nature genetics

# Genetic variation in human *HBB* is associated with *Plasmodium falciparum* transmission

Louis Clement Gouagna[1,7], Germana Bancone[2,7], Frank Yao[3], Bienvenue Yameogo[3], Kounbobr Roch Dabiré[3], Carlo Costantini[4], Jacques Simporé[5], Jean Bosco Ouedraogo[3] & David Modiano[2,6]

**Genetic factors are known to have a role in determining susceptibility to infectious diseases[1,2], although it is unclear whether they may also influence host efficiency in transmitting pathogens. We examine variants in *HBB* that have been shown to be protective against malaria[3] and test whether these are associated with the transmission of the parasite from the human host to the *Anopheles* vector. We conducted cross-sectional malariological surveys on 3,739 human subjects and transmission experiments involving 60 children and 6,446 mosquitoes in Burkina Faso, West Africa. Protective hemoglobins C (HbC, β6Glu→Lys)[4,5] and S (β6Glu→Val)[6–8] are associated with a twofold *in vivo* (odds ratio 2.17, 95% CI 1.57–3.01, $P = 1.0 \times 10^{-6}$) and a fourfold *ex vivo* (odds ratio 4.12, 95% CI 1.90–9.29, $P = 7.0 \times 10^{-5}$) increase of parasite transmission from the human host to the *Anopheles* vector. This provides an example of how host genetic variation may influence the transmission dynamics of an infectious disease.**



Genetic epidemiology studies have shown the importance of genetic variation in infectious diseases[9]. Since Haldane put forward new theories on infectious diseases and evolution[10], a number of genetic association studies have shown a role for human genetic variation in resistance to infections[1,2]. However, little attention has been devoted to the possible influence of human genetic variation on the transmission dynamics of pathogens from host to host.

Malaria is the infectious disease for which the role of human genetics has been best dissected[11]. The protective role of genetic variants of the *HBB* gene, such as those for hemoglobin C (HbC) and S (HbS), on susceptibility to malaria is well documented[4–8]. The HbS allele has a multifocal origin and a widespread geographic distribution in sub-Saharan Africa and Asia. Its prevalence reaches more than 10% in many parts of Africa. The HbC allele is present in the population of central West Africa, reaching its highest frequency (about 12%) in Burkina Faso[12].

To investigate whether HbC and HbS, besides providing a known protection against clinical malaria, do also influence parasite transmission from the human host to the *Anopheles* vector, a large-scale epidemiological study was carried out in areas of Burkina Faso, West Africa, that are hyperendemic for malaria. We compared individuals carrying the HbAA, HbAS, HbAC and HbCC β-globin genotypes in regard to their efficiency in transmitting *P. falciparum* to the *Anopheles* vector. We performed genetic, parasitological and entomological investigations involving a total of 3,799 human subjects and 6,446 *Anopheles* mosquitoes. In particular, we assessed the following parameters dealing with the potential of human-mosquito parasite transmission according to β-globin genotypes: (i) gametocyte rates and densities—that is, the percentage of the human population carrying *P. falciparum* sexual infectious stages responsible for the transmission of the parasite from humans to mosquitoes and its relative concentration in the blood; and (ii) oocyst rates and densities—that is, the percentage of mosquitoes infected by the malaria parasite following genotype-controlled blood meals (*in vivo* and *ex vivo*).

Two cross-sectional parasitological surveys were conducted in 2001 and 2007 in different epidemiological contexts and study periods with respect to the season with the highest malaria transmission rates. The first survey, performed in rural villages in the center of the country near Ouagadougou just after the end of the high-transmission season, involved a total of 1,737 children aged 0–15 years (1,219 AA (mean age ± s.e.m.): 8.6 ± 0.1; 143 AS: 8.5 ± 0.4; 320 AC: 8.8 ± 0.2; 28 CC: 8.6 ± 1.1; 25 SC: 10.4 ± 1.1; and 2 SS: 3.5 ± 1.5 children). The second survey, performed in the southwest of the country in the rural village of Soumousso near Bobo Dioulasso, was conducted in the middle to end of the dry, low-transmission season (March–May) on a total of 2,002 subjects from all age groups (1,399 AA: 17.9 ± 0.4; 162 AS: 19.6 ± 1.4; 398 AC: 18.2 ± 0.9; 37 CC: 14.4 ± 2.1; and 6 SC: 11.3 ± 3.1 subjects).

