Europe PMC Funders Group
Author Manuscript
Hum Mol Genet. Author manuscript; available in PMC 2009 March 19.

Published in final edited form as:
Hum Mol Genet. 2008 February 15; 17(4): 567–576. doi:10.1093/hmg/ddm331.

Europe PMC Funders Author Manuscripts

Common variation in the ABO glycosyltransferase is associated with susceptibility to severe *Plasmodium falciparum* malaria

Andrew E. Fry[1,*], **Michael J. Griffiths**[1,2], **Sarah Auburn**[1], **Mahamadou Diakite**[1], **Julian T. Forton**[1], **Angela Green**[1], **Anna Richardson**[1], **Jonathan Wilson**[1,†], **Muminatou Jallow**[3], **Fatou Sisay-Joof**[3], **Margaret Pinder**[3], **Norbert Peshu**[2], **Thomas N. Williams**[2,4], **Kevin Marsh**[2,4], **Malcolm E. Molyneux**[5,7], **Terrie E. Taylor**[6,8], **Kirk A. Rockett**[1], and **Dominic P. Kwiatkowski**[1,9]

[1]The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK

[2]Kenya Medical Research Institute Centre for Geographical Medicine Research (Coast), P.O. Box 230, Kilifi, Kenya

[3]Medical Research Council, PO Box 273, Banjul, The Gambia

[4]Nuffield Department of Medicine, John Radcliffe Hospital, Oxford OX3 9DS, UK

[5]Malawi–Liverpool–Wellcome Trust Programme of Clinical Tropical Research, College of Medicine, PO Box 30096, Blantyre, Malawi

[6]Blantyre Malaria Project, College of Medicine, PO Box 30096, Blantyre, Malawi

[7]Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK

[8]Department of Internal Medicine, College of Osteopathic Medicine, Michigan State University, East Lansing, Michigan 48824, USA

[9]Wellcome Trust Sanger Institute, Hinxton, CB10 1SA, UK

Abstract

There is growing epidemiological and molecular evidence that ABO blood group affects host susceptibility to severe *Plasmodium falciparum* infection. The high frequency of common ABO alleles means that even modest differences in susceptibility could have a significant impact on the health of people living in malaria endemic regions. We performed an association study, the first to utilize key molecular genetic variation underlying the ABO system, genotyping >9000 individuals across 3 African populations. Using population- and family-based tests we demonstrated that alleles producing functional ABO enzymes are associated with greater risk of severe malaria phenotypes (particularly malarial anemia) in comparison with the frameshift deletion underlying blood group O: Case-control allelic odds ratio (OR) 1.2, 95% confidence interval (CI) 1.09 – 1.32, P=0.0003; Family-studies allelic OR 1.19, CI 1.08 – 1.32, P=0.001; Pooled across all studies allelic OR 1.18, CI 1.11 - 1.26, $P=2\times10^{-7}$. Analyzing the family trios we found suggestive evidence of a parent-of-origin effect at the ABO locus. Non-O haplotypes inherited from mothers, but not fathers, are significantly associated with severe malaria (likelihood ratio test of Weinberg, P=0.046). Finally we used HapMap data to demonstrate a region of low F_{ST} (–0.001) between the three main HapMap population groups across the ABO locus, an outlier in the empirical distribution of F_{ST} across chromosome 9 (~99.5 – 99.9th centile). This low F_{ST} region may be a

*To whom correspondence should be addressed at: The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK. Tel: +441865287536, Fax: +441865287533, Email: afry@well.ox.ac.uk.
†Deceased.

Conflict of Interest Statement. None declared.

Europe PMC Funders Author Manuscripts

signal of longstanding balancing selection at the ABO locus, caused by multiple infectious pathogens including *P. falciparum*.

Introduction

A link between the ABO blood group system and malaria susceptibility has long been suspected. Significant associations between blood group and *P. falciparum* malaria have been reported from cross sectional and case control studies in Brazil, Gabon, India, Sri Lanka and Zimbabwe (1-7) (see also recent reviews and references therein (8, 9)). However other studies in Colombia, India, Sudan and Nigeria could not find an association between malaria and blood group (10-15). The positive association studies have consistently suggested that blood group O individuals are relatively protected from severe malaria.

Human erythrocytes infected with mature forms of the *Plasmodium falciparum* parasite adhere to uninfected red blood cells, endothelia and other components of the vascular space (16). This adhesive behavior is mediated by *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which is encoded by a family of highly variant parasite genes and subject to switching during the course of an infection (17). A range of human host molecules binding to PfEMP1 have been identified including CD36 (18), Intercellular adhesion molecule (ICAM)-1 (19) and Complement receptor-1 (CR1) (20). The ABO A and B antigens have been implicated in the formation of 'rosettes' the process by which infected red blood cells (iRBCs) surround themselves with uninfected erythrocytes (21, 22). PfEMP1 has been identified as the rosetting ligand of the parasite (20) and the blood group A antigen has been shown to bind to the semiconserved head structure of PfEMP1 (23). Erythrocyte rosetting is linked to the pathogenesis of severe malaria phenotypes such as cerebral malaria (CM) and severe malarial anemia (SA) (24, 25). Indeed fresh isolates of *P. falciparum* from Kenyan children with severe malaria bind A antigen more frequently than strains from children with mild disease (26). Recent work on Kenyan and Malian isolates suggests a significant reduction in rosetting in blood group O individuals compared with non-O blood groups, furthermore parasites required rosetting activity for blood group O to offer protection from severe disease (27). Wild and laboratory parasite strains demonstrate blood group preferences, which appear to vary geographically. In general rosette formation has been shown to occur preferentially with blood group A, B or AB erythrocytes, and particularly groups A and AB in studies of African strains (21, 22, 28, 29).

Previous epidemiological studies have employed the phenotype of serology to determine host ABO genotype. Although convenient serological studies of the ABO system are unable to discriminate all genotypes e.g. AO heterozygotes from AA homozygotes, and cannot assess subtler coding or non-coding variation. We took the novel approach of investigating the molecular genetic variation underlying this system. The ABO glycosyltransferase performs the terminal step in the biosynthesis of the ABO macromolecule, adding sugar residues to the precursor H antigen. The enzyme adds either N-acetylgalactosamine, to form the A antigen, galactose to form the B antigen, or is functionless leaving the H (or O) antigen unmodified. A variety of polymorphisms have been reported in the ABO glycosyltransferase gene (30). The common key functional variants are: (i) a one nucleotide deletion in exon 6 (codon 87) leading to a reading frameshift and premature termination of the polypeptide before the N terminal catalytic domain, producing the functionless O allele (31). Alternative 'non-deletional' O alleles exist but are rare in African populations (32). (ii) Four non-synonymous single nucleotide polymorphisms (SNPs) (altering residues 176, 235, 266 and 268) which switch enzyme function from A transferase to B transferase activity. The third and fourth SNPs (codons 266 and 268) have the greatest effect on determining the nucleotide-sugar donor used by the transferase, the second amino acid (235) also affects

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

nucleotide-sugar specificity but to a lesser degree, finally the most 5′ SNP (codon 176) has very little influence on donor specificity (33).

We designed genotyping assays for the O allele frameshift mutation (rs8176719) and the three key A/B nonsynoymous SNPs in codons 235 (rs8176743), 266 (rs8176746) and 268 (rs8176747) in the catalytic domain. We conducted a multi-centre study design employing samples from three African regions: Gambia (West Africa), Malawi (South Central Africa) and Kenya (East Africa). We used two study types, first population-based association studies in each of the regions employing children with severe malarial disease and local cord blood controls, and second, family-based association studies at each site looking for transmission distortion of ABO alleles between parents and affected offspring, an approach which has the advantage of being robust to population stratification. Our aims were: (i) given the presence of substantial linkage disequilibrium (LD) between the functional SNPs in the ABO locus to define the most efficient marker set for large-scale genotyping; (ii) to perform SNP- and haplotype-based tests for association with severe malarial phenotypes; (iii) to employ our family-based genetic data to check for parent-of-origin effects, an approach not possible with previous case-control and cross-sectional study designs; and (iv) to consider the population genetic implications of an association between ABO variation and severe falciparum malaria.

