Interferon regulatory factor-1 polymorphisms are associated with the control of *Plasmodium falciparum* infection

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Abstract

We describe the haplotypic structure of the *Interferon Regulatory Factor-1* (*IRF-1*) locus in two West African ethnic groups, Fulani and Mossi, that differ in their susceptibility and immune response to *Plasmodium falciparum* malaria. Both populations showed significant associations between *IRF-1* polymorphisms and carriage of *P. falciparum* infection, with different patterns of association that may reflect their different haplotypic architecture. Genetic variation at this locus does not therefore account for the Fulani-specific resistance to malaria while it could contribute to parasite clearance's ability in populations living in endemic areas. We then conducted a case-control study of three haplotype-tagging Single Nucleotide Polymorphisms (htSNPs) in 370 hospitalized malaria patients (160 severe and 210 uncomplicated) and 410 healthy population controls, all from the Mossi ethnic group. All 3 htSNPs showed correlation with blood infection levels in malaria patients, and the rs10065633 polymorphism was associated with severe disease (p=0.02). These findings provide the first evidence of the involvement in malaria susceptibility of a specific locus within the 5q31 region, previously shown to be linked with *P. falciparum* infection levels.

Keywords

Malaria susceptibility; Fulani; Mossi; *IRF-1*; 5q31; association study

INTRODUCTION

Interferon Regulatory Factor 1 (IRF-1) is a transcription factor that regulates the expression of a number of genes whose products play crucial roles in innate as well as adaptive immunity (reviewed in ref. 1).

IFN-gamma is the strongest IRF-1 inducer known and IRF-1 promotes transcription of a number of genes, acting as an important mediator of IFN-gamma activity. The expression of IRF-1 can also be induced by TNF-alpha [2] and antagonized by IL-4 [1]. IRF-1 activity is essential for recognition of micro-organisms and antigen presentation, as it regulates the

expression of genes such as *Toll-like Receptor 9*[3], MHC class I and class II genes [1], *LMP2* [1] and *MECL1*[4]. IRF-1 is required for maturation of natural killer cells by regulating the transcription of *IL-15*[1], and it has a pivotal role in monocyte/macrophage differentiation [5]. In macrophages it triggers the expression of *iNOS*[1], a principal mediator of cytotoxicity against pathogens, and *Bf*[6], part of the alternative complement cascade. Moreover, IRF-1 levels seem to be critical for T cell development in the thymus [1] as well as for B cell growth [7]. IRF-1 triggers *IL-2p40* expression [1] and represses *IL-4* transcription [8], and it is therefore central in modulating the balance between Th1 and Th2-type T cell responses [1]. IRF-1 is involved at many points of the immune response and as a consequence is likely to affect susceptibility to infectious diseases.

A role for this transcriptional regulator in both malaria mortality and parasite density has been suggested by studies of *Plasmodium berghei* infection conducted in IRF-1 knockout mice [9], [10]. Interestingly, the *IRF-1* locus lies in the 5q31 human genome region, which contains a cluster of immunological genes encoding cytokines and growth factors , and has been therefore thought to play a role in the response to parasitic diseases. This has been investigated by linkage analysis studies that demonstrated the involvement of this gene region in the control of *Plasmodium falciparum* blood parasite densities [11] as well as of intensity of *Schistosoma mansoni* infection [12], but the *P. falciparum infection level locus (Pfil)* has not yet been narrowed down to a specific gene.

To determine whether genetic variation at the *IRF-1* locus affects resistance to malaria infection in humans, and might therefore underlie the *Pfil* locus, we studied *IRF-1* genetic diversity in two West African ethnic groups that show striking differences in their susceptibility and immune response to malaria, and conducted a candidate gene association study with carriage of *P. falciparum* infection. Despite equivalent exposure to infection and comparable use of protective measures, the Fulani are known to have lower malaria-parasite and fever-attack rates, and higher immune reactivity than sympatric Mossi and Rimaibé from Burkina Faso [13], [14], [15] and Dogon from Mali [16]. The Fulani are genetically distinct from their neighbours [17] but carry known malaria-resistance alleles at a similar or even lower frequency than either sympatric groups [18]. As a consequence these factors cannot be responsible for the increased malaria resistance observed in the Fulani. These ethnic groups therefore provide a unique model for the investigation of novel genetic determinants of the human response to malaria.