Although no differences were observed in asexual parasite rates and densities among β-globin genotypes (**Table 1** and **Supplementary Table 1**), indications of higher infectious reservoirs were observed in HbC carriers. As shown in **Table 1**, higher gametocyte carriage (rates and/or densities) in association with HbC were observed during both surveys. In the first survey, the HbCC genotype was characterized by a fourfold higher gametocyte rate than the rest of the population

[1]Institut de Récherche pour le Developpement (IRD), Unité de Recherche 16, Bobo Dioulasso, Burkina Faso. [2]Department of Public Health Sciences, University of Rome 'La Sapienza', Rome, Italy. [3]Institut de Récherche en Sciences de la Santé (IRSS), Direction Régionale de Bobo-Dioulasso, Bobo Dioulasso, Burkina Faso. [4]IRD, Unité de Recherche 016, Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale, Yaoundé, Cameroon. [5]Centre Medical Saint-Camille, Ouagadougou, Burkina Faso. [6]Istituto Pasteur, Fondazione Cenci Bolognetti, University of Rome 'La Sapienza', Rome, Italy. [7]These authors contributed equally to this work. Correspondence should be addressed to D.M. (david.modiano@uniroma1.it).

Received 17 June 2009; accepted 19 February 2010; published online 21 March 2010; doi:10.1038/ng.554

**Table 1** ***Plasmodium falciparum*** **infection according to β-globin genotype**

| β globin | | | *Plasmodium falciparum* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Frequency | | Parasite rate | | Geometric mean of positive asexual parasite density/μl | | Gametocyte rate | | Geometric mean of positive gametocyte density/μl | |
| Genotype | I survey, *n* (percent of total)[a] | II survey, *n* (percent of total)[b] | I survey | II survey | I survey | II survey | I survey | II survey | I survey | II survey |
| AA | 1,219 (70.2) | 1,399 (69.9) | 0.793 (967/1219) | 0.438 (610/1393) | 606.7 (12–158,489) | 266.1 (3–63,096) | 0.058 (71/1219) | 0.162 (225/1393) | 30.1 (16–631) | 8.8 (3–200) |
| AS | 143 (8.2) | 162 (8.1) | 0.783 (112/143) | 0.340 (55/162) | 507.0 (16–1,000,000) | 207.0 (6–5,012) | 0.042 (6/143) | 0.142 (23/162) | 26.1 (16–158) | 7.0 (3–50) |
| AC | 320 (18.4) | 398 (19.9) | 0.781 (250/320) | 0.467 (185/396) | 534.6 (16–125,892) | 219.3 (3–63,096) | 0.063 (20/320) | 0.212 (84/396) | 23.7 (16–100) | 12.2 (3–794) |
| CC | 28 (1.6) | 37 (1.8) | 0.714 (20/28) | 0.432 (16/37) | 335.0 (16–31,623) | 115.6 (6–6,310) | 0.214 (6/28) | 0.243 (9/37) | 25.1 (16–32) | 15.5 (3–126) |
| SC | 25 (1.4) | 6 (0.3) | 0.880 (22/25) | 0.333 (2/6) | 423.6 (16–10,000) | 100.0 (50–200) | 0.040 (1/25) | 0.000 (0/6) | 16.0 | – |
| SS | 2 (0.1) | 0 (0.0) | 100.000 (2/2) | – | 1778.3 (1,260–2,512) | – | 0.000 (0/2) | – | – | – |

Infection in 3,739 human subjects recruited during two malariological surveys, near Ouagadougou ($n = 1737$) and Bobo Dioulasso ($n = 2002$), Burkina Faso, West Africa. Pairwise comparisons between genotypes are shown in **Supplementary Table 1**.
[a]Out of a total of 1,737 study participants. [b]Out of a total of 2,002 study participants.



(21.4% versus 5.7%; odds ratio (OR) 4.48, 95% CI 1.57–12.11, $P = 0.005$), whereas no effect was observed in individuals carrying the HbAC genotype. The association between the HbCC genotype and higher gametocyte rates was observed in three out of the four villages where HbCC subjects were found (Mantel-Haenszel weighted OR after correction by age, gender and village: 4.75, 95% CI 1.89–11.98, $P = 0.001$; **Fig. 1**).

In contrast, in the second survey (**Table 1** and **Supplementary Table 1**), gametocyte rates were higher in HbAC as compared to HbAA subjects (21.2% versus 16.2%; OR 1.42, 95% CI 1.05–1.92; $P = 0.029$), whereas the differences between HbCC and HbAA subjects did not reach statistical significance (24.3% versus 16.2%; OR 1.67, 95% CI 0.72–3.78, $P = 0.27$). The comparisons between individuals carrying the HbC allele (AC or CC) and normal HbAA subjects showed higher gametocyte rates in the presence of the adaptive C allele (21.5% versus 16.2%; OR 1.42, 95% CI 1.07–1.88, $P = 0.013$), whereas the difference was not significant when HbC carriers (HbAC or HbCC) were compared to HbAS subjects (21.5% versus 14.2%; OR 1.65, 95% CI 0.98–2.81, $P = 0.060$). In the second survey (**Table 1**), a 45% higher gametocyte density was observed in HbC (HbAC or HbCC) gametocyte carriers compared to subjects with HbAA ($P = 0.008$) and HbAS genotypes ($P = 0.031$). A clear trend toward sexual differentiation of *P. falciparum* in the presence of HbC was also indicated by the sexual to asexual differentiation ratio during both surveys; during the first survey, a 50% higher sexual to asexual differentiation ratio was observed in HbCC subjects compared to subjects with HbAA, HbAS and HbAC genotypes (**Table 1**). In the second survey, a 400% and a 65% higher sexual to asexual differentiation ratio was observed respectively in HbCC and HbAC subjects compared to those with HbAA or HbAS genotypes.

