Genome-Wide Linkage Analysis of Malaria Infection Intensity and Mild Disease

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Although balancing selection with the sickle-cell trait and other red blood cell disorders has emphasized the interaction between malaria and human genetics, no systematic approach has so far been undertaken towards a comprehensive search for human genome variants influencing malaria. By screening 2,551 families in rural Ghana, West Africa, 108 nuclear families were identified who were exposed to hyperendemic malaria transmission and were homozygous wild-type for the established malaria resistance factors of hemoglobin (Hb)S, HbC, alpha+-thalassemia, and glucose-6-phosphate-dehydrogenase deficiency. Of these families, 392 siblings aged 0.5–11 y were characterized for malaria susceptibility by closely monitoring parasite counts, malaria fever episodes, and anemia over 8 mo. An autosomal-wide linkage analysis based on 10,000 single-nucleotide polymorphisms was conducted in 68 selected families including 241 siblings forming 330 sib pairs. Several regions were identified which showed evidence for linkage to the parasitological and clinical phenotypes studied, among them a prominent signal on Chromosome 10p15 obtained with malaria fever episodes (asymptotic z score = 4.37, empirical p-value = 4.0 × 10⁻⁵, locus-specific heritability of 37.7%; 95% confidence interval, 15.7%–59.7%). The identification of genetic variants underlying the linkage signals may reveal as yet unrecognized pathways influencing human resistance to malaria.

Introduction

Malaria caused by Plasmodium falciparum is one of the leading causes of human morbidity and mortality worldwide, predominantly affecting populations of resource-poor countries in the south [1]. Drawbacks in developing effective control measures have stressed the demand for research aiming at a better understanding of basic elements of parasite biology and disease pathology.

The blood stages of the parasite comprise asexual forms, which maintain the infection and cause disease, and sexual forms, which transmit the infection [2]. Asexual blood parasite counts are the established measure of infection intensity [3], whereby reports on substantial variations over a short period of time indicated that many measurements may be required for appropriate estimates [4].

Clinically, malaria presents as a mild form of acute febrile episodes and anemia, or as a severe form, which comprises a complex syndrome of life-threatening complications [5]. While the severe form causes an enormous humanitarian burden, it does not affect more than 1%–2% of the residents of endemic areas [6], whereas the mild form predominates in terms of quantitative morbidity and economic reasoning [1,7,8]. While the non-specific symptoms of fever, headache, and nausea make the diagnosis of malaria fever episodes difficult to ascertain, a simple case definition proposed by the World Health Organization (WHO) based on fever and parasitemia is generally accepted due to its high sensitivity and specificity in endemic areas, where the vast majority of such episodes are in fact caused by malaria [9].

A second clinical feature of mild malaria is anemia. It affects an enormous number of children in endemic areas [10] and may present as a chronic, subacute, or acute, sometimes life-threatening form [5]. Its pathogenesis is considered multifactorial and may include the destruction of infected and uninfected erythrocytes and bone-marrow dysfunction, whereby the relative contributions of these factors and their roles in the various forms of malarial anemia have not yet been resolved [11].

The effect of human genetics on malaria has long been recognized when the theory of balancing selection was substantiated for thalassemias, sickle-cell anemia, and other
Author Summary

In tropical Africa, virtually all children become infected with malaria parasites. Most of them experience several malaria attacks per year, and over a million die from disease complications. Sickle-cell anemia, thalassemias, and other inherited red blood cell disorders indicate that malaria has selected for human genetic variants, but no attempts have so far been reported to systematically screen the human genome for malaria-resistance factors. We describe a genome-wide linkage analysis performed in children living in rural Ghana, West Africa, including approaches to select an informative study cohort and to assess, over a period of 8 mo, individual disposition to malaria parasitemia, fever episodes, and anemia. Families carrying the known malaria-protective red blood cell disorders were excluded, infection intensities were adjusted to the use of mosquito-protection devices, and parasitological and clinical findings were corrected according to the state of partial malaria immunity, which, under constant exposure, gradually develops over the first 10 y of life. The study revealed several genomic regions showing evidence for linkage to the various malaria phenotypes recorded, among them a prominent signal on Chromosome 10 correlated to the frequency of fever episodes. Future identification of genes involved is expected to reveal previously unrecognized pathways that may protect children against malaria.