In parallel to this inter-ethnic study we also conducted a classical intra-ethnic case-control study where we compared the allele frequency of three haplotype-tagging SNPs (htSNPs) between healthy population controls, mild malaria cases and severe malaria cases of Mossi ethnicity, in order to evaluate the effect of *IRF-1* polymorphisms on disease severity. We further assessed the involvement of *IRF-1* genetic variation in the control of *P. falciparum* infection by looking at association with blood parasite densities in malaria patients.

RESULTS

Ethnic groups study

We have genotyped 14 Single Nucleotide Polymorphisms (SNPs) in a region extending from 5 kb upstream to 5 kb downstream of the *IRF-1* gene in subjects from the Fulani and Mossi groups (parasite prevalence $31.0 \pm 5.0\%$ vs $60.4 \pm 4.9\%$ respectively, Yate's-corrected $X^2 = 14.8$ p=0.0001). All SNPs were polymorphic in both populations, with high minor allele frequencies (MAF) (Table 1) with the average heterozygosity consequently also very high ($H_M = 0.49$ and $H_F = 0.48$). Two SNPs showed different frequencies among Fulani and Mossi (rs10213701: $MAF_M = 0.42$; $MAF_F = 0.27$, P = 0.004; rs2549005: $MAF_M = 0.49$ $MAF_F = 0.34$, P = 0.006). It is worth noting that the Fulani and Mossi have different

geographical origins and migration history, and are likely to have been exposed to different environmental pressures, so demographic factors and selective pressure are both potential contributors to differences in allele frequency between the two populations.

Using the 14 typed SNPs (Table 1), we generated haplotypes across *IRF-1* . In both the Fulani and Mossi two haplotype blocks (i.e. regions of high linkage disequilibrium (LD)) are identified within the gene (Figure 1): the first corresponding to the coding and the downstream regions, and the second to the upstream region. LD between the two blocks is slightly higher in the Mossi than in the Fulani (average $r^2_M = 0.58$ vs. $r^2_F = 0.54$) and that this is mainly due to marker rs2549005 (average $r^2_M = 0.50$ vs. $r^2_F = 0.32$). The haplotypic diversity (Table 2) across the region is described by a smaller number of haplotypes in the Fulani with respect to the Mossi ($K_F = 12$ vs. $K_M = 19$) and is captured by different sets of haplotype-tagging SNPs (htSNPs). Furthermore, the most common haplotypes (frequency > 0.5) have different frequencies in the two populations, and haplotypes exclusive to just one ethnic group also exist.

We analysed association between these polymorphisms and carriage of *P. falciparum* infection, based on a cross-sectional epidemiological survey of 190 unrelated individuals over 10 years old, of which 85 were Fulani and 105 Mossi. As shown in Table 3, significant associations with carriage of *P. falciparum* infection were found in both populations but each showed a different pattern of association. This might possibly reflect the different haplotype architecture in the two groups. Interestingly, the two SNPs with greatest difference in frequency between Fulani and Mossi were not associated with carriage of *P. falciparum* infection.

One *IRF-1* promoter polymorphism, rs2706384, was associated with protection against *P. falciparum* infection in both populations (Table 4). Although the rs2706384 polymorphism appears to be a marker for protection against *P. falciparum* infection, it is evidently not responsible for the increased malaria resistance of the Fulani compared to the Mossi, as it has a similar allele frequency in both groups (0.41 and 0.51 respectively, Table 1) and when stratified for rs2706384 genotype the parasite rate remains lower in the Fulani than in the Mossi. Furthermore the pattern of association differs between the two populations: while in the Mossi the C allele is associated with a higher risk of carrying a *P. falciparum* infection (OR=1.92, 95% CI 1.02-3.63, p=0.04), this is not the case in the Fulani (OR=1.08, 95% CI 0.53-2.22, p=0.95). In fact in the latter group both CC and AA homozygous individuals are more frequently parasitized than heterozygous CA subjects. This observation further suggests that this polymorphism is not itself the cause of association but could be a marker for a non-genotyped functional SNP.

Genetic variation at this locus does not therefore account for the Fulani-specific resistance to malaria while it could contribute to parasite clearance's ability in populations living in endemic areas.

