CHAPTER 5:
A study of erythrocyte receptor polymorphisms in relation to falciparum malaria in the ethnic populations of Assam, North east India
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5.1 Abstract

Several Red Blood Cell polymorphisms, for example thalassemias, glucose-6-phosphate dehydrogenase (G6PD) deficiency pyruvate kinase, complement receptor-1 and haemoglobinopathies, blood group antigens etc. have a role in the clinical outcome of malaria and they have shown to vary with geographical areas and malaria is documented to be a factor in their selection.

Fyb was found to be more prevalent at 96.09% than Fya at 49.66% of the study population. It was noted that both Fya and Fyb were found to be negatively associated with fever (p=0.016 and p<0.0001 respectively). There was an association with headache but was not statistically significant. Fya and Fyb were found to be protective where they were positively associated with infrequent malaria episodes (Fishers test p=0.010 and p=0.016 respectively). Kpa was found to be negatively associated with fever (p=0.016). No association of malaria episodes with K, Kpa and Kpb was observed. Although, an association of Kpa and Fya (i.e. Fya*Kpa) was seen to be positively associated with frequency of malaria disease (p=0.01). Higher prevalence of homozygous mutant (H/H) of the SNP of CR1 (Q981H) was associated with in malaria disease (Fishers test p=0.003). Among AA, TB and Khasis, it was noted that the alleles of CR1 (Q981H) were seen predominantly in heterozygosity. The homozygous mutant genotype was seen to be absent in the Khasis (Fishers Test p=0.040).

In conclusion, it was seen that there was an association of Fya and Fyb antigens with malaria symptomology. An interaction between Fya*Kpa was observed where they are positively associated with frequent malaria episodes. Our data indicated that CR1 981H/Q genotype to be protective from having falciparum malaria. Homozygous
mutant (H/H) was completely absent in the Khasis. The shift from the wild (QQ) to the heterozygous type (QH) in our study population indicates that this mutation of the CR1 gene may be still evolving in these areas.

5.2 Introduction

There is a dynamic interaction between the merozoite, the asexual blood stage of the malaria parasite, with the red blood cell since it has to gain entry into the cell, grow and divide within it. A number of human genetic polymorphisms affecting the structure, function and physiology of the red cell membrane are known to influence the parasites ability to invade and survive in it. Haemoglobinopathies (Haemoglobin (Hb) C and Hb S), thalassemias, glucose-6-phosphate dehydrogenase (G6PD) deficiency were found to be protective against *P. falciparum* malaria and were selected under malaria pressure in different populations 27, 30, 35-37.

The parasite, *Plasmodium vivax*, utilizes Duffy antigens (Fya and Fyb) present on the surface of red blood cell (RBC) and utilizes them during RBC invasion 288 and individuals negative for these antigens were observed to be resistant to *Plasmodium vivax* infections. Fya and Fyb, are codominantly expressed, and the alleles, FyA and FyB, are encoded on chromosome 1 289, 290. The distribution of these antigens varies in different ethnic groups because the null phenotype, Fy (a-b-), is more common in African populations than in Caucasians 291. Unlike *P. vivax*, *P. falciparum* can invade Duffy negative and positive erythrocytes equally well and is known to utilize a number of distinct receptors 292.

During erythrocyte invasion by merozoite, proteases are known to act at two levels, one at the host cell surface and at the surface or within the apical organelles of the invading parasite 293. Some genes of *P. falciparum* encoding subtilisin-like serine and cysteine proteases have been identified (pfsub-1, pfsub-2, pfsub-3, falcipain 1 etc.) 294, 295.
The Duffy glycoprotein was found to bind a variety of chemokines and is known as the Duffy Antigen Receptor for Chemokines (DARC) \(^{296}\). The function of DARC is yet to be clearly defined. It has been suggested that DARC may permit the erythrocyte to serve as a chemokine “sink” or scavenger, thus limiting activation of leukocytes in the systemic circulation \(^{217,218,296}\). However, it is unclear how long chemokines remain bound to the cell surface or what happens to the chemokines at the end of the erythrocyte lifespan \(^{214}\). In addition, it is unclear as to the importance of this function in inflammatory or infectious disease as Fy (a–b–) erythrocytes do not bind chemokines, although Fy(a–b+weak) erythrocytes bind reduced amounts compared with Fy (a–b+) cells \(^{214}\).