Materials and Methods, 377 individuals of 68 families were selected for genotyping, including 136 parental individuals and 241 siblings, who formed 330 sib-pairs. Applying the Affymetrix Human Mapping 10K array yielded an overall autosomal calling fraction of 94.5% for the raw genotypes. These were defined as SNPs for which definitive genotypes were obtained. After application of the quality control procedure, 1,524 autosomal markers (15.2%) were excluded from further analysis. The remaining markers yielded a mean information content of 0.976 (SD ± 0.029, range 0.510–1.000).

Linkage Analysis

The nonparametric linkage analysis (NPL) and Haseman-Elston multipoint linkage analysis (HE) were applied (Figure 2A–2D). Parasite prevalence, parasite density, fever episodes, and anemia were analyzed as quantitative phenotypes. The most prominent result was a linkage signal for malaria fever episodes on Chromosome 10p15.3–10p14, which reached statistical significance in both the NPL and HE analyses. NPL showed an asymptotic p score of 4.37 (empirical p-value = 4.0 × 10⁻²) between SNP markers rs952153 and rs1964428 marking the interval of 5.9–12.0 cM and 2.5–3.5 Mb of the genetic and physical chromosomal maps, respectively (Figure 2D). HE showed a maximum asymptotic logarithm of odds (LOD) score of 3.03 (empirical p-value = 2.1 × 10⁻³) at marker rs1964428 corresponding to 12.0 cM/3.5 Mb (Figure 2D). The locus-specific heritability was estimated to be 37.7% (95% confidence interval, 15.7%–59.7%) at 11.2 cM. The linkage region was termed PFFE-1 for P. falciparum-fever episode 1. The signal was robust to variations in data analysis, including the use of a raw phenotype without adjustments for covariates (p score of 4.52), or the use of a wider definition of malaria fever episodes that included afebrile malaria episodes diagnosed by the study physicians (p score of 4.04). The 2.2 z score support interval (corresponding to a 1-LOD support) encompassed a 27.4 cM/11.0 Mb distance containing 71 annotated or hypothetical genes. Functional candidates include genes encoding a platelet-type phosphofructokinase (PFKP) also expressed in red blood cells, an inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (iPFK-2/ PFKB3), the alpha chain of the interleukin-2 receptor (IL2RA), the alpha chain of the interleukin 2 receptor (IL2RA), protein kinase C theta (PRKCO), the GATA-binding protein 3 (GATA3), and a gene similar to that of the interleukin 1 receptor precursor (LOC459945).

A further region with evidence for linkage was found using parasite density as the phenotype. The NPL analysis yielded a signal on Chromosome 13q with a maximum asymptotic z score of 3.73 (empirical p-value = 2.3 × 10⁻³) between rs2147363 at chromosomal position 55.0 cM/51.4 Mb and rs726540 at 55.5 cM/52.3 Mb (Figure 2B). HE resulted in a LOD score of 1.19 at this position (Figure 2B). The locus-specific heritability was estimated to be 33.7% (95% confidence interval, 9.8%–57.6%). The region was termed PFPD-2 for P. falciparum-parasite density 2, whereby another linkage region with parasite density had previously been reported [17–19]. The 2.2 z support interval of PFPD-2 encompassed 24.2 cM/32.4 Mb containing 158 annotated or hypothetical genes. Possible functional candidates include genes encoding the lymphocyte cytotoxic protein 1 (LCP1), S-formylglutathione hydrodrolase (esterase D, ESD), the cysteinyl proteinase inhibitor-2 (CPI-2) and the IL-21 receptor (IL21R).