Case-control study

We genotyped three *IRF-1* haplotype-tagging SNPs (htSNPs) in unrelated children of Mossi ethnicity comprising severe malaria cases (SMC), mild malaria cases (MMC), and healthy population controls (HPC). Among the 14 SNPs included in the Ethnic groups study we selected 3 markers that are htSNPs in the Mossi population: rs10065633, rs10213701 and rs2706384 (Table 2). They have been chosen on the basis of their gene location (5′ upstream, intronic, 3′ downstream respectively; Table 1) as well as on the basis of previous association results: association with carriage of parasite infection observed in the Mossi population in the Ethnic groups study (rs10065633 and rs2706384; Table 3) and association with severe malaria observed in Gambian trios (rs10213701; Mangano VD, manuscript in

preparation). The three markers were in Hardy Weinberg equilibrium within each group of children (Table 5).

We compared the minor allele frequency of each polymorphism between the 3 groups to look for marker association with disease status (Figure 2). We observed that the C allele of rs10065633 has a higher frequency in the severe cases group than within the uncomplicated cases (p=0.03) or the healthy children (p=0.04) while the frequency does not vary between uncomplicated cases and healthy children (p=0.64). We could therefore compare the group of children with severe malaria with the group of children with no severe malaria (uncomplicated plus healthy). The C allele at this SNP is associated with severe disease, OR=1.36 (95% CI 1.04-1.78, p=0.02). A similar pattern is shown by the SNP rs10213701, where the T allele shows a trend of association with severe disease, OR=1.3 (95% CI 0.99-1.7, p=0.06). No association with disease status was observed for the SNP rs2706384.

In the Ethnic groups study, in association analysis with carriage of malaria infection within the Mossi population, we observed that the C allele at SNP rs10065633 has higher frequency in infected individuals, OR=2.12 (95% CI 1.12-4.02, p=0.02); a similar trend was observed for the T allele at SNP 10213701 (although this marker shows no significant association, OR=1.52, 95% CI 0.81-2.86, p=0.2) and for the C allele at SNP rs2706384, OR=1.92 (95% CI 1.02-3.63, p=0.04).

In order to verify the contribution of *IRF-1* genetic variation to the individual ability to control *P. falciparum* infection, we compared the blood infection levels of malaria patients (both severe and mild) between carriers and non-carriers of the susceptible alleles, adjusting for the effect of disease status and age. We defined as susceptible the alleles associated with severe disease in the Case-control study and/or with carriage of infection within the Mossi population in the Ethnic group study. These are allele C at SNP rs10065633, allele T at SNP rs10213701 and allele C at SNP rs2706384.

For each of the polymorphisms under study we observed that subjects carrying one or two copies of the susceptible alleles have higher mean parasite density, around two fold that of subjects who are homozygous for the resistant alleles (Figure 3). These differences were statistically significant for rs10065633 and rs10213701 SNPs only.

The same variants associated with higher risk of carrying a malaria infection in the Ethnic group study are associated with higher *P. falciparum* density in the Case-control study. We are aware that the phenotypes analysed in the two studies are different: in the first we looked at parasite prevalence as the study subjects are adults and therefore no much variability can be observed with regard to parasite density in this population group, while in the latter we looked at parasite density as all the malaria cases are by definition parasite positive. Nonetheless, as the results of the two studies are consistent with each other, the overall data suggest that *IRF-1* genetic variation affects the individual ability to control *P. falciparum* infection.

Since the three polymorphisms show a very similar pattern of correlation with parasite density, and on the basis of the data from the Ethnic groups study we expect LD to be quite high between those markers, we applied linear regression analysis to evaluate the independence of the association signals. None of the markers was identified as an independent factor in the control of parasite density (data not shown).

We therefore built multi-marker haplotypes to look for haplotype association with blood infection levels (Table 6). We noted that carriage of Haplotype B (CTC), which we expected to be a risk haplotype as it is a combination of all susceptible alleles, results in an increased

parasite load (mean parasite density [log par/µl] ± SE; non carriers: 3.9 ± 0.1; carriers: 4.2 ± 0.1; p=0.008).

DISCUSSION

Human populations exposed to malaria show high variability as regards to both clinical outcome and development of immune protection. We know that this heterogeneity depends on many factors such as age, transmission intensity, different parasite strains virulence, co-infections with other pathogens and socio-economic status as well as on the genetic background of the human host [19]. A recent study to determine the relative contribution of genetic and other factors to the variability in malaria incidence in a cohort of Kenyan children, estimated that 25% of the total variation is explained by additively acting host genes and that haemoglobin S, the strongest known resistance genetic factor, explained only 2% of the total variation, suggesting the existence of many unknown protective genes, each individually having small population effects [20].