There are some antigens present on the erythrocyte that have proteolytic activity \(^{225}\) and these proteases may aid in the invasion process of the merozoite. The Kell blood group system is one of the most polymorphic antigenic systems in human red blood cells and is composed of 23 Kell antigens including K (K1), k (K2), Kpa (K3), kp (K4), Kpc (K21), Jsa (K6) and Jsb (K7) \(^{220}\). Kell protein has a striking sequence homology with metalloendopeptidases which activate or inactivate bioactive peptides and was recently shown to have proteolytic activity \(^{225,297-299}\). Kell antigens are highly immunogenic and the resulting antibodies can cause severe reactions to transfusion of incompatible as well as causing foetal anaemia and haemolytic disease in new borns \(^{220}\). Depression of the Kell system antigens have been reported in individuals with the rare Kpa (a+b-) phenotype \(^{300}\). An amino acid substitution of Arg281Trp is associated with Kpa antigen causing conformational changes throughout the glycoprotein and may affect its stability or its expression \(^{301}\).

Another red blood cell polymorphism, complement receptor1 (CR1) polymorphism has been noted to influence severity of malaria disease. Human CR1 binds to a major malarial adhesin, the \(P.falciparum\) erythrocyte membrane protein-one (PfEMP-1) \(^{236}\). In malaria, CR1 has been reported to play an important role of both rosette formation phenomenon called resetting in which malaria parasitized erythrocytes bind to
nonparasitized ones and immune complex clearance during malaria infection leading to the pathogenesis of cerebral malaria and severe malaria anaemia respectively. A number of polymorphisms in CR1 gene have been implicated in the pathogenesis and protection of various diseases. However, conflicting results have been obtained from studies in different malaria-endemic regions regarding association of CR1 genetic variants that influences the level of CR1 expression with disease severity.

Study of polymorphisms in ligand binding domains at amino acid I643T (nucleotide T2078C) at the end of short consensus repeats (SCR) 10 at long homologous repeats (LHR) B and at Q981H (nucleotide G3093T) in SCR16 in LHRC showed that the low expression allele encodes a CR1 with greater binding activity due to increase in the positive charge in the ligand binding domain. Another study showed correlation of the Q981H polymorphism with the Hind III RFLP suggesting it to be part of a low CR1 expression haplotype, the gene frequency for this haplotype being highest in the malaria-endemic areas of Asia.

Erythrocyte-associated antigenic polymorphisms or their absence have evolved in the human population to protect against malarial infection. The blood group variants are characteristic of population groups, they have shown to vary with geographical areas, and malaria is documented to be a factor in their selection. In this study we have examined the association of some rare red blood cell antigens like Fya, Fyb, K, Kpa, Kpb and also SNP (Q981H) of CR1 in relation to malaria and ethnicity in Assam State of Northeast India which is endemic for malaria.

5.3 Materials and methods

5.3.1 Study sites

The study was conducted at two study sites: Guabari, a village of Baksa district which lies at the foothills of Bhutan, and at Kondoli in Karbi Anglong foothills of Nagaon
district of Assam. The two study sites may be classified as mesoendemic for malaria. The characteristics of the two study sites have been in chapter 3.

5.3.2 Study participants

Patients were enrolled into the study after they or their accompanying relatives gave full consent. Active as well as passive case detection was followed which was carried out by local health workers in Guabari while at Kondoli it was done by hospital staff. Based on linguistic group affinities, the study population was stratified into two groups namely the speakers of Tibeto-Burman (TB) that included the Bodo-Kachari and Nepalis, the Austro-Asiatic (AA) that comprised the tea tribes who are Mundari speakers and the Indo Europeans (IE) comprised of nontribal Assamese, Bengalis and few Biharis who are the speakers of Indo Aryan languages.\textsuperscript{232, 233} Sampling was done such that the samples were drawn independently and randomly from each group particularly at Guabari that has ethnically mixed population. The characteristics of the study population have been described in detail in Chapter 3.