Results

Phenotypes

392 siblings of 108 families resident in West Africa were followed over a period of 31 wk, which covered an entire rainy season. Prevalences of P. falciparum blood trophozoites, parasite densities, and interim or present malaria fever episodes were monitored weekly and anemia as indicated by the packed blood-cell volume (PCV) was determined biweekly. Compliance was as follows: 98.8% of 12,152 parasitemia assessments, 95.4% of 6,272 PCV assessments, and 98.5% of 12,152 assessments for malaria fever episodes were recorded with a maximum of data missing per planned visits of single participants of 13/31, 6/16, and 18/31 records, respectively. Results from regression models for analyzing the effect of age, bednet use, and intake of antimalarials on the various phenotypes are summarized in Figure 1. Gender had no significant effect on any of the phenotypes, and therefore was not included in the final regression models used for phenotype corrections.

Genotyping

Based on a ranking that favored high levels of parasite densities in conjunction with high intrafamilial variability (see Materials and Methods), 377 individuals of 68 families were selected for genotyping, including 136 parental individuals and 241 siblings, who formed 330 sib-pairs. Applying the Affymetrix Human Mapping 10K array yielded an overall autosomal calling fraction of 94.5% for the raw genotypes. These were defined as SNPs for which definitive genotypes were obtained. After application of the quality control procedure, 1,524 autosomal markers (15.2%) were excluded from further analysis. The remaining markers yielded a mean information content of 0.976 (SD ± 0.029, range 0.510–1.000).
leukotriene receptor 2 (CYSLTR2), and the endothelin receptor, nonselective type, (EDNRB).

Furthermore, a signal on Chromosome 1p36 at 18 cM/9 Mb provided evidence for linkage with both parasite prevalence (LOD score of 2.31; empirical \( p \)-value = \( 5.3 \times 10^{-4} \)), and PCV (LOD score of 2.45; empirical \( p \)-value = \( 3.9 \times 10^{-4} \)) at adjacent marker positions (rs205474 and rs966134, respectively; Figure 2A and 2C). The NPL \( z \) scores were low in both instances (2.75 and 2.36, respectively).

Finally, no evidence was obtained for linkage of parasite density or malaria fever episodes to 5q31-q33 and to the MHC region on 6q23, respectively, which had previously been reported. In the present study, weak evidence was obtained that malarial anemia might be linked to 5q31-q33 (\( z \) score = 2.7, LOD score = 1.8) (Figure 2C).

Discussing

To our knowledge, this is the first genome-wide approach to identify human genetic variants influencing susceptibility and resistance to malaria. Since the seminal observations on balancing selection with inborn red blood cell disorders, malaria is a prominent element in human genetics. The importance of the classic malaria-protective red blood cell traits is in the present study highlighted by the large proportion of 86% of families found to be affected in the initial survey of our study population. These were excluded from the study in order to concentrate the search on as yet unrecognized human genetic variants [16,20]. As genetic influences were reported to be of particular relevance in childhood malaria [14], we limited our study to children aged 0.5–11 y.

Assessing the phenotype of malaria infection intensity

Figure 1. Phenotype Adjustments of the Study Group (392 Siblings)
(A) Presence of asexual blood forms of *P. falciparum* in 31 weekly blood smears.
(B) Log of the 75th percentile of 31 parasite density values per individual.
(C) Median of 16 PCV assessments per individual.
(D) Malaria fever episodes were defined following WHO recommendations whereby multiple malaria attacks within 3 wk were considered recrudescences and counted as one episode (see Materials and Methods).

Gender did not significantly influence any of the phenotypes. For the phenotype of parasite density, the effects of antimalarial treatments were addressed by exclusion of density values following 3 wk after treatment (see Materials and Methods). The phenotype of fever episodes was not corrected for the number of antimalarial treatments because of the direct causal relationship between disease episodes and treatments.

31 weekly regression models.