In the present investigation, in order to dissect the role of *IRF-1* gene in susceptibility to malaria, we retrospectively analysed epidemiological data of two complementary studies conducted in Burkina Faso and based on cross-sectional parasitological surveys and case-control clinical studies. Both in Mossi and Fulani, the parasitological survey showed that *IRF-1* genetic variation entails different abilities to control *P. falciparum* infection. The clinical study, while confirming the role of the *IRF-1* gene in the control of malaria infection, did not provide coherent and convincing evidence of the involvement of this locus in the evolution of malaria as a disease. Indeed, only a trend of association with small differential risk of severe disease was suggested. Moreover, the possible effect on the clinical outcome did not involve the polymorphism that showed the strongest association signal in the parasitological study.

To our knowledge, this work provides the first evidence of a specific locus within the 5q31 region that is associated with the control of malaria infection, and raises the possibility that *IRF-1* could be the *Pfil* locus. Linkage disequilibrium mapping of the Th2 cluster in the same ethnic groups sample-set also pinpoints to *IRF-1* as the most relevant gene (Sadighi Akha E, manuscript in preparation). Nevertheless a recent genome-wide linkage analysis of malaria infection intensity yielded a signal on Chromosome 13q and did not obtain evidence for linkage of parasite density to 5q31 [21], suggesting that a different locus, *PFPD-2,* could be the major genetic determinant of parasite density.

Although the effect of polymorphism at this locus is not large on its own, it may contribute to the complexity of each individual's susceptibility to malaria. Further dissecting this complexity will help to elucidate the mechanisms of immune protection against malaria and/or immunopathology.

Recent genetic association studies suggested a role for Interferon Regulatory Factor 1 in resistance to viral infections. An *IRF-1* haplotype has been shown to protect from Hepatitis C in a Japanese population, and to modify promoter activity as measured by luciferase reporter assay [22]. The authors suggested that this haplotype could affect the secondary structure of the promoter region, and therefore its affinity for transcription factors. *IRF-1* transcriptional regulation is potentially critical given the short half-life of the IRF-1 protein [1]. Actually it has been reported that this variant correlates with IFN-gamma and IL-10 levels produced by Peripheral Blood Mononuclear Cells (PBMCs), as well as with the percentage of Th1 CD4+ cells in patients with chronic Hepatitis C [23]. *IRF-1* polymorphisms have also been found to be associated with resistance to HIV-infection in a sample of Kenyan sex-workers [24]; PBMCs isolated from subjects with protective genotypes showed a reduced

expression of the gene, both before and after IFN-gamma stimulation, as measured by Western Blot analysis.

These observations provide possible functional explanations for our association findings, nevertheless none of these studies could identify the true causative polymorphism/s. Long-range haplotype analysis of Allele-Specific Transcript-Quantification data [25] is being used to uncover regulatory polymorphisms, and targeted functional experiments will be performed to determine how genetic variation affects the molecular mechanisms of immune responses to malaria.

The role of *IRF-1* as a genetic determinant of human susceptibility to malaria is worthy of investigation in larger multi-centre studies of resistance/susceptibility to severe malaria [19].

PATIENTS AND METHODS

Study subjects

Ethnic groups study—The sample comprised 190 unrelated individuals aged > 10 years, 85 belonging to the Fulani (mean age ± SE; 29.8 ± 2.0 years) and 105 to the Mossi (39.2 ± 1.7 years) ethnic groups, who were recruited during a cross-sectional epidemiological survey conducted in August 1994 in the villages of Barkoumbilen and Barkoundouba, Northeast of Ougadougou, Burkina Faso [13]. Rimaibè individuals were not included in the present study, because of their phenotypic and genetic similarity to the Mossi [13], [17]. In spite of the lower mean age of the present Fulani sub-sample, higher *P. falciparum* infection rates were recorded amongst the Mossi.