5.3.3 Sample collection

Blood samples were obtained from individuals irrespective of their \textit{P.falciparum} positive (\textit{Pf}+) status at the time of collection. Inclusion criteria were individuals with \textit{Pf} positivity or with history of \textit{falciparum} malaria. Exclusion criteria were children with age less than 1 year, pregnant women and individuals suffering from any other diseases. \textit{P. falciparum} positivity was checked using Rapid Diagnostic Kits and confirmed by microscopy of thin and thick blood smears. 200ul of blood was collected in EDTA-tubes from both \textit{Pf}+ positive patients as well as from healthy controls. Individuals were categorized according to their disease symptoms and frequency of malaria incidence which were simultaneously recorded. Complicated malaria was defined following WHO guidelines (2000). Uncomplicated malaria included fever, headache, body ache and other mild symptoms or asymptomatic cases with parasitaemia of \(\leq 5000/\mu l\) of blood. On the basis of number of clinical episodes of
malaria, the participants were classified as infrequent (≤ 2 episodes) or frequent malaria (> 2 episodes) groups as detailed in our earlier work. The study was approved by the Tezpur University Ethical Committee (Resolution number 3 dated 13/06/06).

5.3.4 Serological tests for the presence of RBC antigens Fya, Fyb, K, Kpa and Kpb

Fresh drawn blood samples were collected in anticoagulated tubes (n=304). The red blood cells were washed in PBS. The washed RBCs were then incubated with respective antibody (anti Fya, anti Fyb, anti K, anti Kpa and anti Kpb; DiaMed, Switzerland). Agglutination was viewed microscopically.

5.3.5 Validation of the micro agglutination assays of Fya and Fyb by molecular genotyping

200μl of blood was collected in EDTA-tubes and genomic DNA extraction was carried out using Qiagen blood extraction kit (Qiagen, Germany). The Fy gene was then amplified by polymerase chain reaction and then digested using BstNI restriction enzyme as described. After digestion the fragments were then analysed on 3% agarose gels pre-stained with Ethidium Bromide.

5.3.6 CR1 Q981H genotype determination

Genomic DNA was prepared from 200μl of EDTA blood using using Qiagen blood extraction kit according to the manufacturer’s instructions (Qiagen, Germany). The frequencies of the CR1 Q981H polymorphism in the study population was determined by following the protocol of Thomas et al. (2005). PCR amplification for the Q981H SNP was carried out using allele specific primers. Sequences for primer pair used for amplification are 5’-GCTACATGCACGTTGAGACCT-TAC-3’ (forward primer) and 5’-AGCAAGCATACA-GATTTTCCCC-3’ (reverse primer). Amplification using these primers yielded a 366 base pair product which was analyzed
by electrophoresis in a 2% agarose gel stained with ethidium bromide and observed by ultraviolet transillumination. These PCR products then were further restricted digested. For restriction fraction length polymorphism determination, 18 ml PCR product, 2.5 ml buffer, 2.5 ml bovine serum albumin and 2.0 ml of BstNI were digested at 60°C for 2 hours and analyzed on a 3% ethidium bromide gel. Using this protocol, the wild-type 3093G residue yielded fragments of 54, 91 and 221 base pairs, while the 3093T mutant yielded fragments of 54 and 312 base pairs.

5.3.7 Data analysis

The data was analysed using Excel Stat Software, 2012 version. Statistical analysis for the differences in distribution of blood group antigens and genotypes between malaria cases and controls was carried out using Fisher's Exact test. Statistical significance was denoted by P < 0.05. The chi-square test was performed to evaluate whether the allele frequencies of the populations are in Hardy-Weinberg equilibrium.

5.4 Results

Total of 304 samples were analysed for microagglutination assays for the presence or absence of Fya, Fyb, K, Kpa, Kpb (Figure 5.1). The average age of the study population was 24.1 years (range: 2-70 years). The characteristics of the study population have been given in Table 5.1. In the samples typed for ABO blood groups (n=434), it was seen that blood group B was the most prevalent (33.87%), group A being the next prevalent blood group (27.88%; Table 5.1).