Anemia (PCV) was normally distributed, Shapiro-Wilk \( W \) test, \( p > 0.2 \).
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remains a challenge because it is uncertain to which extent any limited number of parasite counts truly reflect the infection intensity [4]. In addition, infection intensities may strongly depend on exposure, which is a variable difficult to assess in field studies. In the present study, the use of bednets and window screens to reduce exposure by preventing mosquito bites was addressed by data adjustments and exclusions of families, respectively (see Materials and Methods). It may be considered an advantage that the NPL and HE methods applied are based on intra-familial evaluations because malaria exposure is likely to be homogeneous within families living in the same households.

As expected, antimalarial treatments had an effect on all phenotypes studied. The influence on parasite prevalences and parasite densities was found to be limited to the two subsequent assessments, therefore it was addressed by correcting the respective values of prevalences and by excluding the corresponding densities (see Materials and Methods).
Methods). In contrast, the influence on anemia was corrected for by adjusting the overall phenotype because epidemiological observations on the effect of drug resistance on anemia suggest possible long-term effects [22,23]. Concerning the number of fever episodes, no adjustments were made because they might have neutralized the essential phenotypic information due to the direct causal relationship between disease episodes and treatments.

Of all covariates tested, age had the strongest effect and was included in all phenotype adjustments. In children older than 6 mo as in the present cohort, the age effect on malaria in endemic areas is dominated by the gradual development of a certain degree of adaptive immunity, termed semi-immunity. This is reflected by a successive decrease over age of the number of fever episodes, the degree of anemia, parasite densities, and, at relatively high age, parasite prevalences [24–27]. Therefore, the phenotypes addressed may be influenced by both innate resistance and adaptive immunity, whereby innate resistance may have a predominant influence in younger children and adaptive immunity in older ones. This may focus the linkage signals obtained in this study on variants that are relevant under both conditions.

The phenotypes studied showed significant correlations between each other. This is in agreement with the general understanding that all signs and symptoms of malaria result from parasitemia. The explained variances in most instances were low, however, leaving room for separate genetic influences. As expected, the correlation between parasite prevalences and parasite densities was exceptionally high. Despite this, both were included as separate phenotypes because there is evidence to suggest that they are under distinct genetic influences. First, epidemiological findings including those of the present study (unpublished data) indicate that semi-immunity suppresses high parasite densities significantly more efficiently than low parasite densities [24], which suggest distinct elements of adaptive immunity. More importantly, HbS has been shown to protect from high parasite density but not from parasitemia itself [28], indicating that mechanisms of genetic resistance may affect high parasite density specifically.

Evaluation of the data using established linkage methods revealed several prominent linkage signals. Interestingly, locus-specific heritability calculations performed for two of these linkage regions indicated that, in both cases, approximately 35% of the total phenotype variability was attributable to these loci in families who did not carry any of the established malaria resistance factors. These estimates allow us to postulate the effect of a major locus in both instances, which would support a recent conclusion that susceptibility and resistance to infectious diseases may be governed by single major genes rather than by a large number of genes each exerting a small influence [29].

The region showing strongest and significant linkage concerned the phenotype of malaria fever episodes (PFFE-I). Notably, the signal was found in both model-free approaches. Furthermore, it was robust to variations in phenotype definitions, which may be of particular importance because the non-specific symptoms of malaria fever episodes make the clinical diagnosis uncertain. That we found the strongest linkage signal with this particular phenotype may relate to the fact that fever regulation might be similar regardless of whether it is influenced by innate resistance or adaptive immunity, with respect to the age-dependent bias introduced into our study cohort by these two factors, as described above. The underlying genetic variant may be of more general interest because it may relate to the regulation of the systemic inflammatory response.

A number of additional regions with evidence for linkage were identified which did not reach statistical significance. Therefore, they are not discussed in any detail, although experiences in other complex diseases have shown that weaker linkage signals may as well lead to the identification of relevant genetic variants [30]. The linkage regions described comprise a number of genes which may be classified as functional candidates because their products are operative in immune regulation or red blood cell metabolism. However, regarding their established functions, we consider none of them a prime candidate.

