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Single nucleotide polymorphisms of *ADRB2* gene and their association with susceptibility for *Plasmodium falciparum* malaria and asthma in an Indian population

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ABSTRACT

The essential route to blood parasitaemia in malaria, erythrocyte invasion is facilitated by activation of the G-protein coupled receptor signaling pathway mediated by the β2-adrenoreceptor as one of the proteins on the surface of red blood cells. The effectiveness of bronchodilators and inhaled corticosteroids in the clinical treatment for asthma patients also depend on polymorphisms in the β2-adrenoreceptor gene (*ADRB2*). In a case control study, individuals affected by *Plasmodium falciparum* malaria, asthma and controls were tested for association of six *ADRB2* single nucleotide polymorphisms (SNPs) *viz.* rs1042711, rs1801704, rs1042713, rs1042714, rs1042717 and rs1042718, by direct DNA sequencing. The rs1801704 locus was significantly associated with malaria when compared against controls. The rs1042713 polymorphism was associated with forced expiratory flow between 25% and 75% of the FVC in asthma patients, pre ($p = 0.048$) and post ($p = 0.038$) treatment measurements. Predicted haplotype of the six SNPs computed from genotype data showed T-T-A-C-G-C conferred significant risk of malaria ($p = 0.02$) whereas T-T-A-C-G-A was associated with risk of asthma ($p = 0.02$). The haplotype T-T-G-C-G-C was protective against both malaria ($p = 0.02$) as well as asthma ($p = 0.026$) and C-C-G-G-G-C was protective uniquely for asthma ($p = 0.04$). A significant outcome was that all variant alleles at the SNP loci were part of the haplotype conferring resistance to malaria disease and asthma, except rs1042713 and rs1042718 which showed very high frequency in asthma. The pairwise linkage disequilibrium (LD) estimates showed a distinct LD block of all SNP loci ($D' = 1$ or >0.8) in malaria patients. This characteristic haplotype block was disrupted in the controls due to non-significant pairwise LD of the SNP loci; and a more extensive disruption of the block was noted in asthma patients. The study provides evidence for the proposed role of β2-adrenoreceptor mediated molecular mechanisms in etiology of malaria, as well as asthma disease and drug response, for further clinical and therapeutic application studies.

1. Introduction

Malaria is a major cause of mortality and morbidity in Southern India. The total number of malaria cases reported in the state of Karnataka, India, during the year 2010 was 44,122; with 7771 of the cases were infection by *Plasmodium falciparum* (*Pf*) (NVBDCP, 2011). Extensive investigations on the role of host genetics in susceptibility to *Pf* malaria and the strong selective forces exerted on the human genome by the parasite in endemic regions, have identified association of several genes with different malarial phenotypes and interacting factors promoting severity of malaria i.e., parasitaemia, parasite induced inflammation, anemia and sequestration of parasitized erythrocytes in brain microvasculature among others (Driss et al., 2011). Genetic variations of human RBCs that appear to have evolved due to natural selection by malaria which include: (a) the sickle cell phenotype (due to HbS), β-thalassaemias caused by mutations in β-globin gene, and a variant form

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of hemoglobin HbC, (b) variations in a red blood cell enzyme glucose-6-phosphate dehydrogenase, (c) ovalocytosis – a defect of a structural protein that helps to maintain the normal shape of the red blood cell, and (d) the Duffy antigen, play a role in disease susceptibility (Kwiatkowski, 2005; Fortin et al., 2002). There is also evidence that the disease severity of malaria is determined by genetic factors that regulate the production of inflammatory mediators such as tumor necrosis factor and nitric oxide which are critical for antiparasitic immunity but harmful in excess (Weatherall and Clegg 2002; Sinha et al., 2008). The evidence that the malarial infection of *Pf* is linked to human chromosomal region 5q31–q33 was first provided by linkage analysis in a sub-Saharan population, a study performed in nonparametric format which included 153 sibs from 34 families with sib-pair linkage analyses between malaria parasite blood infection levels and chromosome 5q31–q33; suggesting complex genetic factors in the locus playing a central role in the mechanisms controlling *Pf* parasitaemia (Rihet et al., 1998). The 5q31–q33 region contains nine candidate genes for immune responses and pathogenesis by *Plasmodium* species. Among them *IL9*, *IL13*, and *ADRB2* were close to the peaks provided by linkage analyses and were of particular interest. The study of proteins involved in erythrocyte mechanisms of malarial infection and the cellular signaling via the erythrocyte β2-adrenergic receptor (β2-AR) and heterotrimeric guanine nucleotide-binding protein (G-alpha-s) in the entry of merozoites into erythrocytes (Harrison et al., 2003) demonstrated the role of host factors during the blood stage infection. It has been demonstrated that the entry of the human malarial parasite *Pf* into the erythrocyte and its internalization in a parasitophorous vacuolar membrane (PVM) was regulated by signaling of the β_2-AR and GαS on its surface (Harrison et al., 2003; Murphy et al., 2006).