Case-control study—The sample of severe (N=160, 4.4 ± 0.2 years) and uncomplicated malaria cases (N=210, 4.6 ± 0.2 years) from the Mossi ethnic group was recruited at the 158-bed Paediatric ward of the Ouagadougou University Hospital [25]. In line with WHO guidelines, severe malaria was defined by the presence of *Plasmodium falciparum* in the thick blood film associated with at least one of the following conditions: prostration (incapacity of the child to sit without help in the absence of coma), unrousable coma (score between 0 and 2 on the Glasgow modified coma scale), repeated generalized convulsions (more than two episodes in the preceding 24 hours), severe anaemia (hemoglobin <5 g /dL), hypoglycemia (<40 mg/dL), pulmonary oedema/respiratory distress, spontaneous bleeding and renal failure (plasma creatinine > 3 mg/dL). Uncomplicated malaria was defined as a clinical illness characterised by an axillary temperature >37.5 °C associated with a *P. falciparum* positive blood film in the absence of clinical signs and symptoms of severe malaria. The sample of healthy control children also belonging to the Mossi ethnic group (N=410, 2.8 ± .06 years) was collected during malaria cross-sectional surveys performed in the Ouagadougou area [25]. Both for malaria patients and healthy controls only unrelated children whose both parents were of Mossi ethnicity have been included in the study.

Plasmodium falciparum detection and density determination

Thick and thin blood smears were prepared following the standard procedures. According to WHO guidelines for the microscopic diagnosis of malaria (Bench Aids for the Diagnosis of Human Malaria; Plate 8, Methods of counting malaria parasites in Giemsa-stained thick blood films), 100 microscopic fields (ca. 20 leukocytes/field at 1000x = ca. 0.25 μl of blood) of the thick blood smear were examined for the determination of parasite density. The *Plasmodium* species was identified on the thin blood smear.

Genotyping and allele frequencies

SNPs in the region 5 kb upstream to 5 kb downstream of the *IRF-1* gene were identified from public databases (dbSNP build 126, Ensemble release 40) and the literature.

For the ethnic groups study 14 polymorphisms were selected on the basis of the following criteria: validated status, overall coverage of the locus with regular spread (density of 1 SNP every 2 Kb on average), encompassing exonic, intronic, regulatory regions of the gene, possible functional effect of the allelic variant [20], [21]. The genotypes for the selected polymorphisms were determined through the SEQUENOM© MassARRAY™ System [26], a high through-put procedure based on an allele-specific primer-extension reaction and mass spectrometry. Primers and multiplexes were designed using the dedicated software SpectroDESIGNER™ (SEQUENOM©).

For the case-control study 3 markers were selected among the initial 14 for being haplotype-tagging SNPs and/or showing an association signal in the Mossi population, as well as on the basis of their gene location (one 5′ upstream, one intronic, one 3′ downstream). Genotyping was performed by Amplification Refractory Mutation System (ARMS). Primers were designed using a dedicated on-line software (http://cedar.genetics.soton.ac.uk/public_html/primer1.html) [27]. DNA samples whose genotype was determined by Sequenom MassArray System were used as positive controls for method validation. Primers and PCR conditions are available on request.

SNPs whose assays had a failure rate higher than 10% or were monomorphic (MAF < 0.01) have been excluded from further analysis. Hardy-Weinberg equilibrium has been tested using a threshold p value of 0.001, which aims to represent a compromise between the safe exclusion of most genotyping errors and the retention of potentially interesting (i.e. under selection) SNPs.

Haplotype structure and Linkage Disequilibrium architecture

Haplotypes have been constructed using the Stephens-Donnelly method [28] (PHASE v.2). Haplotype tagging SNPs (htSNPs) have been determined by an unstructured approach using the ENTROPY algorithm [29], which selects those SNPs that best describe the whole haplotypic diversity of the region. The algorithm starts by picking the most informative marker, which will be that with allele frequency closest to 0.5. It then repeatedly adds new markers from the set, each time choosing the one marker which most increases the logical entropy of the system. HtSNPs are defined by an Entropy value > 0.

Graphs showing Linkage Disequilibrium (LD) patterns have been generated using MARKER (http://www.gmap.net/perl/marker/marker_entry). Typed SNPs are represented on the horizontal axis and dots darkness represents the level of LD (r^2) between each pair of markers.

Haplotypes blocks across the gene have been defined as regions in which pair-wise D′ values between all SNP pairs exceed the threshold of 0.8, using the program HaploBlockFinder [30].

Statistical Analysis

Statistical analysis has been performed using SPSS v14.00 and EpiInfo 2000. A p value of 0.05 has been considered as threshold for significance. Allele, genotype and haplotype distributions between groups have been compared by Yates-corrected χ^2 test. If contingency tables had less than 5 expected events per cell, Fisher's exact test was used. To test marker and haplotype association with blood infection levels in malaria patients we used linear

regression adjusted for disease status and age, after log-transformation of parasite densities. Linear regression analysis was also applied to evaluate the independence of association signals.