When the different blood groups were analysed it was observed that none of them were found to be associated with Pf status. However, regression analysis done for the different blood groups along with Pf status and sex showed that Pf negative females were positively associated with blood group B (p=0.05).
<table>
<thead>
<tr>
<th>Age</th>
<th>24.1 (Range 1.5-70 years)</th>
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<tbody>
<tr>
<td>Frequent malaria episodes</td>
<td>55</td>
</tr>
<tr>
<td>Infrequent malaria episodes</td>
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<td>Ethnic groups</td>
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<td>TB</td>
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<tr>
<td>IE</td>
<td>57</td>
</tr>
<tr>
<td>Blood group (n=434)</td>
<td>A+</td>
</tr>
<tr>
<td>Overall population</td>
<td>121</td>
</tr>
<tr>
<td>AA</td>
<td>49</td>
</tr>
<tr>
<td>TB</td>
<td>56</td>
</tr>
<tr>
<td>IE</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 5.1: Characteristics of the study population

Blood group B was the most prevalent (33.87%) and group A the next prevalent blood group (27.88%) in the study population. AA-Austro-Asiatic; TB- Tibeto-Burman; IE- Indo Europeans
Figure 5.1: Microagglutination of RBCs (A) Agglutination occurred with anti Kpa (B) No agglutination occurred with anti Kpa

5.4.1 Fya and Fyb with malaria

Fyb was found to be more prevalent at 96.09% than Fya at 49.66% of the study population (Figure 5.2). One third of the samples were validated by genotyping studies and 91.04% corresponded with the microagglutination assays. From genotyping data, Fyb was also found to be more prevalent (96.26%) than Fya (54.20%) in the studied samples.

From microagglutination data, the two phenotypes Fy(a+b+) and Fy(a-b+) were observed to occur more frequently (45.4% and 44.6% respectively) than Fy(a+b+) and Fy(a-b-) phenotypes. The frequency of Fy antigens was analysed in relation to malaria symptomology: fever and headache, and with frequency of malaria episodes. It was
noted that both Fya and Fyb were found to be negatively associated with fever (p=0.016 and p<0.0001 respectively). There was an association with headache but was not statistically significant. Fya and Fyb were found to be protective where they were positively associated with infrequent malaria episodes (Fishers test p=0.010 and p=0.016 respectively).

Fy(a+b-) was the least prevalent phenotype. It was observed that 9.7% of the study population had the Fy(a-b-) phenotype. When data was further stratified into different ethnic groups: AA, TB and IE, an interaction among the three ethnic groups and the different phenotypes was observed (Fishers test p=0.008). Fy(a+b+) and Fy(a-b+) were observed to occur more frequently in all the three ethnic groups. Fy(a+b-) phenotype was present marginally for AA, but was absent for both TB and IE (Figure 5.3).

Figure 5.2: Comparison of the frequency of Fya and Fyb antigens with malaria episodes.
Fya and Fyb were found to be protective where they were positively associated with infrequent malaria episodes (Fishers test $p=0.010$ and $p=0.016$ respectively). Frequent malaria episodes; Infrequent malaria episodes.

**Figure 5.3:** Frequency of the four phenotypes [Fy (a+b+), Fy (a+b-), Fy(a-b-) and Fy(a-b+)] in the three ethnic groups: AA, TB and IE

The phenotypes Fy(a+b+) and Fy(a-b+) were the most prevalent in the three ethnic groups. Fy(a+b-) phenotype was present marginally for AA, but was absent for both TB and IE. AA-Austro-Asiatic; TB- Tibeto-Burman; IE- Indo Europeans;

5.4.2 K, Kpa and Kpb with malaria

K+ was seen in most of the samples (87.66%), also when stratified into frequent and infrequent malaria episodes (Figure 5.4). Kpa+(24.66%) was more prevalent than Kpb+ (13.37%) in the study samples. Kpa+ prevalence did not change with frequency of malaria episodes but a lowered percentage for Kpb+ in the infrequent malaria group.
was noted. K antigen was seen to be negatively associated with symptoms of malaria, fever (p=0.0220) and headache (p=0.033). Kpa was found to be negatively associated with fever (p=0.016). No association of malaria episodes with K, Kpa and Kpb was observed. An interaction of Kpa with the three ethnic groups was noted, Kpa being the least in AA group (Fishers test p<0.001). Kp(a-b-) phenotype was the most prevalent, whereas Kp(a+b+) was the least observed in the study population (Figure 5.5). Kp(a-b-) phenotype was also observed the maximum in the three ethnic groups, with AA having the maximum prevalence (83.07%).