The mechanism of signal transduction from β2-AR and the receptor dysfunction in asthma pathogenesis has been extensively investigated. Asthma is a common chronic inflammatory disease of airways that affects about 300 million people worldwide. It has been reported that India has approximately 15–20 million asthma patients and 10–15% of Indian children between the ages of 5 and 11 years show symptoms of asthma. A median prevalence of about 2.4% is also reported in adults of over 15 year of age (Jindal, 2007). *ADRB2* gene is one of the 108 genes that are studied for their association with asthma in Indian population and is also extensively studied worldwide (Bijanzadeh et al., 2011). Two single nucleotide polymorphisms (SNPs) in *ADRB2* rs1042713 and rs1042714 corresponding to amino acid change Arg16Gly and Gln27Glu, and their haplotypes have been extensively investigated because of their high prevalence in the populations and because of their potential clinical correlation with bronchial hyper responsivity. *In vitro* studies have suggested that these polymorphisms have potential clinical correlations with bronchial hyper responsiveness and functional significance (Green et al., 1993, 1994, 1995; D'amato et al., 1998). The homozygosity at the Arg16 allele is associated with a worsening of lung function in patients who regularly use short or long acting β agonists (Israel et al., 2000, 2004; Wechsler et al., 2006). The β2-AR is regulated by a negative feedback loop that reduces the cell's responsiveness for the long term occupation by an agonist and also the number of receptors is reduced after prolonged exposure. Several *ADRB2* polymorphisms are in linkage disequilibrium and various haplotypes have been reported to be associated with the pathogenesis and also with drug response with often conflicting reports (Liggett, 2002; Dishy et al., 2001; Drysdale et al., 2000; Goldstein et al., 2003; Kukreti et al., 2005; Thomsen et al., 2012). Other SNPs in *ADRB2* rs1042711 and rs1801704 that are in tight linkage disequilibrium significantly alter the receptor expression and has been implicated in human metabolic disorders and asthma. The SNP rs1042711 located in the short open reading frame of *ADRB2* gene, termed the 5′ leader cistron causes the missense mutation leading to the change of wild type amino acid cysteine to arginine at the 19th position of the polypeptide. In this background, the possibility that the *ADRB2* gene polymorphisms may alter the disease susceptibility to malaria assumes significance (Fu et al., 2011). Towards this, we have designed and present a case control study of the *Pf* malaria patients on the one hand and a group of asthma patients also recruited from the same ethnic background, along with matched normal controls to investigate the effect of the alleles, genotypes, and haplotypes of six *ADRB2* gene coding region SNPs in the pathogenesis and susceptibility to the diseases.

2. Materials and methods

2.1. Recruitment of participants and collection of samples

The study was conducted after obtaining due clearance of the Institutional Ethical Committee of Kasturba Hospital, Manipal. A total of 450 participants, that included 150 patients diagnosed with malaria due to infection with *Pf* and 150 healthy controls and 150 asthma patients were recruited for the study. All participants were drawn from the population of Dakshin Kannada and adjoining districts in Karnataka, south India and were recruited after due procedures of obtaining their informed consent. The patients were recruited from among the malaria cases reported- at the Kasturba Hospital, Manipal; Kasturba Hospital, Mangalore and their affiliated hospitals/clinics. The asthma patients were recruited from among the patients admitted at the Department of Pulmonary Medicine at Mangalore with severe stable bronchial asthma. The pulmonary function tests were performed using ATS standardized spirometers with inclusion of the following markers: forced vital capacity (FVC%), forced expiratory volume (FEV_1%), ratio between the FEV_1 and the FVC and forced expiratory flow between 25% and75% of the FVC (FEF 25–75%). All patients underwent spirometry test before and after the drug administration. The age of the participants ranged from 12 to 65 years. The mean age was 31.13 years for malaria patients, 33.4 years for asthma patients and 35.21 years for controls. The proportion of males was 126/150 (84%) in the malaria patients group, 62/150 (41.3%) in the asthma patients and 121/150 (80.7%) in the control group. Malaria was defined as fever with a measurable temperature of $\geqslant 37$ °C, a platelet count lower than that of the normal range 150,000–400,000/mm^3, a hemoglobin concentration less than that of the normal levels (males: 13–17 g/dl, females: 12–15 g/dl). The presence of parasites was detected by microscopy examination by using the new quantitative buffy coat technique as developed by Becton–Dickinson and validated by performing PCR based method of genes specific for *Pf* and *P. vivax* (*Pv*) respectively (Berwal et al., 2006; Londono et al., 2009). Age - matched non-malarial controls i.e., healthy individuals from the same ethnic background living in the same environment were recruited on the basis of an interview on their clinical history that excluded asthma. Controls for the study were screened with PCR based assays for the presence of malaria parasite infection at the time of sample collection. Other criteria for controls were good physical fitness, no symptoms of malaria and asthma, as well as other diseases in past six months, and the personal history that excluded malaria and asthma. The ethnicity of participants was determined on the basis of shared language, history, food habit and the shared endemic habitat region. Five milliliters of venous blood was collected from each participant in ethylenediaminetetraacetic acid vacutainers to isolate genomic DNA and to screen for variations in the *ADRB2* gene.

2.2. Genotyping

Genomic DNA was extracted following phenol/chloroform extraction method after isolating peripheral lymphocytes from