We applied a method to correct for multiple testing of SNPs in LD with each other [32], using an interface available online (http://genepi.qimr.edu.au/general/daleN/SNPSpD/). Based on spectral decomposition of matrices of pairwise LD between SNPs, the method determines the effective number of independent markers in the set tested (M_{eff}). The corrected significance threshold required to keep Type I error rate at 5% is equal to 0.05/ M_{eff}.

Acknowledgments

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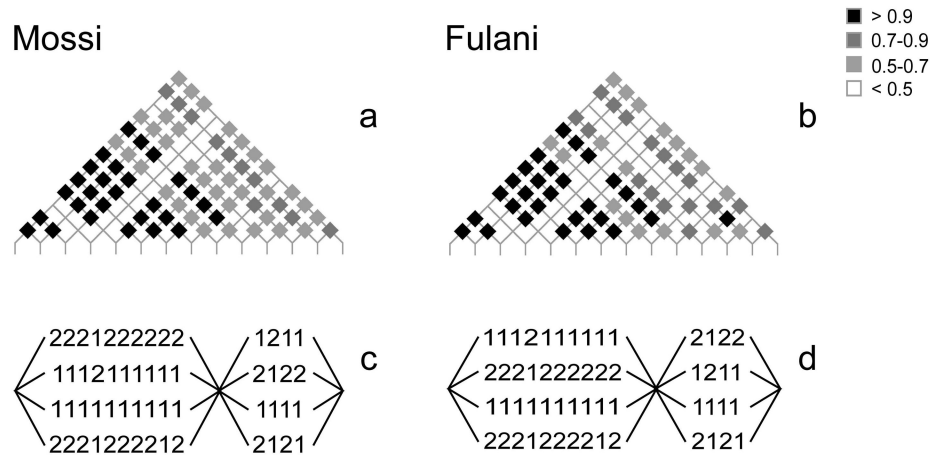
Figure 1. Linkage disequilibrium patterns and haplotype block structure of the *IRF1* locus in Mossi and Fulani of Burkina Faso

(a,b) LD patterns in the Mossi and Fulani samples respectively. Typed SNPs are represented on the horizontal axis and ordered by chromosome position as in Table 1. The linkage disequilibrium (r^2) between each pair of markers is represented by differently coloured diamonds.

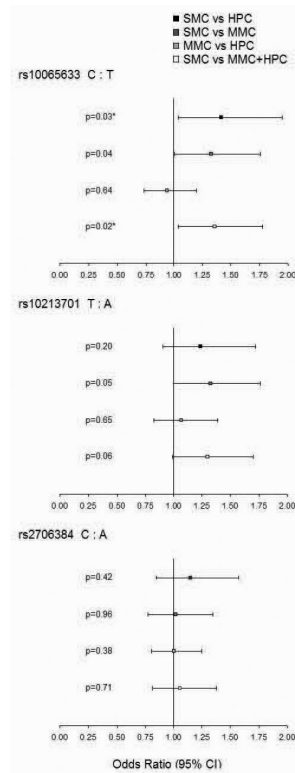(c,d) Haplotype blocks in the Mossi and Fulani samples respectively.

Figure 2. Association of *IRF-1* SNPs with disease severity in Burkina Faso subjects
Comparison of Odds Ratios and p values for Yate's-corrected Chi-Squared test at three
IRF-1 loci in: Severe Malaria Cases and Healthy Population Controls (black square); Severe
Malaria Cases and Mild Malaria Cases (dark gray square); Mild Malaria Cases and Healthy
Population Controls (light gray square); Severe Malaria Cases and children with no severe
malaria (Mild Malaria Cases plus Healthy Population Controls; white square).
Asterisk (*) indicates a significant association after correction for multiple testing (p<0.033).
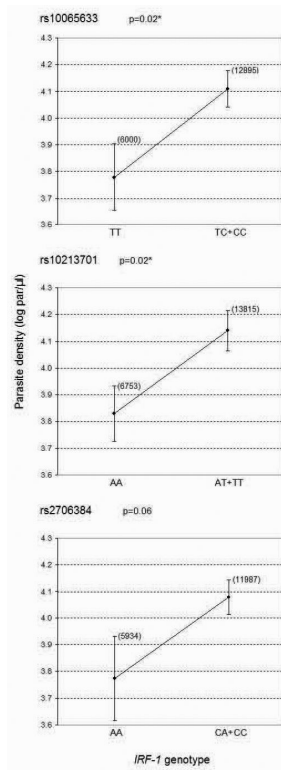
Figure 3. Association of *IRF-1* SNPs with *Plasmodium falciparum* blood infection levels in malaria patients from Burkina Faso