When the different antigens (Fya, Fyb, K, Kpa and Kpb) were analysed with frequency of malaria episodes, an interaction between Fya and Kpa was observed (p=0.01). By itself, Fya was seen to be negatively associated with frequent malaria episodes, but together with Kpa (i.e. Fya*Kpa) was seen to be positively associated with frequency of malaria disease.

![Figure 5.4: Frequency of K, Kpa and Kpb antigens in the different malaria groups.](image-url)
K was seen in 87.66% samples. Kpa (24.66%) was more prevalent than Kpb (13.37%). No association of malaria episodes with K, Kpa and Kpb was observed. Freq M- Frequent malaria episodes; InFreqM-Infrequent malaria episodes.

Figure 5.5: Frequency of the four phenotypes [Kp(a+b+), Kp(a+b-), Kp(a-b-) and Kp(a-b+)] in the three ethnic groups.

Kp(a-b-) phenotype was also observed the maximum in all the ethnic groups, with AA having the maximum prevalence (83.07%). AA-Austro-Asiatic; TB- Tibeto-Burman; IE- Indo Europeans;
5.4.3 CR1 polymorphism

5.4.3.1 Overall frequency of Single Nucleotide Polymorphism of CR1 (Q981H) gene

A total of 282 samples were studied, where we found median age of the participants was 27.6 (Range: 2-70 years). These samples were examined for the frequency of SNP in CR1 (Q981H) (Table 5.2). When the genotype frequencies of the CR1 locus were compared among AA, TB and Khasis, it was noted that the alleles of CR1 (Q981H) were seen predominantly in heterozygosity. The homozygous mutant genotype was seen to be absent in the Khasis, though the frequency of the wild genotype (Q/Q) was comparable with that of the other two populations (Fishers Test p=0.040; Table 5.2).

<table>
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<th>Genotypes</th>
<th>Ethnicity</th>
<th>n</th>
<th>Q/Q</th>
<th>H/H</th>
<th>Q/H</th>
<th>p-value</th>
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<tr>
<td></td>
<td>AA(plains)</td>
<td>63</td>
<td>11</td>
<td>12</td>
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</tr>
<tr>
<td></td>
<td>TB</td>
<td>63</td>
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<td>12</td>
<td>35</td>
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<tr>
<td></td>
<td>Khasis</td>
<td>30</td>
<td>9</td>
<td>0</td>
<td>21</td>
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Table 5.2: Comparison of the genotype frequencies of the CR1 (Q981H) polymorphism in the three ethnic groups: AA, TB and Khasis

Three ethnicities were considered (AA, TB and Khasis) and the others which consist of mixed ethnicities were not taken. Analysis was performed to check the interactions in
the three genotypes of the CR1 gene by Fishers exact test. AA-Austro-ASIatic; TB-Tibeto-Burmans; n-number of samples

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>n</th>
<th>Q/Q</th>
<th>H/H</th>
<th>Q/H</th>
<th>p-values</th>
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<tbody>
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<td>31</td>
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<tr>
<td>Control</td>
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<td>92</td>
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<td>ns</td>
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<tr>
<td>Non severe malaria</td>
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<td></td>
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<tr>
<td>Frequent malaria</td>
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<td>51</td>
<td>ns</td>
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<td>13</td>
<td>3</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.3: Comparison of the genotypes of CR1 (Q981H) between the different malaria groups

Comparison of the frequency genotypes of the SNP of CR1 (Q981H) between disease and control between by Fisher’s test, it noted as that homozygous mutant (H/H) was found to be significantly higher and Q/H genotype lower in disease group than control (Fishers test p=0.003). ns- Not significant
5.4.3.2 Association of SNP of CR1 (Q981H) with malaria

We analysed the frequency of SNP in patients having malaria against healthy controls, disease severity: complicated malaria and uncomplicated malaria and also with respect to frequency of malaria episodes.