Results

Substantial LD between functional SNPs in the ABO glycosyltransferase gene

The four key functional polymorphisms that we tested in the ABO locus are reported to be in substantial LD (30). To confirm this we genotyped 1320 Gambian parent-offspring trios, and 30 Yoruba parent-offspring trios from Ibadan in Nigeria (cell line DNA used by International Haplotype Map (HapMap) Project) (34). This allowed us to look at the fine structure of the ABO locus, combining our functional SNPs with the high density HapMap marker dataset (Fig. 1).

Genotyping in both sample sets demonstrated near perfect LD (r^2 0.9 – 1.0) between the three nonsynonymous SNPs that distinguish A and B haplotypes. The frameshift deletion underlying O alleles is in moderate LD with the three nonsynonymous SNPs ($D' \sim 0.9$, $r^2 \sim 0.3$). The majority of O alleles occur on an A haplotypic background, although the minority of recombinants with B haplotypes are still expected to produce truncated products. The implication of this haplotypic structure is that 2 markers: rs8176719 and one of the three nonsynonymous SNPs can generally distinguish the three ABO alleles.

The common frameshift mutation underlying blood group O is associated with protection from severe malaria phenotypes, particularly severe malarial anemia

Two markers, rs8176719 and rs8176746, were genotyped in 3906 cases of severe malaria plus population and family controls from The Gambia, Kenya and Malawi. Case-control analyses were performed on 2127 cases of severe malaria and 1931 population controls, and family-based association tests were performed on a different set of 1779 cases of severe malaria and their parents. Further details of severe malaria phenotypes and sample sizes from each population are given in Material and Methods.

Our analysis took the statistical convention of using the commonest category (i.e. most frequent allele, haplotype or blood group) as reference, and comparing other alleles, haplotypes and inferred blood groups against this group. Single SNP analysis revealed that the minor allele of rs8176719, an insertion relative to the reference sequence (although almost certainly the ancestral allele), is associated with increased risk of severe malaria phenotypes. Consistent trends were found in both family and case-control studies, and

across study sites (Table 1). However some individual studies did not reach statistical significance; probably due to lack of power (see Materials and Methods for power calculations). Overall the case-control allelic odds ratio (OR) was 1.2, 95% confidence interval (CI) 1.09 – 1.32, P=0.0003; Family-studies allelic OR 1.19, CI 1.08 – 1.32, P=0.001; Pooling data across all our studies, both family- and population-based, suggested an allelic OR of 1.18, CI 1.11 - 1.26, P = 2×10^{-7} for severe disease (Fig. 2). The results suggest that the full-length allele of rs8176719 may be associated with a particular risk of anemia during severe malaria infections. For the phenotype of severe malarial anemia the allelic OR was 1.30, CI 1.12 - 1.5, P = 0.0004 in the case-control study and 1.34, CI 1.1 - 1.64, P = 0.004 in the family-based study.

The situation with rs8176746 is more complex. The major allele, although defining the putatively ‘high risk’ A haplotype, occurs with the ‘low risk’ frame-shift deletion upstream in about two thirds of chromosomes. Thus no simple genetic model is significantly associated with disease when this SNP is considered in isolation.

ABO haplotypes producing A and B antigens are associatied with susceptibility to severe malaria

We inferred 2 SNP haplotypes using rs8176719 and rs8176746. In comparison with O haplotypes, both A (case-control OR 1.27, CI 1.12 – 1.44, P = 0.0003; family OR 1.16, CI 1.03 – 1.31, P = 0.018) and B (case-control OR 1.13, CI 0.99 – 1.28, P = 0.06; family OR 1.20, CI 1.05 – 1.37, P = 0.009) haplotypes are significantly associated with severe malaria (Table 1).

Using an individual's haplotypes we inferred their ABO blood group (A and B haplotypes codominant, O haplotype recessive). Blood group A, B and AB individuals appear to be at significantly greater risk of severe malaria in comparison with blood group O (e.g. blood group A individuals: case-control OR 1.33, CI 1.13 – 1.56, P = 0.00065; family OR 1.29, CI 1.09 – 1.53, P = 0.003) (Table 2). Trends in the data suggest blood group B individuals may be at subtly lower risk than blood group A, while blood group AB individuals are probably at the greatest risk of severe disease (case-control OR 1.59, CI 1.15 – 2.21 P = 0.006; family OR 1.46, CI 1.05 – 2.04, P = 0.025).

Non-O blood groups demonstrate particularly high risk of severe malarial anemia (e.g. blood group A: case-control OR 1.54, CI 1.22 – 1.96, P = 0.00039; family OR 1.51, CI 1.09 – 2.09, P = 0.014). The higher risk of SA experienced by individuals with non-O blood groups may reflect a pathophysiological effect, for example accelerated clearance of erythrocytes bound to iRBC. Frequencies of genotypes, haplotypes and blood groups for all cases, controls and parents are documented in Supplementary Material, Table S1.

ABO glycosyltransferase alleles and a possible parent-of-origin effect in disease susceptibility

Previous association studies of the ABO system in severe malaria have examined serological data from unrelated individuals. In this study, having analyzed genetic data from pedigrees, we are in the unique position of being able to look for parent-of-origin effects. We were surprised to find a substantial difference in transmission of high risk alleles relating to their parent of origin. Full-length alleles of rs8176719 (marking A and B haplotypes) transmitted to offspring are associated with greater risk of severe disease if the allele was transmitted from a mother (OR 1.38, P = 0.0002) rather than a father (OR 1.05, P=0.6), conditional logistic regression fitting separate effects for maternal and paternal alleles supports the existence of a parent-of-origin effect (χ^2 = 3.96, P= 0.047) (Table 3).

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Interactions between maternal genotype and child's genotype can masquerade as parent of origin effects. To investigate this scenario we performed a parent-of-origin likelihood ratio test of Weinberg (PO-LRT) (35), which can allow for maternal genotype effects. The likelihood ratio test demonstrates evidence of a parent-of-origin effect (P=0.046), but did not support a maternal genotype effect (P=0.21) (Table 4). Using 2-SNP haplotypes to identify the three ABO alleles, and repeating the conditional logistic regression we found that both A and B haplotypes are associated with greater risk when transmitted by a mother although only the difference between maternal and paternal A haplotypes is significant ($\chi^2 = 5.19$, P= 0.023).

There was no significant difference in risk estimate for severe malaria between AO and AA genotypes in either the case-control study (AO, OR 1.35, CI 1.13 - 1.60; AA OR 1.23, CI 0.84 - 1.79; Wald test P = 0.64) or family study (AO, OR 1.31, CI 1.10 - 1.55; AA OR 1.13, CI 0.76 - 1.67; Wald P = 0.45). The same was true for BO versus BB genotypes, and grouping both non-O haplotypes. The phase of AO genotypes was distinguishable in the family study and, as noted above, suggested different risks of severe disease ($A^{mat}O^{pat}$, OR 1.64, CI 1.27 - 2.12; $O^{mat}A^{pat}$, OR 1.04, CI 0.81 - 1.33; Wald P = 0.01). Differences between these genotypes and the AA genotype were not significant ($A^{mat}O^{pat}$ versus AA; Wald P = 0.07; $O^{mat}A^{pat}$ versus AA; Wald P = 0.92).

Low levels of population differentiation at the ABO locus represent a signal of longstanding balancing selection

Given that one ABO allele is protective against a major selection pressure such as *P. falciparum* malaria, it is important to consider the reasons why the ABO system remains polymorphic in Africa. The answer may relate to the balance of protection offered by specific ABO alleles to other infectious disease. ABO antigens have been implicated not only in malaria, but also to the pathogenesis of *Escherichia coli* (36), *Helicobacter pylori* (37), Norwalk virus (38), Hepatitis C (39), and respiratory infections (40). There has also been speculation about whether historical agents such as smallpox and plague may have shaped the global distribution of ABO alleles (41, 42).

Blood group A, B and O alleles occur in other primate species. Sequence analysis has suggested that the common ancestral enzyme had A transferase activity and that the presence of species-specific mutations underlying the non-human B and O alleles suggests functional polymorphism of the ABO gene has occurred through convergent evolution (43). High levels of nucleotide diversity at the ABO locus are considered to be significant evidence of non-neutral evolution in primate lineages.