Means of parasite density (log transformed values) ± SE for three SNPs in the *IRF-1* gene in carriers and non carriers of the susceptible allele. The geometric means of the parasite counts are shown in brackets. P value calculated by linear regression analysis adjusting for disease status and age. Asterisk (*) indicates a significant association after correction for multiple testing (p<0.033).

Table 1

IRF-1 Single Nucleotide Polymorphisms (SNPs) genotyped for the ethnic groups study and their frequency in Mossi and Fulani populations of Burkina Faso.

SNP	Chr coord	Location	Alleles	FR %	HWE M	HWE F	MAF M	MAF F	P
rs3846731	131842163	3down	T/C	4.2	0.85	0.66	0.51	0.42	0.105
rs10065633	131844615	3down	T/C	9.5	0.92	0.69	0.49	0.42	0.276
rs839	131847025	3utr	C/T	8.4	0.92	0.32	0.51	0.41	0.094
rs2070729	131847820	intronic	A/C	5.2	0.70	0.71	0.31	0.39	0.174
rs2070728	131847876	intronic	C/T	5.2	0.43	0.71	0.48	0.41	0.196
rs2070727	131848174	intronic	C/A	7.4	0.84	0.80	0.50	0.42	0.204
rs2070725	131849687	intronic	C/T	9.5	0.84	0.83	0.50	0.42	0.151
rs2070724	131849971	intronic	A/G	5.3	0.84	0.71	0.51	0.41	0.078
rs10213701	131851963	intronic	A/T	5.3	0.53	0.81	0.42	0.27	0.004
rs2070722	131852385	intronic	T/G	9.5	0.70	0.81	0.52	0.42	0.074
rs2706384	131854779	5up	C/A	5.3	0.42	0.06	0.41	0.51	0.069
rs2549005	131855090	5up	G/A	4.2	0.76	0.70	0.49	0.34	0.006
rs2549004	131855724	5up	G/C	3.2	0.59	0.19	0.44	0.53	0.142
rs2549002	131857477	5up	G/T	5.3	0.96	0.38	0.41	0.47	0.293

Abbreviations: Chr coord, chromosome coordinate (as from dbSNP build 126); Location, location with respect to the *IRF-1* gene; FR %, genotyping failure rate; HWE, Hardy-Weinberg Equilibrium test p value; MAF, Minor Allele Frequency; P, p value for the difference in allele frequency between the two populations; F, Fulani; M, Mossi.

Table 2

Haplotypes and haplotype-tagging SNPs of the *IRF-1* locus in Mossi and Fulani of Burkina Faso

a) Haplotype frequencies in Mossi and Fulani. The number of chromosomes carrying a given haplotype is shown in brackets. b) Entropy algorithm values for *IRF-1* SNPs in Mossi and Fulani. HtSNPs are defined by en Entropy algorithm value > 0.

a

Hap name	Hap	Freq % M	Freq % F
A	22212222221211	37.6 (79)	25.3 (41)
B	11121111112122	22.9 (48)	32.1 (52)
C	11111111112122	11.9 (25)	14.8 (24)
D	22212222121111	7.6 (16)	14.2 (23)
E	11121111111211	5.2 (11)	6.2 (10)
F	11111111112121	4.3 (9)	3.7 (6)
G	11121111111122	2.4 (5)	0.6 (1)
H	22211222221211	1.0 (2)	0.6 (1)
I	11111111111121	0.5 (1)	0.6 (1)
J	22212222121211	1.9 (4)	0.0 (0)
K	22212222221212	1.0 (2)	0.0 (0)
L	11111111111122	0.5 (1)	0.0 (0)
M	11111111112222	0.5 (1)	0.0 (0)
N	11111112112122	0.5 (1)	0.0 (0)
O	11121222221211	0.5 (1)	0.0 (0)
P	21121111122122	0.5 (1)	0.0 (0)
Q	22211122221211	0.5 (1)	0.0 (0)
R	22212222122111	0.5 (1)	0.0 (0)
S	22212222221222	0.5 (1)	0.0 (0)
T	11111111112221	0.0 (0)	0.6 (1)
U	22111222221211	0.0 (0)	0.6 (1)
V	22212222112122	0.0 (0)	0.6 (1)