No support for our data can be derived from previous linkage studies in mouse malaria. Studying parasite density in murine Plasmodium chabaudi infection, evidence has been obtained for linkage regions on Chromosomes 3, 5, 9, 11, and 17 [31] but not on Chromosome 14, which covers the synteny of the linkage region on human 13q we obtained for P. falciparum-parasite density (NCBI, http://www.ncbi.nlm.nih.gov/Homology/). This is not unexpected because P. falciparum-infected red blood cells adhere to the vascular endothelium [2], which may have a strong influence on parasite biology. Further linkage studies on murine malaria are limited to the phenotype of cerebral manifestations in Plasmodium berghei infections [31], which cannot be compared to our clinical phenotypes of uncomplicated malaria, and identified regions on Chromosomes 1, 11, and 17 but not on 13 and 2, which cover the regions syntenic to PFPE-I on 10p.

To our knowledge, this is the first time that the Affymetrix HMA10k chip was used for genotyping individuals of African descent. The raw genotypes yielded a call rate of 94.5%, which nearly reached 95% considered sufficient for optimal assay performance [32] and was comparable to 96.9% reported for Caucasians [33]. This provides a basis for using the chip in African populations.

Materials and Methods

Participants. The study was conducted in the Asante Akim North District, Ashanti Region of Ghana, West Africa, a region classified as hyperendemic for malaria by a cross-sectional prevalence of 0.54 for P. falciparum.

Ethical approval was obtained from the Committee for Research, Publications and Ethics of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. All procedures were explained in the local language, and consent was obtained from both parents. Parents of 2,551 families were recruited who had three or more children below the age of 12 y and agreed to participate. Venous blood samples of 2 ml were obtained from both parents and preserved by addition of an equal volume of 8 M urea. The genetic variants of hemoglobin (Hb)S, HbC, alpha thalassemia deletion 3.7, and glucose-6-phosphate-dehydrogenase (G6PD) deficiency A-, which were considered to possibly influence susceptibility to P. falciparum parasitemia and mild malaria, were determined, and 346 (13.6%) families were identified not segregating any of the traits.

Of the 346 families, a study group of 392 siblings of 108 families was selected based on (i) the logistic criterion that their homes clustered in 16 of the 30 villages included in the initial survey and (ii) that they did not live in homes equipped with window screens, which a posteriori were found to significantly reduce parasite prevalences.

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from 0.54 to 0.35 (p < 0.001) and other parameters of malaria infection intensity. All families belonged to the ethnic group of Akan. A subset of 377 members of 68 families were selected from the study group by excluding siblings who were absent at more than two assessments and by a ranking that favored high levels of parasite densities of *P. falciparum* in conjunction with high intrafamilial variability. The complete individual score was hence based on a combination of log parasite densities of *P. falciparum* within sib-ships multiplied by the standard deviation of log parasite densities within sibships. Families with highest scores were selected until 377 individuals were identified for genotyping. The genetic study group comprised 136 parents with 241 children, 52.5% boys and 47.5% girls, who, with a mean of 3.34 siblings per family, formed 330 sib pairs. Their median age was 5 y (range 0.5–11 y; IQR 3–8 y).

**Phenotype assessments.** The children were phenotyped from May 20 to December 20, 2002. Weekly assessments by the visit of a trained physician included a medical history, measurement of body temperature by an infrared ear thermometer, a blood sample by finger prick or heel prick (approximately 100 μl), and, in case of disease symptoms, a physical examination. The installation of window screens in homes and the use of bed-nets were recorded.