Comparing the genotype frequencies of the SNP of CR1 (Q981H) between disease and control, it was seen that homozygous mutant (H/H) was found to be significantly higher and Q/H genotype lower in disease group than control (Fishers test p=0.003; Table). Stratifying the data on the basis of disease severity, it was seen that the genotypes were comparable in the two groups with the mutant allele being in the heterozygous condition was more predominant (Table 5.3). H/H genotype was found to be higher in participants having frequent malaria episodes (18.51%) than those that are infrequent (6%) but was not found to be significant (Fishers test p=0.154). It was also noted that when data was stratified on the basis of ethnicity (AA and TB) with respect to frequency of malaria episodes, H/H genotype was significantly lower (Fishers test p=0.05) in infrequent malaria group than in frequent malaria group in both the ethnic groups.

The frequency of mutant allele (H) was noted to increase as one moves away from Africa towards Asia, and we also found that the frequency of mutant allele in Assam is also high (H=0.75). Many alleles were not in Hardy-Weinberg Equilibrium this implies that they are still in the process of evolving so as to reach equilibrium.
5.5 Discussion

Several RBC polymorphisms, including those linked to glucose-6-phosphate dehydrogenase, pyruvate kinase, complement receptor-1 and haemoglobinopathies, have a role in the clinical outcome of malaria. It has been suggested that the presence of erythrocyte-associated antigenic polymorphisms or their absence have evolved in the human population to protect against malarial infection. The blood group variants are characteristic of population groups, and can show conspicuous geographic patterns.

The spatial distribution of the Fy antigens was found to differ in different geographical areas. Frequencies of FY*A prevalence was shown to increase with distance from Africa and Europe, becoming dominant across south-east Asia, including those areas where *P. vivax* endemicity is highest. In our study we observed that Fyb was more prevalent of the two antigens in our population. The global maps in a study by Howes et al. (2011) showed that the distribution and frequency of the FY*B allele is highly restricted, with highest prevalence found in Europe and parts of the Americas, with further patches of increased prevalence in areas buffering the region of FY*BES (erythroid silent), predominance in sub-Saharan Africa.

Fy (a+b+) phenotype was found to be the most prevalent in 45.4% of the cases in the present study and a similar finding by Mohanty et al. (2011) also found that this phenotype was the most prevalent but the frequency was much higher in tribal (Bhil group; 70.4%) than in non tribal group (54.8%) of India. In Europe, this phenotype was seen to be the most prevalent but in Asia Fy (a+b-) was the most prevalent. Erythrocytes expressing Fya had 41-50% lower binding of *P. vivax* compared with Fyb cells. Individuals with the Fy(a+b-) phenotype have a 30-80% reduced risk of clinical *vivax* but not *falciparum* malaria.

We have found an association of Fya and Fyb with fever, one of the symptoms of
malaria but it was a negative association. It has been suggested that DARC may permit the erythrocyte to serve as a chemokine “sink” or scavenger, reducing the levels of circulating inflammatory chemokines, thus dampening systemic leucocyte activation. In DARC-transfected cells, DARC is internalized following ligand binding and this led to the hypothesis that expression of DARC on the surface of erythrocytes, endothelial, neuronal cells and epithelial cells may act as a sponge and provide a mechanism by which inflammatory chemokines may be removed from circulation as well as their concentration modified in the local environment. So, with the levels of cytokines being reduced, assuming pyrogens IL-1β and IL-6 are those cytokines getting reduced, accordingly fever also gets reduced. Although, activation of TLRs by GPI anchors lead to proinflammatory cytokines being produced including IL-1β and IL-6, and because of these pyrogens, fever occurs. Therefore, probability of having fever is dependent on various factors.

There are two conflicting observations in DARC-deficient mice exposed to various inflammatory stimuli. In one study DARC knockout (KO) animals received systemic LPS and responded by a marked increase in neutrophil infiltrate in the lungs and livers as compared to the wild type controls. Lee et al. (2003), showed that DARC KO mice have significantly less leucocyte infiltrate in the bronchoalveolar lavage in response to chemokine instilled into pulmonary airspace. In the present study, Fya and Fyb were found to be protective from having frequent falciparum malaria episodes.