b

SNP	Entropy M	Entropy F
rs3846731	0.03	0.00
rs10065633	1.00	0.00
rs839	0.00	0.01
rs2070729	0.45	0.78
rs2070728	0.09	0.15
rs2070727	0.01	0.00
rs2070725	0.00	0.00
rs2070724	0.03	0.00

b

SNP	Entropy M	Entropy F
rs10213701	0.13	0.00
rs2070722	0.00	0.00
rs2706384	0.19	1.00
rs2549005	0.59	0.46
rs2549004	0.01	0.00
rs2549002	0.24	0.13

Abbreviations: Hap, haplotype; Freq, frequency; M, Mossi; F, Fulani.

Table 3

Association of *IRF-1* SNP genotypes with carriage of *Plasmodium falciparum* infection in Mossi and Fulani subjects from Burkina Faso (3×2 tables Yate's-corrected χ^2 test p values).

SNP	Mossi	Fulani
rs3846731	0.064	0.300
rs10065633	0.043	0.190
rs839	0.136	0.294
rs2070729	0.354	0.096
rs2070728	0.042	0.234
rs2070727	0.071	0.240
rs2070725	0.033	0.394
rs2070724	0.038	0.234
rs10213701	0.163	0.697
rs2070722	0.140	0.135
rs2706384	0.017[*]	0.012[*]
rs2549005	0.080	0.523
rs2549004	0.054	0.062
rs2549002	0.444	0.001[*]

[*] Asterisk indicates a significant association after correction for multiple testing (p<0.020 and p<0.022 in the Mossi and Fulani respectively).

Table 4

Plasmodium falciparum parasite rate (PR) according to *IRF-1* rs2706384 genotype in Mossi and Fulani subjects from Burkina Faso.

Genotype	PR Mossi	PR Fulani
CC	78.4% (29/37)	47.8% (11/23)
CA	47.7% (21/44)	13.3% (4/30)
AA	56.2% (9/16)	44.0% (11/25)
P	0.017[*]	0.012[*]

Number of *P. falciparum* positive subjects and group sizes are shown in brackets. P values calculated by Yate's-corrected χ^2 test.

[*] Asterisk indicates a significant association after correction for multiple testing (p<0.020 and p<0.022 in the Mossi and Fulani respectively).

Table 5

IRF-1 Single Nucleotide Polymorphisms (SNPs) genotyped for the case-control study and their frequency in Healthy Population Controls, Mild Malaria Cases and Severe Malaria Cases from Burkina Faso.

SNP	Alleles	FR %	Healthy Population Controls		Mild Malaria Cases		Severe Malaria Cases	
			MAF	HWE	MAF	HWE	MAF	HWE
rs10065633	T/C	5.9	49.4±1.8	0.48	47.7±2.5	0.91	56.5±2.9	0.38
rs10213701	A/T	4.7	33.6±1.7	0.78	35.1±2.4	0.52	40.2±2.8	0.71
rs2706384	C/A	8.5	42.7±1.8	0.64	45.6±2.5	0.60	42.3±2.9	0.12

The 3 markers were selected among the initial 14 for being haplotype-tagging SNPs and/or showing an association signal in the Mossi population, as well as on the basis of their gene location (one 5′ upstream, one intronic, one 3′ downstream, see Table 1).

Abbreviations: FR %, genotyping failure rate; MAF, Minor Allele Frequency; HWE, Hardy-Weinberg Equilibrium test p value.

Table 6

Association of *IRF-1* haplotypes with *Plasmodium falciparum* blood infection levels in malaria patients from Burkina Faso.

Hap name	Hap	Freq	β	P
A	TAA	43.6	−0.144	0.231
B	CTC	30.4	0.288	0.008
C	CAC	10.6	0.109	0.429
D	TAC	8.2	−0.104	0.493
E	TTA	3.1	−0.043	0.851
F	CAA	1.8	−0.370	0.248
G	TTC	1.9	−0.115	0.680
H	CTA	0.4	0.000	1.000

Haplotypes identified in the case-control study (rs10065533, rs10213701, rs2706384) and their frequency within the group of malaria patients. Beta coefficients and p values obtained by linear regression analysis adjusting for disease status and age.

Abbreviations: Hap, haplotype; Freq, frequency.