Weekly malaria smears were prepared at the study site, and in the laboratory they were stained with Giemsa and examined [34]. Parasite species were assessed, and parasite counts were recorded per 200 leukocytes (if >10 parasites/200 leukocytes) or 500 leukocytes (if <10 parasites/μl blood cell volume). The two independent examinations of each individual were calculated assuming a leukocyte count of 8,000/μl [34]. If the densities as determined in the two counts differed by a factor of three or more, a third independent count was obtained. The median parasite density of two or three counts was included in the analysis.

Weekly point prevalences of malaria parasitemias showed a median prevalence of 53.1% and a 75th percentile of 68.0% in 12,011 weekly malaria smears of 27% of persons with malaria parasitemias (and four with other malaria parasites). Fever attacks were treated by a standard dose of chloroquine or antimalarial drugs (Amodiaquine following national guidelines, other illnesses as deemed appropriate) and the use of bed-nets were recorded.

Anemia was on the spot assessed as PCV by capillary hematocrit centrifugation using 70 μl EDTA anticoagulated capillary tubes (Becton Dickinson, Germany) and mobile centrifuges. To reduce iron-deficiency as a possible confounder, prior to phenotyping, all children were treated against hookworm infection with 400 mg albendazole followed by oral iron supplementation of 2 mg/kg Fe2+ (Becton Dickinson, Germany) and mobile centrifuges. To reduce variability, assessments were performed by the same physician included a medical history, measurement of body temperature by an infrared ear thermometer, a blood sample by finger prick or heel prick (approximately 100 μl), and, in case of disease symptoms, a physical examination. The installation of window screens in homes and the use of bed-nets were recorded.

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A high density SNP genome scan was performed using a whole-genome sampling analysis (WGSA) approach [32] with the Affymetrix GeneChip Human Mapping 10K v2 Array (early access) comprising 10,689 SNPs markers with an average heterozygosity in Caucasians of 58% [54]. The mean length of 258 Kb for 640,000 Affymetrix Annotation files, http://www.affymetrix.com). Mapping order and genetic distances of markers were obtained from Affymetrix, the genetic position of 86 markers was unavailable, 295 were X-linked, and 10,279 were from autosomes. Allele frequencies were estimated from 134 founders. The physical positions of the markers were aligned to human DNA sequence information available from NCBI/NIH (http://www.ncbi.nlm.nih.gov/mapview/maps.cgi).

Genotype calling was done using a single determination of a specific genotype. A longitudinal survey in Mali. Trop Med Int Health 5: 404–412.

WHO (2000) Severe falciparum malaria. World Health Organization, Communicable Diseases Cluster. Trans R Soc Trop Med Hyg 94 (Suppl 1): 9–59.

Greenwood B, Marsh K, Snow B (1991) Why do some African children develop severe malaria. Parasitol Today 7: 277–281.

Foster S, Philpits M (1998) Economics and its contribution to the fight against malaria. Ann Trop Med Parasitol 92: 391–398.

Chima RI, Goodman CA, Mills A (2003) The economic impact of malaria in Africa: A critical review of the evidence. Health Policy 63: 17–36.

WHO (2000) Expert Committee on Malaria, Twentieth Report. WHO Technical Report Series No. 892. Available: http://www.wrb.who.int/docs/er20.pdf. Accessed 22 February 2007.

Schellenberg D, Delaliadze I, Mushi A, Savigny D, Mgalula L, et al. (2001) The silent burden of anaemia in Tanzanian children: A community-based study. Bull World Health Organ 79: 41–50.

Robertson DJ, Casals-Pascual C, Weatherall DJ (2005) The clinical and pathophysiological features of malaria in children. Curr Top Microbiol Immunol 295: 137–167.

Flint J, Harding RM, Boyce AJ, Clegg JB (1998) The population genetics of the haemoglobinopathies. Bailliere Clin Haematol 11: 1–51.

Jepson AP, Banya WA, Sixay-Joos F, Hassan-King M, Bennett S, et al. (1995) Genetic regulation of fever in Plasmodium falciparum malaria in Gambian twin children. J Infect Dis 172: 316–319.