Surveys for signatures of evolutionary selection in the Human genome have demonstrated evidence of balancing selection at the ABO locus (44, 45). Using our merged haplotype data from the HapMap Yoruba population and our additional genotyping of four functional polymorphisms (rs8176719, rs8176746, rs8176747, rs8176743), we investigated whether the long range haplotype (LRH) test (46), which detects signals of recent positive selection, was associated with the functional ABO variants (Fig. 3). Neither of our key SNPs appears to be associated with an extended haplotype homozygosity signal, which is consistent with a longstanding process of balancing selection. A recently reported high LRH score from the ABO locus (45) may reflect positive selection of nearby regulatory sequence controlling expression patterns rather than coding sequence itself. Interestingly we observed very low levels of population differentiation across the ABO locus in HapMap populations (Fig. 4A). The ABO locus including sequences 20-30 kb upstream, a region of 85 SNPs, has an F_{ST} (using a sample-size weighted metric of population differentiation between African, Asian and European populations) of −0.001, compared with a genome-wide average of around 0.1. Although more attention is generally given to high F_{ST} values, as signals of region-specific

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

positive selection (e.g. the Duffy blood group locus under selection in Africa from *P. vivax* malaria (47)), here the low F_{ST} might reflect simultaneous balancing selection in all three populations, and would be consistent with a model of longstanding selection. The extension of the low F_{ST} region 20 – 30kb upstream of the ABO coding sequence (while stopping just 3kb downstream) may indicate that the balancing selection is also shaping the cis-acting regulatory sequences immediately upstream of the gene. Although not a formal test of deviation from neutrality we measured the empirical distribution of F_{ST} in windows across chromosome 9 and found the ABO region to be around the 99.5 – 99.9th centile in this distribution (Fig. 4B).

Discussion

Our analysis strongly supports the hypothesis that blood group O individuals are relatively protected from severe malaria in comparison to other blood groups, particularly blood group A and AB. In addition to population-based studies we conducted the first family-based association studies of ABO variation, with severe malaria. Analysis of parent-offspring trios is robust to artifacts of population stratification and allowed us to examine the possibility of parent-of-origin effects. We found that full-length alleles at rs8176719, particularly A haplotypes, inherited from a mother lead to greater risk for offspring than similar alleles inherited from a father. The analysis suggests a phenomenon such as genomic imprinting rather than an effect of maternal genotype. The tissue- and cell-type specific expression of ABO is controlled by epigenetic signals, particularly at a 5′CpG island in the promoter of the gene (48). Loss of ABO expression mediated by promoter hypermethylation is common in certain forms of malignancy (49). There has also been a small case series documenting preferential loss of the maternally derived ABO allele in adult leukemia (50). Our results would be consistent with altered expression of the paternal ABO allele due to imprinting, leaving less target ligand for iRBCs to bind to, or shifting the pattern of sequestration to a ‘safer’ distribution. Paternal allele expression is not abolished, as demonstrated by the existence of blood group AB. However it is interesting to speculate that during gestation it could be beneficial to modulate or suppress the paternal ABO allele, so as to minimize maternal-fetal ABO incompatibility and the risk of hemolytic disease of the newborn. To our knowledge this is the first report suggestive of genomic imprinting in the genetics of malaria susceptibility. However despite our sample size, we have limited power to detect the subtle difference in risk between maternal and paternally derived alleles, it remains possible that this suggestive result is in fact type I error. Further experiments are required to confirm this finding, and could include family-based studies of ABO variation in malaria and other complex diseases, or functional experiments with family-derived samples to determine if ABO gene expression is affected by parent of origin.

The sizes of our individual study datasets gives us limited power to analyze the differences between regions. The differences in odds ratio between a family study and case-control study in a single population are of similar magnitude to the differences seen between regions, suggesting much of the variation observed is due to statistical fluctuation or methodological issues. Factors possibly contributing to the diversity of risk estimates reported between our populations, and in comparison with other reports include variation in phenotype definition and geographic differences in parasite strain e.g. differing affinities for ABO antigens. The use of cord blood controls instead of age-matched controls could affect association results. Cord blood samples represent the distribution of genotypes in the populations at birth; since a minority will go on to develop severe disease, this will tend to underestimate the true odds ratio of any genetic susceptibility or resistance allele. In contrast, if blood group O individuals are depleted from the population between birth and mid-childhood (the age of the cases) due to non-malarial pathogens this would lead to an over-estimation of protection from blood group O. However given the consistent results

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

between the population- and family-based studies, the similar allele frequencies between cord blood samples and untransmitted parental alleles, and the limited effect size found, such biases are probably limited.

Our study employed samples from children with severe malaria phenotypes and it has been noted that the ABO effect is harder to detect in samples incorporating adults with malaria (8). The gradual accumulation of antibodies against a range of iRBC surface antigens, is thought to explain a component of the immunity to severe life-threatening malaria which develops in late childhood in endemic regions (51). It is possible that a limited repertoire of parasite epitopes binding A and B antigens, could lead to a high prevalence of effective antibodies against these variants in adults, reducing the significance of host ABO blood group in older age groups. It is interesting to speculate whether vaccination, leading to effective immunity to A and B antigen binding PfEMP1 variants, could reduce morbidity among blood group A, B and AB children.

The modest effect size found is typical of validated associations with complex disease in humans. The effect size is likely, in part, to explain why some previous studies have been unable to demonstrated significant results, despite trending towards protection from blood group O, particularly when sample size was low. However the global health impact of the frame-shift deletion, underlying blood group O, needs to be put in context by considering its high frequency. Roughly half the peoples of Sub-Saharan Africa, and many other human populations, at risk of life-threatening disease caused by *P. falciparum*, are homozygotes for this null mutation and are protected by being blood group O (52).

Materials and Methods

Subjects

Patient samples were collected as part of ongoing epidemiological studies of severe malaria at the Royal Victoria Hospital, Banjul, The Gambia; the Queen Elizabeth Central Hospital, Blantyre, Malawi; and Kilifi District Hospital, Kilifi, Kenya. Populations in these study sites are exposed to endemic malaria, with the burden of life-threatening disease being experienced by children (< 5 years old). Nuclear family trios comprised two parents and one affected child. All DNA samples were collected and genotyped following approval from the relevant research ethics committees and informed consent from participants. Controls were cord blood samples obtained from birth clinics in the same locality as the cases. See Supplementary Material, Table S2 for further demographics of patients and controls.

Phenotype definition

All cases were children admitted to hospital with evidence of *P. falciparum* on blood film and clinical features of severe malaria (53, 54). In our analyses of sub-phenotypes we use Blantyre coma score of 2 as a criterion of cerebral malaria (CM), and hemoglobin <5g/dl or packed cell volume <15% as a criterion of severe malaria anaemia (SA). Some individuals had both CM and SA. Of the severe malaria cases that were not CM or SA by these criteria, most had lesser degrees of coma (Blantyre coma score 3) or anemia (Hb 5-6g/dl), or other complications such as respiratory distress. Our samples comprised:

i) 701 Gambian cases (324 with CM and 217 with SA) and 624 controls.

ii) 718 Malawian cases (640 CM, 101 SA) and 405 controls.

iii) 708 Kenyan cases (216 CM, 270 SA) and 902 controls.

iv) 1320 Gambian trios (512 probands with CM and 343 with SA).

v) 225 Malawi trios (216 CM, 39 SA).

Europe PMC Funders Author Manuscripts

vi) 234 Kenyan trios (114 CM, 85 SA).

Power calculations

Power calculations were performed using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/) (55). A single regional case-control study had ~61% power, based on a sample size of 700 cases, 700 unselected controls, allelic odd ratio of 1.2 (our risk estimate for non-O alleles compared with O alleles), high risk allele frequency of 0.3 and a type I error rate of 0.05. Across all case-control studies we would expect 97% power. The smaller family trio studies (N=230 trios) had ~25% power, while the larger Gambian study (N=1320 trios) had ~87% power.

Sample preparation and genotyping

Genomic DNA samples underwent whole genome amplification through either Primer Extension Pre-amplification (PEP) (56) or Multiple Displacement Amplification (MDA) (57), before genotyping on a Sequenom MassArray genotyping platform (58), All assays achieved high rates of genotyping success and no deviations from Hardy-Weinberg equilibrium were encountered (Supplementary Material, Table S2).

