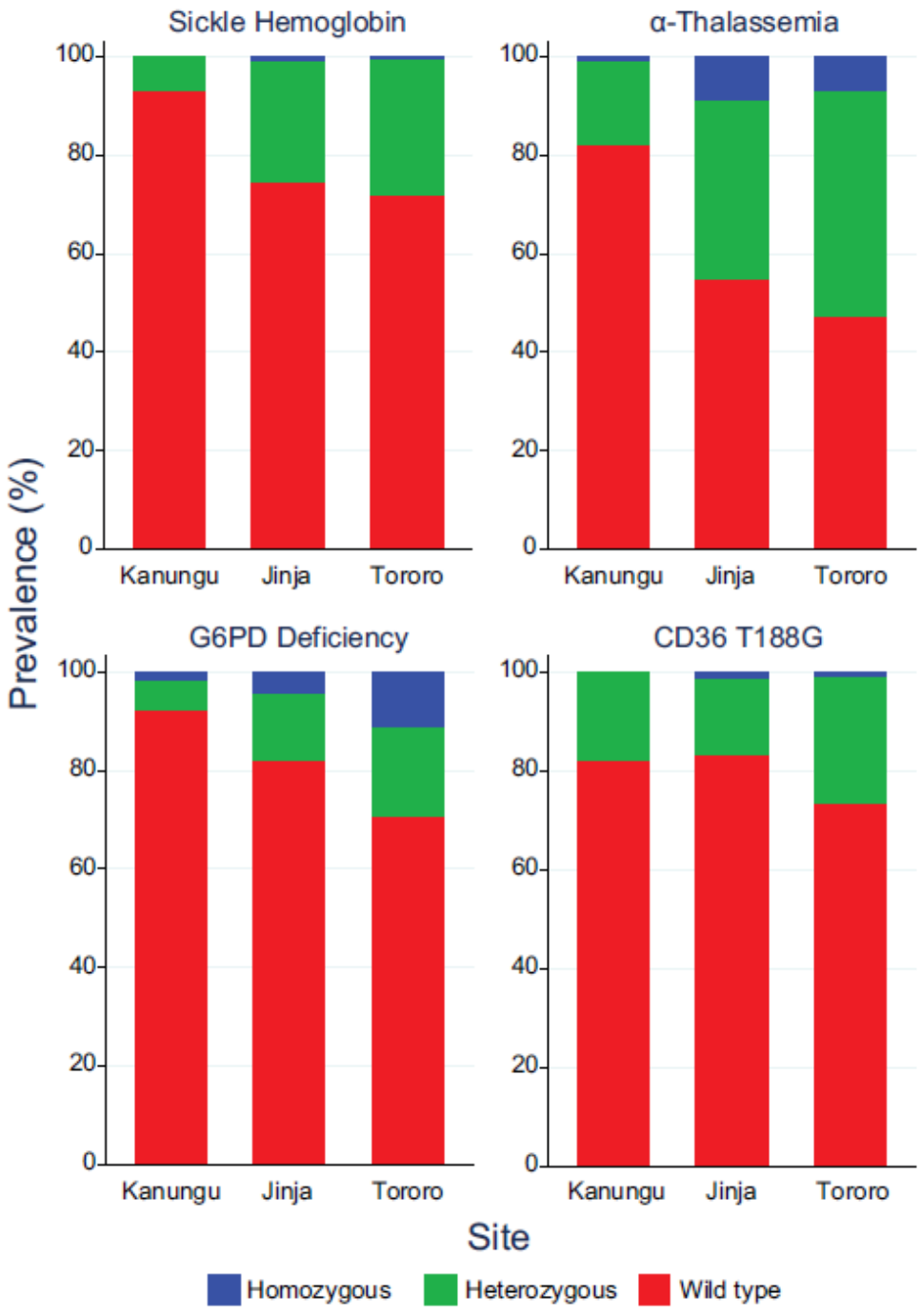

Fig. 2

Highlights for Review

Prevalence of 4 malaria-protective human genetic polymorphisms varied across Uganda.

Prevalence of mutations was lowest in southwestern Uganda.

Prevalence of mutations was lower in Bantu compared to non-Bantu language speakers.

Genetic differences might be explained by ethnic variation and varied malaria risk.