Though many studies have found no association of duffy antigens with P.falciparum infections, however in a study by Beiguelman et al. (2003) observed lesser mean number of malarial episodes in Fy(a-b-) individuals as compared to other positive Duffy phenotypes. Very less is known about K, Kpa and Kpb antigens and their association with P.falciparum malaria. K antigen was prevalent in most of the study.
participants though other studies have shown that a small percentage of the population have this antigen. Fya was seen to be negatively associated with frequent malaria, however, it was seen that there is an interaction between Fya*Kpa where they are positively associated with frequent malaria episodes indicating that Kpa is the deciding antigen. We proposed that kell antigens may facilitate in merozoite invasion process by providing the proteolytic activity required for cleavage. When Kpa allele is not transported to the surface of the erythrocyte, it does not get expressed and so therefore no enzymatic activity in aiding the cleavage occurs and hence it is important in invasion process. The role of the kell antigen in erythrocyte invasion by falciparum malaria parasite needs to be further investigated.

Selection favouring heterozygosity conferred advantages that have been documented as in case of sickle cell haemoglobinopathy, which affords protection against mortality from falciparum malaria in heterozygous individuals, balancing the severe consequences of the disease in homozygous individuals. Our data indicated that CR1 981H/Q genotype protected from falciparum malaria. It was also observed in our earlier work on TLR which indicated TLR9 1486T/C heterozygosity to be protective, where it was negatively associated with complicated malaria. Increased frequency of the CR1 homozygous mutant genotype (H/H) may also increase the probability of having falciparum malaria and also frequent malaria episodes, suggesting balancing selection on CR1 (Q981H) allele.

In the present study, the heterozygous genotype (Q981H) was seen to be the most prevalent. Similar finding was observed where a high rate of the heterozygous (Q981H) gene state in PNG. Also, when our data was stratified into different ethnic groups, it was seen here that the heterozygous genotype was the most prevalent, but what was unusual was that homozygous mutant (H/H) was completely absent in Khasis. This shift from the wild (QQ) to the heterozygous type (QH) indicates that this mutation of the CR1 gene may be still evolving in these locations.
CR1 Q981H has been identified in Caucasians linking to constitutive Erythrocyte-CR1 (E-CR1) expression levels. Erythrocytes having low CR1 expression have been shown to form reduced number of rosettes with Pf-infected cells. In Africa, rosetting has been shown to correlate with disease severity. Since erythrocytes having low CR1 copy numbers form fewer rosettes, it has been postulated that low E-CR1 might protect from severe malaria. Though in the present study, we did not observe any association of the CR1 SNP with severe malaria.

Frequency of blood group B is the highest in our study population, though studies from different parts of India have reported that frequency of blood group O is the highest. It has been hypothesized that blood group O offered a survival advantage during infection with P.falciparum because it has been demonstrated that there is an association between O blood group and lower rosetting capacity. Group O has been observed to be more prevalent in sub Saharan Africa, Turkey, Persia etc. where falciparum malaria is endemic. Many studies on patients from Zimbabwe, Gabon and Ethiopia showed a significant association of group A with severe malaria, in contrast a study by Panda et al (2012) in Odisha India, showed that the frequency of blood group B was associated with severe malaria. We did not find any association of A, B or O groups with falciparum malaria though Assam is endemic for P.falciparum malaria, this variation may be a population specific phenomenon. Though, we did observe that females who were Pf negative are positively associated with group B.

In conclusion, it was seen that there was an association of Fya and Fyb antigens with malaria symptomology. An interaction between Fya*Kpa was observed where they are positively associated with frequent malaria episodes. The frequency of the various RBC antigens varied with different ethnicities of our study population. Our data indicated that CR1 981H/Q genotype to be protective from having falciparum malaria. It was also observed that increased frequency of the CR1 homozygous mutant
genotype (H/H) may also increase the probability of having *falciparum* malaria and also frequent malaria episodes. Homozygous mutant (H/H) was completely absent in the Khasis. The shift from the wild (QQ) to the heterozygous type (QH) in our study population indicates that this mutation of the CR1 gene may be still evolving in these areas.