Statistical analysis

Analysis was performed using STATA (v9.2 for windows) and the genassoc package (http://www-gene.cimr.cam.ac.uk/clayton/software/) written for STATA by David Clayton. In general results for the multiplicative model are presented. Although full-length alleles are dominant with regard to blood group phenotype, we found a trend towards increased susceptibility for blood group AB individuals (homozygotes for the full-length allele at rs8176719), which makes this model valid. Case-control association analysis was undertaken by logistic regression and included the covariates of ethnic group, gender and Sickle status. DNA Sequenom genotyping for the Hemoglobin S (HbS) variant was performed for all samples. The HbS results for a proportion of the Gambian samples have previously been published (59). Gender was included in the regression analysis to control for sex-linked traits (e.g. G6PD). With regards to other variation thought to affect malaria susceptibility: Hemoglobin C was absent from our study populations and the range of deletions underlying the Thalassemias are not easily amenable to the high-throughput genotyping technology used.

Family-based association analysis was performed using a case-pseudo-control approach and conditional logistic regression (60). Trios were drawn from a larger pool of samples checked for relationship misspecification. All family samples were genotyped for 48 SNP markers and 15% of trios (indicated by their Mendelian errors rates) excluded from further analysis.

In the population-based studies two SNP haplotypes (and therefore the ABO alleles) were reconstructed using the snp2hap function, while in family studies phase can often be tracked from parent and child in the creation of the case-pseudo-control dataset. Data from the case-control studies was pooled in a single logistic regression analysis including covariates of ethnic group, gender and Sickle status, while family studies were combined in a single case-pseudo-control analysis. Pooling across all case-control and family-studies is less straightforward. Here we used the UNPHASED application version 3.0 (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/) (61, 62) which employs a retrospective likelihood framework for performing genetic association analysis, and can be used to combine data from nuclear families and unrelated subjects. Ethnic origin was found to be a significant confounder and was retained as a covariate in the UNPHASED analysis.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Integrating phased genotypes from the HapMap project

Phased ABO locus haplotypes for the 30 Yoruba parent-offspring trios from Ibadan in Nigeria were downloaded from the HapMap website (www.hapmap.org) (34). Cell lines derived from the Yoruba individuals (obtained from the Coriell Cell Repositories, http://ccr.coriell.org/) were genotyped for the four key SNPs. The additional SNP data was combined with the known phased genotypes using PHASE version 2.1 (63, 64). LD patterns between our genotyped SNPs and between these genotypes and HapMap markers were visualized using HAPLOVIEW version 3.32 (http://www.broad.mit.edu/mpg/haploview/) (65). Extended haplotype homozygosity values (46) were calculated using SWEEP version 1.0 (http://www.broad.mit.edu/mpg/sweep/index.html).

F_{ST} calculation and window-based screen of chromosome 9

SNP genotyping data for chromosome 9 was downloaded from the HapMap website (Release 21a/Phase 2, Jan 2007). The dataset was derived from three populations: the 30 Yoruba trios; 30 U.S. parent-offspring trios of northern and western European origin, collected by the Centre d'Etude du Polymorphisme Humain (CEPH); and a combined dataset of 45 unrelated individuals from the Tokyo, Japan and 45 unrelated individuals from Beijing, in China(34). 94,678 SNPs across chromosome 9 were polymorphic in all three populations. F_{ST} between the three populations was estimated, in windows across chromosome 9, using the formula for F_{ST} described in the supplementary information to the HapMap Consortial publication (34).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

A.E.F. and M.J.G. were funded by Wellcome Trust Clinical Research Training Fellowships and T.W. by a Wellcome Trust Senior Fellowship. The sample collections and resources used in this study received funding from the UK Medical Research Council, the Wellcome Trust, the Bill and Melinda Gates Foundation, the National Institutes of Health and the European Union Network 6 BioMalPar Consortium. This manuscript is published with the permission of the director of the Kenya Medical Research Institute.

Abbreviations

PfEMP1	*P. falciparum* erythrocyte membrane protein 1
SNP	single nucleotide polymorphism
LD	linkage disequilibrium
CM	cerebral malaria
SA	severe malarial anemia
OR	odds ratio
CI	confidence interval
EHH	extended haplotype homozygosity
LRH	long-range haplotype
HapMap	International Haplotype Map Project

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

References

1. Beiguelman B, Alves FP, Moura MM, Engracia V, Nunes AC, Heckmann MI, Ferreira RG, da Silva LH, Camargo EP, Krieger H. The association of genetic markers and malaria infection in the Brazilian Western Amazonian region. Mem. Inst. Oswaldo. Cruz. 2003; 98:455–460. [PubMed: 12937753]
2. Fischer PR, Boone P. Short report: severe malaria associated with blood group. Am. J. Trop. Med. Hyg. 1998; 58:122–123. [PubMed: 9452303]
3. Lell B, May J, Schmidt-Ott RJ, Lehman LG, Luckner D, Greve B, Matousek P, Schmid D, Herbich K, Mockenhaupt FP, et al. The role of red blood cell polymorphisms in resistance and susceptibility to malaria. Clin. Infect. Dis. 1999; 28:794–799. [PubMed: 10825041]
4. Migot-Nabias F, Mombo LE, Luty AJ, Dubois B, Nabias R, Bisseye C, Millet P, Lu CY, Deloron P. Human genetic factors related to susceptibility to mild malaria in Gabon. Genes Immun. 2000; 1:435–441. [PubMed: 11196674]
5. Pant CS, Gupta DK, Sharma RC, Gautam AS, Bhatt RM. Frequency of ABO blood groups, sickle-cell haemoglobin, G-6-PD deficiency and their relation with malaria in scheduled castes and scheduled tribes of Kheda District, Gujarat. Indian J. Malariol. 1992; 29:235–239. [PubMed: 1291344]
6. Pathirana SL, Alles HK, Bandara S, Phone-Kyaw M, Perera MK, Wickremasinghe AR, Mendis KN, Handunnetti SM. ABO-blood-group types and protection against severe, Plasmodium falciparum malaria. Ann. Trop. Med. Parasitol. 2005; 99:119–124. [PubMed: 15814030]
7. Singh N, Shukla MM, Uniyal VP, Sharma VP. ABO blood groups among malaria cases from district Mandla, Madhya Pradesh. Indian J. Malariol. 1995; 32:59–63. [PubMed: 7589729]
8. Uneke CJ. Plasmodium falciparum malaria and ABO blood group: is there any relationship? Parasitol. Res. 2007; 100:759–765. [PubMed: 17047997]
9. Cserti CM, Dzik WH. The ABO blood group system and Plasmodium falciparum malaria. Blood. 2007; 110:2250–2258. [PubMed: 17502454]
10. Martin SK, Miller LH, Hicks CU, David-West A, Ugbode C, Deane M. Frequency of blood group antigens in Nigerian children with falciparum malaria. Trans. R. Soc. Trop. Med. Hyg. 1979; 73:216–218. [PubMed: 382466]
11. Kassim OO, Ejezie GC. ABO blood groups in malaria and schistosomiasis haematobium. Acta Trop. 1982; 39:179–184. [PubMed: 6126100]
12. Bayoumi RA, Bashir AH, Abdulhadi NH. Resistance to falciparum malaria among adults in central Sudan. Am. J. Trop. Med. Hyg. 1986; 35:45–55. [PubMed: 2936261]
13. Akinboye DO, Ogunrinade AF. Malaria and loaisis among blood donors at Ibadan, Nigeria. Trans. R. Soc. Trop. Med. Hyg. 1987; 81:398–399. [PubMed: 3318017]
14. Thakur A, Verma IC. Malaria and ABO blood groups. Indian J. Malariol. 1992; 29:241–244. [PubMed: 1291345]
15. Montoya F, Restrepo M, Montoya AE, Rojas W. Blood groups and malaria. Rev. Inst. Med. Trop. Sao. Paulo. 1994; 36:33–38. [PubMed: 7997771]
16. Flick K, Chen Q. var genes, PfEMP1 and the human host. Mol. Biochem. Parasitol. 2004; 134:3–9. [PubMed: 14747137]
17. Smith JD, Chitnis CE, Craig AG, Roberts DJ, Hudson-Taylor DE, Peterson DS, Pinches R, Newbold CI, Miller LH. Switches in expression of Plasmodium falciparum var genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. Cell. 1995; 82:101–110. [PubMed: 7606775]
18. Baruch DI, Ma XC, Singh HB, Bi X, Pasloske BL, Howard RJ. Identification of a region of PfEMP1 that mediates adherence of Plasmodium falciparum infected erythrocytes to CD36: conserved function with variant sequence. Blood. 1997; 90:3766–3775. [PubMed: 9345064]
19. Smith JD, Craig AG, Kriek N, Hudson-Taylor D, Kyes S, Fagen T, Pinches R, Baruch DI, Newbold CI, Miller LH. Identification of a Plasmodium falciparum intercellular adhesion molecule-1 binding domain: a parasite adhesion trait implicated in cerebral malaria. Proc. Natl. Acad. Sci. USA. 2000; 97:1766–1771. [PubMed: 10677532]