Riet P, Abel L, Traore Y, Traore-Leroux T, Aucan C, et al. (1998) Human malaria: Segregation analysis of blood infection levels in a suburban area and a rural area in Burkina Faso. Genet Epidemiol 15: 435–450.

Mackinnon MJ, Mwangi TW, Snow RW, Marsh K, Williams TN (2005) Heritability of malaria in Africa. PLoS Med 2: e340. Epub.

Kwiatkowski DP (2005) How malaria has affected the human genome and what human genetics can teach us about malaria. Am J Hum Genet 77: 171–192.

Kwiatkowski P, Traore Y, Abel L, Aucan C, Traore-Leroux T, et al. (1999) Malaria in humans: Plasmodium falciparum blood infection levels are linked to Chromosome 3q11-3q3. Am J Hum Genet 63: 498–505.

Garica A, Marquet S, Bucheton B, Hillaire D, Cot M, et al. (1998) Linkage analysis of blood Plasmodium falciparum levels: Interest of the 3q11-3q3 chromosome region. Am J Trop Med Hyg 58: 705–79.

Floir L, Kumulungui B, Aucan C, Einaudi C, Traore AS, et al. (2003) Linkage and association between Plasmodium falciparum blood infection levels and Chromosome 3q11-3q3. Genes Immun 4: 265–268.

Nowell PJ, C Cremer F, Scharf H, Wipt M (2001) Genomewide scans of complex human diseases: True linkage is hard to find. Am J Hum Genet 69: 936–950.

Lengeler C (2004) Insecticide-treated bednets and curtains for preventing malaria in children. Cochrane Database Syst Rev 2: CD00247. Updated 11 May 2004. doi: 10.1002/14651858.CD00247.pub2.

Evans JA, May J, Tomasini D, Egeltte T, Marks F, et al. (2005) Pre-treatment with chloroquine and parasite chloroquine resistance in Ghanian children with severe malaria. QJM 98: 798–796.

Meermann L, Ord R, Beutner-Navierkom M, Osman E, et al. (2005) carriage of chloroquine-resistant parasites and delay of effective treatment increase the risk of severe malaria in Gambian children. J Infect Dis 192: 1651–1657.

Roper C, Gommenges D, Trape JF (1996) Evidence for an age-dependent pyrogenic threshold of Plasmodium falciparum parasitaemia in highly endemic populations. Am J Trop Med Hyg 54: 613–619.

Trape JF, Rogier C, Konate L, Diagne N, Bougonali H, et al. (1994) The
Linkage Analysis of Mild Malaria

Dielmo project: A longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. Am J Trop Med Hyg 51: 123–137.

26. Cox MJ, Kum DE, Favul I, Narara A, Raiko A, et al. (1994) Dynamics of malaria parasitaemia associated with febrile illness in children from a rural area of Madang, Papua New Guinea. Trans R Soc Trop Med Hyg 88: 191–197.

27. Bloland PB, Ruebush TK, McCormick JB, Ayisi J, Borgia DA, et al. (1999) Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission I. Description of study site, general methodology, and study population. Am J Trop Med Hyg 60: 635–640.

28. Williams TN, Mwangi TW, Wambua S, Alexander ND, Kortok M, et al. (2005) Sickle cell trait and the risk of Plasmodium falciparum malaria and other childhood diseases. J Infect Dis 192: 178–186.

29. Casanova JL, Abel L (2007) Human genetics of infectious diseases: A unified theory. EMBO J. In press.

30. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, et al. (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn’s disease. Nature 411: 603–606.

31. Hernandez-Valladares M, Naessens J, Iraqi FA (2005) Genetic resistance to malaria in mouse models. Trends Parasitol 21: 352–355.

32. Kennedy GC, Matsuzaki H, Dong S, Liu WM, Huang J, et al. (2003) Large-scale genotyping of complex DNA. Nat Biotechnol 21: 1233–1237.