Even with these discrepancies, possibly related to the differing mean ages (± s.e.m.) of the populations between the two surveys (8.7 ± 0.11 versus 18.0 ± 0.4) and the differing epidemiological contexts and study periods with respect to the high malaria transmission season and year of the study, we found consistent association of a higher gametocyte reservoir in individuals carrying the HbC allele within both surveys (**Table 1** and **Supplementary Table 1**).

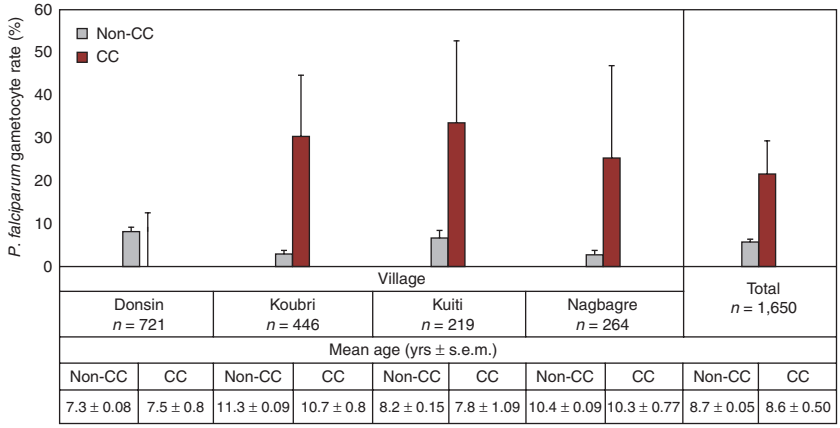


**Figure 1** *Plasmodium falciparum* gametocyte rate according to hemoglobin C homozygosis in children living in rural villages of Burkina Faso, West Africa. Error bars, standard error of the frequency; s.e.m., standard error of the mean age. The possible confounding effect of age and village was considered through a Mantel-Haenszel summary $\chi^2$ test and maximum likelihood estimate (MLE) of OR. Mantel-Haenszel OR 4.75, 95% CI 1.89–11.98, $P = 0.001$. MLE of OR 5.00, 95% CI 1.59–13.44; probability that MLE ≥ 5.00 = 0.003. CC, HbCC genotype.

To assess the efficiency of parasite transmission from the human host to the *Anopheles* vector according to the β-globin genotype, we used two complementary approaches based on natural (*in vivo*) and experimental (*ex vivo*) conditions during the 2008 high malaria transmission season (July–October) in the rural village of Soumousso.

For the *in vivo* study, based on collection of indoor-resting mosquitoes after having fed on subjects with a known β-globin genotype (HbAA, HbAS, HbAC and HbCC) and determination of the mosquito infection rate (oocyst rate) at day 7, we analyzed a total of 3,869 mosquitoes. To estimate the background level of mosquito infection before the β-globin genotype-controlled blood meal, we dissected 10% of the surviving mosquitoes from each capture at day 3 ($n = 406$). Although homogeneous infection rates were recorded in mosquitoes dissected on day 3 ($P = 0.99$), higher

**Table 2** ***In vivo Anopheles*** **infection (oocyst rate and densities) after blood meals on human subjects**

| Blood meal genotype | Mosquito infection rate[a] | Mosquito infection rate[b] | Estimate of mosquito infection rate[c] | Arithmetic mean of oocyst density[d] (range) |
|---|---|---|---|---|
| AA | 14.4 (13/90) | 19.1 (153/802) | 5.6 (47/802) | 1.51 (0–150) |
| AS | 12.9 (12/93) | 22.2 (229/1031) | 8.7 (92/1031) | 1.98 (0–170) |
| AC | 13.2 (14/106) | 25.9 (243/939) | 12.4 (118/939) | 1.47 (0–75) |
| CC | 13.7 (16/117) | 27.5 (302/1097) | 14.0 (156/1097) | 2.49 (0–90) |
| AC + CC | 13.5 (30/223) | 26.8 (545/2036) | 13.3 (271/2036) | 2.02 (0–90) |
| AS + AC + CC | 13.3 (42/316) | 25.2 (774/3067) | 11.9 (365/3067) | 2.01 (0–170) |
| TOTAL | 13.5 (55/406) | 24.0 (927/3869) | 10.67 (413/3869) | 1.91 (0–170) |