20. Rowe JA, Moulds JM, Newbold CI, Miller LH. P. falciparum rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1. Nature. 1997; 388:292–295. [PubMed: 9230440]
21. Carlson J, Wahlgren M. Plasmodium falciparum erythrocyte rosetting is mediated by promiscuous lectin-like interactions. J. Exp. Med. 1992; 176:1311–1317. [PubMed: 1402677]
22. Barragan A, Kremsner PG, Wahlgren M, Carlson J. Blood group A antigen is a coreceptor in Plasmodium falciparum rosetting. Infect. Immun. 2000; 68:2971–2975. [PubMed: 10768996]
23. Chen Q, Heddini A, Barragan A, Fernandez V, Pearce SF, Wahlgren M. The semiconserved head structure of Plasmodium falciparum erythrocyte membrane protein 1 mediates binding to multiple independent host receptors. J. Exp. Med. 2000; 192:1–10. [PubMed: 10880521]
24. Carlson J, Helmby H, Hill AV, Brewster D, Greenwood BM, Wahlgren M. Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies. Lancet. 1990; 336:1457–1460. [PubMed: 1979090]
25. Newbold C, Warn P, Black G, Berendt A, Craig A, Snow B, Msobo M, Peshu N, Marsh K. Receptor-specific adhesion and clinical disease in Plasmodium falciparum. Am. J. Trop. Med. Hyg. 1997; 57:389–398. [PubMed: 9347951]
26. Heddini A, Pettersson F, Kai O, Shafi J, Obiero J, Chen Q, Barragan A, Wahlgren M, Marsh K. Fresh isolates from children with severe Plasmodium falciparum malaria bind to multiple receptors. Infect. Immun. 2001; 69:5849–5856. [PubMed: 11500463]
27. Rowe JA, Handel IG, Thera MA, Deans A-M, Lyke KE, Koné A, Diallo DA, Raza A, Kai O, Marsh K, et al. Blood group O protects against severe Plasmodium falciparum malaria through the mechanism of reduced rosetting. Proc. Natl. Acad. Sci. USA. 2007; 104:17471–17476. [PubMed: 17959777]
28. Udomsangpetch R, Todd J, Carlson J, Greenwood BM. The effects of hemoglobin genotype and ABO blood group on the formation of rosettes by Plasmodium falciparum-infected red blood cells. Am. J. Trop. Med. Hyg. 1993; 48:149–153. [PubMed: 8447516]
29. Rowe A, Obeiro J, Newbold CI, Marsh K. Plasmodium falciparum rosetting is associated with malaria severity in Kenya. Infect. Immun. 1995; 63:2323–2326. [PubMed: 7768616]
30. Yip SP. Sequence variation at the human ABO locus. Ann. Hum. Genet. 2002; 66:1–27. [PubMed: 12014997]
31. Yamamoto F, Clausen H, White T, Marken J, Hakomori S. Molecular genetic basis of the histo-blood group ABO system. Nature. 1990; 345:229–233. [PubMed: 2333095]
32. Franco RF, Simoes BP, Zago MA. Relative frequencies of the two O alleles of the histo-blood ABH system in different racial groups. Vox. Sang. 1995; 69:50–52. [PubMed: 7483492]
33. Yamamoto F, Hakomori S. Sugar-nucleotide donor specificity of histo-blood group A and B transferases is based on amino acid substitutions. J. Biol. Chem. 1990; 265:19257–19262. [PubMed: 2121736]
34. International HapMap Consortium. A haplotype map of the human genome. Nature. 2005; 437:1299–1320. [PubMed: 16255080]
35. Weinberg CR, Wilcox AJ, Lie RT. A log-linear approach to case-parent-triad data: assessing effects of disease genes that act either directly or through maternal effects and that may be subject to parental imprinting. Am. J. Hum. Genet. 1998; 62:969–978. [PubMed: 9529360]
36. Blackwell CC, Dundas S, James VS, Mackenzie DA, Braun JM, Alkout AM, Todd WT, Elton RA, Weir DM. Blood group and susceptibility to disease caused by Escherichia coli O157. J. Infect. Dis. 2002; 185:393–396. [PubMed: 11807723]
37. Boren T, Falk P, Roth KA, Larson G, Normark S. Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens. Science. 1993; 262:1892–1895. [PubMed: 8018146]
38. Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, Baric R. Human susceptibility and resistance to Norwalk virus infection. Nat. Med. 2003; 9:548–553. [PubMed: 12692541]
39. Poujol-Robert A, Boelle PY, Wendum D, Poupon R, Robert A. Association between ABO blood group and fibrosis severity in chronic hepatitis C infection. Dig. Dis. Sci. 2006; 51:1633–1636. [PubMed: 16927132]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

40. Raza MW, Blackwell CC, Molyneaux P, James VS, Ogilvie MM, Inglis JM, Weir DM. Association between secretor status and respiratory viral illness. BMJ. 1991; 303:815–818. [PubMed: 1932971]

41. Adalsteinsson S. Possible changes in the frequency of the human ABO blood groups in Iceland due to smallpox epidemics selection. Ann. Hum. Genet. 1985; 49:275–281. [PubMed: 3865623]

42. Doughty BR. The changes in ABO blood group frequency within a mediaeval English population. Med. Lab. Sci. 1977; 34:351–354. [PubMed: 340834]

43. Kermarrec N, Roubinet F, Apoil PA, Blancher A. Comparison of allele O sequences of the human and non-human primate ABO system. Immunogenetics. 1999; 49:517–526. [PubMed: 10380696]

44. Akey JM, Eberle MA, Rieder MJ, Carlson CS, Shriver MD, Nickerson DA, Kruglyak L. Population history and natural selection shape patterns of genetic variation in 132 genes. PLoS Biol. 2004; 2:e286. [PubMed: 15361935]

45. Sabeti PC, Schaffner SF, Fry B, Lohmueller J, Varilly P, Shamovsky O, Palma A, Mikkelsen TS, Altshuler D, Lander ES. Positive natural selection in the human lineage. Science. 2006; 312:1614–1620. [PubMed: 16778047]

46. Sabeti PC, Reich DE, Higgins JM, Levine HZ, Richter DJ, Schaffner SF, Gabriel SB, Platko JV, Patterson NJ, McDonald GJ, et al. Detecting recent positive selection in the human genome from haplotype structure. Nature. 2002; 419:832–837. [PubMed: 12397357]

47. Hamblin MT, Di Rienzo A. Detection of the signature of natural selection in humans: evidence from the Duffy blood group locus. Am. J. Hum. Genet. 2000; 66:1669–1679. [PubMed: 10762551]

48. Kominato Y, Hata Y, Takizawa H, Tsuchiya T, Tsukada J, Yamamoto F. Expression of human histo-blood group ABO genes is dependent upon DNA methylation of the promoter region. J. Biol. Chem. 1999; 274:37240–37250. [PubMed: 10601288]

49. Gao S, Worm J, Guldberg P, Eiberg H, Krogdahl A, Liu CJ, Reibel J, Dabelsteen E. Genetic and epigenetic alterations of the blood group ABO gene in oral squamous cell carcinoma. Int. J. Cancer. 2004; 109:230–237. [PubMed: 14750174]

50. Dobrovic A, O'Keefe D, Sage RE, Batchelder E. Imprinting and loss of ABO antigens in leukemia. Blood. 1993; 82:1684–1685. [PubMed: 8364219]

51. Bull PC, Marsh K. The role of antibodies to Plasmodium falciparum-infected-erythrocyte surface antigens in naturally acquired immunity to malaria. Trends Microbiol. 2002; 10:55–58. [PubMed: 11827798]

52. Cavalli-Sforza, LL.; Menozzi, P.; Piazza, A. The History and Geography of Human Genes. Abridged edition ed.. Princeton University Press; 1996.

53. Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, Marsh V, Newton C, Winstanley P, Warn P, Peshu N, et al. Indicators of life-threatening malaria in African children. N. Engl. J. Med. 1995; 332:1399–1404. [PubMed: 7723795]

54. Severe and complicated malaria. World Health Organization, Division of Control of Tropical Diseases. Trans. R. Soc. Trop. Med. Hyg. 1990; 84(Suppl 2):1–65.

55. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics. 2003; 19:149–150. [PubMed: 12499305]

56. Zhang L, Cui X, Schmitt K, Hubert R, Navidi W, Arnheim N. Whole genome amplification from a single cell: implications for genetic analysis. Proc. Natl. Acad. Sci. USA. 1992; 89:5847–5851. [PubMed: 1631067]

57. Gonzalez JM, Portillo MC, Saiz-Jimenez C. Multiple displacement amplification as a pre-polymerase chain reaction (pre-PCR) to process difficult to amplify samples and low copy number sequences from natural environments. Environ. Microbiol. 2005; 7:1024–1028. [PubMed: 15946299]

58. Ross P, Hall L, Smirnov I, Haff L. High level multiplex genotyping by MALDI-TOF mass spectrometry. Nat. Biotechnol. 1998; 16:1347–1351. [PubMed: 9853617]

59. Ackerman H, Usen S, Jallow M, Sisay-Joof F, Pinder M, Kwiatkowski DP. A comparison of case-control and family-based association methods: the example of sickle-cell and malaria. Ann. Hum. Genet. 2005; 69:559–565. [PubMed: 16138914]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

60. Cordell HJ, Barratt BJ, Clayton DG. Case/pseudocontrol analysis in genetic association studies: A unified framework for detection of genotype and haplotype associations, gene-gene and gene-environment interactions, and parent-of-origin effects. Genet. Epidemiol. 2004; 26:167–185. [PubMed: 15022205]
61. Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. Genet. Epidemiol. 2003; 25:115–121. [PubMed: 12916020]
62. Dudbridge, F. Technical Report 2006/5. MRC Biostatistics Unit; Cambridge, UK.: 2006. UNPHASED user guide.
63. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 2001; 68:978–989. [PubMed: 11254454]
64. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am. J. Hum. Genet. 2003; 73:1162–1169. [PubMed: 14574645]
65. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]
66. Spielman RS, Ewens WJ. The TDT and other family-based tests for linkage disequilibrium and association. Am. J. Hum. Genet. 1996; 59:983–989. [PubMed: 8900224]

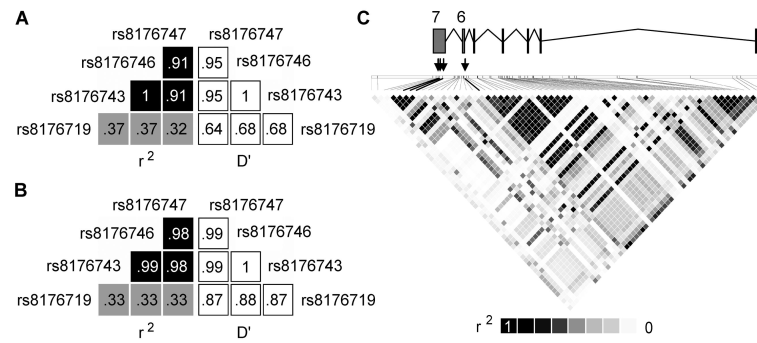

Figure 1.

Linkage disequilibrium around the ABO glycosyltransferase gene. Strong linkage disequilibrium (LD) exists between the four key functional SNPs (A) Yoruba HapMap parent-offspring trios from Ibadan in Nigeria. (B) 1320 Gambian parent-offspring trios. Both of these West-African populations have near perfect LD between the three nonsynonymous SNPs that differentiate A and B ABO alleles, and moderate but lower LD with the common deletion which generates the O allele. (C) r^2 values for the Yoruba population across the ABO gene. Phased genotypes (HapMap, July 2006) with additional genotyping for four functional polymorphisms in ABO. The ABO gene (total region shown chromosome 9, ~24kb, 133156822 – 133180999, NCBI Build 35) is illustrated 3′ to 5′ with exons 6 and 7 on the left. The SNPs differentiating A and B blood groups are in a high LD block, and indicated by three arrows under exon 7. The frameshift mutation responsible for the O allele (arrow under exon 6) is on the 3′ edge of another high LD block.

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

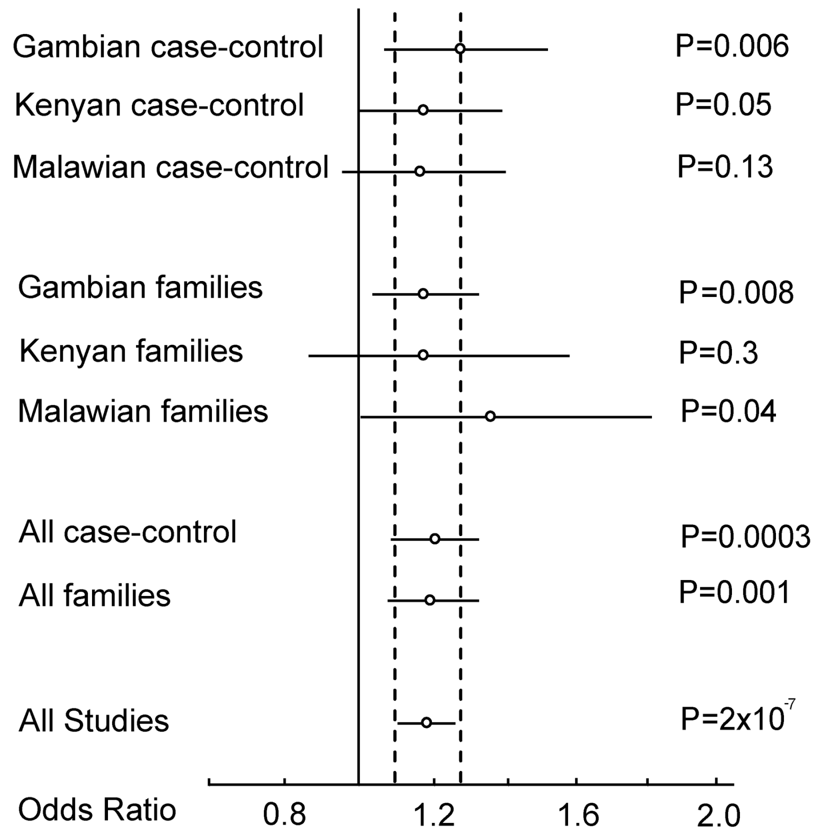

Figure 2.
Estimated risk for ABO rs8176719 in severe malaria. For each study the figure represents the estimated odds ratios (circular marks) and 95% confidence interval (CI, horizontal lines) for the full-length allele, in severe malaria. The P-values were derived from regression analysis with covariates of ethnic group, gender and HbS genotype (for population-based studies), case-pseudo-control approach (for family studies) or UNPHASED analysis (pooled data across all studies). The interrupted lines represent the CI of the pooled data for all studies, both family- and population-based, using the UNPHASED application.