33. Middleton FA, Pato MT, Gentile KL, Morley CP, Zhao X, et al. (2004) Genome-wide linkage analysis of bipolar disorder by use of a high-density single nucleotide polymorphism (SNP) genotyping assay: A comparison with microsatellite marker assays and finding of significant linkage to Chromosome 6q22. Am J Hum Genet 74: 886–897.

34. Greenwood BM, Armstrong JR (1991) Comparison of two simple methods for determining malaria parasite density. Trans R Soc Trop Med Hyg 85: 186–188.

35. Kenney RD, Fortenberry JD, Suratt SS, Ribbeck BM, Thomas WJ (1999) Evaluation of an infrared tympanic membrane thermometer in pediatric patients. Pediatrics 85: 854–858.

36. Flori L, Sawadogo S, Elnouhara N, Nicolae DL, Chen FF, et al. (2003) A polymorphic dinucleotide repeat in the human IL-10 promoter. Immunogenetics 42: 444–445.

37. Sauerbrei W, Royston P (1999) Building multivariable prognostic and diagnostic models: Transformation of the predictors by using fractional polynomials J R Statist Soc A 162: 71–94.

38. Royston P, Ambler G, Sauerbrei W (1999) The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 28: 964–974.

39. Tobin J (1959) Estimation of relationships for limited dependent variables. Econometrica 26: 24–36.

40. Fernandez JR, Etzel C, Brasley TM, Shete S, Amos CI, et al. (2002) Improving the power of sib pair quantitative trait loci detection by phenotype winsorization. Hum Hered 55: 59–67.

41. Herrmann MG, Dobrowolski SF, Wittwer CT (2000) Rapid beta-globin genotyping by multiplexing probe melting temperature and color. Clin Chem 46: 425–428.

42. Timmann C, Moenkemeyer F, Evans JA, Foerster B, Tannich E, et al. (2005) Diagnosis of alpha+thalassemia by determining the ratio of the two alpha-globin gene copies by oligonucleotide hybridization and melting curve analysis. Clin Chem 51: 1711–1713.

43. Beutler F (1994) G6PD deficiency. Blood 84: 3613–3636.

44. Ruvende C Hill (1998) Glucose-6-phosphate dehydrogenase deficiency and malaria. J Mol Med 76: 581–588.

45. Omi K, Ohashi J, Patarapotikul J, Hananantachai H, Naka I, et al. (2003) CD36 polymorphism is associated with protection from cerebral malaria. Am J Hum Genet 72: 364–374.

46. Eskdale J, Gallagher G (1995) A polymorphic dinucleotide repeat in the human IL-10 promoter. Immunogenetics 42: 742–743.

47. O’Connell JR, Weeks DE (1998) PedCheck: A program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 65: 259–266.

48. Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2001) GRR: Graphical representation of relationship errors. Bioinformatics 17: 742–743.

49. Fernandez JR, Etzel C, Brasley TM, Shete S, Amos CI, et al. (2002) Improving the power of sib pair quantitative trait loci detection by phenotype winsorization. Hum Hered 55: 59–67.

50. Kruglyak L, Lander ES (1995) A nonparametric approach for mapping quantitative trait loci. Genetics 139: 1421–1428.

51. Haseman JK, Elston RC (1972) The investigation of linkage between a quantitative trait and a marker locus. Behav Genet 2: 3–19.

52. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: A unified multipoint approach. Am J Hum Genet 58: 1547–1563.

53. Kleesang A, Franke D, Konig IR, Ziegler A (2005) Haplotype-sharing analysis for alcohol dependence based on quantitative traits and the Mantel statistic. BMC Genet 30 (Suppl 1): 75.

54. Zinn-Justin A, Ziegler A, Abel L (2001) Multipoint development of the human IL-10 promoter. Immunogenetics 42: 444–445.

55. Ott J (1999) Analysis of human genetic linkage. 3rd ed. Baltimore: Johns Hopkins University Press.