Subjects had varying β-globin genotypes and were from malaria-hyperendemic areas of Burkina Faso, West Africa. Pairwise comparisons between genotypes are shown in **Supplementary Table 2**.
[a]At day 3 (as a percentage), 'X'. [b]At day 7 (as a percentage), 'Y'. [c]Due to the last blood meal, calculated as 'Y–X' and given as a percentage. [d]At day 7.

infection rates were observed at day 7 when the blood meal came from subjects carrying HbC (HbAC or HbCC) as compared to HbAA (OR 1.39, 95% CI 1.13–1.72, $P = 0.002$; **Table 2** and **Supplementary Table 2**). Higher oocyst densities were recorded in mosquitoes fed on blood meals from HbCC subjects compared to HbAC ($P = 0.004$) or HbAA ($P = 0.008$) subjects. No differences were observed in either prevalence or oocyst density between mosquitoes fed on blood from HbAS and HbAA subjects. Considering the difference between the infection rate observed at day 7 for mosquitoes fed on blood from each genotype (which reflects the background infection rate plus the force of infection of the respective genotype-controlled blood meal) and the homogeneous mean background mosquito infection rate at day 3, we estimate a more than twofold increased risk of mosquito infection following feeding on blood from HbC subjects (HbAC or HbCC) compared to subjects with the normal HbAA genotype (OR 2.47, 95% CI 1.72–3.33, $P = 1.0 \times 10^{-7}$; **Table 2** and **Supplementary Table 2**). Taking into account the mosquito background infection rates, the estimated risk of infectivity of blood meals from HbAS subjects is significantly higher than that of blood meals from HbAA wild-type individuals (OR 1.57, 95% CI 1.08–2.30, $P = 0.018$).

For the *ex vivo* study, based on artificial membrane feeding of naive insectary-reared mosquitoes collected at the larval stage from the same study area as the *in vivo* mosquitoes and at their first blood meal, a total of 2,171 mosquitoes were analyzed. We found a fourfold higher risk of infection compared to the HbAA genotype for mosquitoes fed on blood from subjects with the HbAS (OR 4.46, 95% CI 1.89–10.89, $P = 0.00015$), HbAC (OR 4.08, 95% CI 1.77–9.76, $P = 0.0003$) and HbCC genotypes (OR 3.89, 95% CI 1.68–9.37, $P = 0.0005$) (**Table 3** and **Supplementary Table 3**). These membrane-feeding experiments also showed markedly higher oocysts densities in mosquitoes fed on blood from subjects with the HbAS, HbAC and HbCC genotypes compared to the HbAA genotype ($P < 0.0001$). Oocysts counts were particularly high in mosquitoes that had taken their blood meal from HbAS subjects; there was a significant difference in oocyst count in mosquitoes fed from HbAS blood as compared to HbAC ($P = 0.001$) and HbCC blood ($P = 0.001$) (**Table 3**).

**Table 3** ***Ex vivo Anopheles*** **infection (oocyst rate and densities) after two replicates of membrane feeding experiments**

| Blood meal genotype | Mosquito infection rate (%)[a] | Arithmetic mean of oocyst density (range) |
|---|---|---|
| AA | 1.4 (8/573) | 0.02 (0–2) |
| AS | 5.9 (26/438) | 0.45 (0–28) |
| AC | 5.5 (32/586) | 0.15 (0–9) |
| CC | 5.2 (30/574) | 0.14 (0–11) |
| AS + AC + CC | 5.5 (88/1598) | 0.23 (0–28) |
| TOTAL | 4.4 (96/2171) | 0.173 (0–28) |

Experiments were performed on blood from 14 volunteers from each β-globin genotype group (HbAA, HbAS, HbAC and HbCC) from malaria-hyperendemic areas of Burkina Faso, West Africa. Pairwise comparisons between genotypes are shown in **Supplementary Table 3**.
[a]At day 7.

The parasitological and entomological *in vivo* and *ex vivo* investigations both provide evidence that the transmission of *P. falciparum* from humans to *Anopheles* is enhanced in individuals carrying HbC. Although parasitological surveys did not show any trend of an increased infectious reservoir for individuals carrying HbS, the *in vivo* entomological investigations suggested an increased infectivity compared to HbAA subjects and lower infectivity compared to HbC subjects. The *ex vivo* assays, consistent with previous indications obtained with a similar approach[13], also showed a markedly higher transmission potential for individuals with HbS.