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

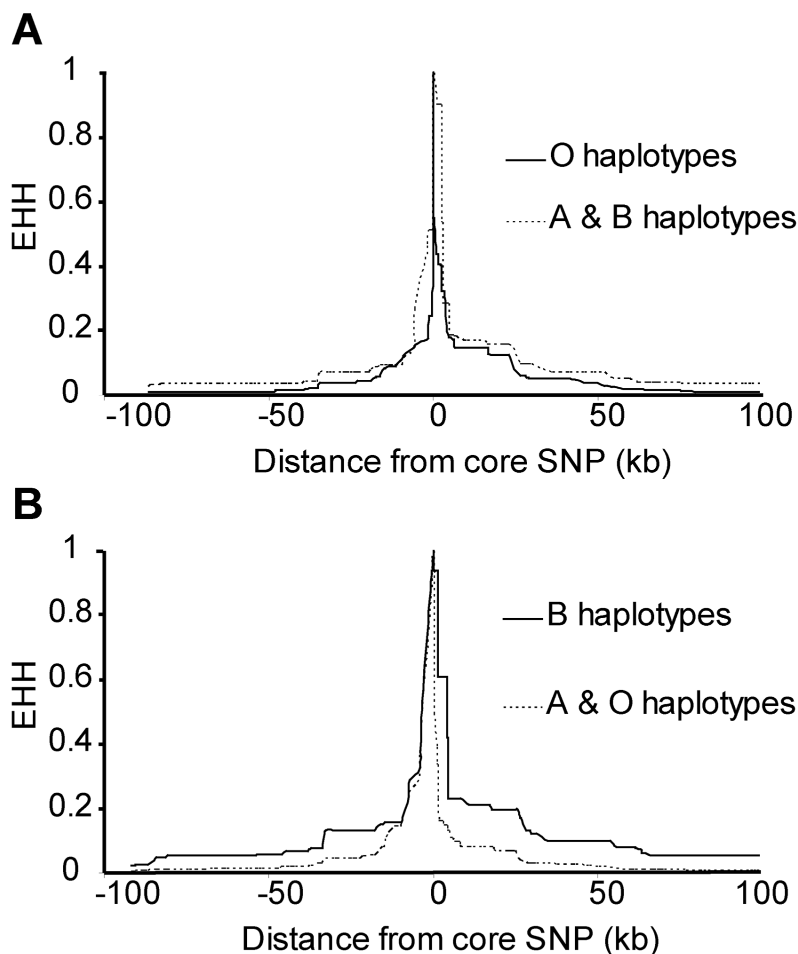

Figure 3.

Extended haplotype homozygosity plots around functional SNPs in the ABO locus. Alleles that have risen rapidly in frequency due to recent positive or balancing selection (e.g. a partial selective sweep) can be surrounded by a region of similar haplotypes that can extend for hundreds of kilobases (45, 46). This occurs because recombination has had insufficient time to swap variation between the selected haplotype and others in the population. The decay of homozygosity (EHH) on phased haplotypes partitioned by the alleles of the functional ABO SNPs. (A) rs8176719, the frameshift mutation in exon 6 of the ABO gene, marking O haplotypes, which is associated with protection from severe malaria. (B) rs8176746, a nonsynonymous coding SNP in the N-terminal catalytic domain of ABO, one of the functional variants determining A/B glycosyltransferase activity. The lack of a pronounced EHH signal suggests that the balanced selection affecting variation at the ABO gene is longstanding.

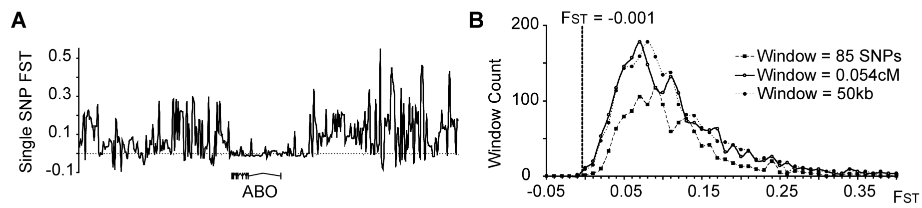

Figure 4.

A region of Low F_{ST} region around the ABO gene. (A) 400 Single SNP F_{ST} values for three HapMap populations (CEU, YRI and combined Asian) surrounding the ABO gene. F_{ST} drops to a level of −0.001 in an 85 SNP window across the gene and the noncoding sequence ~20-30kb upstream. (B) Histogram representing an empirical distribution of F_{ST} determined by screening similarly sized windows across chromosome 9. Three different window sizes were used based on either marker numbers (85 SNPs), genetic distance (0.054 cM) or physical distance (50kb), only windows containing more than 2 markers were included. By all three distributions the region around the ABO locus is a relative outlier ~99.5 – 99.9th centile for low F_{ST}.

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1

Single SNP and Haplotype association analysis for case-control and family studies.

		SNP						Haplotype					
		rs8176719			rs8176746			A			B		
Region	**Phenotype**	**OR**	**CI**	**P**	**OR**	**CI**	**P**	**OR**	**CI**	**P**	**OR**	**CI**	**P**
Population-based study													
Gambia	CM	1.21	0.98 - 1.49	0.08	0.97	0.75 - 1.25	0.81	1.37	1.04 - 1.79	0.025	1.09	0.83 - 1.42	0.54
Kenya	-	1.11	0.87 - 1.42	0.39	1.01	0.74 - 1.37	0.97	1.18	0.87 - 1.6	0.29	1.02	0.74 - 1.41	0.89
Malawi	-	1.18	0.97 - 1.43	0.09	1.02	0.81 - 1.29	0.84	1.31	1.02 - 1.68	0.034	1.07	0.83 - 1.37	0.60
All	-	1.17	1.04 - 1.32	0.011	1.00	0.86 - 1.16	0.99	1.29	1.1 - 1.51	0.001	1.06	0.91 - 1.24	0.46
Gambia	SA	1.29	1.02 - 1.65	0.037	1.01	0.77 - 1.34	0.94	1.41	1.04 - 1.91	0.028	1.17	0.86 - 1.58	0.31
Kenya	-	1.37	1.1 - 1.7	0.005	1.32	1.02 - 1.72	0.037	1.31	0.99 - 1.73	0.06	1.39	1.06 - 1.83	0.018
Malawi	-	1.16	0.83 - 1.6	0.38	0.96	0.63 - 1.46	0.86	1.29	0.85 - 1.97	0.23	1.04	0.67 - 1.6	0.87
All	-	1.30	1.12 - 1.5	0.0004	1.13	0.95 - 1.34	0.17	1.34	1.11 - 1.61	0.002	1.24	1.03 - 1.49	0.023
Gambia	Severe	1.27	1.07 - 1.5	0.006	1.06	0.87 - 1.29	0.55	1.40	1.12 - 1.74	0.003	1.16	0.94 - 1.44	0.17
Kenya	-	1.17	1 - 1.38	0.05	1.16	0.95 - 1.41	0.14	1.16	0.94 - 1.42	0.17	1.16	0.94 - 1.42	0.17
Malawi	-	1.16	0.96 - 1.39	0.13	1.01	0.8 - 1.27	0.95	1.28	1 - 1.63	0.047	1.05	0.82 - 1.34	0.71
All	-	1.20	1.09 - 1.32	0.0003	1.08	0.96 - 1.22	0.18	1.27	1.12 - 1.44	0.0003	1.13	0.99 - 1.28	0.06
Family-based study													
Gambia	CM	1.24	1.02 - 1.49	0.03	1.40	1.12 - 1.76	0.003	1.09	0.87 - 1.38	0.45	1.42	1.12 - 1.81	0.004
Kenya	-	1.26	0.84 - 1.87	0.27	1.10	0.67 - 1.82	0.70	1.22	0.76 - 1.97	0.41	1.21	0.69 - 2.12	0.50
Malawi	-	1.36	1.02 - 1.81	0.037	0.92	0.64 - 1.32	0.65	1.37	0.99 - 1.88	0.06	1.18	0.8 - 1.73	0.41
All	-	1.27	1.1 - 1.47	0.001	1.23	1.03 - 1.47	0.02	1.19	1 - 1.41	0.06	1.34	1.1 - 1.62	0.003
Gambia	SA	1.28	1.02 - 1.61	0.033	1.06	0.8 - 1.41	0.67	1.25	0.96 - 1.64	0.10	1.25	0.92 - 1.7	0.15
Kenya	-	1.27	0.76 - 2.12	0.36	0.95	0.51 - 1.78	0.87	1.29	0.73 - 2.28	0.37	1.04	0.54 - 1.99	0.91
Malawi	-	2.17	1.09 - 4.29	0.027	0.88	0.43 - 1.79	0.72	2.35	1.15 - 4.78	0.019	1.30	0.6 - 2.83	0.51
All	-	1.34	1.1 - 1.64	0.004	1.02	0.8 - 1.3	0.85	1.34	1.07 - 1.69	0.011	1.21	0.93 - 1.57	0.16
Gambia	Severe	1.17	1.04 - 1.32	0.008	1.15	0.99 - 1.33	0.06	1.13	0.98 - 1.3	0.10	1.23	1.05 - 1.44	0.01
Kenya	-	1.17	0.87 - 1.56	0.30	1.05	0.73 - 1.53	0.78	1.10	0.78 - 1.55	0.57	1.08	0.72 - 1.61	0.71
Malawi	-	1.35	1.01 - 1.78	0.04	0.91	0.64 - 1.29	0.60	1.36	1 - 1.87	0.05	1.15	0.79 - 1.69	0.46
All	-	1.19	1.08 - 1.32	0.001	1.10	0.97 - 1.25	0.13	1.16	1.03 - 1.31	0.018	1.20	1.05 - 1.37	0.009