Our study suggests that human genetic variation at the β-globin locus can influence the efficiency of malaria transmission, providing an example of how host genetics may influence the transmission of an infectious disease. In this case, we found that the same genetic variants that are protective against infection also showed an association to transmission efficiency. In the specific case of HbC and HbS, extensive biochemical studies have clearly shown that these mutations result in marked effects on the *P. falciparum*-infected eythrocyte[14–18] and have potential implications in the pathogenesis of severe malaria[16,19–21]. HbC and HbS might also promote the sexual differentiation of *P. falciparum* as a downstream phenotypic event. Previous *in vitro* investigations have suggested that in HbCC and HbSS erythrocytes, the production of *P. falciparum* gametocytes could be increased[22,23]. HbC and HbS could also trigger alternative immune responses[24–26] that enhance the efficiency of parasite transmission from the vertebrate host to the *Anopheles* vector or induce less efficient transmission-blocking immunity[27]. Moreover, it should be considered that the higher infectivity of individuals with β-globin variants observed in this study could also be a consequence of their less frequent use of antimalarial drugs, as these individuals carry more chronic asymptomatic infections. These infections, characterized by low parasite density, are well known to contribute substantially to malaria transmission because of the extended duration of gametocyte carriage[28].

## METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

*Note: Supplementary information is available on the Nature Genetics website.*

ACKNOWLEDGMENTS

We are grateful to the study participants for their understanding and cooperation and to the laboratory and field staff of the IRSS/Centre Muraz of Bobo Dioulasso and of the Centre Medical Saint Camille of Ouagadougou, Burkina Faso, for skilled work, logistics and collaboration. We are indebted to the staff of the IRD Antenne de Bobo Dioulasso for logistic support. We thank G. Modiano, V. Petrarca, B. Arcà and M. Coluzzi for continuous advice and L. Ranford-Cartwright and E. Ferraro for statistical support. This work was funded by grants from the EU, Sixth Framework Programme, BioMalPar Network of Excellence, LSHP-CT-2004-503578 and from the Istituto Pasteur–Fondazione Cenci Bolognetti, University of Rome 'La Sapienza'.

AUTHOR CONTRIBUTIONS
L.C.G. organized and supervised the parasitological and entomological surveys in the Bobo Dioulasso area. G.B. performed parasitological, entomological and genetic surveys in the Bobo Dioulasso area. F.Y. and B.Y. participated in parasitological, entomological and genetic surveys. K.R.D. participated to entomological surveys. J.S. organized and performed the parasitological and genetic survey in the Ouagadougou area. J.B.O. coordinated the study in the Bobo Dioulasso area. D.M. proposed the scientific hypothesis and organized and coordinated the study. L.C.G., C.C., J.B.O. and D.M. designed the research procedure. L.C.G., G.B., J.B.O. and D.M. analyzed and interpreted the data. D.M. and L.C.G. wrote the paper. All authors discussed the results and commented on the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

Published online at http://www.nature.com/naturegenetics/.
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/.

1. Hill, A.V. Aspects of genetic susceptibility to human infectious diseases. *Annu. Rev. Genet.* **40**, 469–486 (2006).
2. Alcaïs, A., Abel, L. & Casanova, J.L. Human genetics of infectious diseases: between proof of principle and paradigm. *J. Clin. Invest.* **119**, 2506–2514 (2009).
3. Williams, T.N. Red blood cell defects and malaria. *Mol. Biochem. Parasitol.* **149**, 121–127 (2006).
4. Agarwal, A. *et al.* Hemoglobin C associated with protection from severe malaria in the Dogon of Mali, a West African population with a low prevalence of hemoglobin S. *Blood* **96**, 2358–2363 (2000).
5. Modiano, D. *et al.* Haemoglobin C protects against clinical *Plasmodium falciparum* malaria. *Nature* **414**, 305–308 (2001).
6. Allison, A.C. The distribution of the sickle-cell trait in East Africa and elsewhere, and its apparent relationship to the incidence of subtertian malaria. *Trans. R. Soc. Trop. Med. Hyg.* **48**, 312–318 (1954).
7. Allison, A.C. Protection afforded by sickle-cell trait against subtertian malarial infection. *BMJ* **1**, 290–294 (1954).
8. Hill, A.V. *et al.* Common west African HLA antigens are associated with protection from severe malaria. *Nature* **352**, 595–600 (1991).
9. Quintana-Murci, L., Alcais, A., Abel, L. & Casanova, J.L. Immunology in natura: clinical, epidemiological and evolutionary genetics of infectious diseases. *Nat. Immunol.* **8**, 1165–1171 (2007).
10. Haldane, J.B.S. Disease and evolution. *Ric. Sci.* **Suppl. 19**, 68–76 (1949).
11. Kwiatkowski, D.P. How malaria has affected the human genome and what human genetics can teach us about malaria. *Am. J. Hum. Genet.* **77**, 171–192 (2005).
12. Modiano, D. *et al.* Haemoglobin S and haemoglobin C: 'quick but costly' versus 'slow but gratis' genetic adaptations to *Plasmodium falciparum* malaria. *Hum. Mol. Genet.* **17**, 789–799 (2008).
13. Robert, V. *et al.* Effect of the sickle cell trait status of gametocyte carriers of *Plasmodium falciparum* on infectivity to anophelines. *Am. J. Trop. Med. Hyg.* **54**, 111–113 (1996).
14. Arie, T., Fairhurst, R.M., Brittain, N.J., Wellems, T.E. & Dvorak, J.A. Hemoglobin C modulates the surface topography of *Plasmodium falciparum*-infected erythrocytes. *J. Struct. Biol.* **150**, 163–169 (2005).
15. Fairhurst, R.M., Fujioka, H., Hayton, K., Collins, K.F. & Wellems, T.E. Aberrant development of *Plasmodium falciparum* in hemoglobin CC red cells: implications for the malaria protective effect of the homozygous state. *Blood* **101**, 3309–3315 (2003).
16. Fairhurst, R.M. *et al.* Abnormal display of PfEMP-1 on erythrocytes carrying haemoglobin C may protect against malaria. *Nature* **435**, 1117–1121 (2005).
17. Tokumasu, F. *et al.* Band 3 modifications in *Plasmodium falciparum*-infected AA and CC erythrocytes assayed by autocorrelation analysis using quantum dots. *J. Cell Sci.* **118**, 1091–1098 (2005).
18. Ayi, K., Turrini, F., Piga, A. & Arese, P. Enhanced phagocytosis of ring-parasitized mutant erythrocytes: a common mechanism that may explain protection against falciparum malaria in sickle trait and beta-thalassemia trait. *Blood* **104**, 3364–3371 (2004).
19. Friedman, M.J. Erythrocytic mechanism of sickle cell resistance to malaria. *Proc. Natl. Acad. Sci. USA* **75**, 1994–1997 (1978).
20. Pasvol, G., Weatherall, D.J. & Wilson, R.J. Cellular mechanism for the protective effect of haemoglobin S against *P. falciparum* malaria. *Nature* **274**, 701–703 (1978).
21. Cholera, R. *et al.* Impaired cytoadherence of *Plasmodium falciparum*-infected erythrocytes containing sickle hemoglobin. *Proc. Natl. Acad. Sci. USA* **105**, 991–996 (2008).
22. Trager, W. & Gill, G.S. Enhanced gametocyte formation in young erythrocytes by *Plasmodium falciparum in vitro*. *J. Protozool.* **39**, 429–432 (1992).
23. Trager, W., Gill, G.S., Lawrence, C. & Nagel, R.L. *Plasmodium falciparum*: enhanced gametocyte formation in vitro in reticulocyte-rich blood. *Exp. Parasitol.* **91**, 115–118 (1999).
24. Hayward, R.E., Tiwari, B., Piper, K.P., Baruch, D.I. & Day, K.P. Virulence and transmission success of the malarial parasite *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. USA* **96**, 4563–4568 (1999).
25. Williams, T.N. *et al.* An immune basis for malaria protection by the sickle cell trait. *PLoS Med.* **2**, e128 (2005).
26. Verra, F. *et al.* Haemoglobin C and S role in acquired immunity against *Plasmodium falciparum* malaria. *PLoS One* **2**, e978 (2007).
27. Vermeulen, A.N. *et al.* Sequential expression of antigens on sexual stages of *Plasmodium falciparum* accessible to transmission-blocking antibodies in the mosquito. *J. Exp. Med.* **162**, 1460–1476 (1985).
28. Price, R. *et al.* Risk factors for gametocyte carriage in uncomplicated falciparum malaria. *Am. J. Trop. Med. Hyg.* **60**, 1019–1023 (1999).

## ONLINE METHODS

**Study populations, cross-sectional parasitological surveys.** The first parasitological survey was performed during November–December 2001 in five rural villages (Donsin, Bassy, Koubry, Kuiti and Nagbagré) near Ouagadougou, Burkina Faso. The area has a rainy season lasting from June to mid-October, which corresponds to the period of highest malaria transmission, and a long dry season from November to May. Malaria transmission levels in this arearange between 50 and several hundred infective bites per person per year. The main malaria vectors are *A. gambiae* s.s., *A. arabiensis* and *A. funestus*. A total of 1,737 children aged 0–15 years (mean age ± s.e.m., 8.7 ± 0.1) were recruited. The second survey was conducted in March–May 2007 in the rural village of Soumousso, situated at about 35 km southeast of Bobo-Dioulasso, Burkina Faso. The area has a rainy season lasting from May to November and a dry season from December to April. Malaria transmission is perennial, with annual entomological inoculation rates in the order of hundreds of infective bites per person per year. The main malaria vectors are *Anopheles gambiae* and *A. funestus*. A total of 2,002 subjects from all age groups (18.0 ± 0.4) were recruited. During both cross-sectional surveys, the frequency of individuals declaring the assumption of any prescription drug intake within the 4 weeks preceding the survey was less than 1% (0.6% in the first survey and 0.3% in the second one).