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

NOTE.- OR=odds ratio; CI=95% confidence intervals; P=P-value from logistic regression with covariates of ethnic group, gender and HbS genotype (case-control) or case-pseudo-control approach and conditional logistic regression based on parental genotypes (family). Results for the multiplicative model. Commonest category (deletion allele and O haplotype) taken as reference. Phenotypes: CM=cerebral malaria; SA=severe anaemia; Severe=all severe cases.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 2

Association analysis between severe malaria phenotypes and inferred blood group.

		Blood Group	A			B			AB	
Study/Region	**Phenotype**	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**
Case-control										
Gambia	CM	1.13	0.78 - 1.61	0.52	0.96	0.67 - 1.37	0.83	2.14	1.12 - 4.07	0.021
Kenya	-	1.42	0.98 - 2.07	0.07	1.17	0.8 - 1.72	0.42	0.93	0.34 - 2.54	0.89
Malawi	-	1.53	1.11 - 2.12	0.01	1.09	0.8 - 1.5	0.58	1.32	0.75 - 2.34	0.34
All	-	1.36	1.11 - 1.66	0.003	1.07	0.87 - 1.31	0.53	1.51	1.02 - 2.22	0.04
Gambia	SA	1.52	1.02 - 2.26	0.041	1.22	0.82 - 1.83	0.33	1.76	0.79 - 3.91	0.17
Kenya	-	1.51	1.06 - 2.16	0.024	1.55	1.1 - 2.19	0.013	2.05	0.98 - 4.29	0.06
Malawi	-	1.66	0.96 - 2.86	0.07	1.15	0.65 - 2.02	0.63	1.33	0.5 - 3.5	0.57
All	-	1.54	1.22 - 1.96	0.00039	1.36	1.07 - 1.72	0.012	1.76	1.1 - 2.81	0.019
Gambia	Severe	1.27	0.95 - 1.7	0.10	1.07	0.8 - 1.42	0.65	2.04	1.16 - 3.58	0.013
Kenya	-	1.28	0.99 - 1.65	0.06	1.22	0.95 - 1.57	0.12	1.43	0.79 - 2.6	0.24
Malawi	-	1.49	1.08 - 2.04	0.014	1.06	0.78 - 1.45	0.70	1.31	0.75 - 2.3	0.34
All	-	1.33	1.13 - 1.56	0.00065	1.13	0.96 - 1.33	0.15	1.59	1.15 - 2.21	0.006
Family-based study										
Gambia	CM	1.14	0.83 - 1.57	0.42	1.56	1.11 - 2.21	0.011	2.66	1.4 - 5.05	0.003
Kenya	-	1.49	0.76 - 2.94	0.25	1.18	0.57 - 2.45	0.65	1.64	0.38 - 7.09	0.51
Malawi	-	1.60	1 - 2.57	0.05	1.09	0.63 - 1.86	0.77	1.85	0.79 - 4.35	0.16
All	-	1.30	1.01 - 1.66	0.038	1.37	1.05 - 1.8	0.02	2.23	1.38 - 3.61	0.001
Gambia	SA	1.56	1.06 - 2.29	0.025	1.43	0.94 - 2.17	0.10	1.46	0.69 - 3.09	0.32
Kenya	-	1.27	0.6 - 2.68	0.53	1.12	0.47 - 2.66	0.80	1.91	0.1 - 36.04	0.67
Malawi	-	1.60	0.53 - 4.8	0.41	0.98	0.31 - 3.12	0.97	2.13	0.49 - 9.16	0.31
All	-	1.51	1.09 - 2.09	0.014	1.32	0.92 - 1.88	0.13	1.58	0.83 - 3	0.16
Gambia	Severe	1.26	1.03 - 1.54	0.025	1.36	1.09 - 1.69	0.006	1.46	1 - 2.14	0.05
Kenya	-	1.20	0.75 - 1.9	0.45	1.02	0.6 - 1.72	0.94	1.23	0.39 - 3.84	0.72
Malawi	-	1.59	1 - 2.52	0.05	1.13	0.66 - 1.91	0.66	1.70	0.74 - 3.92	0.21
All	-	1.29	1.09 - 1.53	0.003	1.28	1.06 - 1.54	0.01	1.46	1.05 - 2.04	0.025

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

NOTE.- OR=odds ratio; CI=95% confidence intervals; P=P-value from logistic regression with covariates of ethnic group, gender and HbS genotype (case-control) or case-pseudo-control approach and conditional logistic regression based on parental genotypes (family). Commonest category (O blood group) taken as reference. Phenotypes: CM=cerebral malaria; SA=severe anaemia; Severe=all severe cases.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 3

Evidence of a parent-of-origin effect at the ABO locus.

Variation/ Region	Origin	CLR			Wald	TDT			
		OR	95% CI.	P		Observed	Expected	TDT χ^2	P
rs8176719									
Gambia	Maternal	1.35	1.1 - 1.65	0.004	-	233	202	9.51	0.002
-	Paternal	1.05	0.86 - 1.28	0.65	0.13	225	212	1.59	0.2
-	All	1.17	1.04 - 1.32	0.008		598	554	6.99	0.008
Malawi	Maternal	1.51	0.94 - 2.4	0.09	-	49	40	4.05	0.044
-	Paternal	1.20	0.74 - 1.93	0.46	0.55	43	37.5	1.61	0.2
-	All	1.35	1.01 - 1.78	0.04		113	98.5	4.27	0.039
Kenya	Maternal	1.48	0.92 - 2.35	0.1	-	46	39	2.51	0.11
-	Paternal	0.89	0.53 - 1.5	0.67	0.21	31	31	0.00	1
-	All	1.17	0.87 - 1.56	0.3		98	91	1.08	0.30
All	Maternal	1.38	1.16 - 1.65	0.0002	-	328	281	15.72	0.00007
-	Paternal	1.05	0.88 - 1.24	0.6	0.047	299	280.5	2.44	0.12
-	All	1.19	1.08 - 1.32	0.001		809	743.5	11.54	0.0007
ABO Haplotype									
A	Maternal	1.45	1.16 - 1.8	0.001	-	-	-	-	-
A	Paternal	0.99	0.8 - 1.22	0.894	0.023	-	-	-	-
B	Maternal	1.30	1.03 - 1.63	0.024	-	-	-	-	-
B	Paternal	1.17	0.92 - 1.49	0.203	0.58	-	-	-	-

NOTE.- CLR=conditional logistic regression; Wald=P-value for the Wald test comparing estimated parameters for maternal and paternal transmission; TDT= Transmission Disequilibrium Test (66). Observed and expected rates of transmission of the minor allele along with classical TDT statistics are presented for comparison.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 4

Parent-of-origin likelihood ratio test for rs8176719.

Test	Model	−2LL	χ^2	df	P
-	Child and maternal genotypes, and PO	1365.582	-	-	-
Parent-of-origin effect	Child and maternal genotypes only	1369.58	3.998	1	0.046
Maternal genotype effect	Child genotype and PO only	1368.714	3.133	2	0.21

NOTE.-LL=log likelihood; χ^2=Log ratio test chi-squared value; df=degrees of freedom, and P=P value for the likelihood ratio test; PO= parent-of-origin effect included in model.