The first survey was performed in the context of a large program for the screening of hemoglobinopathies in Burkina Faso, carried out by the Saint Camille Health Center of Ouagadougou in 2001, and the study protocol was approved by the Ministry of Health of Burkina Faso. The study protocol of the project conducted in the Bobo Dioulasso area was approved by the institutional review board of the Centre Muraz, Bobo Dioulasso. In accordance with the guidelines of the ethical review committee, a village meeting was conveyed to explain the purpose of the survey to the population, after which oral consent was obtained from the area chief and village elders to conduct the study in the area. Written, informed consent was explicitly obtained from individual participants; for participating children, assent was requested and their parents or guardians signed the informed consent form on their behalf.

**Blood examinations.** During both surveys, a 2–3-ml venous blood sample was collected from each subject to perform hematological analyses. Thick and thin blood smears were prepared according to the WHO guidelines for the microscopic diagnosis of malaria (Bench Aids for the Diagnosis of Human Malaria; Plate 8), and 100 microscopic fields (~20 leukocytes per field at ×1,000 corresponding to ~0.25 µl of blood) of the thick blood smear were examined. The *Plasmodium* species was identified on the thin blood smear. The status of *Plasmodium falciparum* gametocyte carrier was defined as the presence of at least one *P. falciparum* gametocyte on 100 microscopic fields (~20 leukocytes per field at ×1000 corresponding to ~0.25 µl of blood, that is, 4 gametocytes/µl of blood). Each smear was evaluated independently by two expert microscopists. Any discordance was resolved by a third microscopist. Subjects with a positive smear were treated following the guidelines of the Ministry of Health of Burkina Faso.

**Hemoglobin typing.** Hemoglobin typing was performed by cellulose acetate electrophoresis on samples from the first survey and by PCR/RFLP on samples from the second survey. DNA extraction from filter paper was performed by the Chelex100 method[29]. Characterization of the A, C and S alleles for the sixth codon of the β chain of hemoglobin has been performed by using a new pentaprimer ARMS-PCR protocol and a modified RFLP protocol. Tetraprimer ARMS-PCR allows the detection of two alleles at the same polymorphic site in a unique PCR reaction by producing allele-specific fragments of different size. In this case, we introduced a fifth primer designed to have a base mismatch at position 2 bp from the 3′ terminus to increase specificity of reaction for the C allele amplification[30].

*Pentaprimer ARMS-PCR for C and A alleles*. PCR conditions: for each sample, 50 ng of DNA were amplified in a total volume of 10 µl using reaction buffer (Bioline), 0.2 mM dNTPs each, 1.5 mM $MgCl_2$, 0.2 µmol of primers Hbext-FOR, Hbext-REV, 0.1 µmol of primers armsC-FOR and BETAC-FOR, 0.4 µmol of primer arms-AREV (primers sequences are listed in **Supplementary Table 4**) and 1 unit of Taq polymerase (Bioline). The following touchdown PCR program was used: 12 cycles, with the annealing temperature decreasing 0.5 °C each cycle, starting at 95 °C for 5 min, 95 °C for 50 s, 64 °C for 50 s and 72 °C for 40 s; followed by 25 cycles of 95 °C for 40 s, 58 °C for 40 s and 72 °C for 30 s, with a final step of elongation at 72 °C for 7 min.

PCR fragments (433 bp for control, 292 bp for A alleles and 183 bp for C alleles) were separated by electrophoresis in 1.5% agarose gel.

*RFLP for the S allele*. The S allele has been characterized by amplification with Hbext-FOR and Hbext-REV primers with the same PCR conditions and program of pentaprimers ARMS-PCR. The amplified fragments were digested at 37 °C for 4 h with DdeI restriction enzyme and analyzed by 1.5% agarose gel electrophoresis.

**Transmission experiments.** Transmission studies were carried out in the rural village of Soumousso during the 2008 high malaria transmission season (July–October). To assess the possible influence of the β-globin genotype on parasite transmission from the human host to mosquitoes, two different and complementary procedures based on *in vivo* and *ex vivo* approaches were applied. Both the *in vivo* and *ex vivo* investigations were performed in blind, that is without checking for the presence of gametocytes in peripheral blood during exposure to mosquitoes (*in vivo* experiments) or before blood collections (*ex vivo*). A total of 6,446 mosquitoes were examined. For each β-globin genotype, the human and mosquito samples analyzed in the *in vivo* and *ex vivo* experiments during each month of the survey are shown in **Supplementary Table 5**. Subjects declaring the assumption of any prescription drug intake within the 4 weeks preceding the *in vivo* or *ex vivo* investigations were excluded. The mosquito maintenance and experimental infections were performed in the laboratory and insectary of IRSS, Bobo-Dioulasso. The team working for transmission experiments (both *in vivo* and *ex vivo*) and the microscopists were independent and not aware of the β-globin genotype of subjects recruited in the study.

*Natural conditions (in vivo)*. A total of 15 groups of 4 children aged 3–12 years, with each child representing one of the four HbAA, HbAS, HbAC and HbCC β-globin genotypes, were selected and ranked by sex and age. Each experimental series involved two groups of four children. Children slept individually in four different sentinel houses for four consecutive nights from 6 p.m. to 6 a.m. Participants exchanged positions between sentinel houses every night so that children with different β-globin genotypes received equal exposure during the round of four nights. Eight groups completed three rounds of experiments (12 nights per child), five groups performed two rounds (8 nights per child) and the remaining two groups were exposed for only one round (that is, 4 nights per child). Following each night, all indoor resting female mosquitoes were collected by aspirator catch in each house, placed separately in small holding cages that were labeled with anonymous identification codes and transported alive in an isotherm box to the laboratory. Non-malaria vectors were discarded, and anophelines were classified into species using morphological criteria. Only freshly fed *Anopheles* mosquitoes from each daily sample were provided with 5% glucose solution and kept in the insectary under constant humidity and temperature. At day 3, a randomly selected subsample of 10% of surviving mosquitoes was dissected to estimate the background infection rates before the genotype-controlled blood meal. To measure the impact of the last blood meal, the remaining mosquitoes were dissected at day 7. Mosquito infection rates were estimated by midgut staining with 2% mercurochrome solution and detection of oocysts by light microscopy (×10). Mosquitoes having at least one oocyst were considered infected and their parasite load was counted. Dissections and microscopic analyses were performed in blind by two expert microscopists not involved in the field mosquito collections.

*Direct membrane feeding assay (ex vivo)*. The same subjects involved in the *in vivo* analyses volunteered to donate 5 ml of venous blood for membrane feeding experiments. Two replicates of experimental infections with a total of 14 subjects for each genotype were performed. Each subject was bled twice with a minimum interval of 45 d between the two bleedings. Mosquito feedings were done using 3–5-day-old female mosquitoes which emerged from larvae collected in productive anopheline breeding sites in the Soumousso study area. On each occasion, mosquito larvae were collected using a standard dipping technique until a sufficient number of larvae had been collected. These larvae were transported to the laboratory and reared to adult age. Developing larvae were fed with Tetramin until all the larvae pupated. Pupae were transferred to holding cages ($30 \times 30 \times 30$ $cm^3$), and after emergence, the adult mosquitoes,

exposed to ambient conditions in the insectary (26 ± 1 °C and relative humidity at approximately 80%), were provided with 5% glucose solution and kept in cages for 2–3 d before being used in blood-feeding experiments with mini-feeders covered with Parafilm membrane[31]. Batches of 50–100 female mosquitoes drawn from the same larval cohort were randomly put in paper cups and starved for 6 h before experimental feeding[32]. Blood samples from individual study participants were collected into a heparinized tube and immediately transferred into prewarmed (37 °C) artificial membrane glass mini-feeders. Two batches of mosquitoes allocated to each genotype were allowed to feed for 20 min, after which only fully fed mosquitoes were kept in 30 × 30 × 30 $cm^3$ holding cages under ambient insectary conditions. Seven days later, surviving mosquitoes were dissected and their midgut was examined for the presence of *P. falciparum* oocysts. Microscopic analyses were performed as described for the *in vivo* experiments.

**Statistical analyses.** *P* values of the comparisons were obtained by Yates-corrected $\chi^2$ test. Unadjusted ORs were calculated with 95% CIs (**Tables 1–3** and **Fig. 1**). Mantel-Haenszel weighted ORs (95% CI) and MLE of ORs (95% CI) were also calculated after stratification by age (0–3, >3–6, >6–10 and >10 years for the first cross-sectional survey; 0–3, >3–6, >6–10, >10–20 and >20 years for the second survey), gender, village, and, for the transmission experiments, by age (3–6, >6–9 and >9–12 years), gender and month of the high malaria transmission season during which the study was conducted. Student's *t*-test was used for the comparisons of the geometric means of asexual and sexual parasite densities. Pairwise comparisons of arithmetic means of oocyst densities, based on the negative binomial distribution, were performed using generalized linear models in SPSS 17.0[33].


29. Walsh, P.S., Metzger, D.A. & Higuchi, R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10**, 506–513 (1991).
30. Ye, S., Dhillon, S., Ke, X., Collins, A.R. & Day, I.N. An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Res.* **29**, E88–E8 (2001).
31. Gouagna, L.C. *et al.* Stage-specific effects of host plasma factors on the early sporogony of autologous *Plasmodium falciparum* isolates within Anopheles gambiae. *Trop. Med. Int. Health* **9**, 937–948 (2004).
32. Tchuinkam, T. *et al.* Experimental infections of *Anopheles gambiae* with *Plasmodium falciparum* of naturally infected gametocyte carriers in Cameroon: factors influencing the infectivity to mosquitoes. *Trop. Med. Parasitol.* **44**, 271–276 (1993).
33. Wilson, K. & Grenfell, B.T. Generalized linear modelling for parasitologists. *Parasitol. Today* **13**, 33–38 (1997).





doi:10.1038/ng.554